1.a. Full Title: Blood metabolomic analytes and their association with cross-sectional age: a Consortium of Metabolomics Studies (COMETS) analysis

b. Abbreviated Title (Length 26 characters): metabolomics and age

2. Writing Group: COMETS Age Working Group
   Writing group members: COMETS Age Working Group members including Steve C. Moore, Marinella Temprosa and others; the ARIC investigators including Bing Yu and Eric Boerwinkle.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ___SM___ [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 (this must be an ARIC investigator).
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3. Timeline: Manuscript to be completed in <1 year after approval.

4. Rationale:
   Human aging is a multifactorial process involving many biological changes, some of which contribute to increased morbidity and, ultimately, risk of death. Understanding biologic factors that contribute to the aging process is a high scientific priority, as it could help identify novel targets for treatment and prevention of a variety of disease endpoints and perhaps to slow the aging process itself. While accumulated oxidative stress and inflammation are accepted contributors to the aging process, many other biologic factors could be relevant, particularly metabolomic factors.

   Metabolomics enables the assessment of hundreds or even thousands of metabolites concurrently in blood or other biospecimens, and is thus well suited to broad-based assessment of metabolic
factors related to aging. Metabolomics has been previously applied to identify metabolites correlated with chronological age (1-3), and associations were reported for G-glycosyltryptophan, creatinine (and other markers of kidney function), sex steroid hormones (e.g. DHEAS), and markers of incomplete fatty acid beta-oxidation in mitochondria (e.g. acylcarnitines). To date, these studies evaluated only relatively small subsets of metabolites (100-300 metabolites) and not all findings were examined in replication datasets. Evaluating an expanded list of metabolites across multiple populations would help to identify new factors related to aging and to confirm associations previously identified. Toward that end, we propose a consortium-based analysis of blood metabolites and their relationship with chronologic age.

An additional rationale for this study is that it is intended to serve as proof of principle analysis for consortium-based metabolomics studies. Age is an ideal outcome for such a study because 1) age is easy to harmonize; 2) age is highly relevant to incidence of most diseases; and 3) few investigators are targeting age as a primary outcome, thus minimizing potential conflicts.

5. Main Hypothesis/Study Questions:

[Note: There are other study questions for other COMETS participants. These are the questions for the ARIC study.]

*Aim 1*: To evaluate relationships between metabolite levels and age in ARIC European and African Americans.

*Aim 2*: To evaluate the gender and race heterogeneity for the findings in Aim 1.

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).

Design and analysis plan:

The analysis will include only participants with metabolomics data. Metabolite profiling in baseline serum samples was done in 1,977 ARIC African and in an additional ~2,000 ARIC participants in 2014 using an untargeted, gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry-based metabolomics quantification protocol. The second sample set includes primarily European-Americans.

For batch normalization, COMETS will use the normalization scheme that each individual cohort has determined to be best for analysis. For the ARIC study, this will be achieved by limiting the analysis to those named metabolites with acceptable reliability (r>0.6), no obvious batch effect, low missing rate (< 25% in both batches) and shared between African Americans and European Americans. It is estimated that there are to be 245 named metabolites that satisfy these criteria.

For aim 1, we will examine Spearman correlations between each metabolite and age using sequentially adjusted models. Model 1 will examine gender- and/or study site- adjusted associations. Model 2 will adjust for smoking status, body mass index, race, education, alcohol consumption, and multivitamin use.

For each of aims 1 and 2, we will stratify upon history of prevalent heart-disease, diabetes, or kidney disease at the baseline examination. That is, age and metabolite associations will be
examined separately in participants who have no history of these conditions versus those who do have such a history, with each condition examined separately. Group-specific results will be compared using the Wald test for homogeneity.

COMETS will use a Bonferroni-corrected threshold to determine statistical significance of associations, with an expected total of ~2,000 metabolites across all the studies. Based on the prior studies, we estimate that dozens, and potentially hundreds, of metabolites will have Spearman correlations greater than 0.2. Given our consortium-based approach, we have near 100% power for detecting associations of this magnitude even after Bonferroni correction.

For aim 2, heterogeneity across studies will be evaluated by Cochran’s Q. Heterogeneity across different platforms and participant subgroups will be meta-analyzed within each group and group-specific results will be compared using the Wald test for homogeneity.

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_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? ____ 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_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:  http://www.cscc.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)?

MS #2380 Metabolomics and Mortality (Yu)

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* 2008.16 and 2014.20)
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/

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 http://www.csc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central. Agreed.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes __X__ No.

Reference List

