Higher serum phosphate concentrations are independently associated with cardiovascular events and all-cause mortality.\(^1,2\) Although these associations have largely been attributed to an increased risk for large-vessel calcification, our recent work demonstrated that higher morning serum phosphate level is associated with microvascular dysfunction, even in persons without kidney disease.\(^3\) However, fasting serum phosphate level may not be a good reflection of total phosphate intake, and the driver of this microvascular dysfunction may be dietary phosphate.\(^4,5\) Considering the growing evidence suggesting that phosphate may induce microvascular dysfunction, we evaluated the association between 24-hour urinary phosphate excretion (UPE; a surrogate for dietary phosphate) and microvascular dysfunction. We hypothesized that higher UPE would be associated with impaired microvascular function in community-dwelling adults.

The Maastricht Study, described in detail elsewhere,\(^6\) is an observational prospective population-based cohort study focused on the cause, pathophysiology, and complications of type 2 diabetes mellitus. A total of 3,116 participants underwent UPE measurement. Subsets of the cohort underwent finger skin capillaroscopy, laser-Doppler flowmetry, or flicker light–induced retinal vessel dilation, 3 validated markers of microvascular function.\(^7\) We used linear regression to evaluate the association between UPE and (1) postocclusive finger skin capillary recruitment, (2) capillary recruitment during venous congestion, (3) heat-induced skin hyperemic response (using laser-Doppler flowmetry), (4) retinal arteriolar dilation in response to flicker light, and (5) retinal venular dilation in response to flicker light.

We created 2 models. Our initial model adjusted for age and sex. The second model additionally adjusted for body mass index, diabetes status, smoking status, systolic blood pressure, antihypertensive use, lipid-lowering medication, serum calcium level, and estimated glomerular filtration rate (eGFR; 4-variable Modification of Diet in Renal Disease Study equation).\(^8\) This study was approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sport of the Netherlands (permit 131088-105234-PG). All participants gave written informed consent.

Participant demographic data for this population have been described previously.\(^9\) Briefly, mean age was 60 ± 8 years, 48% were women, and mean serum phosphate level was 3.2 mg/dL (Table 1). Mean eGFR was 81 ± 17 mL/min/1.73 m\(^2\) and 7% had eGFR < 60 mL/min/1.73 m\(^2\). Mean UPE was 874 ± 315 mg/d. We found an inverse relationship between UPE and serum phosphate level (\(r = -0.26; P < 0.001\)). Results were similar in linear regression models that adjusted for eGFR.

In both our initial and our fully adjusted model, UPE was not associated with any of the microvascular outcomes (Table 2) and there were no significant interactions between UPE and sex, diabetes status, or eGFR on any of the outcomes (\(P > 0.43\)). This is in contrast to the associations evaluating serum phosphate level, which was associated with 2 of the markers of impaired microvascular function, as previously reported (Table 2).\(^3\) In addition, higher serum phosphate level was associated with worse heat-induced skin hyperemic response in men, but not women.\(^7\) We then performed sensitivity analyses, excluding any 24-hour urine collection that had a total urinary creatinine level that was >30% discordant with the expected urinary creatinine excretion because these samples may bias toward the null.\(^9\) In this subcohort, our findings were essentially unchanged.

In this analysis of a large well-characterized cohort of community-dwelling men and women, we demonstrate that higher UPE was not associated with diminished microvascular function. This stands in contrast to serum phosphate level, for which higher concentrations are associated with worse microvascular function in the same individuals. Although higher dietary phosphate intake is often assumed to drive higher serum phosphate concentrations, we found a weak and inverse correlation between 24-hour UPE and serum phosphate concentration, findings similar to those reported in other large cohorts with and without chronic kidney disease (CKD).\(^4,10\) Collectively, these results suggest that a high-phosphate diet alone is not the main driver of the association between higher serum phosphate concentration and microvascular dysfunction. Other factors regulating serum phosphate concentrations are likely to be the main drivers.

Multiple studies of animals and humans have suggested that increased dietary phosphate intake induces microvascular dysfunction. In a rodent model of CKD and endothelial dysfunction, a low-phosphate diet improved vasodilation of the thoracic aorta through induction of increased endothelial nitric oxide synthase activation.\(^11\) Shuto et al\(^12\) gave 11 healthy volunteers high- and low-phosphate diets in a crossover design. The high-phosphate diet induced an increase in postprandial serum phosphate levels and a reduction in brachial artery flow-mediated dilation.\(^12\) Thus, we had hypothesized that a higher-phosphate diet (as indicated by UPE) would be associated with impaired microvascular function assessed by direct measurements of the microvasculature. However, we found no such association, implying that the relationship between morning serum phosphate level and microvascular dysfunction in the Maastricht Study is not diet mediated.

The null results of this study have some important potential implications. In addition to our original Maastricht Study evaluating fasting serum phosphate level, multiple other studies have demonstrated an association
between fasting serum phosphate level and poor vascular outcomes. However, our current work supports the idea that the mechanism of this association is not diet mediated. This is not to say that dietary phosphate restriction is without benefit, only that it may not be the driver of the associations demonstrated in these mentioned studies.

This analysis has several important strengths. First, the Maastricht Study provided several distinct microvascular-imaging techniques performed on more than 3,000 participants with a range of comorbid conditions. The availability of concurrent information on demographics, cardiovascular risk factor status, kidney function, and 24-hour urinary collections are additional key strengths. Limitations of this study include the cross-sectional design and limited number of individuals with CKD in this cohort.

In conclusion, we found no relationship between UPE and microvascular function in community-living individuals predominantly with normal kidney function. Higher UPE was also not associated with higher morning phosphate concentrations. Further studies, including feeding studies, are needed to further explore the relationship between dietary phosphate intake and its effects on serum phosphate concentrations and microvascular function.

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Table 1. Baseline Characteristics of Participants by 24-Hour Urinary Phosphate Quartiles

| Urinary Phosphate Quartile | Q1 (n = 779) | Q2 (n = 781) | Q3 (n = 777) | Q4 (n = 779) |
|---------------------------|-------------|-------------|-------------|-------------|
| Urinary phosphate range, mg/d | 93-690 | 691-874 | 875-1,082 | 1,083-3,447 |
| Age, y                      | 60 (9)     | 60 (8)     | 60 (8)     | 59 (8)     |
| Male sex                   | 208 (26%)  | 326 (41%)  | 443 (57%)  | 629 (81%)  |
| Body mass index, kg/m²     | 26 (5)     | 27 (4)     | 27 (5)     | 28 (4)     |
| Smoking                     |            |            |            |            |
| Never                      | 263 (35%)  | 275 (36%)  | 263 (34%)  | 247 (32%)  |
| Former                     | 380 (50%)  | 394 (51%)  | 401 (52%)  | 424 (55%)  |
| Current                    | 119 (16%)  | 97 (13%)   | 100 (13%)  | 97 (13%)   |
| Diabetes status            |            |            |            |            |
| Normal                     | 423 (54%)  | 452 (58%)  | 438 (56%)  | 435 (56%)  |
| Prediabetes                | 86 (11%)   | 115 (15%)  | 110 (14%)  | 143 (18%)  |
| Type 2 diabetes            | 270 (34%)  | 214 (27%)  | 229 (29%)  | 201 (26%)  |
| Hemoglobin A₁c, %          | 6.0 (1.0)  | 5.9 (0.9)  | 5.9 (0.9)  | 5.9 (0.9)  |
| Retinopathy                | 14 (6%)    | 13 (7%)    | 11 (5%)    | 8 (4%)     |
| Cardiovascular disease     | 147 (20%)  | 120 (16%)  | 110 (15%)  | 117 (15%)  |
| 24-h systolic BP, mm Hg    | 116 (12)   | 118 (12)   | 120 (11)   | 122 (11)   |
| 24-h diastolic BP, mm Hg   | 72 (7)     | 73 (7)     | 74 (7)     | 76 (7)     |
| BP medications             | 335 (46%)  | 309 (40%)  | 300 (39%)  | 286 (37%)  |
| Hyperlipidemia medications | 318 (40%)  | 270 (35%)  | 284 (37%)  | 262 (34%)  |
| eGFR, ml/min/1.73 m²       | 79 (20)    | 80 (16)    | 82 (16)    | 83 (15)    |
| Chronic kidney disease     | 79 (10%)   | 68 (9%)    | 49 (6%)    | 30 (4%)    |
| Urinary albumin-creatinine ratio, mg/g | 5 [3-9] | 5 [3-9] | 4 [2-8] | 4 [2-8] |
| Calcium, mg/dL             | 9.4 (0.4)  | 9.4 (0.3)  | 9.3 (0.3)  | 9.3 (0.3)  |
| Phosphate, mg/dL           | 3.4 (0.5)  | 3.3 (0.5)  | 3.2 (0.5)  | 3.0 (0.5)  |

Note: Variables are presented as number (percent) for binary variables. For continuous variables, they are presented as mean (standard deviation) if normally distributed and median (interquartile range) if not normally distributed. Certain variables were not reported in some participants.

Abbreviations: BP, blood pressure; eGFR, estimated glomerular filtration rate; Q, quartile.
Table 2. Association of 24-Hour Urinary Phosphate Excretion and Serum Phosphate Concentration With Microvascular Measurements

| % Capillary recruitment during postocclusive reactive hyperemia (N = 641) | Range, mg/dL Per 100 mg/d Higher (Urinary) | P | Range, mg/dL Per 1 mg/dL Higher (Serum) | P |
|---|---|---|---|---|---|
| Model 1 | 0.3 (−0.4 to 1.0) | 0.32 | −5.2 (−10.1 to −0.4) | 0.04 |
| Model 2 | 0.1 (−0.7 to 0.9) | 0.82 | −5.0 (−10.0 to −0.1) | 0.04 |
| % Capillary recruitment during venous congestion (N = 641) | | | | |
| Model 1 | 0.49 (−0.28 to 1.3) | 0.21 | −4.7 (−9.8 to 0.5) | 0.08 |
| Model 2 | 0.2 (−0.7 to 1.0) | 0.65 | −4.5 (−9.8 to 0.7) | 0.09 |
| % Heat-induced skin hyperemic response (N = 1,282) | | | | |
| Model 1 | −5.0 (−18 to 8) | 0.45 | −53 (−140 to 35) | 0.24 |
| Model 2 | −9 (−24 to 5) | 0.20 | −25 (−113 to 63) | 0.57 |
| % Retinal arteriolar dilation (N = 2,008) | | | | |
| Model 1 | 0.03 (−0.01 to 0.08) | 0.10 | −0.18 (−0.43 to 0.07) | 0.15 |
| Model 2 | 0.00 (−0.04 to 0.04) | 0.88 | −0.12 (−0.39 to 0.15) | 0.39 |
| % Retinal venular dilation (N = 2,046) | | | | |
| Model 1 | 0.01 (−0.02 to 0.05) | 0.48 | −0.26 (−0.47 to −0.05) | 0.01 |
| Model 2 | 0.001 (−0.03 to 0.04) | 0.94 | −0.23 (−0.44 to −0.02) | 0.03 |

Note: Model 1 is adjusted for age and sex. Model 2 is additionally adjusted for body mass index, smoking status, blood pressure, use of antihypertensive medications, use of lipid-modifying medications, glucose metabolism status, estimated glomerular filtration rate, and serum calcium level.

Data for serum phosphate analysis from prior study.

REFERENCES

1. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol*. 2004;15(8):2208-2218.

2. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G. Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. *Circulation*. 2005;112:2627-2633.

3. Ginsberg C, Houben AJHM, Malhotra R, et al. Serum phosphate and microvascular function in a population based cohort. *Clin J Am Soc Nephrol*. 2019;14(11):1626-1633.

4. Palomino HL, Rifkin DE, Anderson C, Criqui MH, Whooley MA, Ix JH. 24-hour urine phosphate excretion and mortality and cardiovascular events. *Clin J Am Soc Nephrol*. 2013;8(7):1202-1210.

5. Portale AA, Halloran BP, Morris RC Jr. Dietary intake of phosphorus modulates the circadian rhythm in serum concentration of phosphorus (implications for the renal production of 1,25-dihydroxyvitamin D). *J Clin Invest*. 1987;80:1147-1154.

6. Schram MT, Sep SJ, van der Kallen CJ, et al. The Maastricht Study: an extensive phenotyping study on determinants of type 2 diabetes, its complications and its comorbidities. *Eur J Epidemiol*. 2014;29(6):439-451.

7. Li W, Schram MT, Sorensen BM, et al. Microvascular phenotyping in the Maastricht Study: design, and main findings, 2010-2018. *Am J Epidemiol*. 2020;189(9):873-884.

8. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Modification of Diet in Renal Disease Study Group*. *Clinical Journal of the American Society of Nephrology*. 2001;6(7):1867-1872.
of Diet in Renal Disease Study Group. *Ann Intern Med.* 1999;130:461-470.

9. Ix JH, Wassel CL, Stevens LA, et al. Equations to estimate creatinine excretion rate: the CKD Epidemiology Collaboration. *Clin J Am Soc Nephrol.* 2011;6(1):184-191.

10. Isakova T, Gutierrez OM, Smith K, et al. Pilot study of dietary phosphorus restriction and phosphorus binders to target fibroblast growth factor 23 in patients with chronic kidney disease. *Nephrol Dial Transplant.* 2011;26:584-591.

11. Van TV, Watari E, Taketani Y, et al. Dietary phosphate restriction ameliorates endothelial dysfunction in adenine-induced kidney disease rats. *Clin Biochem Nutr.* 2012;51(1):27-32.

12. Shuto E, Taketani Y, Tanaka R, et al. Dietary phosphorus acutely impairs endothelial function. *J Am Soc Nephrol.* 2009;20(7):1504-1512.

13. Foley RN, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. *J Am Soc Nephrol.* 2009;20:397-404.