Urinary Sulfate, Kidney Failure, and Death in CKD: The African American Study of Kidney Disease and Hypertension

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Key Points
- Higher urine sulfate levels were associated with more favorable outcomes in Blacks with kidney disease attributed to hypertension.
- These findings are independent of dietary protein intake, suggesting that sulfate has an effect on health above and beyond protein intake.

Abstract

Background Sulfur is an important mineral element whose principal source is animal protein. Animal protein contributes to the daily acid load, which is associated with poor outcomes in individuals with chronic kidney disease (CKD). We hypothesized that higher urinary sulfate, as a reflection of the daily acid load, is associated with a greater risk of death and CKD progression.

Methods Urinary sulfate was measured in 1057 African American Study of Kidney Disease and Hypertension (AASK) participants at baseline. Participants were categorized by tertiles of daily sulfate excretion. The longitudinal outcome of interest was the composite of death, dialysis, or 50% reduction in measured glomerular filtration rate (GFR). Multivariable adjusted Cox regression models were fit to relate the composite outcome to daily sulfate excretion using the lowest tertile as the reference.

Results Participants in the highest urinary sulfate tertile were more likely to be men and have a higher body mass index, protein intake, measured GFR, and urinary ammonium and phosphate excretion, and lower urinary protein/creatinine. Compared with those in the lowest tertile of sulfate, those in the highest tertile had a 44% lower hazard (95% CI, 0.37 to 0.84), and those in the middle tertile had a 27% lower hazard (95% CI, 0.55 to 0.96) of death, dialysis, or 50% reduction in measured GFR during follow-up after adjusting for demographics, GFR, protein intake, and other potential confounders. Protein intake was not associated with risk of these events.

Conclusions Higher urinary sulfate excretion is associated with more favorable outcomes in Blacks who have CKD attributed to hypertension.

Introduction

Sulfur is the third most abundant mineral element in the human body after calcium and phosphorus. Many important molecules contain sulfur, including the amino acids methionine and cysteine, hydrogen sulfide, and glutathione (1,2). Sulfur is also required for sulfation of drugs and toxins, including uremic toxins such as p-cresol. Dietary protein is the principal source of sulfur; inorganic sulfate (SO₄) from water, food preservatives, and other sources contributes to a lesser extent. Animal protein has a higher proportion of the sulfur-containing amino acids cysteine and methionine than plant protein and is the main source of sulfur in Western society. Although sulfur is an important mineral element, animal protein contributes to the daily dietary acid load (3–5), which is associated with poor outcomes in individuals with CKD, including bone demineralization, muscle wasting, insulin resistance, and more rapid loss of kidney function (6–10).

The potential benefits of restricting animal protein are many and include reducing the daily acid load, improving bicarbonate and phosphorus levels, and attenuating glomerular hyperfiltration and albuminuria (11–21). In most cases, dietary protein restriction involves reducing animal protein intake and, by default, sulfur intake. Hence, protein restriction may have adverse effects on sulfur metabolism and patient outcomes. We therefore evaluated the association between urinary SO₄ levels, the end product of sulfur metabolism, and kidney and patient survival among
participants in the African American Study of Kidney Disease and Hypertension (AASK). We hypothesized that higher urinary SO₄ as a reflection of high protein intake and consequently daily acid load is associated with higher risk of death and loss of kidney function.

Materials and Methods

Study Participants

The details of the AASK have been published previously (22–24). Briefly, Blacks aged 18–70 years with CKD attributed to hypertension were eligible for the study. Key exclusion criteria included elevated fasting or random blood glucose, treatment for diabetes, or a urinary protein to creatinine ratio >2.5. Participants (N=1094) were randomized to ramipril, metoprolol, or amlodipine, and 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). Baseline (prerandomization) urine samples were available for 1057 participants, and these individuals were included in this analysis. The AASK was overseen by the Institutional Review Boards of the participating sites and was performed under the principles embodied in the Declaration of Helsinki.

Measurements

Trained personnel obtained baseline demographic, clinical, and laboratory data using standardized forms. The 24-hour urine samples were confirmed to have been collected according to the AASK protocol and were necessary before randomization. Urine SO₄ was measured by barium precipitation on a Beckman AU 680 analyzer (Beckman Coulter, Brea, CA). The analytical measurement range is 2.5–80 mEq/L. Daily urinary SO₄ excretion was determined from 24-hour urine volumes. Serum total CO₂ was measured using either the kinetic ultraviolet method (Roche Hitachi 747 autoanalyzer; Roche, Indianapolis, IN) or a CO₂ electrode (Beckman CX3 Delta autoanalyzer; Beckman Coulter). Urinary protein excretion was expressed as protein/creatinine (PCR) obtained from the 24-hour urine collection. Daily dietary protein intake (g/d) was calculated from 24-hour urine urea nitrogen excretion using the equation: 6.25×[urine urea nitrogen+(weight×0.031)] (25). Net endogenous acid production (NEAP) was calculated using the formula: −10.2+(54.5×protein intake in g/d)/urine potassium in mEq/d (26).

Statistical Analyses

Participants were categorized by tertiles of daily SO₄ excretion. Continuous variables are presented as means with SD unless otherwise specified. Categorical variables are presented as percentages. Significance tests were performed using analysis of variance for continuous variables and chi-squared tests for dichotomous variables.

The longitudinal outcome of interest was a composite of death, dialysis, or 50% reduction in measured GFR. These events were adjudicated by the AASK outcomes committee. Kaplan–Meier curves were constructed to display event-free survival by tertiles of urinary SO₄. A series of Cox regression models were fit to relate the composite outcome to daily urinary SO₄ excretion using the lowest tertile as the reference. Follow-up time was censored at the end of

### Table 1. Baseline characteristics by tertiles of urinary sulfate

| Variable                          | Total Population (N=1057) | Tertile 1 (N=353) | Tertile 2 (N=352) | Tertile 3 (N=352) | P Value |
|-----------------------------------|---------------------------|-------------------|-------------------|-------------------|---------|
| Urine SO₄, mEq/d                  |                           |                   |                   |                   |         |
| Age, yr                           | 54 (11)                   | 55 (11)           | 54 (11)           | 54 (11)           | 0.27    |
| Men                               | 61                        | 46                | 59                | 80                | <0.001  |
| Heart disease                     | 51                        | 50                | 52                | 52                | 0.78    |
| Current smoker                    | 30                        | 35                | 29                | 24                | 0.009   |
| Past smoker                       | 28                        | 23                | 31                | 32                |         |
| Never smoker                      | 42                        | 42                | 40                | 44                |         |
| SBP, mm Hg                        | 150 (24)                  | 152 (26)          | 150 (22)          | 149 (24)          | 0.34    |
| BMI, kg/m²                        | 31 (7)                    | 29 (7)            | 30 (6)            | 32 (7)            | <0.001  |
| Protein intake, g/d               | 69 (26)                   | 48 (13)           | 66 (12)           | 93 (25)           | <0.001  |
| Protein intake, g/kg per day      | 0.78 (0.24)               | 0.59 (0.15)       | 0.77 (0.16)       | 0.97 (0.24)       |         |
| NEAP, mEq/d                       | 82 (37)                   | 86 (44)           | 79 (33)           | 82 (34)           | 0.05    |
| ACE-I/ARB use                     | 39                        | 37                | 36                | 45                | 0.04    |
| Diuretic use                      | 64                        | 61                | 62                | 69                | 0.03    |
| Measured GFR, ml/min per 1.73 m²  | 47 (14)                   | 43 (14)           | 47 (13)           | 50 (13)           | <0.001  |
| GFR <30 ml/min per 1.73 m²        | 16                        | 23                | 14                | 9                 | <0.001  |
| Urine protein/creatinine, mg/g    | 81 (30–350)               | 108 (41–518)      | 74 (30–323)       | 61 (22–244)       | <0.001  |
| Total CO₂, mEq/L                  | 25.1 (3)                  | 24.8 (3.2)        | 25.1 (2.9)        | 25.5 (2.7)        | 0.005   |
| Total CO₂<22 mEq/L, %             | 12                        | 16                | 11                | 9                 | 0.014   |
| K⁺, mEq/L                         | 4.2 (0.6)                 | 4.3 (0.7)         | 4.2 (0.6)         | 4.2 (0.6)         | 0.47    |
| Serum anion gap, mEq/L            | 10 (2.4)                  | 10.3 (2.6)        | 9.9 (2.2)         | 9.9 (2.4)         | 0.02    |
| Urine NH₄, mEq/d                  | 19.5 (13.2–28.1)          | 13.2 (8.7–18.3)   | 19.5 (14.6–25.3)  | 28.7 (21.1–37.2)  | <0.001  |
| Urine PO₄, mg/d                   | 63.3 (30.2)               | 39.9 (16.4)       | 61.6 (19.4)       | 88.6 (30.3)       | <0.001  |

Data presented as median (interquartile range), mean (SD), or %. N/A, not applicable; SBP, systolic BP; BMI, body mass index; NEAP, net endogenous acid production; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.
the trial phase of the AASK or permanent loss to follow-up. The initial model (model 1) was unadjusted, followed by adjustment for age, sex, randomized group, iothalamate measured GFR, urinary PCR, body mass index (BMI), and serum total CO₂ (model 2). A subsequent model also adjusted for protein intake, urinary potassium as a reflection of alkali intake, urinary phosphate, and serum phosphate (model 3). Model 4 also adjusted for systolic BP, history of heart disease, and smoking history and was considered our main model of interest. Similar Cox models were constructed for the outcomes of death alone or a kidney-specific outcome of 50% reduction in measured GFR or ESKD. A cubic spline regression analysis adjusted for variables in model 4 was performed using daily urinary SO₄ excretion as the predictor variable. Knots were placed at quartiles of daily urinary SO₄, and the median value (26.3 mEq/d) was the reference point. The variance in inflation factor of variables potentially correlated with urine sulfate (protein intake, serum and urine phosphate, serum and urine potassium, GFR, BMI, serum total CO₂) was determined, and there was no evidence of multicollinearity (variance inflation factor ≤3.40 for each variable).

Results
Table 1 presents baseline characteristics of the 1057 participants for whom urinary SO₄ measurements were obtained. Participants in the highest urinary SO₄ tertile were more likely to be men and have higher BMI, protein intake, measured GFR, and urinary ammonium and phosphate excretion, and lower urinary PCR. Although protein intake was highest among those in the highest urine SO₄ tertile group, mean daily protein intake was not excessive.

Figure 1 shows baseline values of urinary SO₄, protein intake, urinary potassium, and NEAP by categories of measured GFR. Urinary SO₄, protein intake, and urinary potassium levels were lower among those with lower measured GFR. There was no significant difference in NEAP across the measured GFR categories.

Figure 2 shows correlations between urinary SO₄ and protein intake, urinary potassium, NEAP, and serum total CO₂ at baseline. Urinary SO₄ was strongly correlated with protein intake (r=0.84) and urinary potassium but to a lesser extent (r=0.53). There was no meaningful correlation between urinary SO₄ and NEAP or total CO₂.

Table 2 shows the number of composite events (death, dialysis, or 50% reduction in measured GFR) experienced among AASK participants according to baseline urinary SO₄ excretion. The number of events was lowest among those in the highest urinary SO₄ tertile group. Figure 3 shows that event-free (death, dialysis, or 50% reduction in measured GFR) survival in the lowest urinary SO₄ tertile started to decline at a greater rate than other groups after about 1 year of follow-up.

Table 3 shows unadjusted and adjusted hazard ratios (HR) for the composite outcome of death, dialysis, or 50% reduction in measured GFR by tertile of urinary SO₄. In the
unadjusted model, the hazard of the composite outcome was 37% lower for those in the highest tertile and 28% lower for those in the middle tertile compared with the lowest tertile. Additional adjustment did not substantially change the HR for either group. In our main model (model 4), those in the middle tertile had a 27% lower hazard, and those in the highest tertile had a 44% lower hazard of death, dialysis, or 50% reduction in measured GFR. Table 3 also shows HR and 95% confidence intervals (CI) for the outcomes of (1) death alone and (2) a kidney-specific outcome of ESKD or 50% reduction in measured GFR after adjusting for variables in model 4. For death alone, there was a 25% lower hazard in the middle tertile and a 53% lower hazard in the highest tertile, although neither was statistically significant because of a low number of deaths during follow-up (n=83). For the kidney-specific outcome (ESKD or 50% reduction in measured GFR), the hazard of experiencing these events was 27% lower in the middle tertile and 41% lower in the highest tertile compared with the lowest tertile.

Figure 4 shows the cubic spline regression plot between urinary SO₄ and the composite outcome using median daily urinary SO₄ excretion (26.3 mEq/d) as the reference point. The lowest risk of the composite outcome was with a urinary SO₄ value of around 35–45 mEq/d. There was a linear and inverse association with the composite outcome among those with urinary SO₄ levels below this range.

Table 2. Number and incidence rate (per 1000 patient years) of the composite outcome of death, dialysis, or 50% reduction in measured GFR

| Daily SO₄ Excretion | Number of Events | Follow-Up Time (Patient Years) | Incidence Rate (95% Confidence Interval) (per 1000 Patient Years) |
|---------------------|------------------|-------------------------------|---------------------------------------------------------------|
| Tertile 1           | 137              | 1383                          | 99 (84–117)                                                   |
| Tertile 2           | 109              | 1489                          | 73 (61–88)                                                    |
| Tertile 3           | 94               | 1467                          | 64 (52–78)                                                    |
| Total               | 340              | 4340                          | 78 (70–87)                                                    |

Figure 2. | Urinary sulfate is strongly associated with protein intake but not net endogenous acid production. Correlation between urinary sulfate and (A) protein intake, (B) urinary potassium, (C) NEAP, and (D) serum total CO₂ at baseline.

Figure 3. | Kaplan–Meier event-free survival plot by tertiles of urine sulfate.
tertile of urinary SO4 had around a 45% lower hazard of
AASK participants. On the contrary, those in the highest
individuals with CKD. Although urinary SO4 was directly
be associated with greater risk of adverse outcomes in indi-
viduals with diabetes and higher urinary SO4
levels had lower risk of CKD progression (29,30). Thus,
higher urinary SO4 is associated with more favorable out-
comes in individuals with CKD.

Similarly, individuals with CKD progression or death during follow-up after adjusting
for important confounders such as measured GFR, protein intake, urinary potassium, urinary phosphate, and
serum phosphate. Model 4: adjusted for model 2 variables and
systolic BP, heart disease, and smoking.

Because urinary sulfate is tightly linked with protein intake, we evaluated the association between protein intake tertiles and the composite outcome in these participants. The model used here adjusted for the same variables as in model 4, excluding protein intake. Compared with those in the lowest tertile, there was no appreciable difference in the hazard of the composite outcome among those in the middle tertile (HR 0.86; 95% CI, 0.64 to 1.15) or the highest tertile (HR 0.98; 95% CI, 0.67 to 1.45) of protein intake.

Discussion
Sulfur is an essential mineral; however, sulfur’s primary
dietary source is animal protein. Due to its link with pro-
tein intake, we postulated that higher urinary SO4 would
be associated with greater risk of adverse outcomes in indi-
viduals with CKD. Although urinary SO4 was directly
associated with protein intake, higher urinary SO4 was not
associated with loss of kidney function or mortality among
AASK participants. On the contrary, those in the highest
tertile of urinary SO4 had around a 45% lower hazard of
CKD progression or death during follow-up after adjusting
for important confounders such as measured GFR, protein
intake, and potassium intake. The observation that urinary
SO4 and protein intake were strongly correlated yet only
SO4 was associated with risk of adverse clinical events sug-
uggests that SO4 exerts its effects independently of protein
intake. These findings are similar to those observed in kid-
ey transplant recipients in whom higher urinary SO4 was
associated with lower mortality and graft failure (27,28).
Similarly, individuals with diabetes and higher urinary SO4
levels had lower risk of CKD progression (29,30). Thus,
higher urinary SO4 is associated with more favorable out-
comes in individuals with CKD.

There are several potential reasons why higher urinary
SO4 is associated with more favorable outcomes in CKD. First, urinary SO4 excretion was higher in individuals with higher GFR. Thus, individuals with less severe CKD may not have been advised to reduce dietary protein intake, or may have had preserved sulfate clearance due to higher GFR. On the other hand, those with lower urinary SO4 could have been consuming a low protein diet or have impaired SO4 excretion with SO4 accumulation. Another potential explanation is that those with lower GFR may accumulate uremic toxins and medications that are metabo-
ized through sulfation pathways. Hence, low urinary SO4
could be a sign of increased proximal SO4 reabsorption via
the sodium-sulfate co-transporter (NaS1) to meet these meta-
obolic demands. Nevertheless, we observed a strong and
statistically significant association between higher urinary
SO4 and lower risk of CKD progression or death, even after adjusting for both protein intake and GFR. Importantly, GFR was measured, and not estimated, in AASK partici-
pants, increasing confidence in the findings.

Another possibility is that urinary SO4 reflects total body sulfur abundance, and lower urinary sulfate excretion could signal a state of sulfur deficiency (28). Sulfur is a component of a variety of important molecules, including
the sulfur-containing amino acids methionine and cysteine.
Methionine is an essential amino acid, whereas cysteine
can be synthesized as long as sulfur is available. Sulfur is
principally stored as glutathione (31), and sulfur deficiency
may favor cysteine and protein synthesis at the expense of
other molecules such as glutathione. Glutathione is a pow-
erful antioxidant whose activity decreases with protein
restriction. Lower glutathione levels are associated with
greater reperfusion injury in AKI in animal models and
humans (32-34), and glutathione suppresses prostaglandin
synthesis pathways (35,36). In addition, glutathione plays a

Table 3. Unadjusted and adjusted hazard ratios of the composite outcome of death, dialysis, or 50% reduction in measured GFR; death alone; and dialysis or 50% reduction in GFR by tertiles of urinary sulfate excretion

| Outcome | Urinary SO4 | Tertile 1 | Tertile 2 | Tertile 3 |
|---------|-------------|----------|----------|----------|
| Death, dialysis, or 50% GFR reduction | Model 1 Reference 0.72 (0.56 to 0.92) 0.63 (0.49 to 0.82) | Model 2 Reference 0.77 (0.6 to 1) 0.69 (0.52 to 0.93) | Model 3 Reference 0.73 (0.55 to 0.97) 0.56 (0.37 to 0.84) | Model 4 Reference 0.73 (0.55 to 0.96) 0.56 (0.37 to 0.84) |
| Death | Model 4 Reference 0.75 (0.43 to 1.3) 0.47 (0.22 to 1) | Model 4 Reference 0.73 (0.53 to 1.01) 0.59 (0.37 to 0.95) |

Data shown as hazard ratio (95% confidence interval). The lowest tertile served as the reference group. Model 1: unadjusted. Model 2: adjusted for age, sex, randomized treatment group, measured GFR, urine protein/creatinine, body mass index, and serum total CO2. Model 3: adjusted for model 2 variables and protein intake, urinary potassium, urinary phosphate, and serum phosphate. Model 4: adjusted for model 2 variables and systolic BP, heart disease, and smoking.

![Figure 4](image-url) | Cubic spline regression plot of the association between urinary SO4 and the primary composite outcome of death, dialysis, or 50% reduction in measured GFR. The solid line represents the hazard ratio, and the dashed lines represent the 95% confidence intervals. The median value (26.3 mEq/d) served as the reference point. Adjusted for age, sex, randomized treatment group, measured GFR, urinary protein/creatinine, body mass index, serum total CO2, protein intake, urinary potassium, urinary phosphate, serum phosphate, systolic BP, heart disease, and smoking.
role in regulating cholesterol. For example, diets supplemented with sulfur-containing amino acids increased levels of reduced glutathione and lowered serum cholesterol (37–39). Sulfur is also an elemental component of hydrogen sulfide, an endogenous neuromodulator. In animal models of kidney disease, exogenous administration of hydrogen sulfide reduced oxidative stress and promoted anti-inflammatory pathways (40–44). Thus, lower urinary sulfate levels may signal a state of sulfur deficiency that adversely affects the production and function of important regulatory molecules and pathways. Although important for metabolism, sulfur in excess can cause gastrointestinal side effects and neurotoxicity.

Despite a strong correlation between urinary SO4 and protein intake, there was no correlation of SO4 with NEAP. However, there was, however, a modest association between urinary SO4 and urinary potassium excretion, suggesting that alkali precursors ameliorate the effect of dietary acid load from protein. We calculated protein intake using urinary urea excretion, which does not discriminate between plant and animal protein sources (45). Others have found that lower fruit and vegetable intake correlated with high NEAP and was associated with more rapid progression of CKD when protein intake was assessed by food diaries rather than urinary urea excretion (46–48). Dietary diaries were not performed in the AASK. Nevertheless, quantification of protein intake from urea nitrogen is a standard approach. However, it does not distinguish between animal- and vegetable-derived protein.

Sulfate is the end product of sulfur metabolism, irrespective of whether it comes from organic or inorganic sources. Sulfites are often added to dry produce, processed meats, and other foods, but it is difficult to distinguish the quantity or proportion of SO4 derived from these food additives. However, sulfites are generally thought to be more harmful than beneficial in human health because they can worsen allergies and asthma, and cause intestinal inflammation (49,50), and therefore they are unlikely to explain the findings. Other limitations of this study include its retrospective nature. Despite our best efforts to control for potential confounders, residual confounding may still be present, and we cannot prove causation. The AASK included African Americans with CKD attributed to hypertension, which could affect the generalizability of this study. However, similar findings have been observed in other cohorts such as individuals with diabetes or those who have received a kidney transplant. We are unaware if participants were instructed to be on a low-protein diet. Nevertheless, our findings were observed after adjusting for protein intake, and protein intake itself was not associated with an increased risk of CKD progression or death here. We cannot completely rule out the possibility that low urinary SO4 reflects reduced filtration of SO4 due to lower GFR. Plasma SO4 levels could help determine if this were the case; however, measuring plasma SO4 levels requires advanced techniques that are beyond the scope of this study. Despite these limitations, this study has important strengths. The AASK is a well-characterized cohort with a large sample size, carefully collected prospective data, and long-term follow-up. Furthermore, GFR was directly measured rather than estimated, which is particularly important, given the strong association between kidney function and the outcomes of interest.

In conclusion, higher urinary SO4 levels are associated with more favorable kidney and patient survival in AASK participants. Similar findings have been observed in individuals with diabetes and those who have received a kidney transplant. Future studies investigating the role of sulfur on health in patients with kidney disease are warranted.

Disclosures
S. Beddu reports consultancy for Bayer and Reata; research funding from Bayer, Boehringer Ingelheim, and Novartis; and an advisory or leadership role for CJASN and Kidney Reports. K.L. Raphael reports consultancy for AstraZeneca and research funding from the Department of Veterans Affairs (I01 CX001695). All remaining authors have nothing to disclose.

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Author Contributions
A. Azim wrote the original draft of the manuscript; S. Beddu and K.L. Raphael were responsible for conceptualization, data curation, and project administration; S. Beddu, J. Murray, and K.L. Raphael were responsible for the investigation; J. Murray and K.L. Raphael were responsible for the methodology; K.L. Raphael was responsible for the formal analysis, funding acquisition, resources, software, supervision, validation, and visualization; and all authors reviewed and edited the manuscript.

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