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## Serum Phosphorus and Risk of Cardiovascular Disease, All-Cause Mortality, or Graft Failure in Kidney Transplant Recipients: An Ancillary Study of the FAVORIT Trial Cohort

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## Abstract

**Background**—Mild hyperphosphatemia is a putative risk factor for cardiovascular disease [CVD], loss of kidney function, and mortality. Very limited data are available from sizable multicenter kidney transplant recipient (KTR) cohorts assessing the potential relationships between serum phosphorus levels and the development of CVD outcomes, transplant failure, or all-cause mortality.

**Study Design**—Cohort study.

**Setting & Participants**—The Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) Trial, a large, multicenter, multiethnic, controlled clinical trial that provided definitive evidence that high-dose vitamin B–based lowering of plasma homocysteine levels did not reduce CVD events, transplant failure, or total mortality in stable KTRs.

**Predictor**—Serum phosphorus levels were determined in 3,138 FAVORIT trial participants at randomization.

**Results**—During a median follow-up of 4.0 years, the cohort had 436 CVD events, 238 transplant failures, and 348 deaths. Proportional hazards modeling revealed that each 1-mg/dL higher serum phosphorus level was not associated with a significant increase in CVD risk (HR, 1.06; 95% CI, 0.92–1.22), but increased transplant failure (HR, 1.36; 95% CI, 1.15–1.62) and total mortality risk associations (HR, 1.21; 95% CI, 1.04–1.40) when adjusted for treatment allocation, traditional CVD risk factors, kidney measures, type of kidney transplant, transplant vintage, and use of calcineurin inhibitors, steroids, or lipid-lowering drugs. These associations were strengthened in models without kidney measures: CVD (HR, 1.14; 95% CI, 1.00–1.31), transplant failure (HR, 1.72; 95% CI, 1.46–2.01), and mortality (HR, 1.34; 95% CI, 1.15–1.54).

**Limitations**—We lacked data for concentrations of parathyroid hormone, fibroblast growth factor 23, or vitamin D metabolites.

**Conclusions**—Serum phosphorus level is marginally associated with CVD and more strongly associated with transplant failure and total mortality in long-term KTRs. A randomized controlled clinical trial in KTRs that assesses the potential impact of phosphorus-lowering therapy on these hard outcomes may be warranted.

## INDEX WORDS

Renal transplantation; phosphate toxicity; hyperphosphatemia; kidney transplant recipient (KTR); kidney failure; cardiovascular disease (CVD); graft failure; death; serum phosphorus; chronic kidney disease (CKD)

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Deranged calcium-phosphorus metabolism frequently occurs in chronic kidney disease (CKD),<sup>1</sup> progressively worsens as patients approach end-stage renal disease (ESRD),<sup>1,2</sup> and

is not fully reversed after kidney transplantation.<sup>3–8</sup> The hyperparathyroidism, elevated parathyroid hormone (PTH) concentrations, and increased fractional excretion of urinary phosphorus/decreased tubular reabsorption of phosphorus<sup>4</sup> that result may not resolve even after successful transplantation with a well-functioning kidney transplant. Kidney transplant recipients (KTRs) whose glomerular filtration rate (GFR) subsequently declines to CKD stages 3b, 4, or 5 (GFRs of 30–44, 15–29, or <15 mL/min/1.73 m<sup>2</sup>, respectively) are also liable to experience the hyperphosphatemia, deficiencies of 25-hydroxyvitamin D<sub>3</sub> and/or 1,25-dihydroxyvitamin D<sub>3</sub>, and increased levels of the phosphatonin fibroblast growth factor 23 (FGF-23) that are seen in CKD stages 3b to 5 in non-KTRs with native kidneys.<sup>3–8</sup> Because these markers of deranged calcium-phosphorus metabolism may be in the causal pathway linking “phosphorus toxicity” and its clinical consequences,<sup>9</sup> one might expect them to be over-adjusted for in observational studies of KTRs. However, the relationship between serum phosphorus levels and outcomes can be seen even in such cohorts.<sup>3,8</sup> Regardless, baseline serum phosphorus levels seem to predict total and/or cardiovascular disease (CVD) mortality in CKD and KTR populations,<sup>3,6–8,10</sup> as well as native kidney (or kidney transplant) failure.<sup>3,8,10,11</sup>

Given that only a single large multicenter study of KTRs with northern European ancestry has assessed these relationships,<sup>8</sup> we addressed this paucity of data by using the novel Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) KTR trial cohort<sup>12,13</sup> to examine the potential associations between serum phosphorus level and the development of CVD outcomes, transplant failure, or all-cause mortality.

## METHODS

### Study Population

The completed FAVORIT Study was a large, multicenter, multiethnic, controlled clinical trial<sup>12–14</sup> ([ClinicalTrials.gov](https://clinicaltrials.gov) study number NCT00064753) that provided definitive evidence that high-dose, compared to low-dose, vitamin B—based lowering of levels of plasma homocysteine—a putatively thromboatherogenic amino acid byproduct of methionine metabolism<sup>12,13</sup>—failed to reduce hard centrally adjudicated CVD events (both fatal and nonfatal), transplant failure, or total mortality in long-term stable KTRs.<sup>13</sup> Because the high-dose vitamin intervention did not result in a significant reduction in event rates for any of these outcomes compared to the low-dose vitamin B treatment,<sup>13</sup> groups are combined in all analyses for this report (for which a Rhode Island Hospital phosphorus data analysis ancillary study exemption was granted July 23, 2015).

KTRs were eligible for the parent study if they provided informed consent, were aged 35 to 75 years, and had clinically stable kidney function and elevated total homocysteine levels. Stable kidney function was ascertained by medical chart review to establish that the patient’s current transplant had been functioning for at least 6 months posttransplantation and there was no documented clinical indication of kidney function deterioration. All enrolled participants had a Cockcroft-Gault estimated creatinine clearance (CL<sub>cr</sub>) ≥ 30 mL/min and elevated total homocysteine level (≥ 12.0 μmol/L for men or ≥ 11.0 μmol/L for women) based on central laboratory analysis of screening specimens. For women recruited after July 2005, CL<sub>cr</sub> eligibility criteria were reduced to ≥ 25 mL/min in acknowledgment of the lower CL<sub>cr</sub>

distribution routinely observed in women relative to that in men. Individuals with chronic illness limiting life expectancy to less than 2 years were excluded, as were those with CVD risk modified because of recent CVD-related events or procedures.<sup>12</sup>

### Measurement of Serum Phosphorus

Nonfasting serum phosphorus concentrations were determined at baseline on an automated clinical chemistry analyzer (Olympus AU 400 Beckman Coulter, Inc; Olympus America Inc). With this methodology, inorganic phosphorus and ammonium molybdate reacted in the presence of sulfuric acid to form a phosphomolybdate complex that was measured at 340/380 nm. Intra- and interassay coefficients of variation were equal to 2.1% and 3.4%, respectively.<sup>14</sup>

### Outcomes Determined

The primary outcome was a composite of incident or first recurrent CVD during the study period, comprising: (1) CVD death, (2) myocardial infarction, (3) resuscitated sudden death, (4) stroke, (5) coronary artery revascularization, (6) lower-extremity revascularization or amputation above the ankle for severe arterial disease, (7) carotid endarterectomy or angioplasty, (8) abdominal aortic aneurysm repair, or (9) renal artery revascularization. The first 4 components of the primary outcome noted were centrally reviewed and adjudicated by the Clinical Endpoints Committee unaware of treatment assignment; the remaining outcomes were identified through medical record abstraction. The Clinical Endpoints Committee also reviewed records for unstable angina cases and urgent coronary revascularization procedures in search of myocardial infarctions that were not identified by the clinical site staff. Dialysis-dependent kidney transplant failure, all-cause mortality, and CVD mortality considered separately were secondary outcomes.<sup>13,14</sup> We also examined a composite outcome of transplant failure and all-cause mortality to account for semi-competing risks. Participants were not censored at the time of return to dialysis or at retransplantation. As such, time to first event of either transplant failure (ie, return to maintenance dialysis or preemptive retransplantation) or all-cause mortality essentially means the latter will only be deaths with transplant function because a death after returning to dialysis is preceded by transplant failure. Not censoring for death with transplant function is the preferred approach to avoid a scenario in which death with transplant function becomes a competing event for transplant failure.

### Other Measurements

Data collected at study enrollment<sup>12–14</sup> included the following: demographics (age, sex, and self-designated race/ethnicity), smoking status (current, former, or never), medical history (baseline CVD and diabetes mellitus), transplant characteristics (living donor kidney and time since transplantation), physical examination findings (body mass index and systolic and diastolic blood pressure), and laboratory variables (serum creatinine; total, high-density lipoprotein, and low-density lipoprotein cholesterol; triglycerides; and spot urinary<sup>15</sup> albumin and creatinine). Baseline blood pressure was the average of 2 measurements, and hypertension was defined as systolic blood pressure  $\geq 140$  mm Hg, diastolic blood pressure  $\geq 90$  mm Hg, or antihypertensive medication use at study enrollment. Diabetes was defined as the use of insulin or oral hypoglycemic medications or patient history. Baseline CVD was

characterized as prior myocardial infarction, coronary artery revascularization, stroke, carotid arterial revascularization, abdominal or thoracic aortic aneurysm repair, and/or lower-extremity arterial revascularization.<sup>12–14</sup> Race was defined as white, black, or other, with individuals who identified as other classified as white for GFR estimation by the CKD-EPI (CKD Epidemiology Collaboration)<sup>16,17</sup> creatinine equation. Body mass index was calculated as weight {in kilograms} divided by height (in meters) squared.

## Statistical Methods

Jonckheere-Terpstra tests for ordered alternatives (for continuous variables) and a Wilcoxon-type test for trend (for categorical variables) were used to compare baseline characteristics across quintiles of serum phosphorus concentrations, with the uppermost quintile divided in half (yielding deciles 9 and 10). Cox proportional hazards regression models were used to evaluate the association of baseline serum phosphorus concentrations with time to primary and secondary study outcomes. Competing events for the primary outcome were censored (ie, Cox models estimate cause-specific hazards). Initial models were adjusted a priori for serum phosphorus level, treatment assignment, systolic blood pressure (dichotomized as 140 vs <140 mm Hg), age, sex, race, pre-existing CVD or diabetes, estimated GFR (eGFR), and natural log urinary albumin-creatinine ratio (UACR). Per additional data available from the FAVORIT trial, extended (“fully adjusted”) models were further adjusted a priori for smoking, body mass index, low-density lipoprotein cholesterol level, high-density lipoprotein cholesterol level, natural log triglyceride level, kidney transplant vintage and type (deceased vs living donor), calcineurin inhibitor use, prednisone use, and lipid-lowering drug use. Accordingly, we settled upon 3 main models: unadjusted, that is, serum phosphorus level only, as a continuous variable (model 1); almost fully adjusted, that is, serum phosphorus level plus all aforementioned variables, except the 2 kidney measures: eGFR and natural log of UACR (model 2); and the fully adjusted model that included these kidney measures (model 3). An additional model used the same full set of covariables, but compared the upper decile of serum phosphorus, relative to the first quintile, as the referent. In the semicompeting risk analyses (ie, for the transplant failure or mortality composite), a single failure event variable was constructed for time to the first of transplant failure or pre-transplant failure death. Maintenance of proportional hazards assumptions was assessed for the primary and secondary outcome analyses by examination of log-log plotting of survival probability and the supremum test. Interaction terms were evaluated between serum phosphorus level and treatment randomization arm, as well as pre-existing CVD or diabetes, to detect for potential effect modification within these 2 higher-risk subgroups, in particular. Analyses were performed using SPSS (version 24.0; IBM) and STATA software (version 14.2; StataCorp LP). Two-sided  $P < 0.05$  was considered statistically significant for all analyses.

## RESULTS

### Study Population

There were 3,138 (76.3%) of the 4,110 FAVORIT participants who had complete data and were included in the present analyses (Fig 1). Mean age of the study population was  $51.6 \pm 9.4$  (standard deviation) years, 62.9% were men, 76.0% were white, 19.3% had a history

of CVD, 39.0% had a history of diabetes mellitus, and median time since transplantation was 4.0 years (Table 1). Mean serum phosphorus level was  $3.07 \pm 0.68$  mg/dL. Participants with increased serum phosphorus concentrations had significantly lower eGFRs and greater UACRs. They also had older transplant vintages, as well as higher prevalences of cadaveric transplants and pre-existing diabetes or CVD. In addition, they were significantly older and more likely to be men or current cigarette smokers and more likely to have higher systolic blood pressures, higher high-density lipoprotein cholesterol concentrations, and a greater prevalence of lipid-lowering drug use (Table 1).

## Outcome Analyses

Proportional hazards assumptions were met for all outcome analyses.

**CVD Outcomes**—During a median follow-up of 4.0 years, there were 436 new CVD events (Table 2), including 135 CVD deaths (Table 3). In the unadjusted model and almost fully adjusted model (without adjustment for eGFR and UACR), each 1-mg/dL higher serum phosphorus concentration was associated with 25% greater (hazard ratio [HR], 1.25; 95% confidence interval [CI], 1.08–1.45) and 14% greater (HR, 1.14; 95% CI, 1.00–1.31) CVD risk, respectively. On additional adjustment for eGFR and UACR, this association was no longer statistically significant (HR, 1.06; 95% CI, 0.92–1.22; Table 3).

**Kidney Transplant Failure**—The most robust association observed was between serum phosphorus level and kidney transplant failure ( $n = 238$  events; Table 2). Risk for transplant failure increased by 73% (HR, 1.73; 95% CI, 1.44–2.07) and 72% (HR, 1.72, 95% CI, 1.46–2.01), respectively, per 1-mg/dL higher serum phosphorus level in the unadjusted model and the almost fully adjusted model (without adjustment for eGFR and UACR; Table 3). Risk was attenuated but remained statistically significant in the fully adjusted model (HR, 1.36; 95% CI 1.15–1.62; Table 3). Comparison of the uppermost distribution of serum phosphorus (Table 1), that is, decile 10, 3.93 mg/dL, to the lowest quintile, 2.51 mg/dL, revealed 215% increased risk (HR, 2.15; 95% CI, 1.33–3.46) for transplant failure in the fully adjusted model.

**Mortality**—There were 348 deaths in the study cohort during the follow-up period (Table 2), predominantly from 3 causes: CVD deaths ( $n = 135$ ), deaths due to infection ( $n = 72$ ), and cancer deaths ( $n = 58$ ), but also including deaths from other causes: pulmonary ( $n = 20$ ), gastrointestinal ( $n = 16$ ), unknown ( $n = 19$ ), accidents ( $n = 12$ ), renal ( $n = 8$ ), procedural ( $n = 2$ ), valvular disease ( $n = 2$ ), pulmonary embolus ( $n = 1$ ), infectious endocarditis ( $n = 1$ ), diabetes ( $n = 1$ ), and suicide ( $n = 1$ ). The unadjusted models and almost fully adjusted model (without adjustment for eGFR and UACR) revealed that each 1-mg/dL higher serum phosphorus concentration was associated with 43% greater (HR, 1.43; 95% CI, 1.22–1.67) and 34% greater (HR, 1.34; 95% CI, 1.15–1.56) risk for all-cause mortality, respectively. Total mortality risk remained significant, although attenuated (HR, 1.21; 95% CI, 1.04–1.40) after further adjustment for eGFR and UACR (Table 3). When analyses were restricted to the 135 CVD deaths, the unadjusted model and almost fully adjusted model indicated that each 1-mg higher serum phosphorus level was associated, respectively, with 39% (HR, 1.39; 95% CI, 1.10–1.77) and 34% (HR, 1.34; 95% CI, 1.05–1.71) greater risk. This association was no



longer statistically significant on full adjustment including the kidney measures eGFR and UACR (HR, 1.15; 95% CI, 0.89–1.48; Table 3).

### Competing Risk, Interaction, and Sensitivity Analyses

We examined the composite outcome of kidney transplant failure and all-cause mortality (with a functioning transplant) to assess semicompeting risk factors (534 total events; Table 2). Risk for this composite outcome increased by 54% (HR, 1.54; 95% CI, 1.35–1.76) and 47% (HR, 1.47; 95% CI, 1.31–1.65), respectively, per 1-mg/dL higher serum phosphorus level in the unadjusted model and almost fully adjusted model. On further adjustment for eGFR and UACR, risk was attenuated to 25% (HR, 1.25; 95% CI, 1.13–1.41) but remained significant and was intermediate between the risks associated with transplant failure (36%) and total mortality (21%), as separate individual outcomes, in the same fully adjusted models (Table 3).

As noted, the almost fully adjusted model (without adjustment for eGFR or UACR) strengthened associations between a 1-mg/dL higher serum phosphorus level and CVD (HR, 1.14; 95% CI, 1.00–1.31), mortality (HR, 1.34; 95% CI, 1.16–1.55), transplant failure (HR, 1.72; 95% CI, 1.46–2.01), or the composite outcome of transplant failure and all-cause mortality (HR, 1.47; 95% CI, 1.31–1.65). Comparable findings resulted when these models were restricted to those with eGFRs of 15 to 44 mL/min/1.73 m<sup>2</sup> (CKD stages 3b–4; n = 1,428): CVD (HR, 1.11; 95% CI, 0.94–1.32), transplant failure (HR, 1.57; 95% CI, 1.31–1.89), mortality (HR, 1.21; 95% CI, 1.01–1.47), and transplant failure and all-cause mortality (HR, 1.35; 95% CI, 1.17–1.55).

Interaction analyses found no evidence for significant effect modification by serum phosphorus level with treatment assignment during the clinical trial (n = 1,569 participants each in the high- or low-dose vitamin B treatment groups), history of CVD, or history of diabetes, for any of the outcomes examined.

## DISCUSSION

Overall, we found that each 1-mg/dL higher serum phosphorus level, determined at baseline, was at least marginally associated with CVD (14% greater risk) and more strongly associated with transplant failure (72% greater risk) or total mortality (34% greater risk) in our large multicenter long-term KTR cohort after adjustment for traditional CVD risk factors, transplant type (living vs cadaveric), and transplant vintage. Further adjustment for the kidney measures eGFR and UACR attenuated these associations, rendering the CVD association even smaller (6% greater risk) and nonsignificant, but associations persisted, significantly, for both transplant failure (36% increased risk) and total mortality (21% increased risk). Serum phosphorus concentrations ≥ 3.93 mg/dL, that is, within the uppermost decile, were associated with 215% increased risk for kidney transplant failure relative to concentrations < 2.51 mg/dL (the lowest quintile distribution), even after full adjustment including kidney measures.

Our findings are concordant with most,<sup>6,7,11</sup> but not all,<sup>18</sup> previous small to moderately sized single-center studies, a sizable central European transplantation center study,<sup>3</sup> and the

lone multicenter KTR cohort study.<sup>8</sup> Regarding the latter, Pihlström et al<sup>8</sup> looked at the association of baseline phosphorus concentrations with major CVD events, kidney transplant loss, and all-cause mortality in 1,840 stable KTRs who were participants in the ALERT (Assessment of Lescol [fluvastatin] in Renal Transplantation) trial. During a mean follow-up of 6.7 years, death-censored transplant loss was recorded in 333 patients, 277 patients had a major CVD event, and 342 died, 168 from CVD. In unadjusted models, serum phosphorus level was associated with death from all causes (HR per 1-mg/dL greater serum phosphorus, 1.23; 95% CI, 1.07–1.43) and transplant loss (HR, 2.61; 95% CI, 2.25–3.04). On multivariable modeling (including PTH > 65 pg/mL), the mortality finding lost statistical significance (HR, 1.07; 95% CI, 0.89–1.28), but the transplant loss relationship persisted (HR, 1.52; 95% CI, 1.27–1.82).<sup>8</sup> In another study of stable KTRs, this time in a single center in Hungary, Wolf et al<sup>3</sup> studied 984 patients for a median follow-up of 37 months. During this time, 87 patients died and 101 patients had kidney transplant loss. In fully adjusted models (adjusting for eGFR, age, sex, systolic blood pressure, body mass index, albumin level, calcium level, modified Charlson Comorbidity Index score, and transplant vintage, as well as serum phosphorus, PTH, and FGF-23 levels), the authors found that a 0.9-mg/dL (ie, a 1 —standard deviation) higher serum phosphorus level was associated with the composite end point of death or transplant failure (HR, 1.23; 95% CI, 1.08–1.40).<sup>3</sup>

Our data are supportive of the phosphate toxicity hypothesis,<sup>9,19</sup> which could extend into the normative range of serum phosphorus levels, as Eddington et al<sup>20</sup> have reported in nontransplant CKD. However, there may also be poor clinical outcomes associated with long-term upregulation of the hormonal mechanisms that preserve phosphorus homeostasis, even in the context of normal serum phosphorus concentrations. For instance, higher serum phosphorus concentrations (secondary to dietary intake and/or decreased GFR) appear to trigger synthesis of the bone-derived hormone FGF-23, which stimulates greater renal phosphorus excretion.<sup>1,3</sup> This increase in FGF-23 level might then cause cardiac myocyte hypertrophy, clinically manifest as left ventricular hypertrophy,<sup>1,3</sup> as well as a decrease in calcitriol (1,25-hydroxyvitamin D<sub>3</sub>) synthesis from 25-hydroxyvitamin D<sub>3</sub>.<sup>1,3</sup> In patients with CKD stages 3 to 4, associations have been observed between elevated FGF-23 levels and left ventricular hypertrophy, CVD events, mortality, or progression to ESRD.<sup>1</sup> Thus, when the body attempts to bring serum phosphorus levels into the normal range by effecting a longterm increase in FGF-23 concentrations, the result may be increased risk for left ventricular hypertrophy, CVD, and ESRD.<sup>13</sup> Because higher phosphorus concentrations also trigger excess PTH secretion, clinical hyperparathyroidism may also occur in the long term.<sup>19</sup> Ultimately, the relentless progression of CKD, along with additional nephron loss, proves too much for these compensatory hormonal mechanisms, leading most patients with advanced CKD to display hyperphosphatemia, elevated FGF-23 and PTH levels, and decreased 1,25-hydroxyvitamin D<sub>3</sub> levels.<sup>1,9</sup>

The exact pathways accounting for the cytotoxicity of extracellular phosphate require much further clarification. However, there is evidence that extracellular phosphorus combines with calcium and fetuin A to form insoluble calciprotein particles.<sup>9,21</sup> These highly bioactive ligands can be cytotoxic and can also induce osteogenic transformation of vascular endothelium and renal tubular epithelium. Calciprotein particles have been detected in the circulation of animal models and also patients, including those with CKD, suggesting that



they may play a role in tissue injuries brought about by circulating phosphorus.<sup>9,21</sup> There is now a validated assay of calcification propensity (the time required for transformation of primary calciprotein particles to secondary calciprotein particles), which has been used to accumulate further evidence that disruptions to phosphorus metabolism may lead to conditions favorable to ectopic (including vascular) calcification.<sup>21</sup> Hyperphosphatemia, inadequate vitamin D status, elevations in PTH and FGF-23 levels, and greater calcification propensity have been associated with fatal CVD, transplant failure or rapid decline in eGFR, and total mortality among KTRs in epidemiologic analyses<sup>3,5,6–8,11,21</sup> and may also have clinical relevance. The association between calcification propensity—which is intimately related to serum phosphorus concentration<sup>21,22</sup> and associated with CVD outcomes in ESRD,<sup>22</sup> as well as cardiorenal outcomes or all-cause mortality among KTRs<sup>21</sup>—underscores the critical role of phosphate toxicity,<sup>9,19</sup> which may supersede putative effects of deficient vitamin D status, or excess PTH, and FGF-23 concentrations.<sup>1,3,5,8</sup> Although often neglected,<sup>1,3,5,8</sup> a remarkably consistent series of elegant in vivo mouse knockout studies and a confirmatory rat 5/6 nephrectomy model report have elucidated that irrespective of high or low FGF-23 or PTH concentrations and vitamin D status, hyperphosphatemia promotes ectopic organ and vascular calcification, accompanied by premature death.<sup>19,23–31</sup> Several of these animal model studies have also demonstrated correction of the hyperphosphatemia, uniquely, extended longevity.<sup>19,27,29,31</sup> Most strikingly, in the *Klotho*<sup>(−/−)</sup>/*NaPi2a*<sup>(−/−)</sup> double knockout mouse model, notwithstanding persistent elevations in FGF-23 levels due to the deletion of *Klotho* (ie, the membrane-bound protein that facilitates effects of FGF-23), deleting the sodium-dependent phosphate transporter *NaPi2a* lowered serum phosphorus levels, suppressed ectopic calcification, and prolonged the reduced survival characteristic of the hyper-phosphatemic *Klotho*<sup>(−/−)</sup> single knockout model.<sup>19</sup> High-phosphate diet-induced hyperphosphatemia in this *Klotho*<sup>(−/−)</sup>/*NaPi2a*<sup>(−/−)</sup> double knockout model, conversely, was marked by the reappearance of ectopic calcification and accelerated aging.<sup>19</sup>

From an epidemiologic perspective, ignoring this central mechanistic argument, which rivets on phosphorus itself; absence of ancillary measures of dysregulated phosphorus metabolism such as FGF-23, PTH, vitamin D status; and calcification propensity, is a potential weakness of our study. Despite adjustment for eGFR and UACR, there may be residual confounding by other undetermined kidney measures of kidney function or damage. Furthermore, the data are purely observational, based on a single measurement of serum phosphorus, and cannot address directly whether lowering serum phosphorus concentrations would favorably affect any of the outcomes studied.

A limitation of our study included the fact that trial participants were excluded for lack of phosphate measurement. Also, compared to the general CKD population, our study may have some limitations in terms of generalizability because of restriction to KTRs. Specifically, the selection process for kidney transplantation tends to favor patients with ESRD with greater education, social support, and adherence to medical therapy. Usually KTRs have prolonged derangement of the parathyroid gland with effects on bone disease and potentially the vascular system. Finally, transplant recipients require immunosuppression.

In conclusion, we found that serum phosphorus level was marginally associated with CVD and more strongly associated with transplant failure and total mortality in KTRs. Adjustment for the basic clinical kidney measures eGFR and UACR attenuated these associations. Our data suggest that KTRs merit a randomized controlled clinical trial that assesses the potential impact of phosphorus-lowering therapy on hard outcomes in this CKD population, such as CVD, all-cause mortality, and the development of kidney transplant failure—the last outcome, especially.

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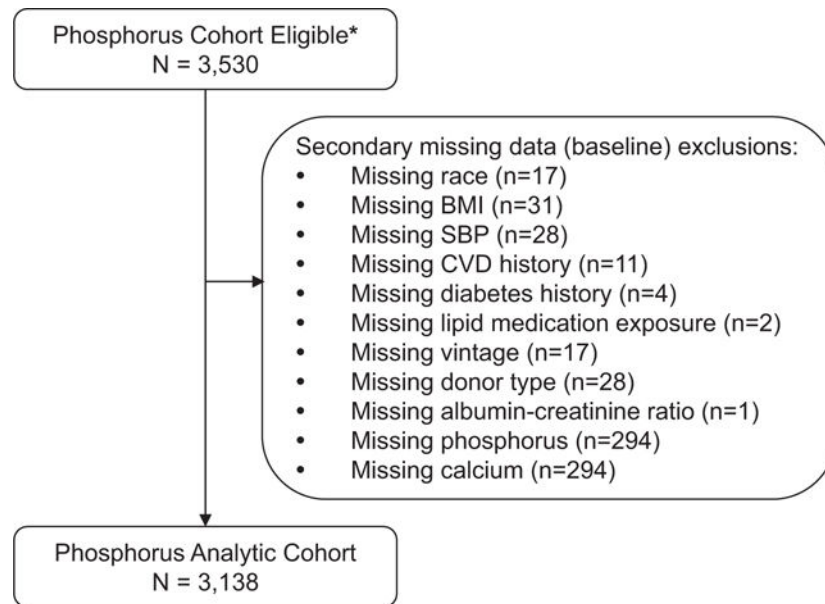
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**Figure 1.**

Derivation of phosphorus analysis cohort. \*Phosphorus cohort– eligible Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) participants were the 3,530 who were not missing baseline creatinine, estimated glomerular filtration rate, cholesterol, triglyceride, or follow-up values and who had urine sent for determination of albumin-creatinine ratio. Abbreviations: BMI, body mass index; CVD, cardiovascular disease; SBP, systolic blood pressure.

Table 1

## Baseline Characteristics by Phosphorus Concentration

Characteristic	Overall: 0.79–8.32 mg/dL (N = 3,138)	Q1: 0.79–2.51 mg/dL (n = 638)	Q2: 2.52–2.90 mg/dL (n = 643)	Q3: 2.91–3.22 mg/dL (n = 615)	Q4: 3.23–3.61 mg/dL (n = 617)	D9: 3.62–3.92 mg/dL (n = 315)	D10: 3.93–8.32 mg/dL (n = 310)	P
Age, y	51.6 ± 9.4	50.5 ± 9.3	51.6 ± 9.8	51.4 ± 9.3	52.2 ± 9.2	52.8 ± 9.3	51.5 ± 9.3	0.001
Male sex	1,973 (62.9%)	461 (72.3%)	405 (63.0%)	400 (65.0%)	367 (59.5%)	178 (56.5%)	162 (52.3%)	<0.001
White race	2,382 (75.9%)	468 (73.4%)	500 (77.8%)	476 (77.4%)	471 (76.3%)	243 (77.1%)	224 (73.5%)	0.8
Transplant vintage, y	4.0 [1.7–7.3]	3.1 [1.3–6.4]	3.6 [1.5–6.9]	4.0 [1.8–7.1]	4.2 [1.9–7.6]	4.8 [2.2–7.7]	4.9 [1.9–8.5]	<0.001
CVD history	606 (19.3%)	108 (16.9%)	96 (14.9%)	133 (21.6%)	129 (20.9%)	68 (21.6%)	72 (23.2%)	0.001
Diabetes history	1,223 (39.0%)	211 (33.1%)	223 (34.7%)	246 (40.0%)	239 (38.7%)	151 (47.9%)	153 (49.4%)	<0.001
Current smoker	340 (10.8%)	57 (8.9%)	72 (11.2%)	55 (8.9%)	72 (11.7%)	37 (11.7%)	47 (15.2%)	0.008
BMI, kg/m <sup>2</sup>	29.0 ± 6.0	29.0 ± 5.8	28.9 ± 6.0	29.2 ± 6.1	28.7 ± 5.9	29.8 ± 6.5	28.9 ± 6.3	0.8
Systolic BP, mm Hg	136.4 ± 19.8	134.3 ± 18.1	136.4 ± 19.3	136.3 ± 20.4	137.4 ± 21.2	137.9 ± 21.1	137.3 ± 20.2	0.05
Living transplant donor type	1,356 (43.2%)	254 (39.8%)	275 (42.8%)	276 (44.9%)	287 (46.5%)	160 (50.8%)	104 (33.5%)	0.6
Calcineurin inhibitor use <sup>a</sup>	2,784 (88.7%)	550 (86.2%)	582 (90.5%)	545 (88.6%)	556 (90.1%)	274 (87.0%)	277 (89.4%)	0.4
Lipid-lowering drug use	1,700 (54.2%)	319 (50.0%)	326 (50.7%)	326 (53.0%)	351 (56.9%)	194 (61.6%)	184 (59.4%)	<0.001
Prednisone use	2,862 (91.2%)	579 (90.8%)	592 (92.1%)	554 (90.1%)	571 (92.5%)	278 (88.3%)	288 (92.9%)	0.8
eGFR, mL/min/1.73 m <sup>2</sup>	49.0 ± 17.5	53.4 ± 18.0	51.0 ± 17.5	49.9 ± 17.3	47.0 ± 16.6	44.5 ± 15.8	42.5 ± 17.0	<0.001
UACR, µg/mg	31.3 ± 522.7 <sup>b</sup>	26.9 ± 475.3 <sup>b</sup>	29.4 ± 555.8 <sup>b</sup>	27.9 ± 458.9 <sup>b</sup>	31.0 ± 322.8 <sup>b</sup>	35.3 ± 526.6 <sup>b</sup>	55.6 ± 848.7 <sup>b</sup>	<0.001
Cholesterol								
Total, mg/dL	185.2 ± 43.8	184.8 ± 40.6	184.9 ± 43.9	184.7 ± 42.5	186.7 ± 46.9	183.5 ± 41.0	187.6 ± 53.7	0.8
LDL, mg/dL	101.4 ± 34.5	100.5 ± 31.4	101.6 ± 35.3	101.6 ± 33.0	102.8 ± 37.2	100.1 ± 33.1	101.8 ± 40.4	0.9
HDL, mg/dL	46.2 ± 14.0	44.5 ± 13.6	46.3 ± 13.0	46.4 ± 14.1	46.8 ± 14.4	46.2 ± 13.1	47.9 ± 16.0	0.007
Triglycerides, mg/dL	199.9 ± 190.2 <sup>b</sup>	217.5 ± 157.7 <sup>b</sup>	191.6 ± 122.9 <sup>b</sup>	192.5 ± 135.8 <sup>b</sup>	194.0 ± 137.7 <sup>b</sup>	188.1 ± 94.3 <sup>b</sup>	217.8 ± 447.2 <sup>b</sup>	0.06
Phosphorus, mg/dL	3.07 ± 0.68	2.16 ± 0.29	2.72 ± 0.12	3.06 ± 0.09	3.41 ± 0.11	3.75 ± 0.09	4.30 ± 0.47	<0.001

Note: Unless otherwise indicated, values for categorical variables are given as number (percentage); values for continuous variables, as arithmetic mean ± standard deviation or median [interquartile range]. P value comparisons across serum phosphorus categories are based on Wilcoxon-type tests for trend for categorical variables and Jonckheere-Terpstra tests for continuous variables. Conversion factors for units: cholesterol in mg/dL to mmol/L, ×0.02586; phosphorus in mg/dL to mmol/L, ×0.3229; triglycerides in mg/dL to mmol/L, ×0.01129.

Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; D, decile; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Q, quintile; UACR, urinary albumin-creatinine ratio.

<sup>a</sup>Calcineurin inhibitors are cyclosporine or tacrolimus.

$b$  Geometric mean  $\pm$  standard deviation.

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**Table 2**

Events and Rates With and Without Stratification by Phosphorus Groups

Phosphorus Group	Total	CVD		Transplant Failure		All-Cause Mortality		CVD Mortality		Transplant Failure/All-Cause Mortality Composite	
		n	Rate	n	Rate	n	Rate	n	Rate	n	Rate
Q1 (0.79–2.51 mg/dL)	638	70	26.8	31	11.5	49	17.8	19	7.0	76	28.0
Q2 (2.52–2.90 mg/dL)	643	80	31.8	35	13.4	67	25.0	21	7.9	96	36.7
Q3 (2.91–3.22 mg/dL)	615	96	40.4	49	19.9	65	25.4	29	11.4	101	40.9
Q4 (3.23–3.61 mg/dL)	617	92	40.5	53	22.3	76	30.8	34	13.6	119	50.0
D9 (3.62–3.92 mg/dL)	315	48	41.3	24	20.4	44	35.7	16	13.2	61	51.6
D10 (3.93–8.32 mg/dL)	310	50	43.9	46	40.2	47	37.9	16	13.1	81	70.5
Total events and rates irrespective of phosphorus group	3,138	436	36.1	238	19.1	348	26.9	135	10.5	534	42.7

*Note:* Rates are per 1,000 participant-years.

Abbreviations: CVD, cardiovascular disease; D, decile; Q, quintile.

Unadjusted and Multivariable-Adjusted Relative Risk Estimates for Clinical Events With Increasing Serum Phosphorus Concentration

Cox Proportional Hazards Model	Outcome				
	Cvd (436 Events)	Transplant Failure (238 Events)	All-Cause Mortality (348 Events)	Cvd Mortality (135 Events)	Transplant Failure Or All-Cause Mortality (534 Events)
Model 1 <sup>a</sup> : Unadjusted	1.25 (1.08–1.45)	1.73 (1.44–2.07)	1.43 (1.22–1.67)	1.39 (1.10–1.77)	1.54 (1.35–1.76)
Model 2 <sup>a</sup> : Multivariable Adjusted, Without Kidney Measures <sup>b</sup>	1.14 (1.00–1.31)	1.72 (1.46–2.01)	1.34 (1.16–1.55)	1.34 (1.05–1.71)	1.47 (1.31–1.64)
Model 3 <sup>a</sup> : Fully Adjusted <sup>c</sup>	1.06 (0.92–1.22)	1.36 (1.15–1.62)	1.21 (1.04–1.40)	1.15 (0.89–1.48)	1.25 (1.11–1.41)

Note: Outcomes are given as hazard ratio (95% confidence interval).

Abbreviation: CVD, cardiovascular disease.

<sup>a</sup>Per 1-mg/dL higher serum phosphorus level.

<sup>b</sup>Kidney measures are estimated glomerular filtration rate and urinary albumin-creatinine ratio; model 2 excludes these.

<sup>c</sup>Fully adjusted model 3 includes serum phosphorus level, treatment allocation, systolic blood pressure (dichotomized as 140 vs <140 mm Hg), age, sex, race, pre-existing CVD or diabetes, estimated glomerular filtration rate, natural log urinary albumin-creatinine ratio, smoking, body mass index, low-density lipoprotein cholesterol level, high-density lipoprotein cholesterol level, natural log triglyceride level, kidney transplant vintage and type (deceased vs living donor), calcineurin inhibitor use, prednisone use, and lipid-lowering drug use.