Title: Low serum bicarbonate as a predictor of kidney function decline and incident chronic kidney disease: The Multi-Ethnic Study of Atherosclerosis

Type of study: Retrospective cohort study

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Background:
Patients with chronic kidney disease (CKD) often develop metabolic acidosis,[1, 2] which is associated with a variety of adverse extrarenal outcomes, including decreased cardiac function, decreased bone mineral density, and muscle wasting.[3, 4] The pathogenesis of metabolic acidosis in patients with CKD is thought to be secondary to tubular dysfunction, causing decreased bicarbonate production in the proximal convoluted tubules.[2] Metabolic acidosis may also play a role in the pathogenesis of CKD by causing tubular damage via a compensatory increase in tubular ammonia production, resulting in complement activation and inflammation.[3, 5, 6]

Numerous studies have found that metabolic acidosis, reflected by a low serum bicarbonate level, is associated with adverse kidney and mortality outcomes in the setting of established CKD. With respect to kidney outcomes, low bicarbonate levels in patients with CKD are associated with CKD progression, rapid kidney function decline, and faster progression to end-stage renal disease.[3, 5, 7] Although low serum bicarbonate levels are associated with an increased risk of mortality in CKD patients,[4, 5, 7] higher serum bicarbonate levels are also associated with increased mortality risk in CKD patients, giving bicarbonate a u-shaped association curve with mortality. However, this may be confounded by coexisting comorbid conditions, such as chronic obstructive pulmonary disease or congestive heart failure.[4, 8]

Correction of low serum bicarbonate levels through supplementation has shown the potential for slowing CKD progression, suggesting that it may be a modifiable risk factor. A pair of small randomized controlled trials found that correcting low serum bicarbonate levels in patients with CKD slowed renal function decline[6, 9] and progression to end-stage renal disease.[6]

Whether bicarbonate levels have prognostic importance in persons without CKD has not been well studied. It remains unknown if bicarbonate levels are associated with kidney, cardiovascular, or mortality outcomes in patients without baseline CKD. Detection of low serum

bicarbonate levels may have the potential to be an early diagnostic tool for kidney tubular dysfunction in CKD and even a target to slow CKD progression or prevent the onset of CKD.

Research question:
Are serum bicarbonate concentrations associated with kidney function decline and incident CKD in a multi-ethnic cohort of adults without baseline chronic kidney disease?

Methods:
Design: Cohort analysis using all participants without baseline CKD (eGFR <60 by cystatin C) with follow-up for kidney function decline and incident CKD.

Target Population:
People without baseline CKD in the Multi-Ethnic Study of Atherosclerosis (MESA).

Accessible Population:
MESA is a population study of community-dwelling adults aged 45-84 years and was designed to determine the characteristics of subclinical cardiovascular disease (CVD) and its progression. From 2000-2002, a total of 6,814 adults without evidence of clinical CVD at baseline were recruited from 6 US communities (Baltimore, MD; Los Angeles, CA; northern Manhattan, NY; and St. Paul, MN). The study sample was diverse across race/ethnicity; there were 2,624 individuals who identified themselves as Caucasian, 1,894 as African American, 1,493 as Hispanic, and 803 as Chinese.[10] Participants were followed for 7 years. The MESA population is appropriate for two reasons: 1) its multi-ethnic population and its low prevalence of baseline CVD, which correlates into a low prevalence of baseline CKD; 2) its 7-year follow-up, which allows ample time for participants to develop CKD.

Subject inclusion Criteria:
- Particpiants who met inclusion criteria for MESA[10]
- Participants without baseline CKD (eGFR <60 by cystatin C)[11]
- All MESA participants with baseline measures of serum bicarbonate and at least 2 measures of creatinine and cystatin C to measure kidney function decline.

Sampling procedures:
- We will have access to a database with recorded concentrations of cystatin C, creatinine, and bicarbonate measured from stored serum samples collected at each MESA visit.

Subject inclusion criteria:
1. Kidney outcomes – All MESA participants with baseline measure of serum bicarbonate and at least two measures of creatinine and cystatin C to measure kidney function decline.

Predictor Variable:

- Serum bicarbonate measured at MESA exam 1

Outcome Variables:

1. Kidney function decline: Linear change in eGFR: For primary analysis, GFR will be calculated from cystatin C. The estimated GFR from serum cystatin C (GFR_{cys}) will be calculated using the CKD-EPI (CKD Epidemiology Collaboration) equations. [12, 13] Secondary analysis will use the creatinine-based CKD-EPI equation.[13]
2. Incident CKD: Defined by reaching an eGFRcys <60 during follow-up with at least an annual eGFR loss of 1 ml/min/1.73 m^2.[14]

Candidate covariates:

Demographics:

- Age (in years)
- Gender (female or male)
- Education
- Clinical site
- Race

Risk Factors (using current MESA definitions):

- Diabetes (type 1 or type 2)
- BMI (kg/m^2)
- Current or former smoker
- HDL, LDL, and triglyceride levels
- Hypertension
- SBP (mmHg)
- Fasting glucose (mg/dL)
- Albumin/creatinine ratio

Sample size calculation:

For the detection of kidney function decline, a conservative effect size was chosen, yielding an estimated sample size of 60.[14] This was derived from a t-test table using a power of 0.9 and a two-sided alpha of 0.05.[15] Similarly, for incident CKD, a conservative detectable increased incidence of 0.05% yielded a sample size of 1252 patients.[16] This was derived from a chi-squared table using a power of 0.9 and a two-sided alpha of 0.05.[15] With a MESA population of greater than 6000 patients, the study population should be adequately powered to detect both kidney function decline and incident CKD.

Statistical Analysis

1. General analytical approach for each outcome: We will run preliminary regressions to determine which variables we need to adjust for in the final model. Specifically, we will adjust for demographic covariates and baseline $eGFR_{cys}$ and other potential confounding variables. Covariates will be included in the final model based on their impact on the primary predictor, bicarbonate. Each covariate will be entered in to a separate regression predicting bicarbonate and if its retention accounts for >5% change in the coefficient (i.e., R^2 increase of at least 5%) for bicarbonate, then it will be retained in the final model.
2. Modeling of bicarbonate: For each outcome, we will evaluate the form of association of bicarbonate using spline analyses or lowess plots. *A priori*, we plan analyses of bicarbonate: a) as quintiles; b) as clinical categories (<21, 21-23, 23-25, >25); c) as a

linear variable. However, the spline analyses will inform these decisions for each outcome.

3. Linear eGFR outcomes: Decline in kidney function will be determined from longitudinal calculations of eGFR based on cystatin C and serum creatinine. We will use linear mixed models with random intercepts and slopes within each category of serum bicarbonate to estimate and compare linear trajectories of mean eGFR. [14, 17]
4. Incident CKD: For bicarbonate concentration and incident CKD, we will use multivariate Cox proportional hazards models. We will have statistical support from the team statistician.

References:

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