ARIC Manuscript Proposal #4210

PC Reviewed: 3/14/23 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Metabolomics and proteomics of the MIND diet and risk of dementia and cognitive decline

b. Abbreviated Title (Length 26 characters): MIND diet biomarkers and dementia

2. Writing Group [please provide a middle name if available; EX: Adam Lee Williams]:
Writing group members: Jiaqi Yang, Jennifer A. Deal, Jinyu Chen, Bing Yu, Eric Boerwinkle, Lyn M. Steffen, Josef Coresh, Casey M. Rebholz

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

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3. Timeline: Analyses will begin after this proposal is approved. We anticipate having the first draft of the manuscript ready within one calendar year of manuscript proposal approval. Jiaqi will be completing the analysis and preparing the manuscript as part of her master's thesis project.

4. Rationale: The Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet is a dietary pattern that emphasizes the consumption of certain foods to promote brain health, such as green leafy vegetables, nuts, and whole grains.[1,2] Previous studies have suggested that

adherence to the MIND diet is associated with a slower rate of cognitive decline and reduced dementia risk.[1,3-6] One study has reported that higher adherence to the MIND dietary pattern was associated with muscle strength and physical function maintenance among older adults.[7] Although the MIND diet has potentially favorable associations with cognitive and physical function, the underlying biological connections remain unknown.

Interrogating the molecular compounds in blood and relating these compounds to dietary intake can help to discover new dietary biomarkers and highlight modifiable mechanisms for the prevention of cognitive decline. Several studies have focused on diet-related metabolites or protein biomarkers and chronic disease risk.[8,9] Those studies mainly focused on several dietary patterns, including plant-based diet, DASH diet, or Mediterranean diet, while there are limited studies investigating the metabolomic or proteomic profile of the MIND diet. Therefore, studying the metabolome and proteome may help to reveal novel biomarkers of MIND diet and underlying biological connections with cognitive function.

5. Main Hypothesis/Study Questions:

Aim #1: We hypothesize that we will be able to identify known and novel metabolites associated with the modified MIND diet.

Aim #2: We hypothesize that we will be able to identify known and novel plasma protein biomarkers associated with the modified MIND diet.

Aim #3: We hypothesize that some of the MIND diet-related metabolites will be associated with risk of incident dementia and cognitive decline.

Aim #4: We hypothesize that some of the MIND diet-related proteins will be associated with risk of incident dementia and cognitive decline.

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 Design: For Aim #1, we will conduct a cross-sectional analysis using visit 1 dietary and metabolomics data. For Aim #2, we will conduct a cross-sectional analysis using visit 3 dietary and proteomic data. For Aim #3, we will conduct a prospective analysis with visit 1 as the baseline, aligning with metabolomic data. For Aim #4, we will conduct a prospective analysis with visit 3 as the baseline, aligning with proteomic data. For the analysis of cognitive decline in Aim #3 and Aim #4, we will use cognitive function data collected at visits 4, 5, 6, and 7.

Eligibility Criteria: Participants who completed valid food frequency questionnaires (FFQ) at visit 1 will be included. A valid FFQ refers to having plausible total energy intake (implausible total energy intake will be defined as <600 or >4500 kcal/d for men and <500 or >3500 kcal/d for women) and missing fewer than 10 items in the FFQ. For those participants who were missing cognitive data at visit 4 (1996–1998), missing metabolomics data, proteomics data, or covariates will also be excluded from the analyses.

Exposures & Outcomes: For Aim #1, the exposure will be modified MIND diet score, and the outcome will be metabolites. For Aim #2, the exposure will be modified MIND diet score, and the outcome will be proteins. For Aim #3, the exposure will be MIND diet-related metabolites, and the outcome will be risk of incident dementia and cognitive decline. For Aim #4, the exposure will be MIND diet-related proteins, and the outcome will be risk of incident dementia and cognitive decline.

Modified MIND diet score: We will use an existing scoring system that was designed to assess adherence to the MIND diet, and modify it according to available dietary data in the ARIC study.[1] Specifically, we will remove berries and olive oil from the score. For Aim #1, we will use visit 1 dietary data. For Aim #2, we will use dietary data from visit 3.

- Ranges from 0 (lowest) to 13 (highest)
- 8 brain healthy components (green leafy vegetables, other vegetables, nuts, beans, whole grains, fish, poultry, and wine)
- 5 brain unhealthy components (red meats, butter and stick margarine, cheese, pastries and sweets, and fried/fast food)
- Each of the components will be scored based on the frequency of consumption of food portion size with a score of 0, 0.5, or 1, according to Table 1.

Table 1. Modified MIND diet component servings and scoring

	Component	0	0.5	1
Brain healthy components	Green leafy vegetables	≤2 servings/wk	> 2 to <6 servings/wk	≥6 servings/wk
	Other vegetables	<5 servings/wk	5 – <7 servings/wk	≥1 servings/day
	Nuts	<1 servings/mo	1/mo – <5 servings/wk	≥5 servings/wk
	Beans	<1 meal/wk	1–3 meals/wk	>3 meals/wk
	Whole grains	<1 serving/d	1–2 servings/d	≥3 servings/d
	Fish (not fried)	Rarely	1–3 meals/mo	≥1 meals/wk
	Poultry (not fried)	<1 meal/wk	1 meal/wk	≥2 meals/wk
	Wine	>1 glass/d or never	1 glass/mo – 6 glasses/wk	1 glass/d
Brain unhealthy components	Red meat and products	7+ meals/wk	4–6 meals/wk	< 4 meals/wk
	Butter, margarine	>2 T/d	1–2 T/d	<1 T/d
	Cheese	7+ servings/wk	1–6 servings/wk	< 1 servings/wk
	Pastries & sweets[a]	7+ servings/wk	5 −6 servings/wk	<5 servings/wk
	Fast/fried foods	4+ times/wk	1–3 /wk	<1 time/wk

Metabolomic Profiling: Metabolites from fasting serum samples stored at -80°C since collection at visit 1 (1987-1989) were detected and quantified using an untargeted, gas chromatography-mass spectrometry and liquid chromatography–mass spectrometry–based metabolomic quantification protocol by Metabolon, Inc. (Durham, North Carolina).[10] The current analysis will focus on approximately 300 named (known), non-drug metabolites.

Proteomic Profiling: Protein analytes from plasma specimens stored at -80°C since collection at visit 3 (1993-1995) were quantified by SomaLogic (Boulder, Colorado).[11,12]

Approximately 5,000 proteins were standardized and normalized by the SOMAscan process. ARIC study investigators performed data cleaning and quality control for the proteomic data.[13]

Incident dementia: Dementia will be identified in 3 ways: 1) adjudicated dementia based on complete neuropsychological battery at visit 5 and visit 6 (ARIC-NCS visit), the informant interviews, and expert review on classification for participants attending the clinic visits; 2) for people with missing clinical visits, dementia based on Telephone Interview for Cognitive Status-Modified (TICSm) with participants at visit 5 or Six Item Screener (SIS) / Eight-item Informant Interview to Differentiate Aging and Dementia (AD8) from visit 6 and forward.; 3) surveillance of a prior discharge hospitalization ICD-9/10 or death certificate code for dementia if the previous information was not avaliable.[14,15]

Cognitive decline: Cognitive testing scores from visit 4 to visit 7 assessed by the Delayed Word Recall Test (DWRT), Digit Symbol Substitution Test (DSST), and Word Fluency Test (WFT) will be used in the analyses. We will use the cognitive factor scores which were created using latent variables methods in ARIC as the outcome variable.[15,16]

Other Variables of Interest: In cross-sectional analyses (Aim #1, Aim #2), we will consider adjusting for the following variables: sex, age, race-study center, body mass index (BMI), education, physical activity, smoking status, alcohol consumption, and total energy intake. In prospective analyses (Aim #3, Aim #4), we will consider adjusting the following variables: sex, age, race-study center, BMI, education, physical activity, prevalent coronary heart disease (CHD), hypertension, and ApoE status.

Statistical Analysis:

Aim #1 (metabolites) and Aim #2 (proteins)

We will perform cross-sectional analyses using multivariable linear regression models to estimate the association between adherence to the MIND dietary pattern (exposure) and metabolites (outcome) for Aim #1 and between adherence to the MIND dietary pattern and proteins for Aim #2. The MIND dietary pattern will be estimated by the modified MIND diet score and analyzed continuously. In multivariable linear regression models, we will adjust for sex, age, race-study center, BMI, education, physical activity, smoking status, alcohol consumption, and total energy intake. Additionally, we will use Pearson's correlation coefficients to measure the linear correlations between the significant metabolites and, separately, between the significant proteins. We will apply false discovery rate (FDR) to account for multiple comparisons.

Aim #3 (incident dementia)

We will conduct a survival (time to event) analysis using Cox proportional hazards regression models to estimate the association between MIND diet-related metabolites (clusters and individual metabolites) or proteome (clusters and individual proteins) and incident dementia. We will use Schoenfeld residuals to assess the proportionality assumption of the Cox model and Efron method in the presence of ties to produce more accurate estimates. The time origin will be visit 1 for metabolite models and visit 3 for protein models. The time scale will be years for both models. Diet-related metabolites and

proteins will be log_2-transformed. In multivariable-adjusted regression models, we will adjust for sex, age, race-study center, BMI, education, physical activity, prevalent CHD, hypertension, and ApoE status. Furthermore, we will perform a mediation analysis using Baron and Kenny's method to examine whether the association between the MIND diet score and dementia/cognitive decline is mediated by metabolites/proteins.[17] In addition, we will conduct stratified analyses to examine the potential differences by sex, age, race, and ApoE status and we will test for interactions using likelihood ratio tests.

Aim #4 (cognitive decline)

We will perform a longitudinal analysis using linear mixed models to estimate the association between MIND diet-related metabolites (clusters and individual metabolites) or proteome (clusters and individual proteins) and cognitive decline 24-year change from visit 4 to visit 7. A two-piece linear spline with a knot at the median time between visit 4 and visit 5 will be used to model time. We will also adjust the following variables in the models: sex, age, race-study center, BMI, education, physical activity, prevalent CHD, hypertension, ApoE status, and the interactions with time spline terms. Additionally, we will use multiple imputation chained equations (MICE) models to impute missing cognitive data and covariates.[18] Stratified analyses will be performed to examine the potential difference by sex, age, race, and ApoE status and we will test for interactions using likelihood ratio tests.

Anticipated Methodologic Limitations or Challenges: One limitation is that we will use dietary data collected using a food frequency questionnaire to assess adherence to the MIND diet, which can introduce self-reporting bias and lead to over- or under-reporting of the absolute intake of food items. In addition, since diets will only be assessed once at visit 1, dietary intake may not reflect dietary changes over time. However, previous research in the ARIC study and other studies have reported that the average change in dietary intake over time is small.[19] Moreover, due to the large number of metabolites and proteins involved in the study, false positive associations may occur. To account for multiple comparisons, we will use the Bonferroni method to adjust the significance threshold.[20]

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes __X__ 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 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 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 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/mantrack/maintain/search/dtSearch.html

___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)?
MP#3855: Proteomic profiling of cognitive function (first author: Adrienne Tin)
This previous proposal did not relate proteins to the MIND dietary pattern.

MP#2145: Nutrition, Healthy Diet and 21-year Cognitive Decline (first author: Jennifer Dearborn)
This previous proposal did not incorporate metabolomic data or proteomic data.

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* _____)

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)

___ 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 https://sites.cscc.unc.edu/aric/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 http://www.cscc.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.

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