

# Urine Anion Gap to Predict Urine Ammonium and Related Outcomes in Kidney Disease

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## Abstract

**Background and objectives** Low urine ammonium excretion is associated with ESRD in CKD. Few laboratories measure urine ammonium, limiting clinical application. We determined correlations between urine ammonium, the standard urine anion gap, and a modified urine anion gap that includes sulfate and phosphate and compared risks of ESRD or death between these ammonium estimates and directly measured ammonium.

**Design, setting, participants, & measurements** We measured ammonium, sodium, potassium, chloride, phosphate, and sulfate from baseline 24-hour urine collections in 1044 African-American Study of Kidney Disease and Hypertension participants. We evaluated the cross-sectional correlations between urine ammonium, the standard urine anion gap (sodium + potassium – chloride), and a modified urine anion gap that includes urine phosphate and sulfate in the calculation. Multivariable-adjusted Cox models determined the associations of the standard urine anion gap and the modified urine anion gap with the composite end point of death or ESRD; these results were compared with results using urine ammonium as the predictor of interest.

**Results** The standard urine anion gap had a weak and direct correlation with urine ammonium ( $r=0.18$ ), whereas the modified urine anion gap had a modest inverse relationship with urine ammonium ( $r=-0.58$ ). Compared with the highest tertile of urine ammonium, those in the lowest urine ammonium tertile had higher risk of ESRD or death (hazard ratio, 1.46; 95% confidence interval, 1.13 to 1.87) after adjusting for demographics, GFR, proteinuria, and other confounders. In comparison, participants in the corresponding standard urine anion gap tertile did not have higher risk of ESRD or death (hazard ratio, 0.82; 95% confidence interval, 0.64 to 1.07), whereas the risk for those in the corresponding modified urine anion gap tertile (hazard ratio, 1.32; 95% confidence interval, 1.03 to 1.68) approximated that of directly measured urine ammonium.

**Conclusions** Urine anion gap is a poor surrogate of urine ammonium in CKD unless phosphate and sulfate are included in the calculation. Because the modified urine anion gap merely estimates urine ammonium and requires five measurements, direct measurements of urine ammonium are preferable in CKD.

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## Introduction

Clinical assessment of kidney function focuses almost exclusively on markers of glomerular function and proteinuria (1). However, tubulointerstitial disease is observed commonly in kidney biopsy specimens, is strongly associated with eGFR decline across different kidney disease etiologies (2–4), and is poorly captured by clinical markers of kidney function. We have been interested in identifying noninvasive markers of kidney tubule dysfunction and injury that assist in predicting CKD progression above and beyond eGFR and proteinuria (5–9). A critical function of kidney tubules is regulation of acid-base homeostasis, which involves production and excretion of ammonium and bicarbonate generation. Decrements in ammonium excretion are observed before low serum bicarbonate develops, and lower urine ammonium strongly associated with ESRD or death independent of measured

GFR, proteinuria, and serum bicarbonate in the African-American Study of Kidney Disease and Hypertension (AASK) participants (10). Similar findings were observed in the NephroTest Cohort (11) and the Chronic Renal Insufficiency Cohort participants with diabetes (12). This suggests that lower urine ammonium identifies patients with impaired tubule function and high risk for CKD progression.

Few clinical laboratories measure urine ammonium, which represents an important barrier to translating these findings into clinical practice. However, the urine anion gap (UAG) has been advocated as a urine ammonium surrogate (13). In clinical practice, the UAG (sodium + potassium – chloride) is used to gauge robustness of ammonium excretion during metabolic acidosis. In metabolic acidosis secondary to diarrhea, ammonium excretion is enhanced, thereby adding “unmeasured cations” and inducing a more

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negative UAG (14). With renal tubular acidosis and the metabolic acidosis of CKD, robust increases in ammonium excretion do not occur, resulting in a more positive UAG (14,15). However, the ability of the UAG to predict urine ammonium concentration and assess clinical risk in patients with CKD is questionable. For example, in 30 individuals with impaired kidney function, the standard UAG had no correlation with urine ammonium concentration. However, the correlation between urine ammonium and the UAG was improved when urine phosphate and sulfate were included in the calculation (16). If confirmed, this finding suggests that the quantity of “unmeasured anions” critically influences the ability of the UAG to estimate ammonium concentrations in CKD. However, the relationship between these UAG measurements and urine ammonium concentration has not been evaluated in a large CKD cohort, and whether they predict clinical outcomes similarly to urine ammonium has not been evaluated.

We designed this study to address two aims. First, we determined the correlation between urine ammonium and the standard UAG and compared it with modified UAG calculations that include sulfate and phosphate in 1044 AASK participants overall and in 128 participants with acidosis. Second, we determined the association of these UAG calculations with risk of ESRD or death among the AASK participants and compared the strengths of these associations with those of directly measured urine ammonium. Our purpose was to determine whether the UAG gives reliable estimates of ammonium and associated outcomes in patients with CKD.

## Materials and Methods

### Study Participants

The details of the AASK have been published (17,18). Briefly, blacks ages 18–70 years old with hypertensive CKD (defined as measured GFR =20–65 ml/min per 1.73 m<sup>2</sup> by clearance of iodine <sup>125</sup>I iothalamate and diastolic BP >95 mm Hg) were eligible for the study. Between April of 1995 and September of 1998, 1094 participants were randomized to ramipril, metoprolol, or amlodipine as well as to one of two BP goals (usual mean arterial pressure goal of 102–107 mm Hg or a low mean arterial pressure goal of ≤92 mm Hg). At the end of the randomized trial phase, participants who did not reach ESRD were eligible to enroll in the nonrandomized AASK Cohort Study. Baseline urine samples were available for 1057 participants. We excluded 13 participants with daily urine ammonium excretion normalized by body weight ≥0.7 mEq/kg out of concern for falsely elevated urine ammonium excretion due to bacterial overgrowth. Hence, 1044 participants were evaluated, corresponding to 99% of participants with available samples and 95% of randomized participants. The AASK was overseen by the institutional review boards of the participating sites and performed under the principles embodied in the Declaration of Helsinki.

### Measurements

Using standardized forms, trained personnel obtained data on baseline demographic, clinical, and laboratory

data. Urine analytes were measured from aliquots of the baseline 24-hour urine collection. Urine ammonium was measured by the glutamate dehydrogenase method. Urine sodium, potassium, and chloride were measured using ion-selective electrodes. Urine phosphorous was measured using the photometric molybdate technique. Urine sulfate was measured using barium precipitation. Daily excretion of these ions was determined by multiplying concentrations with 24-hour urine volumes. The 24-hour urine samples were confirmed to have been collected according to the AASK protocol and were necessary before randomization. Urine collections were preserved with acetic acid at the time of collection, rendering urine pH measurements inaccurate. Thus, we calculated the quantity of phosphate in milliequivalents using the Henderson-Hasselbalch equation with pKa=6.8 for HPO<sub>4</sub><sup>2-</sup>/H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, assuming urine pH of 5.5 (16). Four parameters were evaluated in regard to their correlations with urine ammonium: the UAG, the urine anion gap with inclusion of urine phosphate (UAG<sub>PO4</sub>), the urine anion gap with inclusion of urine sulfate (UAG<sub>SO4</sub>), and the urine anion gap with inclusion of urine phosphate and sulfate (UAG<sub>PLUS</sub>) (Table 1).

Serum bicarbonate was measured using either the kinetic ultraviolet method (Roche Hitachi 747; Roche, Indianapolis, IN) or a CO<sub>2</sub> electrode (Beckman CX3 Delta; Beckman, Brea, CA). Urinary protein excretion was expressed as a protein-to-creatinine ratio from the 24-hour urine collection. Daily dietary protein intake (grams per day) was calculated from 24-hour urine urea nitrogen excretion using the equation 6.25 × [urine urea nitrogen in grams per day + (weight in kilograms × 0.031)] (19). Net endogenous acid production was calculated using the formula -10.2 + (54.5 × protein intake in grams per day) / urine potassium in milliequivalents per day (20).

### Statistical Analyses

Participants were categorized by tertiles of daily UAG excretion and daily urine ammonium. Continuous variables are presented as means with SD, unless otherwise specified. Categorical variables are presented as percentages. Significance tests were performed using ANOVA for continuous variables and chi-squared tests for dichotomous variables. Pearson correlation coefficients between urine ammonium and each UAG measurement were determined in the total cohort and the acidosis subgroup, which was defined as serum bicarbonate <22 mEq/L. Bland-Altman plots quantified the level of agreement of urine ammonium with the additive inverse of UAG<sub>PLUS</sub> in the total cohort and the acidosis subgroup. The additive inverse of UAG<sub>PLUS</sub> was used, because higher urine ammonium correlates with more negative UAG<sub>PLUS</sub>; it would have been inappropriate to compare differences between positive and negative values in the Bland-Altman plot.

The longitudinal outcome of interest was the composite of death or ESRD, which was adjudicated by the outcomes committee. Follow-up time was censored at the administrative end date, at permanent loss to follow-up, or if the participant did not enroll in the cohort phase. Cox

**Table 1. Terminology and calculations of the four urine anion gap measurements in this study**

Term	Calculation, mEq/d
UAG	Urine (sodium + potassium) – (chloride)
UAG <sub>SO<sub>4</sub></sub>	Urine (sodium + potassium) – (chloride + sulfate)
UAG <sub>PO<sub>4</sub></sub>	Urine (sodium + potassium) – (chloride + phosphate)
UAG <sub>PLUS</sub>	Urine (sodium + potassium) – (chloride + phosphate + sulfate)

Because urine pH was not available, the milliequivalents of phosphate were calculated using urine pH of 5.5 in all participants. UAG, urine anion gap; UAG<sub>SO<sub>4</sub></sub>, urine anion gap with inclusion of urine sulfate; UAG<sub>PO<sub>4</sub></sub>, urine anion gap with inclusion of urine phosphate; UAG<sub>PLUS</sub>, urine anion gap with inclusion of urine phosphate and sulfate.

regression models related the composite outcome of death or ESRD to daily urine ammonium, UAG, UAG<sub>PO<sub>4</sub></sub>, UAG<sub>SO<sub>4</sub></sub>, and UAG<sub>PLUS</sub>. In analyses using urine ammonium as the independent variable, the highest tertile was the reference. For the UAG-based measurements, the lowest (most negative) tertile was used as the reference, which should correspond with higher urine ammonium. Cox models were adjusted for age, sex, randomized group, measured GFR, proteinuria, net endogenous acid production, serum potassium, and serum bicarbonate to maintain consistency with our prior report and facilitate comparisons across the independent variables (10). Proportional hazards assumptions were evaluated using a formal significance test on the basis of the unscaled and scaled Schoenfeld residuals and a graphical assessment of log-log survival curves using urine ammonium as the independent variable. Body mass index (BMI) violated the proportional hazards assumption and was included as a stratification variable (<25, 25 to <30, and ≥30 kg/m<sup>2</sup>) in the models.

The analyses were performed using Stata 14 (College Station, TX).

## Results

### Baseline Characteristics

Characteristics of the 1044 study participants are presented in Table 2. The mean age was 54 years old, 62% were men, mean measured GFR was 47±14 ml/min per 1.73 m<sup>2</sup>, and median urine protein-to-creatinine ratio was 80 mg/g. One hundred twenty-eight participants (12%) had metabolic acidosis at baseline, and the mean urine ammonium was 21±12 mEq/d. The mean 24-hour urine phosphate excretion was 21±10 mEq/d, and mean 24-hour urine sulfate excretion was 29±16 mEq/d. Compared with those with higher UAG, participants with lower UAG (and presumably, higher ammonium) had lower BMI, were more likely to smoke, had lower protein intake, and had higher net endogenous acid production, and a greater proportion were women. They also had slightly lower measured GFR and lower bicarbonate. Contrary to our expectation, urine ammonium was higher in individuals with higher (more positive) UAG.

By comparison, higher urine ammonium was associated with men and higher measured GFR, bicarbonate, BMI, protein intake, and net endogenous acid production as previously reported (10). Thus, the baseline characteristics of the cohort were markedly different when UAG was used

as an estimate of ammonium excretion compared with directly measured ammonium.

### Correlations of Urine Ammonium with the UAG Measurements

Table 3 presents values of urine ammonium and the four UAG measurements in the study sample overall and across tertiles of urine ammonium. We expected that a more negative UAG would reflect higher urine ammonium, and therefore, an inverse correlation was hypothesized. By contrast, we observed a slightly positive correlation between UAG and urine ammonium. Whereas UAG was higher with higher urine ammonium, UAG<sub>SO<sub>4</sub></sub>, UAG<sub>PO<sub>4</sub></sub>, and UAG<sub>PLUS</sub> were lower with higher urine ammonium and thus, in the expected direction. Figure 1 shows that UAG<sub>PLUS</sub> had the strongest inverse correlation with urine ammonium in the entire study sample ( $r=-0.58$ ). The correlations between the four UAG measurements and urine ammonium were similar in the subgroup of 128 individuals with acidosis. Among participants with acidosis, all but two had a positive UAG, and there was no correlation of the UAG with urine ammonium in the acidosis subgroup ( $r=0.07$ ).

Bland–Altman plots (Figure 2) show the agreement between urine ammonium with the additive inverse of UAG<sub>PLUS</sub> in the entire cohort and the subgroup with acidosis. Urine ammonium was, on average, 13 mEq/d higher than UAG<sub>PLUS</sub>, indicating positive bias in the cohort. More importantly, the 95% limits of agreement were broad (–18–43 mEq/d). The results were similar in the subgroup of participants with acidosis.

### Association of UAG Measurements with Clinical Outcomes

Next, we determined the associations of each UAG measurement with the composite outcome of ESRD or death. During 7862 patient-years of follow-up, there were 296 ESRD events and 168 deaths. After adjusting for demographics, measured GFR, proteinuria, and other risk factors, the lowest urine ammonium tertile was associated with 46% higher risk of ESRD or death compared with the highest tertile, as previously reported (10). We used this hazard ratio as an indicator of the reliability of the UAG measures to predict the same clinical outcomes. Although the reference group for the urine ammonium analysis was those in the highest urine ammonium tertile, the reference group for the UAG-based measures was the lowest tertile (most

**Table 2. Baseline characteristics presented as mean (SD) or number (percentage) unless otherwise specified**

Characteristics and Measurements	Total Population, n=1044	Daily UAG Tertiles		
		-80 to <29 mEq/d n=348	29 to <50 mEq/d n=348	50–228 mEq/d n=348
<b>Demographics</b>				
Age, yr	54 (11)	54 (10)	55 (11)	53 (11)
Men, no. (%)	645 (62)	178 (51)	202 (58)	265 (76)
<b>Clinical characteristics</b>				
Body mass index, kg/m <sup>2</sup>	30.6 (6.6)	28.8 (6.4)	30.8 (6.6)	32.2 (6.5)
Cardiovascular disease, no. (%)	538 (52)	186 (53)	177 (51)	175 (50)
Current smoker, no. (%)	305 (29)	134 (39)	85 (24)	86 (24)
Past smoker, no. (%)	298 (29)	83 (24)	102 (29)	113 (32)
Never smoker, no. (%)	441 (42)	131 (38)	161 (46)	149 (43)
Systolic BP, mmHg	150 (24)	150 (25)	150 (24)	151 (22)
Protein intake, g/d	69 (25)	51 (16)	68 (18)	88 (25)
Net endogenous acid production, mEq/d	83 (37)	96 (42)	83 (34)	69 (27)
ACE-i/ARB use, no. (%)	397 (39)	125 (37)	135 (40)	137 (40)
Diuretic use, no. (%)	649 (64)	206 (61)	212 (62)	231 (68)
Measured GFR, ml/min per 1.73 m <sup>2</sup>	47 (14)	46 (14)	46 (14)	48 (13)
Measured GFR <30 ml/min per 1.73 m <sup>2</sup> , no. (%)	163 (16)	60 (17)	62 (18)	41 (12)
<b>Serum measurements</b>				
Bicarbonate, mEq/L	25 (3)	25 (3)	25 (3)	25 (3)
Bicarbonate <22 mEq/L, no. (%)	128 (12)	55 (16)	35 (10)	38 (11)
Potassium, mEq/L	4.2 (0.6)	4.2 (0.7)	4.2 (0.6)	4.2 (0.6)
Anion gap, mEq/L	10 (2)	10 (3)	10 (2)	10 (2)
<b>Urine measurements</b>				
Protein-to-creatinine ratio, mg/g <sup>a</sup>	80 (29–342)	77 (31–298)	68 (27–332)	100 (34–373)
Urea nitrogen, g/d	8.0 (3.7)	5.4 (2.2)	8.0 (2.6)	10.8 (3.7)
Sodium, mEq/d	152 (81)	111 (69)	147 (67)	197 (82)
Potassium, mEq/d	43 (24)	27 (16)	40 (13)	63 (25)
Chloride, mEq/d	153 (82)	120 (79)	148 (70)	191 (82)
Ammonium, mEq/d	21 (12)	19 (11)	22 (11)	24 (13)
Sulfate, mEq/d	29 (16)	18 (10)	29 (12)	40 (17)
Phosphate, mEq/d	21 (10)	14 (6)	21 (7)	29 (10)

UAG, urine anion gap; ACE-i, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.  
<sup>a</sup>Presented as median (interquartile range).

negative), which is anticipated to correspond with higher urine ammonium. In comparison with the hazard ratio for the lowest urine ammonium tertile, the highest UAG tertile was not associated with death or ESRD when adjusted for identical covariates. Similarly, neither the highest UAG<sub>SO4</sub> tertile nor the highest UAG<sub>PO4</sub> tertile were associated with ESRD or death. By contrast, the highest UAG<sub>PLUS</sub> tertile was associated with ESRD or death; an association that was comparable in strength with that observed for the lowest tertile of urine ammonium (Table 4).

## Discussion

Lower urine ammonium excretion is a risk factor for poor outcomes in CKD (10–12); however, urine ammonium is uncommonly measured by clinical laboratories. We evaluated whether UAG reasonably estimates urine ammonium and recapitulates the association between low urine ammonium and clinical outcomes in the AASK. UAG had a poor and direct correlation with urine ammonium excretion and hence, was not associated with clinical outcomes. The correlation between UAG and urine ammonium was substantially improved and in the expected inverse

**Table 3. Values of urine ammonium and the four urine anion gap measurements in the total population and by tertiles of urine ammonium**

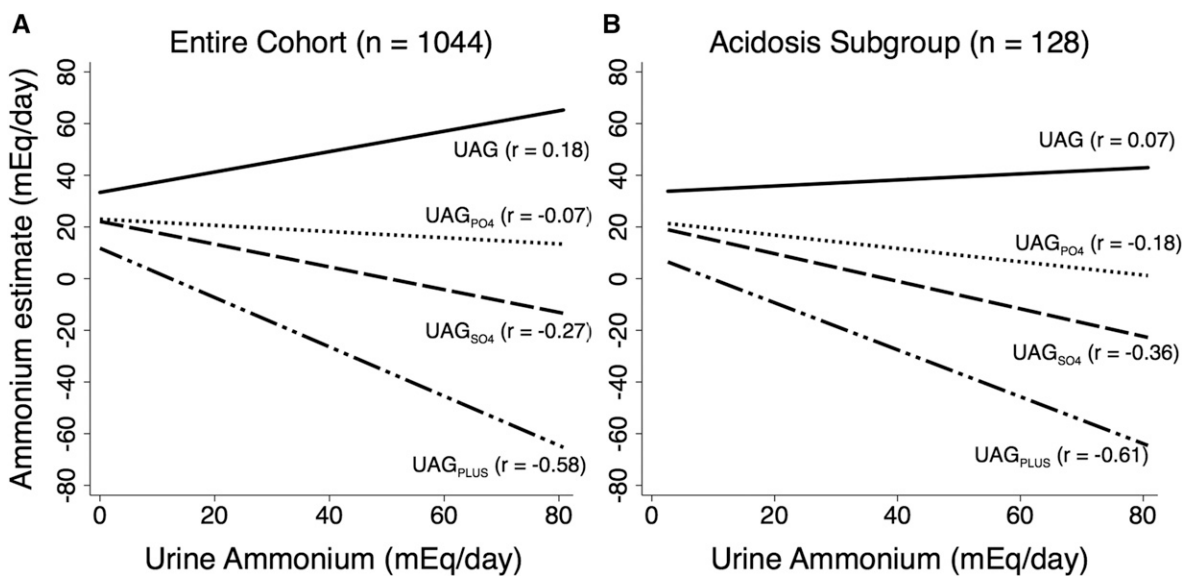
Measurement, mEq/d	Total Population, n=1044	Urine Ammonium Tertile 1 (Range, 0–15 mEq/d), n=348	Urine Ammonium Tertile 2 (Range, 15–24 mEq/d), n=348	Urine Ammonium Tertile 3 (Range, 24–81 mEq/d), n=348	P Value
Urine ammonium	21 (12)	10 (3)	19 (3)	35 (9)	—
UAG	42 (25)	37 (25)	42 (24)	47 (26)	<0.001
UAG <sub>SO4</sub>	13 (19)	18 (18)	14 (19)	6 (19)	<0.001
UAG <sub>PO4</sub>	20 (20)	22 (20)	21 (20)	18 (19)	0.05
UAG <sub>PLUS</sub>	-9 (19)	3 (15)	-7 (16)	-22 (18)	<0.001

Values shown as mean (SD). —, not applicable; UAG, urine anion gap; UAG<sub>SO4</sub>, urine anion gap with inclusion of urine sulfate; UAG<sub>PO4</sub>, urine anion gap with inclusion of urine phosphate; UAG<sub>PLUS</sub>, urine anion gap with inclusion of urine phosphate and sulfate.

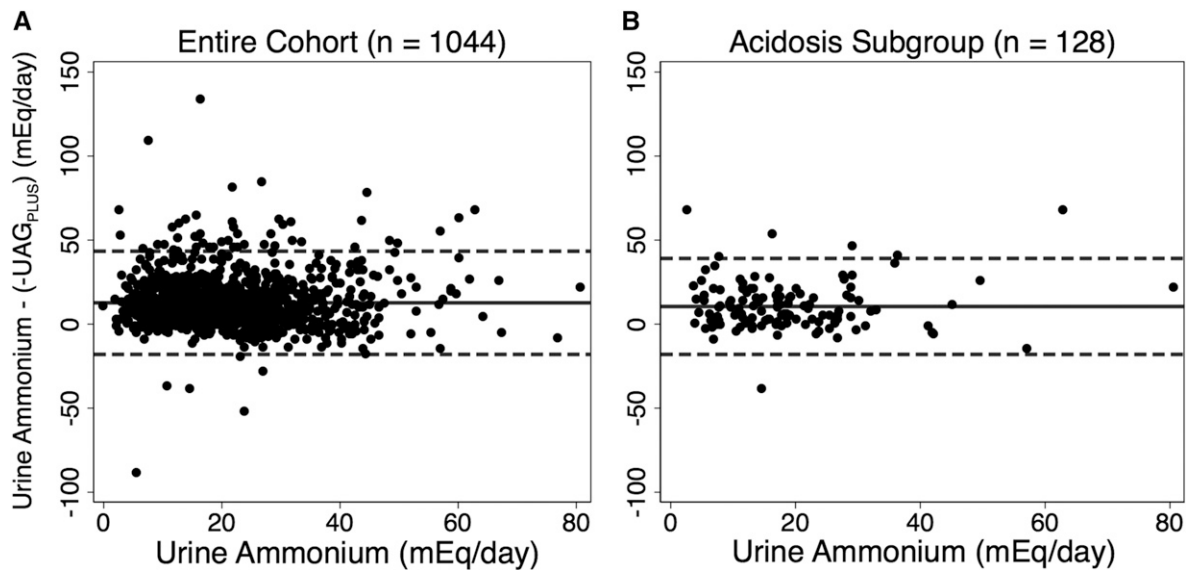
direction when urine phosphate and sulfate were included in the UAG calculation as UAG<sub>PLUS</sub>. Furthermore, UAG<sub>PLUS</sub> approximated the risk of ESRD or death observed when urine ammonium was the independent variable of interest. Thus, UAG is a poor surrogate for urine ammonium in CKD, unless sulfate and phosphate are included in the calculation.

In their seminal paper, Goldstein *et al.* (13) showed a strong inverse correlation ( $r = -0.97$ ) between the standard UAG and urine ammonium in patients with metabolic acidosis and normal GFR. Batlle *et al.* (14) also reported a strong inverse correlation between UAG and urine ammonium in 53 patients with distal renal tubular acidosis, diarrhea, or healthy controls receiving ammonium chloride infusions. Clinically, the UAG is used to gauge whether robust urinary ammonium excretion is present in the setting of metabolic acidosis, signaled by a negative

UAG. In CKD, ammonium excretion is impaired in acidosis. This change, and potentially other changes, in unmeasured urine anions and cations rendered the UAG positive in the majority of the AASK participants with acidosis. In terms of estimating ammonium more precisely in patients with CKD, our results confirm and meaningfully extend those of Kirschbaum *et al.* (16). Specifically, they found that the poor correlation between UAG and urine ammonium was markedly improved when urine phosphate and sulfate were accounted for in individuals with CKD. As in this study, Kirschbaum *et al.* (16) included individuals with and without acidosis. Although they did not report whether these estimates of ammonium excretion vary by acidosis status in CKD, we show that the correlations between the four UAGs assessed in this study and ammonium are similar irrespective of acidosis status. Because UAG had poor correlation with urine ammonium,



**Figure 1. | Whereas UAG was higher with higher urine ammonium, UAG<sub>SO4</sub>, UAG<sub>PO4</sub>, and UAG<sub>PLUS</sub> were lower with higher urine ammonium.** Pairwise correlation between measured urine ammonium (abscissa) and the estimates of urine ammonium (1) urine anion gap (UAG), (2) urine anion gap with inclusion of urine phosphate (UAG<sub>PO4</sub>), (3) urine anion gap with inclusion of urine sulfate (UAG<sub>SO4</sub>), and (4) urine anion gap with inclusion of urine phosphate and sulfate (UAG<sub>PLUS</sub>) in (A) the total cohort and (B) the subgroup with acidosis at baseline.



**Figure 2.** | The limits of agreement between urine ammonium and the additive inverse of  $UAG_{PLUS}$  were broad. Bland–Altman plots showing mean difference and limits of agreement between urine ammonium and the additive inverse of urine anion gap with inclusion of urine phosphate and sulfate ( $UAG_{PLUS}$ ) in (A) the total cohort and (B) the acidosis subgroup. In each case, there is positive bias. That is, mean urine ammonium is higher than the additive inverse of  $UAG_{PLUS}$ . (A) Mean difference = 13 mEq/d, limits of agreement = – 18, 43 mEq/d. (B) Mean difference = 11 mEq/d, limits of agreement = – 18, 39 mEq/d.

it was not surprising that there was no association of UAG with clinical outcomes. However, we show that inclusion of urine phosphate and sulfate in the UAG calculation ( $UAG_{PLUS}$ ) recapitulated these associations with outcomes similar to but weaker than those observed with directly measured urine ammonium.

Goldstein *et al.* (13) recognized that the presence of anions other than chloride, such as phosphate and sulfate, would influence the UAG, and in this study, sulfate (mean = 29 mEq/d) and phosphate (mean = 21 mEq/d) accounted for about 25% of the urine anions. Their inclusion in  $UAG_{PLUS}$  substantially improved the correlation with urine ammonium and rendered it in the expected inverse direction. Differences of urine phosphate excretion occur in persons with and without CKD (21,22), and dietary factors, GFR, and use of intestinal phosphate binders in CKD are factors that account for interindividual urine phosphate variability (23). Across-individual differences in urinary sulfate, largely determined by animal protein intake, have been shown in prior studies also (24,25). Thus, individual variability of urine phosphate and sulfate materially influences the relationship of the UAG with urine ammonium and if not accounted for, renders UAG a poor surrogate of urine ammonium in patients with CKD.

Given the poor performance of the UAG, we conclude that it should not be used as a surrogate for ammonium when estimating CKD progression risk. Although our results show that accounting for urine phosphate and sulfate better approximates urinary ammonium in CKD, the  $UAG_{PLUS}$  calculation requires measurement of five variables, each increasing the likelihood of measurement error. Furthermore, the level of agreement between daily urine ammonium excretion and  $UAG_{PLUS}$  was biased and

broad as shown in Figure 2. In large-scale studies, such as the AASK,  $UAG_{PLUS}$  may be useful to rank order individuals to evaluate its association with clinical end points. However, the use of  $UAG_{PLUS}$  does not seem to be feasible in clinical practice. Instead, we believe that the time has come to directly quantify urine ammonium, which clinical laboratories could perform using diluted urine samples and the standard plasma ammonia assay (26).

A limitation of this study is that urine samples in the AASK were preserved with acetic acid at the time of collection; thus, we could not measure urine pH. We assumed urine pH of 5.5 in study participants, consistent with mean levels reported in patients with CKD (16), which corresponds to 5% of phosphate as  $HPO_4^{2-}$  and 95% as  $H_2PO_4^-$ . The mean milliequivalents of phosphate and its distribution in the population would not be significantly different with lower urine pH. However, higher urine pH would increase the milliequivalents of phosphate in the  $HPO_4^{2-}$  state, generate a more negative  $UAG_{PLUS}$ , and strengthen the inverse correlation with urine ammonium. In sensitivity analyses, the Cox model results were similar, irrespective of whether a urine pH of 5.5, 6.0, or 6.8 was imputed for our study sample. Nevertheless, urine pH measurements could have narrowed the mean difference and levels of agreement between urine ammonium and  $UAG_{PLUS}$ . Hence, the agreement between  $UAG_{PLUS}$  and urine ammonium may have been underestimated. Because urine phosphate is not included in the UAG, this would not influence the poor correlation between UAG and urine ammonium. We did not quantify urine bicarbonate, which could influence the UAG. In general, bicarbonaturia is <10 mEq/L if the urine pH is below 6.8, and urine pH above this is

**Table 4. Associations between urine ammonium and each urine anion gap measurement with the composite outcome of dialysis or death before dialysis**

Composite Outcome (Death or ESRD)	Urine Ammonium	UAG	UAG <sub>SO4</sub>	UAG <sub>PO4</sub>	UAG <sub>PLUS</sub>
<b>Tertile corresponding with low urine ammonium or high UAG</b>					
Hazard ratio (95% CI)	1.46 (1.13 to 1.87)	0.82 (0.64 to 1.07)	0.94 (0.72 to 1.21)	0.95 (0.74 to 1.21)	1.32 (1.03 to 1.68)
Mean (SD) urine ammonium, mEq/d	10.2 (3.3)	23.9 (12.9)	18.8 (10.4)	20.4 (12.4)	14.9 (9.0)
No. of events	188	151	63	153	174
<b>Intermediate urine ammonium or UAG</b>					
Hazard ratio (95% CI)	1.14 (0.89 to 1.46)	0.82 (0.65 to 1.05)	0.93 (0.73 to 1.18)	0.82 (0.65 to 1.04)	1.23 (0.97 to 1.56)
Mean (SD) urine ammonium, mEq/d	19.5 (2.6)	21.6 (10.9)	19.6 (10.3)	21.3 (11.4)	18.8 (8.3)
No. of events	146	150	163	156	159
<b>High urine ammonium or low UAG</b>					
Hazard ratio (95% CI)	Reference	Reference	Reference	Reference	Reference
Mean (SD) urine ammonium, mEq/d	34.6 (9.3)	18.7 (10.5)	25.8 (12.8)	22.5 (12.4)	30.4 (11.3)
No. of events	130	163	138	155	131

Models were adjusted for age, sex, randomized group, measured GFR, proteinuria, net endogenous acid production, serum potassium, and serum bicarbonate and stratified by body mass index. Also shown are the mean (SD) values of urine ammonium within each tertile of UAG and the numbers of events within each tertile (number at risk =348 in each). The concentrations of urine ammonium are in the opposite direction expected in the UAG group. Inclusion of sulfate (UAG<sub>SO4</sub>) and phosphate (UAG<sub>PO4</sub>) yielded the expected pattern of higher urine ammonium concentration with lower (more negative) UAG. UAG<sub>PLUS</sub> showed the best separation of urine ammonium in the expected direction across the tertiles. Only UAG<sub>PLUS</sub> recapitulated the hazard ratios of the composite outcome observed with urine ammonium. UAG, urine anion gap; UAG<sub>SO4</sub>, urine anion gap with inclusion of urine sulfate; UAG<sub>PO4</sub>, urine anion gap with inclusion of urine phosphate; UAG<sub>PLUS</sub>, urine anion gap with inclusion of urine phosphate and sulfate; 95% CI, 95% confidence interval.

uncommon in CKD (16,27). Our findings suggest that excluding urine sulfate and phosphate from the UAG calculation is more important than excluding urine bicarbonate. The urine osmolal gap is an alternative urine ammonium estimate that has previously been reported to have stronger correlation with ammonium than the UAG (16). Urine osmolality was not measured in the AASK; hence, we could not compare the performance of the urine osmolal gap with that of UAG, in particular UAG<sub>PLUS</sub>, in this cohort; future studies are required to evaluate this approach. Systemic pH and pCO<sub>2</sub> were not measured in the AASK; hence, some with low bicarbonate may have had chronic respiratory alkalosis, which also manifests with a positive UAG, rather than metabolic acidosis. Nevertheless, we suspect that the majority of these patients with CKD and low bicarbonate had metabolic acidosis. Strengths of this study include the well characterized, large study sample with carefully collected data. Furthermore, over 95% of randomized participants were included in these analyses. GFR was directly measured by <sup>125</sup>I iothalamate clearance, which is important considering the strong relationship between kidney function, urine ammonium excretion, and long-term outcomes.

In conclusion, urine phosphate and sulfate contribute significant anionic milliequivalents to the urine in CKD and are variable from one individual to the next. These anions are not included in the typical UAG calculation and render it a poor surrogate for urine ammonium concentration in CKD. Although lower urine ammonium excretion was independently associated with risk of ESRD or death, there was no association of UAG with these end points. Including urine sulfate and phosphate in the UAG improves the correlation with urine ammonium and approximates the association of urine ammonium with clinical outcomes. However, a modified UAG that accounts for phosphate and sulfate still leads to significant bias and broad levels of agreement, and it requires measurement of five variables, which increases the risk of measurement error. Direct measurements of urine ammonium by clinical laboratories will help clinicians better assess kidney tubule function as it relates to acid-base regulation and CKD progression risk.

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**Disclosures**

None.

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