# ARIC Manuscript Proposal Form

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| Publication Committee Review Date: 4/8/2025<br>ARIC Manuscript Proposal Number#: 4644 |

**1.a. Full Title**: Validation of ultra-processed foods-related blood metabolites and cardiovascular diseases in ARIC

**b. Abbreviated Title (Length 26 characters)**: UPF, metabolites, and CVD

**2. Writing Group [please provide a middle initial if available; EX: Adam L Williams]:**
Writing group numbers: Lei Wang, Casey Rebholz, Jingsha Chen, Jiaqi Yang, Bing Yu, Danxia Yu

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

**First author [please provide a middle initial; EX: Adam L Williams]:**


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**ARIC sponsor** to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located. The ARIC sponsor is responsible for **carefully reviewing the proposal and ensuring its soundness prior to submission to the ARIC Publications Committee.** (The ARIC sponsor 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**: April - June 2025: Validation analysis; June - December 2025: Manuscript draft

**4. Rationale**:

Diet plays an important role in human health, especially in the development of cardiovascular diseases (CVD). Poor dietary habits, such as high consumption of sodium, sugar-sweetened beverages, and processed meat, or low intake of whole grains, fruit, vegetables, and nuts, have been linked to an increased risk of CVD and related mortality.[1–9] In recent years, ultra-processed foods (UPF), which emerged with industrial food system, have become dominant contributors to daily energy intake in some developed countries, especially in the United States (US), accounting for 50-70% of total energy intake.[10] UPF are usually low in fiber and vitamins but high in sugar, saturated fat, salt, and additives, such as dyes, flavor enhancers, emulsifiers, and humectants, raising concerns about potential harm to human health. Indeed, numerous studies have shown that higher UPF consumption is associated with a worse cardiometabolic risk profile (e.g., obesity, high waist circumference, and low HDL-cholesterol level), as well as 31% increased diabetes risk and 35% increased CVD risk.[11–13]

The biological mechanisms through UPF affect cardiovascular health are complex and not yet well understood. Blood metabolomics, by measuring thousands of small-molecule metabolites, can provide a comprehensive picture of the underlying metabolic responses of dietary intake and link them to disease outcomes; thus, it is a powerful tool for mechanistic investigation. Evidence has shown that CVD was associated with metabolites and metabolic pathways including glycine, serine and threonine metabolism, arginine and proline metabolism, glutamine and glutamate metabolism, alanine, aspartate and glutamate metabolism, synthesis and degradation of ketone bodies, and pyruvate metabolism pathways.[14] These pathways highly reflect the CVD pathogenic process, such as low-grade inflammation, insulin resistance, oxidative stress, mitochondrial dysfunction, and dysbiosis of gut microbiota. Research on UPF and human metabolic processes is needed to better understand the mechanisms underlying UPF-CVD associations. However, the metabolic profiling of UPF has not been well elucidated. In a cross-sectional analysis in British children, UPF intake was associated with 115 metabolic traits, many of which contributed to child obesity risk.[15] We also noticed that 12 metabolites were identified for UPF intake in ARIC cohort, among which mannose, glucose, and N2, N2-dimethylguanosine were positively associated with incident chronic kidney disease.[16] Research on identifying and validating metabolites related to UPF intake and prospectively examining associations between UPF-related metabolites and CVD are still needed.

To fill these research gaps, we examined circulating metabolites related to UPF and CVD outcomes in the Southern Community Cohort Study (SCCS), a prospective cohort of predominantly low-income American adults. In the SCCS, diet information (via a validated 89-item food frequency questionnaire) and blood samples were collected at baseline (2002 –2009). Untargeted plasma metabolite profiling was performed by liquid chromatography-mass spectrometry using the discoveryHD4 platform at Metabolon Inc. We have found **142** metabolites associated with UPF intake, **25** of which were associated with coronary heart disease (CHD) incidence or CVD mortality; all FDR<0.10, please see Appendix.

We aim to leverage the valuable resource in the ARIC to validate SCCS findings. We have consulted Dr. Casey Rebholz and will include involved investigators as coauthors to ensure comprehensive and collaborative research efforts.

## 5. Main Hypothesis/Study Aims:

We hypothesize that the identified circulating metabolites related to UPF and CVD outcomes in SCCS will be reproducible in ARIC. Specifically, to test this hypothesis, we will pursue the following aims:

***Aim 1***

To validate the associations between UPF intake and 142 blood metabolites.

***Aim 2***

To validate the associations between 25 UPF-related metabolites and CHD incidence and CVD mortality.

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

a) **Inclusion/exclusion**

This study will include 4032 participants with metabolomics profiling of fasting serum collected at baseline (1987-1989) in ARIC.
Participants inclusion criteria: 1) no missing in dietary data and daily caloric intake 600 - 8000 kcal; 2) no cancer or CVD before blood sample collection.

b) **Study design**

Aim 1: cross-sectional study
Aim 2: cohort study

c) **Outcome and other variables of interest with specific reference to the time of their collection**

Dietary intake was collected using a semiquantitative 66-item food frequency questionnaire at baseline (1987-1989) in ARIC. All food items were classified based on the Nova food classification according to the extent and purpose of food processing, and UPF intake will be calculated as servings/day or weight percentage (%) of UPF in the total food intake by grams/day.

Outcomes include CHD incidence and CVD mortality.

Covariates (collected at baseline) include age, sex, race center, education, income, smoking, alcohol drinking, physical activity, total energy intake, BMI, and history of diabetes, hypertension, and dyslipidemia.

d) **Summary of data analysis**

***Aim 1: UPF intake and circulating metabolites***

**Model**: linear regression.
**Exposure:** UPF intake (standardize to mean of 0 and variance of 1)
**Outcome:** any metabolites in the list of 142 metabolites (data transformation: impute missing and zero with the half of the minimum of non-missing values, natural log transform, and standardize to mean of 0 and variance of 1).

**Covariates** (collected at baseline): age, sex, race center, education, income, smoking, alcohol drinking, physical activity, total energy intake, BMI, and history of diabetes, hypertension, and dyslipidemia.
**Output:** metabolite name, compound ID, beta coefficients, standard errors, 95%CI, and p values among all eligible participants and by sex and by race.

***Aim 2: UPF-related circulating metabolites and CHD incidence/CVD mortality***
**Model:** Cox regression.
**Exposure:** any metabolites in the list of 25 metabolites (data transformation: impute missing and zero with the half of the minimum of non-missing values, natural log transform, and standardize to mean of 0 and variance of 1).
**Outcome:** 1) CHD incidence (follow-up time will be calculated from enrollment date to the date of CHD diagnosis, death, or the last follow-up visit, whichever occurred first); 2) CVD mortality (follow-up time will be calculated from enrollment date to the date of death or the last follow-up visit, whichever occurred first).
**Covariates** (collected at baseline): age, sex, race center, education, income, smoking, alcohol drinking, physical activity, total energy intake, BMI, and history of diabetes, hypertension, and dyslipidemia.
**Output:** metabolite name, compound ID, HR (95%CI) and p values among all participants and by sex and by race.

Missing imputation for covariates: For covariates with missing rate <=5%, sex-specific median (for continuous variable) or mode (categorical variable) will be assigned to missing data. For covariates with missing rate >5%, a separate "missing" category will be assigned.

e) **Any anticipated methodologic limitations or challenges if present** No
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 PHF data) This project proposes use of metabolomics 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. Will the data be used by a for-profit organization in this manuscript? ☐ Yes* ☒ No**

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

* Please carefully review the instructions and documentation on the ARIC website at: https://aric.cscc.unc.edu/aric9/researchers/Obtain_Submit_Data [ARIC website > Researchers > Obtain/Submit Data] for instructions on proper data use.

9. **The lead author or the ARIC sponsor 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)?**
MP3969 – Metabolomics of Ultra-Processed Food, Incident Chronic Kidney Disease, and Incident Hypertension

This paper has been published (PMID: 36735499). This proposal focuses primarily on a distinct cohort (SCCS) with validation in ARIC.

**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)

*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. The NIH-NHLBI Public Access Brochure about the public access policy from http://publicaccess.nih.gov/ is 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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