1.a. **Full Title**: The associations of dietary copper with neurocognitive outcomes: The ARIC Study

b. **Abbreviated Title (Length 26 characters)**: dietary copper and cognition

2. **Writing Group**:
   Writing group members (by alphabetical order of last name): Erin Bennett, Eun Sug Park, Melinda C. Power (last), Richard L. Smith, James Stewart, Jingkai Wei (first), Eric A. Whitsel, Xiaohui Xu, Qi Ying, others welcome

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

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3. **Timeline**:

4. **Rationale**:
   Copper is an essential nutrient in the human body. It is involved in various biological processes, including iron metabolism, antioxidant defense, neuropeptide synthesis, and immune function.\(^1\) Copper excess or deficiency can lead to serious health issues, such as ischemic heart disease.\(^2\)
anemia, diarrhea, peripheral neuropathy, and anxiety. Copper is contained in rock, soil, water, sediment, and at low concentrations in air. It may therefore enter the human body by breathing air/dust, drinking water, or eating food that contains copper.

Copper may be involved in Alzheimer’s disease pathogenesis. Copper binds with beta amyloid, a peptide as a feature of Alzheimer’s disease, and accelerates the aggregation of beta amyloid and formation of neurotoxic plaque, a pathologic hallmark of Alzheimer’s disease. Similarly, copper binds the tau peptide and APOE gene involved in the development of Alzheimer’s disease. Neurotoxicity of copper can be potentiated by homocysteine that may increase the risk of dementia, and older adults with high copper and/or homocysteine levels may have significant oxidative damage to neurons, which may be associated with even greater risk of dementia.

Results from studies with small samples support a link between copper and neurocognitive outcomes. Agarwal et al. found that patients with Alzheimer’s disease and vascular dementia had significantly higher serum copper compared to those without them. Park et al. found that the level of serum copper is significantly higher among participants with Alzheimer’s disease than controls, and serum copper level is negatively correlated with scores on the Boston naming test. Squitti et al. found that a higher free copper level is associated with a lower MMSE score among patients with Alzheimer’s disease, and Salustri et al. showed that the association also exists in subjects without Alzheimer’s disease. However, these samples were small and the cross-sectional nature of these analyses raise concerns about reverse causation. In addition, the association between serum copper and dementia-related outcomes may be attributable to either differences in copper exposure or differences in copper metabolism.

Diet is a major pathway for copper to get into human body. Dietary copper intake of 1.5 to 3.0 mg per day has been recommended by The Food and Nutrition Board for adults. Copper is abundant in some foods, such as legumes, potato and potato products, nuts and seeds, and beef. Copper content of foods may vary regionally, as it can be affected by soil copper concentration, slurry spreading, use of copper compound-containing bactericides or fungicides on crops, and local copper emissions from industry.

Both animal and human studies suggest that a combination of high dietary copper intake and high cholesterol accelerates cognitive decline. In a rabbit model of Alzheimer’s disease manipulated with high cholesterol diet, a minimal amount of copper added to drinking water exacerbated the impact of high cholesterol diet on neurodegenerative change (i.e., retarding the ability of rabbits to learn a difficult trace conditioning task). In another rabbit model, dietary cholesterol-induced accumulation of beta-amyloid, an important biomarker related to Alzheimer’s disease increased when rabbits were fed with distilled water supplemented with 0.12 ppm copper ion compared to rabbits fed with unaltered distilled water. In a mouse model of Alzheimer’s disease, amyloid accumulation and learning impairment were found among mice fed with 0.1 mg/L copper in drinking water and 2% cholesterol in the food, and it can be reduced with intake of zinc. This dietary copper -cholesterol interaction is supported in the only large-scale epidemiological study on dietary copper with cognitive decline that is available. In the Chicago Health and Aging Project, Morris et al. found that higher dietary copper intake is associated with faster 6-year cognitive decline among subjects with high intake of saturate fats and trans fatty acids among older. However, as the prodromal period of dementia persists for years to decades, it is possible that these results reflect reverse causation, whereby declining cognition leads to increased copper and fat intake.
Besides the copper-cholesterol interaction, intake of iron and zinc may also affect the associations of dietary copper with cognition. Similar to copper, iron has been found involved in oxidative promotion and neuroinflammation in the brain,\textsuperscript{23,24} which increases the risk of dementia. In contrast, zinc may help improve cognitive function by reducing free copper levels.\textsuperscript{25} A lack of zinc may damage neurons,\textsuperscript{26} and zinc deficiency has been found in patients with Alzheimer’s disease.\textsuperscript{27}

Thus, our objective is to examine the relationship between midlife dietary copper intake with late life cognitive health in data from the Atherosclerosis Risk in Communities Study (ARIC), as well whether this relationship is modified by intake of saturated fat.

5. Main Hypothesis/Study Questions:
Aim 1: To examine the associations of dietary copper intake with neurocognitive outcomes.

Hypothesis 1.1. Higher dietary copper is associated with faster 20-year cognitive decline.

Hypothesis 1.1.1. Higher dietary copper is associated with faster 20-year cognitive decline particularly in those with high intake of saturated fat.

Hypothesis 1.2. Higher dietary copper is associated with higher incidence of dementia.

Hypothesis 1.2.1. Higher dietary copper is associated with higher incidence of dementia particularly in those with high intake of saturated fat.

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: prospective cohort study of dietary copper measured in midlife and early older adulthood (visits 1 and 3) with cognitive decline from visits 4 to 6 and incident dementia through visit 6.

Exclusion criteria: Participants without information on dietary copper; implausible total caloric intake or dietary copper intake; participants without information on cognitive tests or dementia at baseline; Asian or Native Indians; White participants in MS; Black participants in MD, MN.

Exposures:
-Dietary copper intake (visit 1 and 3):
Dietary intake was assessed in the full ARIC sample at Visits 1 and 3 using an interviewer-administered, 66-item food frequency questionnaire adapted from the 61-item FFQ developed by Willett et al.\textsuperscript{28} The amount of nutrients calculated from nutrient value in foods was provided by
the Willett group. Copper included in dietary supplements was derived from the medication survey questionnaire.

We propose to average the measures from Visits 1 and 3 as a measure of long-term copper intake in midlife.

**Effect modifiers:**
- Intake of saturated fat (visit 1 and 3)
- Location (NC/MS/MD/MN)

**Outcomes:**

**-Cognitive tests (visit 4, 5, 6):**

Delayed Word Recall Test (DWRT): A test of immediate verbal memory. Participants are asked to learn a 10-word list and should recall as many words as possible after a 5-minute delay.²⁹

Digit Symbol Substitution Test (DSST): A test of executive function and psychomotor speed. Participants are asked to relate numbers to symbols using a key within 90 seconds. The maximum possible score is 93, and the score reflects the number of correct symbol-number matches.³⁰

Word Fluency Test (WFT): A test of language and executive function. The score is the total number of correctly generated words from three letters (“F”, “A”, “S”).³¹

**-Dementia (visit 4, 5, 6):**

Dementia is adjudicated in three levels:

--level 1: based on both reviewer diagnosis and algorithmic syndromic diagnosis from both Visits 5 and 6; reviewer diagnosis is given higher priority.

--level 2a: based on reviewer diagnosis, algorithmic syndromic diagnosis, telephone interview for cognitive status (TICS) and proxy interview. Diagnoses are prioritized, with the reviewer diagnosis being given highest priority, then the algorithmic syndromic diagnosis, TICS and finally the proxy interview.

--level 2b: based on reviewer diagnosis, algorithmic syndromic diagnosis, failed Dementia Screening Interview (AD8), and two failed Six Item Screeners (SIS). Diagnoses are prioritized, with the reviewer diagnosis being given highest priority, then the algorithmic syndromic diagnosis, TICS/proxy, AD8, and finally the SIS.

--level 3: based on reviewer diagnosis, algorithmic syndromic diagnosis, AD8, SIS, dementia codes on the cohort eligibility form (CELB), and dementia codes on the death certificate form (DTH). Diagnoses are prioritized, with the reviewer diagnosis being given highest priority, then the algorithmic syndromic diagnosis, AD8, SIS, CELB dementia codes, and finally the DTH form.³²

We will consider analyses using Level 3 diagnosis.

**Analysis plans**

Quartiles will be created for both dietary copper intake, and descriptive analysis of baseline (visit 4) characteristics will be conducted based on levels of dietary copper intake.
For each cognitive test at a visit, z scores will be calculated based on the means and standard deviations at baseline (visit 4). The global cognition z score will be calculated by averaging the z scores across the three tests.

**Aim 1.1. Longitudinal analysis on dietary copper and cognition:**

Mixed-effect models on dietary copper intake with domain-specific and global cognition with a random intercept, a random slope for spline 1 and a random slope for spline 2. An independence covariance matrix for the random effects will be used. Linear splines will be included to estimate the change in cognition from (a) 0-15 years and (c) 15-20 years to the end of study. An interaction term between categorical dietary copper intake and each time spline will be incorporated to estimate the change separately for years 0-15 and 15-20 years of study.

To address potential bias due to loss to follow up, we will adapt existing methods for use of multiple imputation by chained equation to account for the missing information on z-score of cognitive function at visit 5 and/or visit 6.33,34

**Aim 1.2. Longitudinal analysis on dietary copper and incident dementia:**

Participants with level 3 dementia at baseline (visit 4) will be excluded from analysis.

Cox PH model will be used to examine the association of quartiles of dietary copper intake with incident level-3 dementia.

To address potential bias due to loss-to-follow-up, we will develop and apply inverse probability weights for non-death attrition and death.

**Covariates:** fixed: sex, race-center, education, APOE4; visit 4: age, smoking, alcohol drinking; visit 1 & 3: total fruit and vegetable intake, zinc intake, iron intake, total caloric intake, intake of saturated fat, polyunsaturated fat, monounsaturated fat, dietary patterns (constructed based on the Dietary Approaches to Stop Hypertension (DASH) dietary pattern).

**Effect modification:** Saturated fat and saturated fatty acid intake (upper 25th quartiles as high) will be considered an effect modifier, and the analysis above will be stratified by quartiles of saturated fat and saturated fatty acid. To address potential mis-measurement in copper using the food frequency questionnaire by location due to local determinants of copper food content, the analysis will also be stratified by location (NC/MD/MN/MS) and combined using inverse variance-weighted meta-analysis.

**Methodological limitations:** The food frequency questionnaire may be less accurate than some other means of dietary assessment (e.g., 24-hour dietary recall, dietary record). However, we average two measures to get a better assessment of long-term intake. Copper from other sources is not considered. However, dietary copper is a primary mode of copper intake and is more readily modifiable than other sources. There is significant attrition across visits of the ARIC study, which may or may not be related to copper content. There is variation of concentration of copper in food by location; we will address this through site-specific analysis.

7.a. Will the data be used for non-CVD analysis in this manuscript? _x___ Yes _____ 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? _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? __x__ Yes    ____ 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”? __x__ 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)?
   #3239 Hu et al. Dietary patterns and risk of incident dementia and cognitive decline: Results from the ARIC study.
   #2495 Petruski-Ivleva et al. Association of habitual milk intake with cognitive decline from mid-life to late-life in the ARIC Neurocognitive Study
   #2558 Li et al. Plasma fatty acids and cognitive decline: the ARIC Neurocognitive Study
   #2145 Dearborn et al. Nutrition, Healthy Diet and 21-year Cognitive Decline

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* __2016.20_______)
   ___X___ 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 https://www2.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
REFERENCES