1.a. Full Title: Choline metabolites and cardiovascular disease

b. Abbreviated Title (Length 26 characters): Choline and CVD

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

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3. Timeline:

From time of data receipt:
3 months for analysis
2 months for manuscript preparation
2 months for approval, submission processes

4. **Rationale:**

Choline/L-carnitine metabolite trimethylamine N-oxide (TMAO) has been implicated in the development and progression of cardiovascular disease (CVD). The direct administration of TMAO in Apo E-/- mice has been shown to accelerate atherosclerosis\(^1\). In a large sample of patients (n=4007) undergoing coronary angiography, individuals (mean age 63 yrs) in the highest quartile of TMAO had significantly increased risk of major CVD events (n=513) over 3 years of follow-up [HR=1.43 (95% CI: 1.05-1.94), comparing the 4th to the 1st quartile of TMAO]\(^2\).

Subsequent studies in high-risk samples have confirmed the association with CVD, as well as indicate a role for TMAO in kidney disease\(^3\) and heart failure\(^4\). In a recent meta-analysis of published studies, TMAO was associated with major adverse cardiovascular events [summary RR=1.62 (95% CI: 1.45-1.80)]\(^5\).

Together, these results have garnered much interest as TMAO-associated risk may be limited by decreased consumption of dietary precursors (such as choline) or through pharmacologic interventions aimed at TMAO itself or the gut microbial enzymes upon which trimethylamine production relies. TMAO is produced by oxidation of trimethylamine (TMA) in the liver, which is dependent on FMO3 genetic variants; TMA is generated through conversion of dietary precursors choline and L-carnitine by gut microbial enzymes\(^6\)\(^-\)\(^9\). A GWAS of mouse and human studies indicates that variability in TMAO largely reflects dietary consumption and production of TMA, rather than variability in FMO3 gene variants\(^10\).

There remain several limitations of previous studies, which ARIC data can help address. As pointed out by the authors of the meta-analysis\(^5\) and others\(^11\), there is a lack of data from population-based studies of TMAO and CVD events, and it is not clear whether TMAO is prospectively associated with the development of CVD in samples without clinical disease at baseline. In an analysis of 817 CARDIA participants, TMAO was not associated with coronary artery calcium (CAC) or intimal-media thickness (IMT)\(^12\). This analysis was limited by a relatively small sample size and the lack of hard endpoints. In previous, untargeted, analysis of ARIC broad-spectrum metabolomics data, TMAO precursor choline has not been identified as predictive of CVD-related outcomes, after adjusting for multiple comparisons\(^13\)\(^-\)\(^17\). Other studies of untargeted metabolomics have not identified TMAO as associated with increased CVD risk\(^18\). However, it is possible that untargeted analysis may be low-powered and that a targeted analysis of TMAO and related metabolites would yield additional information.

Population-based data will also to limit potential confounding by renal function—as TMAO is excreted in urine—and contribute to understanding possible etiologic pathways through kidney disease\(^11\). Although previous studies have adjusted for estimated glomerular filtration rate (eGFR), TMAO concentrations are notably higher, and eGFR lower, in clinic-based samples\(^2\)\(^,\)\(^12\), and residual confounding remains possible. Most studies of TMAO have lacked dietary data, though dietary precursors of TMAO include red meat and eggs, which may confound the association with CVD risk. Furthermore,
analysis of dietary precursors choline and betaine have not supported independent associations with CVD, including in the ARIC study, which has implications for dietary recommendations to limit choline for CVD prevention.

The ARIC metabolomics data allow for targeted analysis of TMAO, and circulating precursors choline and betaine, with respect to incident CVD and CVD mortality in a population-based cohort. ARIC includes data on relevant covariates on dietary consumption and renal function.

5. **Main Hypothesis/Study Questions:**

1. TMAO is prospectively associated with CVD incidence and mortality.
2. Serum choline and betaine are not associated with CVD events.

6. **Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).**

**Study population:**
The analysis will include ARIC participants with: 1) serum metabolome analyzed from baseline samples, 2) complete event follow-up, and 3) complete data on study covariates.

We will use data from Dr. Boerwinkle’s metabolomics studies (Phases 1 and 2), which include data from 1,553 whites and 2,479 blacks. Choline and betaine are available on all participants, while TMAO was not quantified in Phase 1 and is available on roughly 1,500 participants.

**Exposure:**
Targeted analysis on serum TMAO, choline, and betaine.

**Outcomes:**
1. Incident CVD (nonfatal MI, stroke, heart failure)
2. CVD mortality (fatal CHD, stroke, heart failure)

**Covariates (baseline or as close to baseline as measured):**
Education, race, gender, age
Dietary data (food frequency questionnaire)
Smoking
Physical activity
Creatinine (eGFR)
Lipids and lipid-lowering medication use
Glucose
Blood pressure and antihypertensive medication use
BMI
Diabetes
Prevalent heart failure, coronary heart disease, and stroke
**Statistical analysis:**
Baseline variables will be compared across categories of TMAO by using chi-square for categorical variables and ANOVA for continuous variables. TMAO, choline, and betaine will be centered by mean and scaled by SD prior to the analysis.
Cox proportional hazards regression will be used to quantify the association between TMAO (and choline and betaine, separately) and CVD events. Multivariable-adjusted regression models will include:
Model 1: age, sex, race, education
Model 2: Model 1 plus health behaviors (diet, physical activity, and smoking)
Model 3: Model 2 plus clinical covariates (body mass index, systolic blood pressure, hypertension medications, diabetes, eGFR\textsubscript{CKD-EPI}).

To assess the possibility that choline/betaine associations differ by TMAO produced\textsuperscript{21}, we will conduct analysis of choline and betaine stratified by TMAO concentration.

7.a. Will the data be used for non-CVD analysis in this manuscript?  ____ Yes  ____ X  ____ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES\textsubscript{OTH} = “CVD Research” for non-DNA analysis, and for DNA analysis RES\textsubscript{DNA} = “CVD Research” would be used?  ____ X  ____ Yes  ____ No
(This file ICTDER has been distributed to ARIC PIs, 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  ____ X  ____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES\textsubscript{DNA} = “No use/storage DNA”?
 ____ Yes  ____ No

9. The lead 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 web site at: \url{http://www.csc.unc.edu/ARIC/search.php}
 ____ X  ____ 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)?
Bing Yu: Associations of serum metabolome and mortality among African American in the ARIC study

Myriam Fornage: Metabolomics studies of incident stroke and vascular brain aging

**11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?**

__X__ Yes    ____ No

**11.b. If yes, is the proposal**

__X__ A. primarily the result of an ancillary study (list number*)
_Boerwinkle metabolomics study_________

__    B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s))* _________ _________ _________

*ancillary studies are listed by number at [http://www.csc.unc.edu/aric/forms/](http://www.csc.unc.edu/aric/forms/)

**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/](http://publicaccess.nih.gov/) are posted in [http://www.csc.unc.edu/aric/index.php](http://www.csc.unc.edu/aric/index.php), under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit_process_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to Pubmed central.

**References**


