Phosphate, fibroblast growth factor 23 and retinopathy in chronic kidney disease: the Chronic Renal Insufficiency Cohort Study

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ORIGINAL ARTICLE

ABSTRACT

Background. Elevated circulating concentrations of phosphate and fibroblast growth factor 23 (FGF23) contribute to the pathogenesis of cardiovascular disease in chronic kidney disease (CKD). Retinopathy is a common manifestation of microvascular disease in CKD, but its associations with phosphate and FGF23 have not been studied. We tested the hypothesis that higher serum phosphate is associated with more severe retinopathy in individuals with CKD, independent of FGF23 and known risk factors for retinopathy.

Methods. We tested the associations of serum phosphate and plasma FGF23 with retinopathy in a cross-sectional analysis of 1800 participants in the Chronic Renal Insufficiency Cohort Study who underwent fundus photography. Retinopathy severity was graded according to the Early Treatment of Diabetic Retinopathy Severity score, and retinal venous and arterial diameters were measured.

Results. Mean estimated glomerular filtration rate (eGFR) was 46.5 ± 15.4 mL/min/1.73 m^2, mean serum phosphate was 3.7 ± 0.6 mg/dl and median plasma C-terminal FGF23 was 133 RU/mL (interquartile range 87.2, 217.8 RU/mL). In multivariable ordinal logistic regression models, higher serum phosphate was associated with greater retinopathy severity independent of hypertension, diabetes, CKD severity and FGF23 [adjusted odds ratio of being in one higher category of retinopathy severity: 1.19 per 1 standard deviation increase; 95% confidence interval (CI) 1.05, 1.36; P = 0.007]. Presence of diabetes or hypertension did not modify the results. Higher serum phosphate was also independently associated with greater retinal venous diameter (multivariable-adjusted 1.70 μm increase per 1 standard deviation increase in phosphate; 95% CI 0.46, 2.93; P = 0.007). FGF23 levels were not independently associated with retinopathy severity or retinal venous diameter, and neither FGF23 nor phosphate was associated with retinal arterial diameter.

Conclusions. Among individuals with moderate-to-severe CKD, higher serum phosphate but not FGF23 was independently associated with more severe retinopathy and microvascular retinal venous dilatation.

Keywords: CKD, FGF-23, phosphate, retinopathy, vascular disease

INTRODUCTION

Abnormalities of phosphate homeostasis contribute to the high burden of cardiovascular disease in patients with chronic kidney disease (CKD). Elevated circulating levels of phosphate and the phosphate-regulating hormone, fibroblast growth factor 23 (FGF23), are each independently associated with increased risk of cardiovascular events and mortality in patients with end-stage renal disease undergoing hemodialysis, those with earlier stages of CKD, and in the community [1–12]. Underlying these associations, higher serum phosphate concentrations contribute directly to the pathogenesis of medium and large arterial calcification, which increases risk of cardiovascular disease and death in CKD [8–11]. In contrast, elevated FGF23 levels are not consistently associated with arterial calcification but may increase the risk of cardiovascular events and death through direct effects on cardiac myocytes that culminate in left ventricular hypertrophy, atrial fibrillation and increased risk of congestive heart failure [11–16].

These results suggest that elevated phosphate and FGF23 levels may induce distinct and perhaps synergistic cardiovascular toxicities in patients with CKD, but few studies have directly compared the relationships of phosphate and FGF23 to vascular disease [17]. Furthermore, data on the effects of phosphate and FGF23 on microvascular disease are scarce. The retina is an ideal vascular bed to investigate microvascular disease in humans because it can be easily visualized *in vivo* using non-invasive techniques. Furthermore, given the similarities between the glomerular and retinal vasculature, understanding mechanisms of fundus pathology may provide novel insight into mechanisms of microvascular disease in the kidney [18]. Previously, we showed that higher serum phosphate was associated with coronary artery calcification in moderate-to-severe CKD, independently of FGF23 [11]. In this study, we tested the hypothesis that higher serum phosphate is similarly associated with more severe retinopathy and with retinal vascular disease in moderate-to-severe CKD, independent of FGF23 and established risk factors for retinopathy.

MATERIALS AND METHODS

Study population

We evaluated the associations of circulating levels of phosphate and FGF23 with retinopathy in individuals with CKD who participated in the Chronic Renal Insufficiency Cohort (CRIC) Study and its ancillary Retinopathy in CRIC Study (RCRIC). The CRIC study is a prospective observational cohort study that enrolled 3612 adults aged 21–74 years with an estimated glomerular filtration rate (eGFR) between 20 and 70 mL/min/1.73 m^2 [19, 20]. Enrollment occurred between June 2003 and August 2008 at seven main clinical centers across the USA. Exclusion criteria included pregnancy, New York Heart Association class III–IV heart failure, cirrhosis, human immunodeficiency virus infection, myeloma, renal cancer, polycystic kidney disease, recent chemotherapy or immunosuppressive therapy, institutionalization, prior treatment with dialysis for at least 1 month, organ transplantation, enrollment in other studies or inability to consent.

A primary goal of the CRIC Study is to evaluate risk factors for cardiovascular disease in patients with moderate-to-severe CKD. The goals of the RCRIC ancillary study are to investigate risk factors for retinopathy and its association with CKD progression and cardiovascular disease [21]. All participants from six of the seven CRIC clinical centers were offered inclusion into the RCRIC ancillary study. Between 2006 and 2008, 1936 of 2605 (74%) participants agreed to undergo ocular photography. Of these 1936, 1820 (94%) had photographs that were of sufficient quality to support grading of retinopathy severity in at least one or both eyes [21–23]. The final population for the current study included the 1800 of these 1820 participants who also had blood samples available to measure serum phosphate and plasma FGF23. The study adhered to the Declaration of Helsinki, was approved by the institutional review boards of the participating institutions, and all participants provided written informed consent.

Retinal photography

Participants were seated for 5 minutes in a darkened room to induce physiologic dilatation of the pupils without use of pharmacologic mydriatic compounds. A set of two 45° digital color fundus photographs were taken from each eye by trained personnel using a Canon CR-DGI, Non-Mydriatic Retinal Camera (Canon Inc., Tokyo, Japan). One image was centered on the optic disc and the other was centered on the macula. Digital fundus photographs were mailed to the University of Pennsylvania's central RCRIC Fundus Photograph Reading Center where they were evaluated by a trained grader and a retinal specialist who were masked to all participant information. The graders assessed fundus pathology, and measured the diameter of the major retinal vessels in each photograph [21, 22].

Retinopathy score

The primary outcome of this study was the retinopathy severity score. The Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol was used to assess retinopathy severity score, as described previously for diabetic and non-diabetic populations [24]. Among individuals with assessable images in both eyes, retinopathy severity was graded in both, and the eye with the more severe score was assigned as participants' overall score. Among individuals with assessable images from only one eye, the score of that eye was assigned as participants' overall score. Retinopathy features that contributed to the severity scoring included photocoagulation scars, microaneurysm and retinal hemorrhage counts, retinal hemorrhage type (flame or blot), hard and soft exudates, intraretinal microvascular abnormality, neovascularization and fibrous proliferation. Participants' overall retinopathy scores were classified on an ordered categorical scale as none (score <14), mild non-proliferative retinopathy (14–20), moderate non-proliferative retinopathy (35–53) or proliferative retinopathy (≥60), as has been done previously [22, 23]. Intergrader and intragrader reliability for retinopathy scoring was assessed in 200 eyes of 100 participants. The weighted kappa for participants' ETDRS score was 0.80 (95% CI 0.69, 0.91) for intergrader agreement and 0.77 (95% CI 0.67, 0.88) for intragrader agreement, consistent with the standard of reproducibility reported by ETDRS [22].

Retinal vessel diameter

The secondary outcomes of the study were mean retinal arterial and venous diameters. Among the 1800 participants with retinopathy severity scores, 1599 also had measurements of retinal arterial and venous caliber, which was assessed by the Atherosclerotic Risk in Communities (ARIC) protocol [25]. Distance from the optic nerve was established by overlaying a grid centered on the optic disc, and vessels were measured within an annulus of 0.5–1 disc diameter from the edge of the disc [22]. Measuring at this distance from the disc reduces potential bias that could be introduced by the presence of optic nerve atrophy. Based on the vessels' sharpness and straightness, graders chose up to six major veins and six major arteries in each eye for measurement [22]. The individual measurements were combined into summary measures that reflected the average diameters of the veins and arteries of each eye, and the averages

across both eyes served as individual participants' overall venous and arterial diameters, as has been done previously [25]. The intragrader and intergrader reliability for measuring retinal vessel caliber was assessed in 98 eyes of 50 participants. The intraclass correlation coefficient for intragrader agreement was 0.99 (95% CI 0.98, 0.99) for venous diameters and 0.96 (95% CI 0.93, 0.98) for arterial diameters. The intraclass correlation coefficient for intergrader agreement was 0.97 (95% CI 0.95, 0.98) for venous diameters and 0.89 (95% CI 0.80, 0.94) for arterial diameters [22, 23].

Exposures

The primary exposures were serum phosphate and plasma FGF23 levels, which were measured after a single thaw of samples that were collected and stored at the annual CRIC study visit most proximate to when fundus photography was performed. In 97% of CRIC participants, fasting samples were collected. The second-generation C-terminus assay (Immutopics, San Clemente, CA) was used to measure FGF23 in duplicate with a mean intra-assay coefficient of variation of <5%. Plasma parathyroid hormone (PTH) was measured using a total intact assay which captures the 1–84 and 7–84 PTH peptides with a mean coefficient of variation <5% (Scantibodies, Santee, CA). Hemoglobin A1C, total cholesterol, serum creatinine, albumin, calcium and phosphate, and urinary albumin and creatinine were measured using standard assays. All assays were performed by the CRIC Study central laboratory at the University of Pennsylvania [4]. Estimated GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [26].

Statistical analysis

We used standard descriptive statistics to summarize and compare demographics and clinical characteristics of the study population according to ascending categories of retinopathy severity. To test the associations between phosphate, FGF23 and retinopathy severity, we used ordinal logistic regression, which estimated the odds of being in one higher category of retinopathy severity (none to mild to moderate to proliferative) per 1 standard deviation increase in phosphate or natural log-transformed FGF23 (transformed to approximate a normal distribution). We fit separate models for phosphate and FGF23, and hierarchically adjusted for demographics (age, sex, race, ethnicity), traditional retinopathy risk factors [presence of diabetes, presence of hypertension, history of cardiovascular disease, body mass index (BMI), smoking, hemoglobin A1C and systolic blood pressure], CKD-specific factors (eGFR, urinary albumin-to-creatinine ratio) and other laboratory covariates (parathyroid hormone, albumin and total cholesterol). In the final models, we further adjusted the full phosphate model for FGF23, and the full FGF23 model for phosphate, to test the independent associations of each. As a sensitivity analysis, we also adjusted for the time between the retinal assessments and when blood was collected for phosphate and FGF23 testing. For ease of interpretation, we repeated these analyses using quartiles of phosphate and FGF23 instead of their continuous measures, and tested the statistical significance of the linear trend

across ascending phosphate and FGF23 quartiles in the full multivariable-adjusted ordinal logistic regression models.

We used linear regression to analyze the associations of phosphate and FGF23 with retinal vascular diameters as the outcome. Separate sets of models were fit for venous and arterial diameters. We identified factors that were significantly associated ($P < 0.05$) with retinal vascular diameter in univariable regression models and carried these factors forward into the multivariable models.

We assessed whether the associations of phosphate with retinopathy severity and retinal venous diameter were modified by sex, race, diabetes, hypertension, smoking, prior history of cardiovascular disease and eGFR by testing the significance of the interaction terms of phosphate with each of these candidate effect modifiers. All analyses were performed using Intercooled Stata 11. P values < 0.05 were considered statistically significant.

RESULTS

In the overall study population of 1800 participants, mean eGFR was 46.5 ± 15.4 mL/min/1.73 m^2, mean serum phosphate was 3.7 ± 0.6 mg/dl, median FGF23 was 133 RU/mL (interquartile range 87.2, 217.8). Sixty-nine percent of participants ($n = 1236$) demonstrated no retinopathy, 8% ($n = 141$) demonstrated mild non-proliferative retinopathy, 13% ($n = 240$) demonstrated moderate non-proliferative retinopathy and 10% ($n = 183$) demonstrated proliferative retinopathy. Clinical and demographic characteristics of study participants are presented in Table 1 according to retinopathy severity. In addition to higher levels of phosphate and FGF23, other characteristics that were associated with greater retinopathy severity included black race, Hispanic ethnicity, hypertension, diabetes, lower eGFR, higher urinary albumin-to-creatinine ratio, higher levels of PTH and lower levels of serum albumin and calcium (Table 1). There were no differences in age or sex according to retinopathy severity. Participants with more severe retinopathy had larger retinal venous diameter, but retinal arterial diameter did not vary by retinopathy severity (Table 1).

Retinopathy score

In univariable analysis, higher serum phosphate was associated with greater severity of retinopathy (Table 2). This association remained significant after adjustment for age, gender, race and ethnicity, and was only partially attenuated with further hierarchical adjustments for other known risk factors for retinopathy including the presence of diabetes and hypertension, history of cardiovascular disease, BMI, smoking, hemoglobin A1C and systolic blood pressure, and for eGFR, urinary albumin-to-creatinine ratio and other laboratory covariates. The association of serum phosphate with more severe retinopathy remained significant after adjusting for plasma FGF23 levels and when we further adjusted for the time between the retinal assessments and when serum was collected for phosphate testing (data not shown). The multivariable-adjusted odds ratio for greater retinopathy severity increased in a stepwise manner with ascending quartiles of serum phosphate (Figure 1A). There was no significant modification of the

Table 1. Characteristics according to retinopathy severity

	No retinopathy (N = 1236)	Mild non-proliferative retinopathy (N = 141)	Moderate non-proliferative retinopathy (N = 240)	Proliferative retinopathy (N = 183)	P[a]
Age, years	57.8 ± 11.0	57.2 ± 10.3	57.7 ± 10.9	56.1 ± 10.7	0.27
Sex, % female	47.1	40.4	40.8	40.4	0.09
Race-ethnicity, %					
Non-Hispanic White	53.2	36.9	32.1	31.7	<0.001
Non-Hispanic Black	38.1	52.5	53.8	53.0	
Hispanic	4.4	2.1	8.3	10.9	
Other	4.3	8.5	5.8	4.4	
Hypertension, %	79.4	92.2	92.1	95.6	<0.001
Systolic BP, mmHg	122.9 ± 19.0	129.5 ± 22.1	135.2 ± 23.0	138.4 ± 22.5	<0.001
Diastolic BP, mmHg	71.5 ± 11.8	75.4 ± 14.3	71.1 ± 13.9	71.6 ± 14.4	0.004
Diabetes mellitus, %	27.6	42.6	86.7	93.4	<0.001
Hemoglobin A1c, %	6.1 ± 1.1	6.5 ± 1.3	7.5 ± 1.7	7.9 ± 1.6	<0.001
Body mass index kg/m^2	31.1 ± 7.3	32.9 ± 8.6	32.6 ± 7.8	32.5 ± 6.8	0.002
Current smoking, %	11.0	15.6	16.7	10.9	0.05
History of CVD, %	24.1	39.0	37.5	42.6	<0.001
Medications[b]					
ACE inhibitor or ARB, %	64.6	68.6	77.7	78.1	<0.001
Diuretic, %	49.9	60.7	73.5	72.1	<0.001
Beta blocker, %	42.2	57.9	56.7	56.8	<0.001
Calcium channel blocker, %	33.6	47.1	46.2	54.1	<0.001
Statin, %	49.7	56.4	62.2	77.1	<0.001
Laboratory and renal parameters					
eGFR, mL/min/1.73m^{2c}	48.4 ± 15.5	45.5 ± 15.7	41.8 ± 14.1	40.5 ± 12.8	<0.001
Urinary albumin–creatinine ratio, mg/g	17.3 (5.5–160.5)	44.5 (9.5–341.9)	190.8 (23.9–892.2)	326.8 (61.7–1392.5)	<0.001
Serum albumin, mg/dl	4.1 ± 0.4	3.9 ± 0.4	3.8 ± 0.5	3.7 ± 0.5	<0.001
Serum calcium, mg/dl	9.2 ± 0.5	9.2 ± 0.5	9.2 ± 0.5	9.0 ± 0.5	<0.001
PTH, pg/mL	45.1 (31.7–72.5)	58.7 (39.0–102.0)	61.0 (38.0–106.0)	70.0 (39.0–125.1)	<0.001
Total cholesterol, mg/dl	184.3 ± 39.4	179.5 ± 41.7	178.3 ± 47.0	172.1 ± 53.2	0.001
Serum phosphate, mg/dl	3.6 ± 0.6	3.7 ± 0.8	3.8 ± 0.7	4.0 ± 0.7	<0.001
Plasma FGF23, RU/mL	118.7 (81.0–192.7)	137.0 (94.4–220.2)	172.7 (108.4–282.8)	194.1 (119.5–315.2)	<0.001
Retinal vascular diameters					
Venous diameter, μm	218.2 ± 22.6	220.1 ± 23.3	226.8 ± 25.7	231.0 ± 32.6	<0.001
Arterial diameter, μm	149.0 ± 14.4	148.6 ± 15.0	151.2 ± 14.2	145.3 ± 15.0	0.05

Results are reported as mean ± standard deviation, medians (interquartile range) or proportions.

BP, blood pressure; BMI, body mass index; CVD, cardiovascular disease; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; FGF23, fibroblast growth factor 23

[a]P for overall differences between groups.

[b]Values based on data available from 1792 of the total 1800 individuals.

[c]By CKD-EPI equation.

Table 2. Unadjusted and adjusted associations of serum phosphate and plasma FGF23 levels with retinopathy severity

Hierarchical models	Phosphate odds ratio (95% CI)	P	FGF23 odds ratio (95% CI)	P
Univariable	1.59 (1.43, 1.76)	<0.001	1.55 (1.40, 1.71)	<0.001
+ age, gender, race	1.57 (1.42, 1.74)	<0.001	1.53 (1.38, 1.69)	<0.001
+ CVD, DM, A1c, HTN, SBP, BMI, smoking	1.27 (1.13, 1.43)	<0.001	1.28 (1.14, 1.43)	<0.001
+ eGFR, ACR	1.15 (1.02, 1.30)	0.024	1.08 (0.95, 1.23)	0.22
+ PTH, albumin, TC	1.20 (1.05, 1.36)	0.006	1.04 (0.92, 1.19)	0.53
+ FGF23 or phosphate	1.19 (1.05, 1.36)	0.007	1.01 (0.89, 1.16)	0.83

Results are reported as odds ratios per 1 standard deviation increase in serum phosphate and natural log-transformed FGF23.

CVD, cardiovascular disease; DM, diabetes mellitus; A1c, hemoglobin A1c; HTN, hypertension; SBP, systolic blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate; ACR, albumin/creatinine ratio; PTH, parathyroid hormone; TC, total cholesterol; FGF23, fibroblast growth factor 23; CI, confidence interval.

association of higher serum phosphate with more severe retinopathy across subgroups of sex, race, diabetes, hypertension, smoking, history of cardiovascular disease or eGFR (P for interactions all >0.3).

Plasma FGF23 levels were associated with greater severity of retinopathy on univariable analysis, but the effect was attenuated after adjusting for known retinopathy risk factors and for eGFR and urinary albumin-to-creatinine ratio (Table 2). The results were unchanged when further adjusted for serum phosphate. Similarly, higher FGF23 quartiles were not independently associated with more severe retinopathy (Figure 1B).

Retinal vessel diameter

In univariable analysis, higher serum phosphate was associated with a graded increase in retinal venous diameter. After
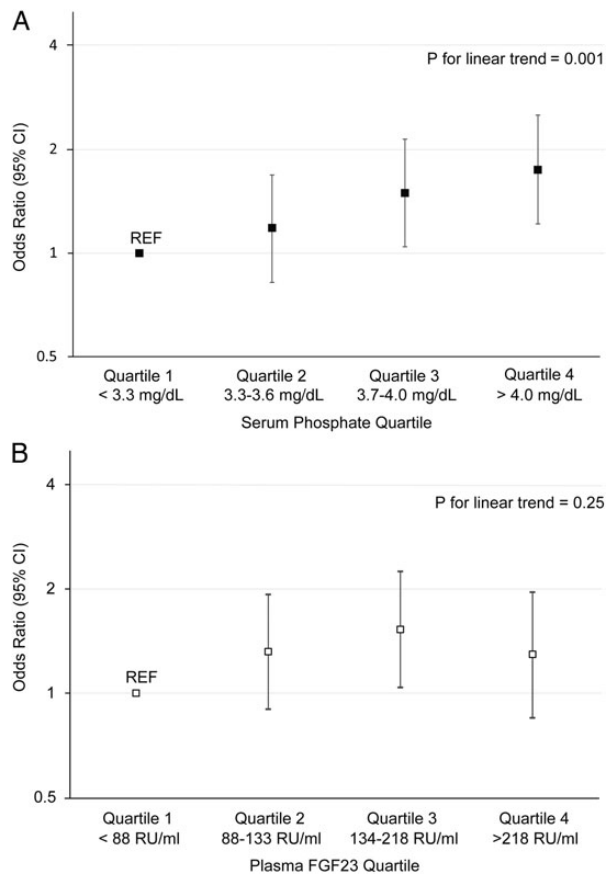
FIGURE 1: Multivariable-adjusted odds ratio for greater retinopathy severity. (**A**) Quartiles of serum phosphate; (**B**) Quartiles of plasma FGF23. For each analysis, quartile 1 served as the referent group (REF). P values represent the tests of linear trend of the ascending quartiles in the full multivariable-adjusted ordinal logistic regression models.

adjusting for other associated factors, higher serum phosphate remained independently associated with greater retinal venous diameter (Table 3). Forcing FGF23 into the multivariable model did not alter the results (Table 3). The association of higher serum phosphate with greater retinal venous diameter was unchanged when we further adjusted for the time between the retinal assessments and when blood was collected for phosphate testing (data not shown), and did not differ across subgroups of sex, race, diabetes, hypertension, smoking or prior history of cardiovascular disease (P for interactions all >0.3). FGF23 levels were not associated with retinal venous diameter in any analysis (Table 3), and neither FGF23 nor phosphate was associated with retinal arterial diameter (data not shown).

DISCUSSION

Retinopathy is a common end-organ microvascular complication of CKD [21, 22] that is associated with more rapid progression of CKD and higher risk of cardiovascular events [27–31]. In a large cohort of individuals with moderate-to-severe CKD, we observed that higher serum phosphate was associated with

more severe retinopathy independent of traditional risk factors for retinopathy, including diabetes and hypertension. Corroborating our primary findings, higher serum phosphate, along with other classic cardiovascular risk factors, was also independently associated with greater retinal venous diameter, which itself is linked to higher risk of cardiovascular events [32–34]. In each of these concordant analyses, the associations of phosphate with retinopathy were independent of FGF23, which was not associated with retinopathy or retinal venous diameter. In aggregate, these findings suggest that the retina may be a novel end organ that is susceptible to injury from higher serum phosphate concentrations. More broadly, these results offer new evidence in support of a role of phosphate excess in the pathogenesis of microvascular complications of CKD independent of FGF23.

Although serum phosphate levels were within the normal range in the majority of participants in the CRIC Study, we observed a monotonic, linear increase in retinopathy severity across the range of serum phosphate levels. This finding is consistent with previous reports of strong independent associations between higher serum phosphate, within the normal range and vascular and valvular calcification, cardiovascular events and death [9, 35, 36]. Higher phosphate concentrations can induce endothelial dysfunction, osteochondrogenic transformation of vascular smooth muscle cells and arterial calcification that contribute to a pattern of accelerated vascular aging [37–41]. It is possible that similar mechanisms also promote ocular pathology in CKD by inducing retinal vascular injury. It is, however, important to acknowledge that the definition of retinopathy included several pathological components, some of which are less likely to be plausibly related to higher serum phosphate. Although it is possible that the direct toxic effects of phosphate on retinal endothelial cells that promote capillary leak, ischemia and release of vasoactive cytokines could contribute to formation of microaneurysms, hemorrhages and other vascular changes of retinopathy, further studies are needed to investigate these possibilities [42].

Most prior studies that investigated the relationship between phosphate and cardiovascular disease focused on the arterial system. Thus, an especially novel finding of the current study is that higher serum phosphate was independently associated with greater retinal *venous* diameter. These results suggest that phosphate-associated vascular toxicity may extend beyond the arterial system to perhaps also include the venous vasculature. Ocular venous toxicity of phosphate is indirectly supported by a previous report that linked higher serum phosphate to significantly increased incidence of retinal vein occlusion [43], and by reports of eye disease in familial tumoral calcinosis, a rare disease in which deficiency of biologically active FGF23 or α-klotho results in hyperphosphatemia due to impaired renal phosphate excretion [44, 45]. Collectively, these data raise the possibility that certain aspects of the vascular toxicity of phosphate occur independently of pressure and shear, which are much lower in veins [46], and that phosphate may specifically injure endothelial cells that are common to arteries and veins. This could result in not only pathological structural changes that are characteristic of retinopathy, but also microcirculatory changes that culminate in retinal venous

Table 3. Unadjusted and adjusted associations of demographic and clinical factors associated with mean retinal venous diameter

	Univariable β (95% CI)	P	Multivariable β (95% CI)	P	Multivariable including FGF23 β (95% CI)	P
Age, per 10 year increase	−4.49 (−5.56, −3.43)	<0.001	−3.63 (−4.81, −2.45)	<0.001	−3.63 (−4.81, −2.54)	<0.001
Sex, female versus male	0.10 (−2.29, 2.49)	0.94	—		—	
Race-ethnicity						
Non-Hispanic white	Reference		Reference		Reference	
Non-Hispanic black	15.74 (13.38, 18.11)	<0.001	14.63 (12.21, 17.06)	<0.001	14.63 (12.21, 17.06)	<0.001
Hispanic	16.87 (11.47, 22.28)	<0.001	12.61 (7.11, 18.11)	<0.001	12.59 (7.09, 18.10)	<0.001
Other	13.28 (7.90, 18.65)	<0.001	10.92 (5.46, 16.37)	<0.001	10.90 (5.44, 16.36)	<0.001
Diabetes mellitus, present versus absent	4.86 (2.42, 7.30)	<0.001	3.65 (1.15, 6.14)	0.004	3.66 (1.16, 6.15)	0.004
SBP, per 10 mmHg	0.20 (−0.38, 0.78)	0.50	—		—	
eGFR, per 10 mL/min/1.73m² increase	0.81 (0.04, 1.57)	0.04	1.11 (0.25, 1.97)	0.012	1.08 (0.18, 1.98)	0.019
Ln ACR, per 1 SD increase	2.78 (1.56, 4.00)	<0.001	0.68 (−0.65, 2.01)	0.316	0.71 (−0.64, 2.07)	0.303
Calcium, per 1 SD increase	−0.92 (−2.13, 0.28)	0.13	—		—	
Phosphate, per 1 SD increase	**2.82 (1.61, 4.04)**	**<0.001**	**1.70 (0.46, 2.93)**	**0.007**	**1.72 (0.47, 2.97)**	**0.007**
Ln FGF23, per 1 SD increase	**0.52 (−0.66, 1.70)**	**0.39**	**—**		**−0.16 (−1.45, 1.13)**	**0.809**
Ln PTH, per 1 SD increase	0.23 (−0.99, 1.45)	0.71	—		—	

Results (β coefficients) refer to the mean μm increase in venous diameter. Factors that were significant in univariable analyses were included in the multivariable model. SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; ACR, albumin/creatinine ratio; FGF23, fibroblast growth factor 23, PTH, parathyroid hormone; SD, standard deviation; CI, confidence interval; Ln, natural log; vs, versus. Phosphate and Ln FGF23 are bolded as they are the primary exposures in the current study.

dilatation. Alternatively, retinal venous dilatation may be an indirect consequence of phosphate-induced arterial toxicity that results in retinal ischemia and subsequent release of vasodilatory cytokines such as nitric oxide, interleukin 1 and tumor necrosis factor α, that induce secondary venous dilatation [47]. Factors such as optic atrophy, venous congestion and vascular wall thinning are other possible alternative mechanisms of retinal venous dilatation. Additional research is needed to investigate these possibilities.

In contrast to serum phosphate, the level of FGF23 was not independently associated with retinopathy severity or retinal venous caliber. FGF23 regulates serum phosphate by stimulating urinary phosphate excretion and modulating renal production and degradation of 1,25-dihydroxyvitamin D [15]. As glomerular filtration of phosphate gradually declines with CKD progression, rising FGF23 levels maintain normal serum phosphate levels. However, chronic elevation of FGF23 may be ultimately maladaptive as shown by its association with increased risk for left ventricular hypertrophy, congestive heart failure and death [3, 12–15]. In contrast, higher FGF23 levels have not been consistently associated with increased risk of atherosclerotic events or coronary artery calcification, and, unlike phosphate, *in vitro* studies confirmed no direct pro-calcification effects of FGF23 on vascular smooth muscle cells or explanted aortic rings [11, 48]. This suggests distinct pathways whereby phosphate promotes cardiovascular disease through vascular toxicity whereas FGF23 contributes via cardiac toxicity [17]. While preclinical data suggest that FGF23 and phosphate may each have adverse effects on microcirculatory function [49, 50], the current study provides further support for the emerging concept of distinct cardiovascular toxicities of phosphate and FGF23.

The large sample size, detailed covariate data, rigorous adjudication of retinopathy severity and novel results are strengths of the current study. We also acknowledge certain limitations. The study design precludes us from determining whether higher serum phosphate predates or follows the development of retinopathy. However, given the lack of prior studies to investigate disordered phosphate homeostasis and microvascular disease of the eye, the current results provide the impetus to extend this line of investigation into prospective studies with repeated, longitudinal evaluations of the fundus. Single measurements of serum creatinine, phosphate and FGF23, and single sets of fundus photographs, occasionally with only one evaluable eye, may have led to misclassification of the severity of retinal disease, the severity of CKD or its associated alterations in mineral metabolites. However, if such misclassification was random, as expected, it would have biased our results towards the null hypothesis of no associations. Another potential limitation is that although retinopathy is a leading cause of vision loss in diabetes, the clinical implications of the quantitative measurements of retinal arterial and venous caliber are currently less clear. Finally, although we adjusted for a wide range of potentially confounding clinical covariates, since we were unable to adjust for vitamin D levels, which have been associated with retinopathy [51], we cannot exclude the possibility of residual confounding.

In conclusion, higher serum phosphate but not FGF23 was independently associated with more severe retinopathy and greater retinal venous diameter independent of diabetes, hypertension and other cardiovascular risk factors. Since phosphate excess is also associated with cardiovascular events and accelerated progression of CKD, perhaps it activates common mechanisms of microvascular injury in the kidney, retina and elsewhere. Additional human studies are needed to validate our results in both CKD and non-CKD populations, and if confirmed, further investigation of potential molecular mechanisms of phosphate-associated retinopathy and retinal venous disease should be pursued. Given the likelihood of shared mechanistic pathways underlying microvascular disease in different organs, such studies could yield novel insights into vascular pathobiology and mechanisms of CKD progression.

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