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CLINICAL STUDY

Early post-transplantation hypophosphatemia is associated with elevated FGF-23 levels

Andrea Trombetti[1], Laura Richert[1], Karine Hadaya[2,3], Jean-Daniel Graf[4], François R Herrmann[1], Serge L Ferrari[1], Pierre-Yves Martin[2,3] and René Rizzoli[1]

[1]*Division of Bone Diseases,* [2]*Division of Nephrology, Department of Internal Medicine,* [3]*Service of Transplantation, Department of Visceral Surgery and* [4]*Central Laboratory of Clinical Chemistry, University Hospitals and Faculty of Medicine of Geneva, Rue Gabrielle-Perret-Gentil 4, 1211 Geneva 14, Switzerland*

(Correspondence should be addressed to A Trombetti; Email: andrea.trombetti@unige.ch)

Abstract

Background: We examined the hypothesis that high FGF-23 levels early after transplantation contribute to the onset of hypophosphatemia, independently of parathyroid hormone (PTH) and other factors regulating phosphate metabolism.

Methods: We measured serum phosphate levels (sPi), renal tubular reabsorption of Pi (TmPi/GFR), estimated GFR (eGFR), intact PTH (iPTH), calcitriol, intact (int) and C-terminal (Cter) FGF-23, dietary Pi intake and cumulative doses of glucocorticoids in 69 patients 12 days (95% confidence interval, 10–13) after renal transplantation.

Results: Hypophosphatemia was observed in 43 (62%) of the patients 12 days after transplantation. Compared with non-hypophosphatemic subjects, their post-transplantation levels of intact and CterFGF-23 were higher (195 (108–288) vs 48 (40–64) ng/l, $P<0.002$ for intFGF-23; 205 (116–384) vs 81 (55–124) U/ml, $P<0.002$, for CterFGF-23). In all subjects, Cter and intFGF-23 correlated inversely with sPi ($r=-0.35$, $P<0.003$; -0.35, $P<0.003$, respectively), and TmPi/GFR ($r=-0.50$, $P<0.001$; -0.54, $P<0.001$, respectively). In multivariate models, sPi and TmPi/GFR were independently associated with FGF-23, iPTH and eGFR. Pre-transplant iPTH levels were significantly higher in patients developing hypophosphatemia after renal transplantation. Pre-transplant levels of FGF-23 were not associated with sPi at the time of transplantation.

Conclusion: In addition to PTH, elevated FGF-23 may contribute to hypophosphatemia during the early post-renal transplant period.

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Introduction

Hypophosphatemia related to decreased renal tubular reabsorption is a common complication following kidney transplantation, and is usually limited to the early post-transplant period (1, 2). However, in some cases, hypophosphatemia persists for more than 10 years after transplantation (3).

Persistently elevated parathyroid hormone (PTH) levels have long been considered to be the cause of post-transplant hypophosphatemia, but hyperparathyroidism does not appear to be the only mechanism. Decreased renal tubular reabsorption may occur, despite low levels of PTH, and hypophosphatemia can persist even after elevated PTH levels have normalized (4–7). Furthermore, even if hypophosphatemia and hyperparathyroidism stimulate calcitriol ($1,25\text{-}(OH)_2\text{-}D_3$) synthesis, calcitriol levels are often inappropriately low following renal transplantation, despite normal or mildly impaired allograft function. FGF-23 decreases renal tubular reabsorption of Pi and inhibits renal 1α-hydroxylase, which leads to decreased calcitriol synthesis. Recent studies suggest that high levels of FGF-23 encountered in terminal renal failure persist after kidney transplantation and may contribute to early post-transplant hypophosphatemia (8–10). It is unclear whether FGF-23 and PTH act alone or together in the development of hypophosphatemia. On the other hand, factors other than PTH and FGF-23 – such as renal function and dietary phosphorus intake – may modulate serum phosphate (sPi) levels (11, 12). This combination of factors was rarely considered in previous studies. We therefore tested the hypothesis that increased FGF-23 levels may be associated with decreased sPi levels and TmPi/GFR, regardless of renal and parathyroid function, dietary phosphate intake and corticosteroid treatment early after renal transplantation. We also tested the hypothesis that involvement of FGF-23 is mostly limited to a short period after transplantation.

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Subjects and methods

Patients

The protocol of this prospective monocenter observational study was approved by the ethics committee of the Geneva University Hospitals. Each patient signed an informed consent form.

Renal transplant recipients during the early post-transplant period and 1 year after transplantation were studied. We investigated 69 renal transplant recipients (37 men and 32 women) 12 days (95% confidence interval (CI), 10–13) post-transplantation, just before their discharge from the Transplant Unit. Renal transplant recipients were consecutively recruited between June 2005 and January 2009. Inclusion criteria were: age >18 years and a functioning renal transplant (creatinine clearance >30 ml/min). There were no exclusion criteria. Seven of the 69 evaluated during the early post-transplantation period could be evaluated before transplantation as well.

Another group of 24 patients were assessed 1 year after transplantation. During this year, all of them received bisphosphonates (pamidronate or alendronate), oral calcium (1 g/day) and vitamin D (800–1000 IU/day) supplements, in a systematic program aimed at preventing glucocorticoid-induced osteoporosis.

Clinical assessment

Height and weight were determined and body mass index was calculated.

Dietary calcium, protein and phosphate intakes were assessed by a dietician, using a record of the food consumed during the 3 days before blood sample was taken. Time from first dialysis to study inclusion was calculated. The daily dose of glucocorticoids at the time of evaluation and the cumulative dose since transplantation were also recorded.

Serum and urinary measurements

Intact (int) (full length) and C-terminal (Cter) FGF-23 were measured (13). The intFGF-23 ELISA Kit (Kainos Laboratories, Inc., Tokyo, Japan) is a two-site ELISA, which recognizes only full-length biologically active FGF-23. The CterFGF-23 assay (Immutopics, San Clemente, CA, USA) detects both intFGF-23 and its Cter fragments. FGF-23 levels were measured in duplicate after a single freeze–thaw cycle in batched assays. Reported inter- and intra-assay coefficients of variation (CV) for the Cter assay were 7.3 and 5.0%, respectively. For intFGF-23, the inter- and intra-assay CV ranged from 6.0–11.2% and 4.9–7.7%, for the higher and lower values of FGF-23, respectively. When the upper limit of detection of this kit was reached, samples were diluted appropriately to ensure adequate measurements. The slope of diluted samples paralleled the standard curves.

Fasting plasma and urinary calcium, phosphate, creatinine and total alkaline phosphatase were measured simultaneously using an automatic analyzer. Plasma calcium was adjusted for albumin levels (14). The following parameters were calculated: i) estimated GFR (eGFR), using the Cockroft and Gault formula; ii) fasting urinary calcium-to-creatinine ratio (bone resorption index, BRI); iii) renal tubular reabsorption of phosphate (TmPi/GFR) and calcium (TRCaI), calculated as described elsewhere (15, 16). Serum intact PTH (iPTH) and osteocalcin were measured using two-site chemiluminescent immunometric assays (Elecsys R, Roche). The specific urinary marker of bone resorption deoxypyridinoline was measured by fluorescence emission after acid hydrolysis and HPLC separation (Bio-Rad System), and expressed as a creatinine ratio. Calcifediol (25-OH-D_3) and calcitriol were determined by chemiluminescence assays (Immunodiagnostic Systems Limited, IDS, Boldon, UK). Regarding the patients studied early after transplantation, samples were collected after transplantation before administration of bisphosphonates and calcium/vitamin D.

All 69 renal transplant recipients underwent pre-transplant evaluation of serum PTH levels. Only seven of them were tested for FGF-23 twice, i.e. before and early after transplantation.

Statistical analysis

The summary descriptive statistics are the median and the 95% binomial CI (95% CI). The binomial methods applied to obtain CI make no assumptions about the underlying distribution of the variable. Continuous variables in hypophosphatemic patients and patients with normal phosphate levels were compared by non-parametric Mann–Whitney *U* test. Proportions were compared by Fisher's exact test. The strength of bivariate association between sPi, TmPi/GFR and recorded variables were determined by Spearman's rank correlation. Associations between sPi, TmPi/GFR and recorded variables were also determined by multiple (backward stepwise) linear regression analyses.

We normalized non-Gaussian variables using simple mathematical transformations. As this method was inefficient for normalizing TmPi/GFR, we performed normalization by ranking. The Shapiro–Francia W test and Skewness/Kurtosis tests for Normality 'sktest' were used to verify the normality of the distributions. Standard multiple regression models were constructed using serum PTH, albumin-corrected calcium levels, dietary phosphate intake, renal function, cumulative doses of glucocorticosteroids and FGF-23 (intact and CterFGF-23, in two different models) as covariates. Adjusted regression coefficients and CI were computed for each covariate. An interaction between FGF-23 levels and serum PTH was sought by adding an

Table 1 Characteristics of the patients evaluated in the early post-renal transplantation (Tx) period (12 days (95% CI, 10–13)) and 1 year thereafter.

Characteristics	At the time of Tx	One year after Tx
Number of subjects (women; *n* (%))	69 (46)	24 (25)
Age (years; median (95% CI))	52 (46–58)	50 (40–57)
Duration of dialysis (years; median (95% CI))	2.3 (1.6–2.9)	2.1 (1.5–2.8)
Dietary phosphate intake (mg/day; median (95% CI))	975 (915–1097)	1070 (1030–1313)
Dietary calcium intake (mg/day; median (95% CI))	903 (781–1019)	995 (730–1136)
Dietary protein intake (g/day; median (95% CI))	67 (61–71)	78 (73–84)
Cumulative dose of glucocorticoids (mg; median (95% CI))	1339 (1291–1414)	3880 (1166–4662)
Main causes of renal failure (*n* (%))		
Chronic glomerulonephritis	23 (33)	5 (21)
Polycystic kidney disease	14 (20)	5 (21)
Vascular disease[a]	26 (38)	6 (25)
Tubular and interstitial disease	8 (12)	6 (25)
Chronic pyelonephritis[b]	9 (13)	0 (0)
Diabetes	9 (13)	4 (17)
Congenital renal anomalies[c]	11 (16)	2 (8)
Others or unknown	5 (7)	6 (25)

[a]Vascular disease includes vasculitis, thromboembolic disease, hemolytic–uremic syndrome or thrombotic thrombocytopenic purpura (HUS/TTP), hypertension, scleroderma, unilateral or bilateral renal artery stenosis, and cholesterol atheroembolic disease.
[b]Includes reflux nephropathy.
[c]Congenital renal anomalies include aplasia, hypoplasia, dysplasia. A given patient could have more than one cause of renal failure.

interaction term. To identify the probability of patients at risk for developing hypophosphatemia or a low TmP/GFR early after transplantation, a univariate logistic regression was used, based on the following pre-transplantation parameters: dialysis duration, age at time of transplantation, preexisting parathyroidectomy, serum levels of iPTH, FGF-23 and sPi. The effect of predictive variables was assessed by odds ratio (OR) and 95% CI. The significance level for two-sided P values was 0.05 in all tests. The data were analyzed using STATA statistical software package (version 11.1; Stata Corporation, College Station, TX, USA).

Results

Early post-transplantation period

Sixty-nine patients were evaluated after a median of 12 days (95% CI, 10–13) post-renal transplantation (Tables 1 and 2). Thirty-two patients (46%) were female. The median age at the time of transplantation was 50 years (95% CI, 46–54). Median time on dialysis was 2.3 years (95% CI, 1.6–2.9). The leading causes of renal failure are listed in Table 1.

The median post-transplantation sPi was 0.75 mmol/l (95% CI, 0.67–0.79) and TmPi/GFR was 0.45 mmol/l GFR (95% CI, 0.35–0.58), i.e. both below the lower limit of normal. Hypophosphatemia (sPi $<$ 0.8 mmol/l) was found in 43 patients (62%). None were considered symptomatic. Hypophosphatemia was severe ($<$0.33 mmol/l) in five (7%) patients. Median post-transplantation levels of CterFGF-23 and intFGF-23 levels were 124 U/ml (95% CI, 97–211) and 95 ng/l (95% CI, 54–164), respectively, i.e. well above the higher limit of normal (Table 2). Cter and intFGF-23 levels were highly correlated (Spearman coefficient correlation (r): 0.76, $P<0.0001$). Compared with normophosphatemic patients, those with hypophosphatemia had significantly higher levels of both intact and CterFGF-23 (195 (95% CI, 108–288) vs 48 (95% CI, 40–64) ng/l for the intact, $P<0.002$; and 205 (95% CI, 116–384) vs 81 (95% CI, 55–124) U/ml for the Cter assay, $P<0.002$) (Table 2). Serum calcium levels tended to be higher in hypophosphatemic patients, as were the levels of bone resorption, as defined by the ratio of fasting urinary calcium to creatinine and urine deoxypyridinoline (Table 2). However, serum levels of iPTH and calcitriol did not differ between hypo- and normophosphatemic groups.

Of note, patients exhibiting low phosphate levels had a significantly lower creatinine value, although the estimated glomerular filtration rate did not differ between the two groups (Table 2). This finding was unrelated to differences in body weight and gender between groups (data not shown).

According to univariate analysis, both CterFGF-23 and intFGF-23 inversely correlated with sPi ($r=-0.35$, $P<0.003$ and -0.35, $P<0.003$ for CterFGF-23 and for intFGF-23, respectively), and with TmPi/GFR ($r=-0.50$, $P<0.0001$ for CterFGF-23, -0.54, $P<0.0001$ for intFGF-23) (Fig. 1a and b). Cter and intFGF-23 were inversely associated with serum calcitriol levels ($r=-0.48$, $P<0.0002$, and -0.65, $P<0.0001$, respectively). This relationship remained significant after adjusting for eGFR. Moreover, serum levels of intFGF-23 were significantly associated with increased serum calcium levels ($r=0.24$, $P<0.05$), but not with turnover markers. There was also a significant negative correlation between serum iPTH, sPi and TmPi/GFR ($r=-0.24$, $P<0.05$ for sPi, and -0.34,

Table 2 Biochemical characteristics (median (95% CI)) of the patients with or without hypophosphatemia during the early post-transplantation period.

			No. of patients (%)					
	Normal range	**All patients** ($n=69$)	Low[a]	High[a]	***P*** [b]	**sPi** <0.8 mmol/l ($n=43$)	**sPi** >0.8 mmol/l ($n=26$)	***P***
Serum calcium	2.25–2.60 mmol/l	2.21 (2.13–2.52)	46 (32)	1 (1)	NS	2.22 (2.14–2.33)	2.15 (2.1–2.25)	NS
Serum albumin-corrected calcium	2.25–2.60 mmol/l	2.42 (2.39–2.45)	12 (8)	7 (5)	NS	2.43 (2.40–2.52)	2.40 (2.34–2.44)	0.04
Serum phosphate (sPi)	0.80–1.40 mmol/l	0.75 (0.67–0.79)	64 (43)	3 (2)	–	0.63 (0.56–0.67)	0.91 (0.86–1.13)	–
Serum creatinine	35–106 μmol/l	132 (117–138)	0 (0)	81 (54)	NS	118 (102–136)	138 (130–157)	0.03
GFR	>60 ml/min	56 (49–60)	61 (42)	NA	0.03	59 (52–65)	49 (40–58)	NS
Serum protein	61–79 g/l	56 (53–58)	78 (52)	0 (0)	NS	56 (52–58)	58 (52–60)	NS
Serum albumin	35–48 g/l	29 (28–30)	99 (66)	0 (0)	NS	28 (28–30)	29 (26–30)	NS
Serum osteocalcin	8.8–29.7 μg/l	12 (8–19)	30 (20)	15 (10)	NS	14 (7–23)	12 (7–15)	NS
Serum total alkaline phosphatases	30–125 IU/l	63 (51–71)	4 (3)	6 (4)	NS	63 (48–85)	61 (44–72)	NS
Serum parathyroid hormone	1.1–6.8 pmol/l	12 (10–16)	0 (0)	85 (57)	NS	14 (11–19)	10 (7–12)	NS
25-Hydroxyvitamin D	75–120 nmol/l	42 (35–48)	94 (65)	0 (0)	NS	42 (35–53)	43 (28–50)	NS
1,25-Dihydroxyvitamin D	40–140 pmol/l	38 (32–56)	54 (32)	0	NS	33 (15–53)	54 (34–97)	NS
Intact FGF-23	10–50 ng/l	95 (54–164)	0 (0)	64 (44)	0.02	195 (108–288)	48 (40–64)	0.002
C-terminal FGF-23	30–57 U/ml	124 (97–211)	0 (0)	84 (57)	0.001	205 (116–384)	81 (55–124)	0.002
Urinary calcium/creatinine	0.10–0.50 mmol/mmol	0.22 (0.19–0.30)	15 (10)	16 (11)	NS	0.32 (0.22–0.43)	0.13 (0.11–0.20)	0.0003
Urinary D-pyr/cr[c]	4.2–18.2 nmol/mmol	14 (12–18)	0 (0)	35 (15)	0.01	18 (13–21)	12 (10–13)	0.02
TRCaI/GFR[d]	2.4–2.9 mmol/l GFR	2.65 (2.56–2.71)	12 (8)	7 (5)	NS	2.65 (2.54–2.71)	2.66 (2.54–2.80)	NS
TmP/GFR[e]	0.8–1.4 mmol/l GFR	0.45 (0.35–0.58)	82 (55)	1 (1)	0.0001	0.29 (0.26–0.39)	0.70 (0.60–0.84)	0.0001

NA, not applicable.
[a]Low and high are defined as less than the laboratory-defined lower limit of normal and more than the upper limit of normal, respectively. Age and gender-adjusted normal values were taken into account.
[b]The *P* value expresses the significance of the differences in the observed proportions of high or low variable values between patients with and without low serum phosphate level, using Fisher's exact test.
[c]For deoxypyridinoline/creatinine (D-pyr/cr), the presented normal range refers to men and pre-menopausal women.
[d]Renal tubular reabsorption of calcium index.
[e]Renal tubular reabsorption of phosphate.

$P<0.005$ for TmPi/GFR). Serum levels of iPTH and FGF-23 were not correlated. Moreover, we found a significant correlation between serum iPTH levels and urine deoxypyridinoline ($r=0.40$, $P<0.002$). A significant negative association was found between eGFR and sPi ($r=-0.28$, $P<0.02$). Dietary phosphate intake, daily and cumulative doses of corticosteroids, serum 25-OH vitamin D levels and serum magnesium did correlate with neither sPi nor TmPi/GFR. Of note, dietary phosphate intake was not associated with FGF-23 levels. Increased urinary deoxypyridinoline levels were significantly associated with increased serum calcium levels ($r=0.25$, $P<0.05$). The latter were also significantly associated with TRCaI ($r=0.47$, $P<0.0001$).

To assess the relative contribution of variables involved in the determination of sPi and TmPi/GFR, multiple regression models were constructed using a backward stepwise process. After adjustment, sPi was significantly associated with eGFR and with both Cter and intFGF-23 levels ($P<0.05$ for all associations, Table 3). The association with serum iPTH was of borderline significance in the model that features CterFGF-23, but was significantly associated with sPi when intFGF-23 was used. TmPi/GFR was significantly associated with intact and CterFGF-23 and serum iPTH ($P<0.05$ for all associations, Table 3). The Spearman correlation coefficients and *P* values indicated that FGF-23, whatever the assay used, seemed to be more significantly correlated with sPi and TmP/GFR than was iPTH. There was no interaction between FGF-23 and iPTH.

As mentioned in Subjects and methods section, all patients studied during the early post-transplantation period were tested for their iPTH levels before transplantation. These were significantly higher in patients who developed hypophosphatemia after renal transplantation: 30 (95% CI, 22–37) vs 19 (95% CI, 11–29) pmol/l in hypophosphatemic and normophosphatemic patients respectively, $P<0.05$.

In seven patients who subsequently underwent renal transplantation, pre-transplant levels of FGF-23 did not correlate with those of sPi at the time of transplantation. But pre-transplant intFGF-23 correlated with post-transplant intFGF-23 ($r=0.92$, $P<0.03$).

To identify the probability of the patients at risk for developing hypophosphatemia or a low TmP/GFR early after transplantation, a logistic regression was used, based on the following pre-transplantation parameters: dialysis duration, age at time of transplantation, preexisting parathyroidectomy, serum levels of iPTH, FGF-23 and sPi. Crude OR showed that patients with higher levels of sPi before transplantation were more likely to have hypophosphatemia or a low TmP/GFR early after transplantation (crude OR: 7.9, 95% CI, 1.8–33.9, $P<0.005$; crude OR: 11.4, 95% CI, 1.5–84.4, $P<0.017$; respectively).

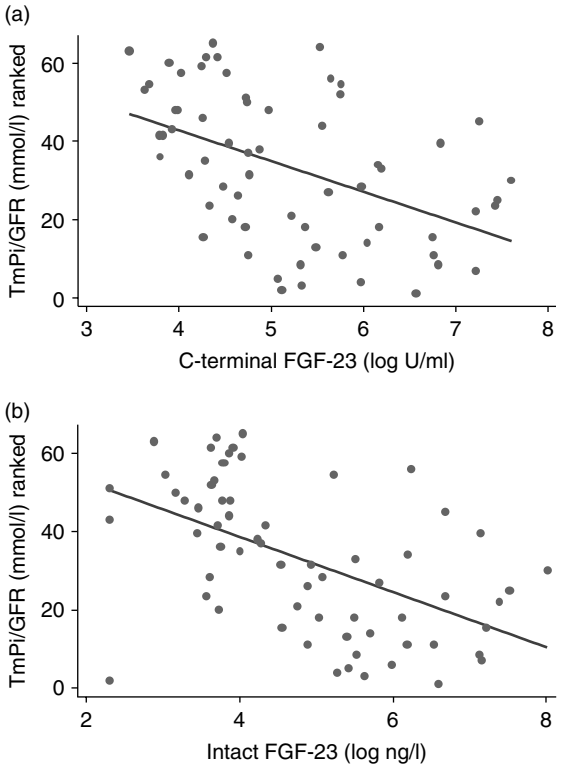

Figure 1 Univariate regression between C-terminal FGF-23 (a), intact FGF-23 (b) and TmPi/GFR during the early post-transplant period.

One year after transplantation

In 24 patients studied 1 year post-transplantation, median sPi level was 1.07 mmol/l (95% CI, 0.93–1.17) (Table 4). There was a significant positive correlation between sPi and CterFGF-23 ($r=0.53$, $P<0.01$). No such association applied to intFGF-23 levels. TmPi/GFR was negatively correlated with serum iPTH levels ($r=-0.45$, $P<0.04$) but not with FGF-23 levels, regardless of the assay used. Only 3 of the 24 patients evaluated had mild hypophosphatemia. In these patients, TmPi/GFR tended to be lower (Table 4). In two of these, serum level of FGF-23 was within the normal range. One of the two had severe vitamin D deficiency with secondary hyperparathyroidism. Consequently, hypophosphatemia was in this case related to elevated iPTH levels. The serum iPTH values of the other two patients were normal and only one of them had decreased levels of sPi that could be attributed to an increased FGF-23 level.

Discussion

The results suggest that FGF-23 may contribute to hypophosphatemia during the early post-renal transplant period, in addition to – but independently from – increased levels of PTH. Conversely, decreased post-transplantation renal function may attenuate the fall in sPi levels. The results also suggest that FGF-23 seemed to be more significantly correlated with sPi and TmPGFR than was iPTH. Patients with high level of sPi before transplantation seem to be more prone to develop hypophosphatemia or a TmPi/GFR early after transplantation.

Despite some suggestions that FGF-23 may play a role in early post-transplant hypophosphatemia, no clear evidence has been reported so far (4, 6, 8–10, 17–19). Indeed, in 41 kidney transplant recipients, no significant association was found between intFGF-23 and sPi levels. Furthermore, mean serum levels of intFGF-23, 3 months post-renal transplantation, were not statistically different between patients with and without hypopophosphatemia (8). In 27 patients, FGF-23 was independently associated with sPi levels and urinary phosphate excretion, whereas PTH was not related to any of these parameters. Although longitudinal and well designed, the latter study had several limitations: only Cter assay was performed, the data were confounded by the administration of immunosuppressive drugs and other factors such as renal function and dietary phosphate intake and a small number of patients was studied (9). In seven patients out of 10 with post-transplant hypophosphatemia, whose CterFGF-23 was measured 1 day pre-transplantation and 4–5 days thereafter, there was a negative correlation between sPi and FGF-23 concentrations (10). No correlation was observed with serum PTH. Potential confounders such as renal function were not taken into account. In 15 out of 22 patients during the early post-transplantation period, serum PTH values were normal despite low mean sPi levels of 0.9 ± 0.003 mg/dl (4, 9). Thus, even if persistent hyperparathyroidism has been implicated in the pathogenesis of post-transplant hypophosphatemia, data substantiating its role are limited (4, 9, 20).

FGF-23 metabolism is strongly modified by reduced glomerular filtration rate. Circulating FGF-23 levels are already elevated in patients with CKD stages 2 and 3, and this rise appears to be the reflection of an increased production rather than accumulation because of impaired renal function (21, 22). Renal transplant recipients represent a subset of patients with CKD. The mean GFR at the early post-transplantation period was 56 ml/min (Table 2) and thus GFR may modulate sPi levels by modifying serum FGF-23 levels at early stages of CKD or may limit phosphate excretion at more advanced stages.

Factors other than FGF-23, PTH and renal function may influence sPi as well, such as glucocorticoids (21, 23–25), magnesium deficiency (26) and dietary phosphate intake (11, 27–29). In our study, these factors were prospectively recorded and we found no association with sPi or TmPi/GFR. Moreover, despite the use of similar immunosuppressive regimens,

Table 3 Factors associated with serum phosphate and rank of TmPi/GFR (multivariate regression model constructed by backward stepwise modeling procedure) in models that feature Cter FGF-23 or int FGF-23 in the early post-transplantation period.

	Dependent variable: serum phosphate (log)			**Dependent variable: TmPi/GFR** (ranked)		
Variables[a]	Regression coefficient (95% CI)	*P*	r^2	Regression coefficient (95% CI)	*P*	r^2
CterFGF-23 (log U/ml)	−0.10 (−0.20 to 0.01)	0.04		−7.1 (−10.7 to 3.4)	0.001	
Serum PTH (log pmol/l)	−0.12 (−0.25 to 0.02)	0.08		−7.0 (−12.5 to 1.5)	0.01	
GFR (ml/min)	−0.006 (−0.01 to 0.0003)	0.04				
Whole model		0.01	17%		0.0001	30%
Int FGF-23 (log ng/l)	−0.1 (−0.18 to 0.03)	0.005		−7.4 (−10.0 to 4.7)	0.0001	
Serum PTH (log pmol/l)	−0.16 (−0.30 to 0.02)	0.02		−7.5 (−12.4 to 2.5)	0.004	
GFR (ml/min)	−0.006 (−0.01 to 0.0002)	0.04				
Dietary phosphate intake	0.0003 (−0.0002 to 0.0008)	0.195				
Whole model		0.005	23%		0.0001	43%

The following parameters were included: GFR, serum PTH, albumin-corrected calcium levels, dietary phosphate intake, cumulative dose of corticosteroids and levels of both C-terminal and intact FGF-23 in two different models. The variables 'cumulative doses of corticosteroids' and albumin-corrected calcium levels were removed from the model automatically, *P* being ≥0.2.
[a]Cter FGF-23, int FGF-23 and serum PTH are log-transformed. GFR is not included in the model for TmPi/GFR, which was already adjusted for renal glomerular filtration rate.

hypophosphatemia is a rare phenomenon following transplantation of other solid organs, highlighting that these factors play, at best, a modest role in the inception of decreased phosphate levels.

We observed, like others, a correlation between serum calcium and FGF-23 levels (8). Increased levels of serum calcium seem to be driven by an increase in both bone resorption and calcium tubular reabsorption. Data suggesting that PTH or calcium instigates stimulation of FGF-23 secretion are scarce. Nevertheless, it seems plausible to hypothesize that increased FGF-23 may in part be related to the persistence of elevated PTH levels and to its effect on bone and kidney. It is well known that FGF-23 inhibits 1α-hydroxylase activity (30, 31). This may explain the strong negative correlation between calcitriol and FGF-23 levels.

It has been shown that a high-phosphate diet increases FGF-23 levels and a low-phosphate diet decreases FGF-23 both in humans and rodents (11, 28, 29). However, the present study failed to detect an association between dietary phosphate intake, FGF-23 and sPi levels. Furthermore, we found that the level of dietary phosphate intake did not influence the relation between FGF-23 and sPi levels. This may be attributable to alteration in GFR. We can also speculate that the in-hospital food intake in the preceding days before sampling during the post-transplantation period did not vary sufficiently to allow us to detect a correlation. The dietary phosphate intake ranged from 915 to 1097 mg daily in 95% of the studied population early after transplantation. A larger patient population may be necessary to detect significant changes.

Table 4 Biochemical characteristics (median (95% CI)). of the patients with or without low TmPi/GFR 1 year after transplantation ($n=24$). Age-adjusted normal values were taken into account.

	Normal range	**All patients** ($n=24$)	**TmPi/GFR** <0.8 mmol/l ($n=7$)	**TmPi/GFR** >0.8 mmol/l ($n=17$)
Serum albumin-corrected calcium	2.25–2.60 mmol/l	2.46 (2.43–2.48)	2.48 (2.35–2.80)	2.44 (2.40–2.47)
Serum phosphate (sPi)	0.80–1.4 mmol/l	1.07 (0.93–1.17)	0.91 (0.75–1.06)	1.16 (1.06–1.29)*
Serum creatinine	35–106 μmol/l	126 (101–140)	141 (92–184)	105 (95–138)
GFR	>60 ml/min	66 (59–76)	60 (37–100)	70 (56–80)
Serum albumin	35–48 g/l	38 (37–39)	38 (34–40)	38 (37–40)
Serum osteocalcin	8.8–29.7 μg/l	27 (17–56)	34 (16–120)	24 (12–68)
Serum total alkaline phosphatases	30–125 IU/l	76 (53–97)	67 (38–136)	62 (49–97)
Serum parathyroid hormone	1.1–6.8 pmol/l	9.9 (6.5–12.3)	13.4 (6.9–27.7)	9.0 (5.8–11.9)
25-Hydroxyvitamin D	75–120 nmol/l	69 (51–90)	61 (22–128)	69 (48–97)
1,25-Hydroxyvitamin D	40–140 pmol/l	79 (57–110)	78 (14–111)	84 (56–114)
Intact FGF-23	10–50 ng/l	63 (49–90)	85 (20–145)	62 (34–90)
C-terminal FGF-23	30–57 U/ml	77 (48–176)	78 (40–194)	76 (45–302)
Urinary calcium/creatinine	0.10–0.50 mmol/mmol	0.15 (0.11–0.22)	0.15 (0.07–0.27)	0.15 (0.10–0.21)
Urinary D-pyr/cr[a]	4.2–18.2 nmol/mmol	8.4 (5.3–13.3)	9.0 (4.8–27.0)	8.6 (4.8–18.3)
TRCaI/GFR[b]	2.4–2.9 mmol/l GFR	2.73 (2.65–2.85)	2.73 (1.41–2.92)	2.75 (2.65–2.87)
TmPi/GFR[c]	0.8–1.4 mmol/l GFR	0.90 (0.73–0.96)	0.67 (0.65–0.72)	0.93 (0.89–0.99)

*$P<0.004$ expresses the significance of the differences in the observed median values between patients with and without low TmPi/GFR.
[a]For deoxypyridinoline/creatinine, the presented normal range applies to men and pre-menopausal women.
[b]Renal tubular reabsorption of calcium index.
[c]Renal tubular reabsorption of phosphate.

It is unclear why excess FGF-23 persisted early after renal transplantation. It has been suggested that the patients with elevated FGF-23 levels after transplantation actually displayed the highest levels beforehand (8). Bone, in particular osteocytes, which produce FGF-23 (32), may not appropriately reduce FGF-23 secretion immediately after transplantation due to prolonged stimulation during the preceding months. Two studies have examined this question (8, 9). Evenepoel *et al.* (8) found that the pre-transplant FGF-23 level was the only significant predictor of the sPi nadir. Bhan *et al.* (9) reported that compared with the subjects whose initial reduction in FGF-23 levels was above the median, those whose FGF-23 levels persisted at higher levels (percent reduction in FGF-23 levels below the median) were more likely to develop hypophosphatemia. In our study, patients who displayed hypophosphatemia during the early post-transplantation period tended to have higher levels of pre-transplant FGF-23, but not significantly so, probably partly because it was measured in a subset of only seven patients. Nonetheless, the high correlation we found between the pre- and early post-transplantation level of FGF-23 suggests an autonomously high FGF-23 production in renal transplant recipients and extends the knowledge published previously (8, 9).

Various findings suggest that serum FGF-23 may be regulated by factors other than serum phosphorus and potentially by activation of the PTH/PTHrP receptor in bone (33). FGF-23 gene expression is up-regulated by PTH receptor activation in osteocytes *in vitro* and *in vivo* (34). This would explain why patients with high pre-transplant levels of serum PTH are more prone to persistently high FGF-23 levels and to develop hypophosphatemia during the early period after transplantation. Thus, an increase in FGF-23 levels may be favored by an elevated level of serum PTH pre- and post-transplantation and by the accompanying increase in bone resorption.

Controversy exists as to whether the renal phosphorus wasting persists on the long term and if so whether this is related to increased PTH and/or FGF-23 levels. In patients 1 year after transplantation, we observed a decrease in serum PTH, FGF-23 and BRI (the latter being favored by the systematic use of bisphosphonates). Only three of the 24 transplanted patients evaluated had hypophosphatemia and seven of the 24 had a decrease in TmPi/GFR. At that time point, TmPi/GFR seemed to be influenced mainly by serum PTH, but not by FGF-23. The current findings are in agreement with the observation that FGF-23-related hypophosphatemia regresses 1 year after successful renal transplantation (35).

One limitation of the study is that we measured only a small number of patients longitudinally. The ancillary data at 1 year post-transplantation are of exploratory nature and should be interpreted with caution. Nevertheless, to our knowledge, the sample of 69 patients studied during the early post-transplantation period is the largest cross-sectional study on this topic. Furthermore, the treatment administered (i.e. calcium, vitamin D and bisphosphonates) may modulate the results at 1 year post-transplantation. All transplanted patients received bisphosphonates, oral calcium and vitamin D supplements, in a systematic program aimed at preventing glucocorticoid-induced osteoporosis. In our study, all participants studied early after transplantation had samples collected before the administration of bisphosphonates and calcium/vitamin D. At that time, vitamin D insufficiency and deficiency (Table 2) were common. Low vitamin D levels were associated with modestly increased PTH levels, which in turn increased the risk of hypophosphatemia. However, we found a significant association between FGF-23 and early post-transplant phosphatemia even after adjustment for PTH. Moreover, the correction of the vitamin D deficiency may have minimized secondary hyperparathyroidism, and allowed one to better analyse the correlation between FGF-23 and sPi levels or TmPi/GFR at 1 year post-transplant.

On the other hand, bisphosphonates may modify bone turnover. FGF-23 is produced by bone cells, and it has been correlated with bone turnover markers in patients with fibrous dysplasia of bone (36) and in healthy young men (11). Data on the effects of bisphosphonate administration on the serum levels of FGF-23 are scarce. In an experimental study examining the role of bone remodeling in the regulation of circulating concentrations of FGF-23 using different mouse models, the potent antiresorptive agent osteoprotegerin or alendronate was administrated. It was confirmed that bone remodeling, and specifically the rate of bone formation, is a potent stimulus for the production and release of FGF-23 (37).

In conclusion, our study strongly suggests that sPi levels early after kidney transplantation results from both increased serum PTH and FGF-23 levels.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

List of each author's specific contributions to the work: A Trombetti: participated in research design, acquisition of data, analysis and interpretation of data and writing of the manuscript. He takes responsibility for the integrity of the overall project. L Richert: participated in the elaboration of the research design, acquisition of data, the analysis of data and revision of the manuscript. K Hadaya: participated in patient recruitment and in revising the article for important intellectual content. J-D Graf: participated in the acquisition

of data and in revising the article for important intellectual content. F R Herrmann: participated in the statistical analysis of data and revision of the manuscript. S L Ferrari: participated in the elaboration of the research design, analysis of data and in the writing and revision of the manuscript. P-Y Martin: participated in the elaboration of the research design and the writing and revision of the manuscript. R Rizzoli: participated in the elaboration of the research design, the analysis of the data and in the writing and revision of the manuscript. We confirm that this manuscript has not been published or submitted elsewhere. All authors have approved the manuscript and agree with its submission to *European Journal of Endocrinology*.

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