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Acid Load and Phosphorus Homeostasis in CKD

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Abstract

Background—The kidneys maintain acid-base homeostasis through excretion of acid as either ammonium or as titratable acids (TA) that primarily use phosphate as a buffer. In CKD, ammoniogenesis is impaired, promoting metabolic acidosis. Metabolic acidosis stimulates phosphaturic hormones, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF-23) in vitro, possibly to increase urine TA buffers, but this has not been confirmed in humans. We hypothesized that higher acid load and acidosis would associate with altered phosphorus homeostasis including higher urinary phosphorus, PTH and FGF-23.

Study Design—Cross-sectional

Setting & Participants—980 participants with CKD enrolled in the Chronic Renal Insufficiency Cohort (CRIC) Study

Predictors—Net acid excretion as measured in 24-hour urine, potential renal acid load (PRAL) estimated from food frequency questionnaire responses, and serum bicarbonate < 22 mEq/L

Outcome & Measurements—24h urine phosphorus and calcium; serum phosphorus, FGF-23, and PTH

Results—Using linear and log-linear regression adjusted for demographics, kidney function, comorbidities, body mass index, diuretic use and 24h urine creatinine, we found that 24h urine phosphorus was higher at higher net acid excretion, higher PRAL, and lower serum bicarbonate (each $p < 0.05$). Serum phosphorus was also higher with higher net acid excretion and lower serum bicarbonate (each $p = 0.001$). Only higher net acid excretion associated with higher 24h urine calcium excretion ($p < 0.001$). Neither net acid excretion nor PRAL were associated with FGF-23 or PTH. PTH, but not FGF-23 ($p = 0.2$), was 26% (95% CI, 13%-40%) higher in participants with a serum bicarbonate $22 < \text{vs } 22 \text{ mEq/L}$ ($P < 0.001$). Primary results were similar if stratified by eGFR categories, or if adjusted for iothalamate GFR ($n = 359$), total energy intake, dietary phosphorus or urine urea nitrogen, where available.

Limitations—Possible residual confounding by kidney function or nutrition; urine phosphorus was included in calculation of the titratable acid component of net acid excretion

Conclusions—In CKD, higher acid load and acidosis associates independently with increased circulating phosphorus and augmented phosphaturia, but not consistently with FGF-23 or PTH. This may be an adaptation that increases TA excretion, and thus helps maintain acid-base homeostasis in CKD. Understanding if administration of base can lower phosphorus requires testing in interventional trials.

Keywords

acid-base; acidosis; phosphorus; fibroblast growth factor 23 (FGF-23); parathyroid hormone (PTH); chronic kidney disease (CKD); phosphorus homeostasis; phosphorus excretion; FEP; phosphaturic hormones; acid load; physiology; potential renal acid load (PRAL)

The major role of the kidneys is to maintain the internal milieu, including preserving acid-base and phosphorus homeostasis. In health, the kidney regulates acid-base balance by excreting fixed acids generated from nutrient metabolism ^{1,2}, and phosphorus balance by excreting the absorbed load of ingested phosphorus ³. As kidney function declines in chronic kidney disease (CKD), both of these functions are compromised. To overcome lower acid excretion in the form of ammonium (NH_4^+), excretion of titratable acid in the form of monovalent phosphate (H_2PO_4^-) may be relatively increased through reduction in urine pH or augmented phosphaturia ⁴⁻⁷. Additionally, to prevent hyperphosphatemia, fractional phosphorus excretion may be augmented by the phosphorus-regulatory hormones parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF-23) ³. Thus, both of these processes occur in tandem during progressive kidney disease and may converge on urine phosphorus excretion as a critically regulated parameter (see Figure S1, available as online supplementary material). Nonetheless, few studies have evaluated the relationship between derangements in acid-base and derangements in phosphorus homeostasis in patients with CKD.

Several animal and *in vitro* models suggest that changes in acid-base status and acid load may affect phosphorus homeostasis ⁷⁻¹². For instance, acid loading in rats increases urine phosphorus, presumably to augment titratable acid excretion ¹¹. However, study conclusions differ about whether this phenomenon is the result of indirect effects of the phosphaturic hormones PTH and FGF-23 ^{8,9,12}, or direct effects on sodium-phosphate transporters ^{7,11}. Furthermore, in studies documenting increased PTH and FGF-23 transcription, the pH used to elicit these changes was below the typical systemic pH of patients with early to moderate CKD ^{8,12}. Thus, few studies in animals or humans have evaluated integrated acid-base and phosphorus physiology relevant to early or moderate CKD when overt metabolic acidosis is uncommon, but adaptations to increase titratable acidity may already be occurring ^{7,13}. Due to well-documented risks associated with even minor changes in FGF-23 and other aspects of mineral metabolism ¹⁴, acidosis and acid load may represent additional targets to prevent early derangements in the phosphorus axis. In this study, we used comprehensive data from a prospective cohort of patients with CKD to test the hypothesis that acid load and acidosis are associated with phosphorus homeostasis and phosphaturic hormones.

Methods

Study Population

The Chronic Renal Insufficiency Cohort (CRIC) Study is an observational cohort study of 3939 individuals with CKD recruited in 2003-2008 at multiple centers in the United States. All participants were aged 21-74 years with an estimated glomerular filtration rate (eGFR) within 20-70 ml/min/1.73 m² at enrollment. By design, approximately 50% of participants

had diabetes mellitus. Participants with multiple myeloma, polycystic kidney disease or requiring active immunosuppressive therapy were excluded. Detailed inclusion and exclusion criteria have been previously reported ^{15,16}. The current report includes 980 CRIC participants who were randomly selected to have net acid excretion measurements performed in 24-hour urine collections from baseline (n=1000) and who had a urine pH of 4.0-7.4 (n=20 excluded). All participants provided written informed consent for participation in the CRIC Study. The CRIC Study was approved by the Institutional Review Board (IRB) at all clinical centers and the net acid excretion ancillary study was deemed exempt by the Duke University Health System IRB under protocol number Pro00056642.

Measurements and Data Collection

We quantified acid load as net acid excretion and potential renal acid load (PRAL) and acidosis based on serum bicarbonate, each as exposure variables. Outcomes include urine and serum phosphorus, urine calcium, FGF-23, and PTH. Net acid excretion was calculated from urine ammonium, pH, phosphorus and creatinine (see end of paragraph). Other urine measurements were used as dietary biomarkers, such as urine urea nitrogen and urine sulfate, or outcome measurements (e.g., urine calcium and phosphorus). Urine ammonium, pH and sulfate were measured in baseline 24-hour urine collections that had been stored at -80°C since collection. Urine pH was measured by an electrode, urine ammonium was measured using enzymatic assays, and urine sulfate by turbidometric assay, each by Litholink Corp® (Chicago, IL). Other clinical urine variables (e.g., urine phosphorus, calcium, creatinine and urine nitrogen) were measured in the central CRIC laboratory in baseline 24-hour urine samples that had been stored at -20°C. Titratable acidity was calculated using the Henderson Hasselbalch equation, urine pH, phosphorus, and creatinine and the pK_a values of the relevant reactions, as previously described by our group ¹³ and others ¹⁷⁻¹⁹. Net acid excretion was calculated as the sum of titratable acidity and urine ammonium, with urine bicarbonate assumed negligible in a urine pH range of 4.0 to 7.4 ¹³.

Bicarbonate, phosphorus, intact PTH (Scantibodies, Santee, California) and carboxy-terminal FGF-23 (Immutopics, San Clemente, California) were measured in baseline serum samples at the CRIC central laboratory. Dietary intake was assessed using the National Cancer Institute Diet History Questionnaire²⁰. The PRAL estimates the contribution of the diet to fixed acid production as follows: $PRAL = 0.49 \times \text{protein (g)} + 0.037 \times P \text{ (mg)} - 0.021 \times K \text{ (mg)} - 0.026 \times Mg \text{ (mg)} - 0.013 \times Ca \text{ (mg)}$ ²¹. PRAL was set to missing for individuals using alkali supplements (n=23).

Additional covariates were measured per CRIC protocol. Demographics and medical history were assessed by self-reported questionnaire. History of cardiovascular disease was defined as self-reported history of congestive heart failure, peripheral vascular disease, stroke, prior myocardial infarction or coronary revascularization. History of diabetes mellitus was defined as fasting glucose ≥ 126 mg/dl, random glucose ≥ 200 mg/dl or use of diabetic medications including insulin or hypoglycemic medications each at the baseline study visit. A history of smoking was defined as lifetime use of ≥ 100 cigarettes. Body composition including body mass index (BMI) was assessed by anthropometry. Kidney function was measured as eGFR using the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation; and

albuminuria quantified as total albumin excretion in a 24 hour urine collection. Per the CRIC protocol, measured iothalamate glomerular filtration rate (iGFR) was performed by urine clearance in 1/3 of the cohort ^{16,22}.

Statistical Analysis

We examined baseline characteristics of study participants by categories of serum bicarbonate. Characteristics according to quartiles of net acid excretion have been previously reported ¹³. We modeled the associations between acid-base parameters (net acid excretion, PRAL and serum bicarbonate) and the expected mean outcome level (urine and serum phosphorus, urine calcium, FGF-23, PTH) using generalized linear models. All models adjusted sequentially for demographics (age, sex, black versus nonblack race), kidney function (eGFR and 24-hour urine albumin), comorbidities (cardiovascular disease, diabetes mellitus, and ever versus never smoking), BMI, diuretic use and 24 hour urine creatinine. Urine calcium, FGF-23 and PTH were log-transformed prior to modeling due to skewed distributions. Thus, results for these outcome variables are reported as percent difference in the geometric mean. All models incorporate robust variance estimation due to heteroscedastic residuals noted during model checking for some outcomes. Adjusted quantile regression was used to model the median of untransformed FGF-23, PTH and urine calcium as an alternative methodology ²³. Confidence intervals (CIs) for these models were calculated by bootstrapping with 1000 replicates.

We performed several secondary, sensitivity and subgroup analyses to ensure robust results. First, because FGF-23 and PTH modulate phosphorus homeostasis in part by altering the fractional excretion of phosphorus (FE_{pi}), we evaluated FE_{pi} as a secondary outcome. FE_{pi} was calculated as (urine phosphorus* serum creatinine)/(serum phosphorus * urine creatinine) * 100. FE_{pi} >100% were excluded from the analysis (n=6). Second, because urine phosphorus is used in calculation of the titratable acid component of net acid excretion, we also performed sensitivity analyses evaluating associations of independently measured components of acid excretion (i.e., urine ammonium and urine pH) with urine phosphorus. Third, urine phosphorus, serum phosphorus and acidification parameters may each be influenced by nutrition; thus, we performed analyses additionally adjusted for nutrition parameters including total energy intake and dietary phosphorus, as well as urine urea nitrogen and urine sulfate as unbiased measures of protein intake. Finally, net acid excretion, serum bicarbonate and mineral metabolites can be affected by kidney function. We evaluated each relationship adjusted for directly measured (using iothalamate clearance) GFR where available (n=359). As secondary analyses, we performed models stratified by sex, level of eGFR, BMI, diuretic use, and history of diabetes. All analyses were performed using Stata SE version 13 (StataCorp LP, College Station, TX).

Results

Study Participants and Overview

Overall, the study population included 57% males and 51% of the participants had diabetes. Mean eGFR was 44.0 ± 14.4 mL/min/1.73 m²; mean net acid excretion was 33 ± 18 mEq/d. Mean PRAL was -3.4 ± 20.0 mEq/day. Eighteen % of the study population had a serum

bicarbonate < 22 mEq/L. Several acid-base exposure measures were modestly to moderately correlated (see Table S1). Lower serum bicarbonate was associated with greater likelihood of diabetes, lower eGFR, greater albuminuria, as well as lower urine pH, potassium and creatinine (Table 1). Clinical characteristics according to quartiles of net acid excretion have been previously published and are notable for associations of higher net acid excretion with higher eGFR, greater total energy intake and larger body size, among other characteristics¹³.

Urine and Serum Phosphorus

24 hour urine phosphorus was significantly higher among those with higher net acid excretion in demographic-adjusted models (p for trend <0.001) and after full adjustment (437 [95% CI, 377-497] mg higher in quartile 4 versus 1; Figure 1). Likewise, higher quartiles of PRAL were also associated with greater 24 hour urine phosphorus in demographic-adjusted models (p for trend=0.002) and fully adjusted models (77 [95% CI, 11-143] mg higher in quartile 4 versus 1; Figure 1). Lower serum bicarbonate was not associated with 24 hour urine phosphorus in unadjusted models; however, after full adjustment, 24 hour urine phosphorus was 52 (95% CI, 10-93) mg higher among those with a bicarbonate level <22 compared to ≥22 mEq/L. Associations between net acid excretion, PRAL, serum bicarbonate, and urine phosphorus were similar after additional adjustment for 25-hydroxyvitamin D or if excluding individuals using active vitamin D sterols.

Higher net acid excretion was also associated with higher serum phosphorus in fully adjusted models (0.21 [95% CI, 0.08-0.34] mg/dl higher serum phosphorus in quartile 4 versus 1 of net acid excretion; Figure 1). Serum phosphorus was not associated with PRAL, but was 0.19 (95% CI, 0.08-0.31) mg/dl higher among those with serum bicarbonate <22 versus ≥22 mEq/L after full adjustment (Figure 1).

Urine Calcium

Because urine phosphorus mobilization could be related to release from bone, urine calcium was also evaluated as an outcome measure. Similar to urine phosphorus, 24 hour urine calcium was significantly higher in higher quartiles of net acid excretion after full adjustment (66% [95% CI, 35%-107%] higher in quartile 4 versus 1). PRAL was not significantly associated with urine calcium after adjustment (p=0.1). 24-hour calcium was significantly lower in patients with low serum bicarbonate in models adjusted for demographics, kidney function and comorbidities (-17% [95% CI, -29% to -2%] among those with serum bicarbonate <22 versus ≥22 mEq/L), but this association mildly attenuated and was not significant after additional adjustment for BMI, diuretic use and 24 hour urine creatinine (-14%; 95% CI, -26% to 0%).

Phosphaturic Hormones

In a demographic adjusted model, FGF-23 trended lower across increasing quartiles of net acid excretion, but this association attenuated after adjustment for eGFR (Table 2). Likewise, FGF-23 levels were 36% (95% CI, 21%-53%) higher in participants with a serum bicarbonate <22 mEq/L in the demographic adjusted model, but these trends attenuated in

the fully adjusted model ($p=0.2$). The PRAL was not associated with levels of FGF-23 in any model (Table 2).

Similar to FGF-23, PTH was lower with increasing quartiles of net acid excretion in a demographic adjusted model. No association was found between PTH and net acid excretion in a fully adjusted model or between PTH and PRAL in any model (Table 2). Levels of PTH were significantly higher when serum bicarbonate was <22 mEq/L with levels 57% (95% CI, 39%-76%) higher in a demographic adjusted model. The association remained significant in a fully adjusted model with a level 26% (95% CI, 13%-40%) higher among participants with bicarbonate <22 mEq/L.

Secondary, Sensitivity, and Subgroup Analyses

Both FGF-23 and PTH increase the FEPi to maintain phosphorus homeostasis. In secondary analyses, we evaluated the associations of net acid excretion, PRAL and serum bicarbonate with FEPi. Higher quartiles of net acid excretion, but not PRAL or serum bicarbonate < 22 mEq/L ($p=0.9$ for each), were associated with higher FEPi (absolute difference of 14% [95% CI, 12%-16%] between quartile 4 versus 1) in fully adjusted models (Figure S2).

Urine phosphorus is a component of the net acid excretion calculation and, thus, these variables may be statistically correlated regardless of their biological relationships. Therefore, in addition to our complementary analyses of PRAL, we utilized urine ammonium and urine pH as alternative indices of acid excretion and modeled their fully adjusted association with 24 hour urine phosphorus and FEPi in sensitivity analyses. Among those with higher urine ammonium, 24 hour urine phosphorus remained higher, similar to in our primary models, although the magnitude of association was reduced (Figure 2). Lower urine pH did not associate with urine phosphorus (p for trend=0.2). Higher FEPi also remained associated with higher urine ammonium (absolute difference of 7.4% between quartile 4 versus 1 of urine ammonium; $p<0.001$).

Dietary intake could affect both acid-base status as well as serum and urine phosphorus. We found similar associations of acid load and acidosis with urine and serum phosphorus when additionally adjusted for total energy intake, dietary phosphorus, urine urea nitrogen, or urine sulfate (figure *a* of Item S1). Kidney function is a critical potential confounder of associations between net acid excretion, serum bicarbonate and mineral metabolites. We found overall similar results when we adjusted analyses for measured GFR in a subset of participants ($n=359$; figure *b* of Item S1). Additional models stratified by clinical covariates were used to evaluate possible effect modification by eGFR, sex, BMI, diuretic use and history of diabetes. There was no evidence of moderation by any of these covariates (figures *b-f* of Item S1). Finally, quantile regression, which models median values of untransformed FGF-23, PTH and urine calcium, yielded similar inferences as primary results from log-linear models (Table S2).

Discussion

In this cross-sectional study of 980 individuals with CKD, we observed that a higher acid load, and each of its components, namely higher net acid excretion, higher PRAL and, lower

serum bicarbonate, were associated with higher 24-hour urine phosphorus excretion. Furthermore, both higher net acid excretion and lower serum bicarbonate also independently associated with higher serum phosphorus level. Although these results *suggest* phosphorus mobilization may help promote titratable acid excretion, the cross sectional nature of our study does not allow us to infer causality between our exposures and outcomes. Furthermore, limitations of our data do not allow us to evaluate whether higher circulating and urine phosphorus could reflect increased intestinal phosphorus absorption, or mobilization of phosphorus from sites of sequestration, such as bone or the intracellular space. We also cannot definitively exclude residual confounding by kidney function or nutrition as factors in these associations although several sensitivity analyses were robust to these factors. Despite these acknowledged limitations, ours is one of few studies evaluating the combined physiology of altered acid-base and phosphorus homeostasis in a CKD cohort. Based on our results, we hypothesize that higher acid load and acidosis may stimulate phosphate excretion in CKD in part by increasing the availability of circulating phosphorus and the filtered phosphorus load.

Prior studies documented increased urine phosphorus excretion in response to an acid load in healthy individuals and animal models ^{11,24}. Many studies find evidence of bone demineralization ²⁵⁻²⁸ and increased calciuria ^{24,29,30} in the setting of an acid load. Similarly, in our study, urine calcium excretion was higher with higher acid load, suggesting bone as a potential source for the increased excretion of phosphorus. Other studies indirectly implicate mobilization of phosphorus from intracellular stores ³¹, or upregulation of intestinal phosphate absorption ¹⁰. However, in these prior studies of healthy individuals, acid-induced phosphaturia results in *lower* serum phosphorus, presumably due to renal losses that exceed absorbed or mobilized phosphorus ²⁴. On the contrary, in our study, we observed that higher acid load and lower serum bicarbonate associated with *higher*, not lower, serum phosphorus. It is possible that in CKD, the limited ability to filter, and thus excrete, a phosphorus load could result in a different balance between acid-induced phosphorus mobilization and excretion that favors higher circulating phosphorus, yielding our results. With growing epidemiologic and pre-clinical evidence of risk associated with higher serum phosphorus in CKD ¹⁴, ongoing interventions to reduce acid load should be tested for impact on serum phosphorus, phosphorus absorption and mobilization from storage sites.

Somewhat surprisingly, although high net acid excretion did associate with higher FEPi, it was not associated with increased levels of phosphaturic hormones. Prior *in vitro* and *in vivo* studies conflict in this regard. In some studies, acidosis induced expression of the phosphaturic hormones FGF-23 ¹² and PTH ^{8,9,32,33}, while altering expression and abundance of sodium phosphorus co-transporters in the proximal tubule ¹¹. Other studies have attributed changes in phosphaturia to direct effects of pH on the flux through these co-transporters, but not their abundance ⁵, which is more consistent with our results. Although we did not observe an association between urine pH and urine phosphorus, final urine pH may not accurately reflect the local pH in the proximal tubule and thus local pH may be a factor driving differences in excretion. Our results related to phosphaturic hormones disagree with some prior studies of physiology. This may be related to the severity of acidosis in patients with early to moderate CKD ³⁴. For instance, prior *in vitro* studies that documented

increased FGF-23 transcription in acidic environments used experimental pH of 7.1–7.2¹², which is lower than would be expected in early to moderate CKD. However, we did find associations of low serum bicarbonate with higher PTH in our study.

Our study has many strengths, including detailed clinical phenotyping, direct dietary reporting and measurement of multiple parameters of acidification and mineral metabolism in a relatively large CKD cohort. However, several limitations affect our conclusions. This study is observational and cross-sectional. Although our results *suggest* possible links between acid-base and mineral metabolism, they cannot prove cause and effect. We evaluated 15 exposure-outcome relationships which can inflate study-wide type 1 error. However, p-values for many of our findings were strong and would have persisted below a more conservative Bonferroni-corrected p-value of 0.003. Additionally, limitations of our measurements include lack of blood pH measurements, estimates derived from dietary questionnaires that may be subject to recall biases (i.e., PRAL), use of urine samples reflecting a single day of exposure (i.e., net acid excretion) and the age of the stored urine samples that could affect testing accuracy¹³. Overall, these measurement errors would typically increase variability and result in false negative, as opposed to false positive, findings.

In our study, general inferences regarding acid load were similar if quantified as net acid excretion, PRAL, or urine ammonium. This is critical because incorporation of urine phosphorus in the calculation of titratable acidity induces mathematical correlation between these variables. Additionally, the dependence of both relative over- and under-collection could induce some correlation between even biologically unrelated variables from timed urine collections. The latter concern was mitigated by adjustment for urine creatinine as a marker of collection adequacy. Although urine ammonium remained associated with urine phosphorus in our sensitivity analyses, urine pH did not. This may be because the higher quantity of urine buffer allows for greater titratable acid excretion without a more dramatic reduction in pH.

Finally, residual confounding by kidney function could contribute to some of the associations between acid-base and phosphorus parameters. It is unlikely to contribute to the direct associations observed between net acid excretion and serum phosphorus or indirect associations between serum bicarbonate and urine phosphorus, because the direction of expected confounding differs from the associations that we observed. For instance, at lower GFR, net acid excretion declines while serum phosphorus levels rise. However, in our results, these variables correlated directly, such that higher net acid excretion correlated with higher serum phosphorus levels. On the contrary, residual confounding by kidney function could theoretically induce spurious associations between net acid excretion, urine ammonium and urine phosphorus, each of which fall with lower kidney function^{13,35}. We have carefully performed sensitivity analyses using measured GFR where available to mitigate this concern.

Finally, diet could link the physiology of both acid-base and phosphorus homeostasis with higher protein intake contributing to both higher acid load and higher phosphorus content^{36,37}. We adjusted for a wide variety of dietary variables, where available, including

estimated dietary phosphorus, total energy intake, urine sulfate and urine urea nitrogen with similar results. Interventional studies with alkali supplements or phosphorus binders will be better able to dissociate dietary phosphorus absorption and acid load to study direct effects of isolated changes.

Recently, both acid-base and mineral homeostasis have garnered attention as possible modifiable risk factors for progression and complications of CKD ^{14,38-41}. Our results suggest physiologic links between these CKD complications that exceed their shared dependence on GFR and diet. Although this paradigm provides a rationale to evaluate for therapeutic synergy when treating both acid-base and mineral metabolism complications in CKD, trials are needed to confirm our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Adjusted absolute difference in mean 24-hour urine phosphorus and serum phosphorus across quartiles of net acid excretion (NAE), quartiles of potential renal acid load (PRAL) and bicarbonate levels

Squares represent the difference between 24 hour urine phosphorus levels and reference category. Circles represent the difference between serum phosphorus levels and reference category. Error bars represent 95% confidence intervals. Models are adjusted for age, sex, black vs. non-black race, estimated GFR, 24h urine albumin, diabetes, cardiovascular disease, smoking, body mass index, diuretic use, and 24h urine creatinine. (*) indicates p-trend < 0.05 from a continuous linear model.

Figure 2. Adjusted absolute difference in mean 24-hour urine phosphorus across quartiles of urine pH and urine ammonium
 Diamonds represent the difference between 24-hour urine phosphorus levels and reference category (quartile 1) across urine ammonium quartiles. Triangles represent the difference between 24-hour urine phosphorus levels and reference category (quartile 4) across urine pH quartiles. Error bars represent 95% confidence intervals. Models are adjusted for age, sex, black vs. non-black race, estimated GFR, 24h urine albumin, diabetes, cardiovascular disease, smoking, body mass index, diuretic use, and 24h urine creatinine. (*) indicates p-trend <0.05 from a continuous linear model.

Table 1

Baseline characteristics of study population according to serum bicarbonate.

	Serum Bicarbonate		P
	22 mEq/L (n=793) [†]	<22 mEq/L (n=178)	
Demographics			
Age (years)	58 ±11	58 ±11	0.7
Male sex	56.6%	57.3%	0.9
Black race	44.1%	38.2%	0.1
Comorbidities			
Diabetes	48.3%	61.2%	0.002
Hypertension	85.4%	89.9%	0.1
Cardiovascular disease	31.9%	32.6%	0.9
Ever smoker	55.9%	56.2%	0.9
Laboratory data			
Urine pH	5.9 ± 0.5	5.6 ± 0.5	<0.001
24 h urine ammonium (mmol/d)	13 (8, 20)	12 (8, 20)	0.4
24 h urine creatinine (g/d)	1.3 (0.9, 1.7)	1.2 (0.9, 1.5)	0.03
eGFR (mL/min/1.73 m ²)	46 ± 15	36 ±13	<0.001
24 h urine albumin (mg/d)	53 (9, 418)	199 (24, 1314)	<0.001
Physical Examination			
Systolic BP (mmHg)	127 ± 22	129 ± 23	0.2
Diastolic BP (mmHg)	71 ± 12	70 ± 13	0.5
BMI (kg/m ²)	32 ± 8	32 ± 7	0.3
Medications			
ACE inhibitor/ARB	68.0%	74.6%	0.09
Aldosterone antagonist	4.7%	5.1%	0.8
Diuretic	58.9%	57.6%	0.8
Anti-acidosis medications	2.5%	3.4%	0.5
Active Vitamin D	2.3%	3.4%	0.4
Phosphorus binder	6.7%	5.7%	0.6
Dietary Data			
Total energy (kcal/d)	1669 (1200, 2259)	1539 (1098, 2282)	0.3
Calories from protein (%)	16 ± 4	16 ± 4	0.6
Calories from fat (%)	34 ± 8	35 ± 9	0.04
Calories from carbohydrates (%)	51 ± 11	49 ± 11	0.09
Phosphorus (mg/d)	1048 (757, 1458)	1045 (676, 1367)	0.5
Calcium (mg/d)	626 (436, 866)	609 (391, 880)	0.3
Sodium (mg/d)	2579 (1836, 3628)	2583 (1665, 3476)	0.6
24 h urine urea nitrogen (g/d)	7.8 (5.7, 10.7)	7.5 (5.2, 10.0)	0.3
24 h urine potassium (mmol/d) ‡	51 (38, 69)	44 (32, 65)	0.003
24 h urine sodium (mmol/d)	149 (108, 203)	148 (103, 198)	0.7

Note: Values for categorical variables are given as percentage; values for continuous variables, as mean $\pm$ standard deviation or median [interquartile range].

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate

[†] serum bicarbonate was missing in 9 individuals

Table 2
Relative difference in geometric mean of PTH and FGF-23 in demographic and fully adjusted models according to quartiles of net acid excretion, quartiles of PRAL, and serum bicarbonate

	Net acid excretion				PRAL				Bicarbonate	
	Q2	Q3	Q4	p*	Q2	Q3	Q4	p*	<22 mEq/L	p
% Difference in PTH										
Demographic c adjusted	-2 (-14, 12)	-3 (-15, 10)	-14 (-25, -3)	0.01	0 (-13, 16)	3 (-10, 18)	3 (-11, 20)	0.7	57 (39, 76)	<0.001
Fully adjusted	0 (-11, 12)	-1 (-11, 11)	-3 (-16, 11)	0.6	3 (-8, 16)	3 (-9, 16)	6 (-6, 21)	0.7	26 (13, 40)	<0.001
% Difference in FGF-23										
Demographic c adjusted	-7 (-19, 7)	-11 (-22, 2)	-20 (-30, -8)	0.002	-12 (-25, 3)	-10 (-21, 5)	-4 (-18, 13)	0.4	36 (21, 53)	<0.001
Fully adjusted	-2 (-13, 10)	-3 (-14, 9)	-4 (-16, 10)	0.9	-8 (-20, 5)	-9 (-19, 2)	-1 (-14, 13)	0.8	7 (-3, 18)	0.2

Note: Values are given as difference (95% confidence interval). For net acid excretion and PRAL quartiles, Q1 is reference category; for bicarbonate, 22 mEq/L is reference category. Demographic adjusted models are adjusted for age, sex, and black vs non-black race. Fully adjusted models are adjusted for demographics as well as estimated glomerular filtration rate, 24-h urine albumin, diabetes, cardiovascular disease, smoking, body mass index, diuretic use, and 24-h urine creatinine

FGF, fibroblast growth factor; PRAL, potential renal acid load; PTH, parathyroid hormone; Q, quartile; Ref, reference;

* p is for linear trend from continuous model