ARIC Manuscript Proposal #4193

PC Reviewed: 02/14/23 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: Association of omega-3 PUFAs with peripheral artery disease in a combined analysis of ARIC and MESA participants

b. Abbreviated Title (Length 26 characters): N-3 PUFAs and PAD

2. Writing Group:

Writing group members: Natalie L. Weir, Sarah O. Nomura, Weihua Guan, Parveen K Garg, Matthew Allison, Amy B. Karger, James S. Pankow, Jeffrey Misialek, and Michael Y. Tsai at the University of Minnesota.

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

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3. Timeline:

We anticipate it taking approximately 2 months to analyze data.

4. Rationale:

Lower extremity peripheral artery disease (PAD) is the chronic accumulation of atherosclerotic plaque in major conduit arteries in the legs and is associated with high morbidity and mortality from atherosclerotic cardiovascular disease (ASCVD).

Accumulating evidence suggests omega-3 polyunsaturated fatty acids (PUFAs) exert favorable effects on several biological processes involved in the development and progression of ASCVD. In particular, the marine omega-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to have various anti-inflammatory, anti-atherosclerotic, and anti-

thrombotic effects. Despite this, as well as previous associations with CVD risk factors and outcomes, studies examining associations between circulating omega-3 PUFA levels and PAD are limited, particularly in large ethnically diverse cohorts.

To address this important research gap, we aim to evaluate whether omega-3 PUFAs are associated with incident clinical PAD events in individuals free of clinical ASCVD at baseline in two cohorts: the Multi-Ethnic Study of Atherosclerosis (MESA) and the Atherosclerosis Risk in Communities (ARIC) study. We hypothesize omega-3 PUFAs, EPA and DHA, would be inversely associated with incident PAD.

5. Main Hypothesis/Study Questions:

Objectives:

To examine the association between N-3 PUFA biomarkers (EPA, DHA) and incidence rate of peripheral artery disease (PAD).

Primary Hypotheses:

EPA and DHA are both associated with a lower incidence of PAD

Secondary Hypothesis:

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: Participants who are adults (≥18 years of age) and who were free of PAD (prevalent or past) at baseline with available data on exposure biomarkers [plasma EPA (20:5ω-3), DPA (22:5ω-3) and DHA (22:6ω-3)] and outcomes are eligible for this project.

Inclusion Criteria

1.) Adults (≥ 18yrs)
2.) No prevalent PAD or history of PAD at the time of fatty acid measurement (a history of brief post-op AF is allowed)
3.) Available exposure measurements

Exposures:

1) Plasma Eicosapentaenoic acid (EPA; 20:5ω-3);
2) Plasma Docosahexaenoic acid (DHA; 22:6ω-3).

Each exposure will be analyzed:

1) as a continuous variable (% total fatty acids, per inter-quintile range [IQR] increment);
2) and as cohort-specific quintiles.

Outcome definition:

The primary outcome will be PAD as defined by 1) ICD-9 codes for peripheral artery disease-related hospitalizations (440.20, 440.21, 440.22, 440.23, 440.24, 440.29, 440.3, 440.8, 443.9) or leg artery revascularization (38.18, 39.25, 39.29, 39.50).

Covariates:

- Age
- Sex
- Race/ethnicity
- Clinical centre / field site
- Education
- BMI
- Waist circumference
- Smoking
- Alcohol intake
- Physical activity
- Prevalent diabetes status
- Prevalent hypertension

Missing data: Participants with missing data on omega-3 fatty acids exposure should be excluded. The missing rate of each covariate will be checked. Missing covariates will be handled as per the usual practice of each cohort and study investigators, e.g. imputation or exclusion.

Statistical analysis and pooling:

Individual cohort analysis

Descriptive statistics for participant demographics, lifestyle, and clinical characteristics will be calculated and univariable associations between population characteristics and omega-3 PUFA levels by incident PAD status will be evaluated using one-way ANOVA for continuous variables and Wald X^2 tests for categorical variables. Omega-3 PUFAs will be modeled as quartiles and continuously. DHA and EPA will be evaluated individually and in combination. Cox proportional hazards regression will used to estimate hazard ratios (HR) for omega-3 PUFAs and incident PAD. Follow-up time will be calculated from baseline to date of event, end of follow-up, loss to follow-up, or death, whichever occurred first. To standardize follow-up time between the two studies ARIC will be restricted to PVD cases through 15 years follow-up. Three different models will be conducted for each association: (1) unadjusted; (2) age, sex, and (in MESA only) race/ethnicity; (3) age, sex, race/ethnicity (in MESA only as ARIC-MN is only White) education, pack-years smoking, physical activity, triglycerides, alcohol intake, systolic blood pressure, blood pressure and lipid-lowering medication usage, total cholesterol, HDL-C, BMI, diabetes status. Models will additionally be evaluated stratified by insulin resistance, diabetes status, sex and race (Black, White) and potential interactions will be evaluated using cross-product terms in models. SAS 9.4 (SAS Institute Inc, Cary, NC) will be used for data analysis.

Meta analysis

The meta-analysis will be conduct by Weihua Guan at the University of Minnesota.

Results from each participating cohort will be combined using inverse-variance weighted meta-analysis. Heterogeneity of the results will be assessed using chi-square test and I^2 values. Random-effect meta-analysis will be considered if significant heterogeneity is observed. Leave-one-out analysis will be carried out to evaluate impact of single studies.

Because fatty acids can be measured in different lipid compartments using differing methods, EPA, DHA and EPA+DHA will be evaluated continuously per study-specific interquartile ranges (the distance between the midpoint of the first and third quartiles) to facilitate pooling.

Potential non-linear relationships will be assessed by pooling the HRs for each study-specific quartile, established as an indicator variable against the lowest quartile as the reference. To statistically test the non-linear relationships, by conducting multivariate inverse-variance weighted meta-regression, modelling the quartile results using restricted cubic splines.

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)?

- **MS 3923:** Phospholipid Eicosadienoic Acid with Incident Peripheral Artery Disease: a pooled analysis in the Multi-Ethnic Study of Atherosclerosis (MESA) and Atherosclerosis Risk in Communities (ARIC) Study

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* ___N/A_____)

___ 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.