ARIC Manuscript Proposal Form

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Publication Committee Review Date: **_12/12/23_**
ARIC Manuscript Proposal Number: **# 4385**

1.a. Full Title: Metabolomic and Proteomic Markers of Dietary Potassium, Chronic Kidney Disease, Hypertension, and Cardiovascular Disease

b. Abbreviated Title (Length 45 characters): Dietary Potassium Metabolomics and Proteomics

2. Writing Group [please provide a middle initial if available; EX: Adam L Williams]:
Writing group members: Lauren Bernard, Jingsha Chen, Valerie K. Sullivan, Bing Yu, Paul A. Welling, Casey M. Rebholz, others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __LB___ **[please confirm with your initials electronically or in writing]**

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ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (The ARIC author should be involved enough in ARIC to be able to point the lead author to appropriate ancillary study PIs and to be able to search ARIC manuscript proposals if the lead author doesn't have the access needed to do such a search).

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3. Timeline: Analyses will begin after the manuscript proposal is approved. We anticipate the first draft of the manuscript will be completed approximately 1 year after approval.

4. Rationale:

Dietary potassium has been implicated in the development of kidney disease, hypertension, and cardiovascular disease (CVD). Previous research conducted with data from the National Health and Nutrition Examination Survey (NHANES) has reported that higher intake of dietary potassium is associated with lower odds of incident chronic kidney disease (CKD).[1] In a cohort of young adults without CKD, each 1 gram higher of dietary potassium intake was associated with 29% lower risk of incident albuminuria, a marker of kidney damage.[2] Urinary potassium excretion, a proxy for dietary potassium, has also been inversely associated with systolic blood pressure, a CVD risk factor.[3] One proposed theory for these observations is that dietary potassium stimulates a decrease in the Na^+-Cl^- cotransporter to increase potassium secretion and sodium excretion.[4] Yet, the exact mechanisms of potassium's role in kidney disease, hypertension, and CVD are not completely understood. Additional insights are needed to disentangle the physiology underlying dietary potassium's impact on chronic kidney disease, hypertension, and CVD.

Metabolomics provides an opportunity to understand the metabolic activity of dietary potassium through the study of small compounds, called metabolites, within biospecimen (e.g., serum, urine, etc.). Metabolites are responsive to dietary intake, which makes them keenly useful to study as potential biomarkers of dietary intake.[5,6] Metabolomic findings can also provide novel insights into pathways perturbed by dietary potassium that are associated with kidney disease, hypertension, and CVD.

This study has four primary aims. First, we aim to discover serum metabolites and plasma proteins that are associated with dietary potassium. Second, we will identify plasma proteins that are associated with dietary potassium. The main implication of these two aims will be to improve existing dietary assessment tools (e.g., self-report questionnaires) with objective measurement of diet biomarkers in the future. Third, we will identify potassium-related metabolites and proteins that are significantly associated with risk of incident kidney disease and cardiovascular outcomes (i.e., incident hypertension and CVD). These findings will drive physiologic understanding of dietary potassium's impact on disease development. Fourth, we want to add to the existing body of literature on the associations between dietary potassium with kidney disease and with cardiovascular outcomes (i.e., incident hypertension and CVD). Thus, the richly phenotyped ARIC study population can help to reconcile whether dietary potassium is associated with chronic disease.

5. Main Hypothesis/Study Aims:

Aim #1: We hypothesize that we will discover serum metabolites associated with total dietary potassium.

Aim #2: We hypothesize that we will identify plasma proteins associated with total dietary potassium. We hypothesize that there will be unique proteomic signatures of dietary potassium.

Aim #3: For metabolites and proteins significantly associated with total dietary potassium, we hypothesize that several metabolites and proteins will be associated with incident CKD and cardiovascular outcomes (i.e., incident hypertension, incident CVD, CVD subtypes).

Aim #4: We hypothesize total dietary potassium will be associated with incident CKD and cardiovascular outcomes (i.e., incident hypertension, incident CVD, CVD subtypes).

6. Design and analysis - please address the following aspects:

a) **inclusion/exclusion**
b) **study design**
c) **outcome and other variables of interest with specific reference to the time of their collection**
d) **summary of data analysis**
e) **Any anticipated methodologic limitations or challenges if present**

Study Design: For Aim #1, the design will be a cross-sectional analysis of dietary potassium and serum metabolites, which were both collected at the baseline visit (1987-1989). For Aim #2, we will perform a cross-sectional analysis of total dietary potassium and plasma proteins collected at visit 3 (1993-1995). For Aim #3-4, the prospective analyses will be conducted through the most recent follow-up data that are available. The baseline will be visit 1 for these analyses, except proteins for Aim 3 will start at visit 3.

Eligibility Criteria: For Aim #1, ARIC study participants must have completed the food frequency questionnaire and provided fasting blood samples at visit 1 for serum metabolomic profiling (N~4000). For Aim #2, ARIC study participants must have completed the food frequency questionnaire and provided blood samples at visit 3 for plasma proteomic profiling (N~11500).

For Aims #3-4, separate study populations will be created for incident CKD, incident hypertension, and incident CVD. In incident CKD analyses, participants must not have prevalent CKD [defined at baseline as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 or end-stage kidney disease identified through USRDS linkage]. In incident hypertension analyses, participants with prevalent hypertension (defined as self-reported anti-hypertensive medication use at baseline or visit 1 readings of a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg) will be excluded. For the proteomic aim of Aim #3, participants with prevalent myocardial infarction, stroke, or heart failure at visit 1 will be excluded. Participants who experienced a CVD event (coronary heart disease, stroke, heart failure) between visit 1 and visit 3 will also be excluded. Participants with missing covariate or follow-up data will be excluded.

Exposures & Outcomes: For Aim #1, the exposure will be dietary potassium and the outcome will be serum metabolites. For Aim #2, the exposure will be total dietary potassium and the outcome will be plasma proteins. For Aim #3, the exposure will be potassium-related metabolites

and proteins, and the outcomes will be incident CKD, incident hypertension, and incident CVD. For Aim #4, the exposure will be total dietary potassium and the outcomes will be incident CKD, incident hypertension, and incident CVD. Each exposure-outcome for Aim #3 and #4 will be separately modeled.

Dietary Potassium: At visit 1 and visit 3, dietary intake was assessed using an interviewer-administered, semi-quantitative food frequency questionnaire (FFQ). This FFQ was developed by modifying the Willett 61-item questionnaire.[7] Dietary intake of potassium was estimated by relating reported intake of food and beverage items to nutrient databases. We plan to analyze total dietary potassium in milligram (mg) as well as using a nutrient density approach, i.e., mg of potassium per 1,000 kilocalories.

Serum Metabolomics: Visit 1 (1987-1989) fasting serum specimens were analyzed by Metabolon, Inc. (Durham, North Carolina) with an untargeted, ultra-high performance liquid chromatography tandem mass spectrometry approach. The metabolomic profiling was completed in two samples at different timepoints (sample 1 was completed in 2010, sample 2 was completed in 2014). For Aim #1, we will perform an untargeted analysis of ~320 metabolites shared across both samples with <80% missing values. For Aim #3, we will study metabolites significantly associated with total dietary potassium in Aim #1.

Plasma Proteomics: Visit 3 (1993-1995) plasma specimen were analyzed by SomaLogic (Boulder, Colorado) using an aptamer-based technology. SomaLogic conducted a standardization and normalization process of proteomic data, and this data was subsequently cleaned and underwent quality control by ARIC investigators. For Aim #2, we will conduct an untargeted analysis of ~5000 proteins that met quality control standards.

Chronic Kidney Disease: Incident CKD will be defined by meeting one of these four criteria: 1) reduced kidney function (eGFR <60 mL/min/1.73 m2) with a 25%+ eGFR decline at any follow-up visit relative to visit 1, 2) ICD-9/10 code for a hospitalization related to CKD stage 3+, 3) ICD 9/10 code for a death related to CKD stage 3+, and 4) end-stage kidney disease identified by linkage to the US Renal Data System (USRDS) registry. This definition of incident CKD has been widely used in the ARIC study and was previously validated.[8]

Hypertension: Incident hypertension will be defined from participants' self-report of a physician's diagnosis of hypertension, self-reported anti-hypertensive medication use, or elevated blood pressure at a follow-up visit (systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg).[9,10] Blood pressure was measured at study visits. Diagnosis of hypertension and medication use was self-reported at each follow-up visit and by annual telephone calls.

Cardiovascular Disease: Incident CVD will be a composite outcome defined as development of coronary heart disease (hospitalized or fatal), stroke, or heart failure. These events have been collected through multiple methods including annual telephone calls, hospital discharge records, obituaries, community death certificates, and linkage to the National Death Index. We will also model each subtype (i.e., coronary heart disease, stroke, or heart failure) individually.

Other Variables of Interest: As potential covariates in these analyses, we are interested in age, sex, race, study center, body mass index, total energy intake, baseline eGFR, serum potassium, smoking status, physical activity, education, and alcohol consumption.

Statistical Analysis: For Aim #1, we plan to run multivariable linear regression models to assess the relationship between dietary potassium (exposure) and serum metabolites (outcome). For Aim #2, we will run multivariable linear regression models estimating associations between total dietary potassium (exposure) and plasma proteins (outcome). For Aim #3-4, we will run Cox proportional hazards regression models to examine prospective relationships with clinical outcomes. We plan to estimate effect sizes per doubling of the metabolite/protein (Aim #3) and per one standard deviation higher in dietary potassium (Aims #1, #2, and #4). We plan to primarily adjust for age, sex, race, study center, body mass index, total energy intake, and baseline eGFR. A combined race-center variable will be used for sample 2. We will consider further adjustment for serum potassium, smoking status, physical activity, education, and alcohol consumption. For Aims #3-4, we plan to additionally adjust for diabetes status (in CKD, hypertension, and CVD analyses) and hypertension status (only for the analysis of CKD). Metabolomic analyses will be on a $\log_2$-transformed scale and run per sample and then meta-analyzed with fixed-effects models. As a secondary analysis for Aim #4, we plan to analyze total dietary intake of potassium as quartiles. The lowest potassium intake quartile will serve as the reference group.

Anticipated Methodologic Limitations or Challenges: A common challenge across untargeted metabolomic and proteomic studies is the possibility of detecting false positive associations (i.e., type 1 error). We plan to apply a conservative approach in Aims #1-3 by accounting for the number of statistical tests being conducted (i.e., Aim 1: 0.05/# of known metabolites shared across samples, Aim #2: 0.05/# of proteins tested, Aim #3: 0.05/# of potassium-related metabolites or proteins tested).[11]

f) Will the author need Limited data to complete the proposed manuscript? ☐ Yes, Limited data is needed (Provide a brief (2-3 sentences) justification for requesting PHI data) . ☒ No, De-identified data will be sufficient.

*Please note, Limited dataset access is strict and rarely provided. Limited data includes identifiable information such as dates (birthdays, visit dates, etc.). CMS, Genomic, Geocoded, Proteomics/Somalogic, and other -omic data all fall under the limited data category. De-identified data does not include dates. All dates are date adjusted to "Days since Visit 1".

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? (Non-ARIC analysis means that the authors are not regarded as ARIC investigators and the "ARIC author" is essentially just a facilitator rather than an integral part of the writing group.) ☐ Yes ☒ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ☐ Yes ☐ No
(The file ICTDER is distributed to ARIC PIs annually, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ☐ Yes ☒ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ☐ Yes ☐ No

9. The lead author or the "sponsoring" ARIC author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the website at: https://aric.cscc.unc.edu/aric9/proposalsearch [ARIC Website□Publications□Proposal Search]

☒ Yes ☐ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

#2034: The human metabolome is associated with dietary intake among African Americans in the Atherosclerosis Risk in Communities Study (lead author: Yan Zheng)

> The manuscript based on this proposal has already been published [Zheng Z, Yu B, Alexander D, Steffen LM, Boerwinkle E. Human metabolome associates with dietary intake habits among African Americans. Am J Epidemiol 2014;179(12):1424-1433]. This manuscript studied serum metabolites associated with food groups (e.g., sugar-rich foods and beverages, fruits and vegetables) and single-food categories (e.g., coffee, eggs, fruit juice, nuts and butter). This present proposal's metabolomic aims focus on a single micronutrient (potassium). Additionally, we plan to include a larger study sample with the inclusion of participant samples analyzed in 2014 that were representative of all four study centers.

#1882: A longitudinal study of metabolomics and kidney function among African Americans in the Atherosclerosis Risk in Communities (ARIC) study (lead author: Bing Yu)

> The manuscript based on this proposal has already been published [Yu B, Zheng Y, Nettleton JA, Alexander D, Coresh J, Boerwinkle E. Serum metabolomic profiling and incident CKD among African Americans. Clin J Am Soc Nephrol 2014;9(8):1410-1417]. This manuscript analyzed 204 compounds and discovered 40 known metabolites were associated with incident CKD. Our proposal's prospective analysis with CKD will only study metabolites associated with potassium, in order to uncover biological mechanisms underlying potassium's impact on kidney function.

#2325: Dietary Acid Load and Incident Chronic Kidney Disease (lead author: Casey Rebholz)

The manuscript based on this proposal has already been published [Rebholz CM, Coresh J, Grams ME, et al. Dietary Acid Load and Incident Chronic Kidney Disease: Results from the ARIC Study. Am J Nephrol. 2015;42(6):427-435]. This analysis focused on the relationship between dietary acid load and incident CKD. Dietary acid load is estimated with multiple components, including dietary potassium as well as protein, phosphorus, magnesium, and calcium. This paper found a positive association between higher dietary acid load and risk of incident CKD. This paper is complementary to our proposal's fourth aim, which will focus more specifically on associations between total dietary potassium with CKD.

#3458: Metabolomics of Dietary Acid Load and Incident Chronic Kidney Disease (lead author: Anam Tariq)

The manuscript based on this proposal has already been published [Tariq A, Chen J, Yu B, et al. Metabolomics of Dietary Acid Load and Incident Chronic Kidney Disease. J Ren Nutr. 2022;32(3):292-300. doi:10.1053/j.jrn.2021.05.005]. This targeted metabolomic analysis studied twelve metabolites previously associated with dietary acid load in the African American Study of Kidney Disease and Hypertension (AASK) and the Modification of Diet in Renal Disease (MDRD) studies. They reported eleven of the twelve metabolites were inversely associated with CKD risk. As dietary potassium is one component of dietary acid load, we hypothesize several of these metabolites will also be significantly associated with CKD risk in our study. However, our proposal again focuses only on total dietary potassium.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or does it use current [or ongoing] ancillary study data (this includes ACHIEVE)? ☒ **Yes** ☐ **No → Skip to question 12**

11.b. If yes to 11.a., is the proposal

☒ **A. primarily the result of an ancillary study**

☐ **B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables)**

11.c. If yes to 11.a., list number

2014.20: Genomics, Metabolomics, and Cardiovascular Disease (PI: Eric Boerwinkle)
2008.16: Metabolomics & Heart Failure: A Novel Approach to Biomarker Discovery (PI: Jennifer Nettleton)
2017.26: Proteomic longitudinal ARIC study: SOMAscan of multiple visits (PI: Josef Coresh)
2020.22: Metabolomic Markers of Dietary Factors Associated with Kidney Health (PI: Casey Rebholz)

*ancillary studies are listed by number
https://aric.cscc.unc.edu/aric9/researchers/ancillary_studies/approved_ancillary_studies [ARIC Website Ancillary Studies Approved Ancillary Studies]

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in https://aric.cscc.unc.edu/aric9/publications/policies_forms_and_guidelines [ARIC Website Publications Publication Policies, Forms, and Guidelines]. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

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