ARIC Manuscript Proposal #2172

PC Reviewed: 7/9/13  Status: A  Priority: 2
SC Reviewed: _________  Status: _____  Priority: ____

1.a. Full Title:  Long-chain omega3 fatty acids and incident CHD

b. Abbreviated Title (Length 26 characters): omega3 and CHD

2. Writing Group:
   Writing group members: Liana Del Gobbo, Dary Mozafarrian, Jason Wu, Rozenn LeMaitre, David Siscovik, Lyn Steffen, Mike Tsai, Brian Steffen, Luc Djousse, and potentially other investigators from the CHARGE fatty acid working group and other cohorts with plasma or tissue fatty acids and incident CHD events.

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

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3. Timeline: 2 years

February, 2013 – January 2014: manuscript preparation including literature review, describing the methods for each study, methods for meta-analysis, results, and writing the Discussion section.

May 2013 – December 2013: data analysis at each center; meta-analysis
4. **Rationale:**

Multiple lines of evidence support a protective effect of long-chain omega-3 polyunsaturated fatty acids (LC-PUFAs) on coronary heart disease (CHD) (1). However, recent meta-analyses have shown inconsistent effects of LC-PUFA supplementation for the prevention of CHD events (2-7). While previous trials were largely short-term, secondary prevention studies in patients concomitantly using cardiovascular drugs, long-term prospective studies of LC-PUFA intake have demonstrated clearer evidence of benefit (8). These prior prospective studies frequently estimated total dietary eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) or fish consumption from self-reported questionnaires, however, which are limited by measurement error. Few studies have examined the association of biomarkers of LC-PUFA with CHD endpoints. Circulating phospholipid concentrations of LC-PUFAs provide objective biomarkers of individual exposure (9), reflecting intake, absorption, desaturase and elongase activity, and membrane incorporation (10), and permit separate assessment of the potential distinct effects of EPA, docosapentaenoic acid (DPA; 22:5n-3), and DHA, which are not well characterized. Available evidence on the association of circulating biomarkers of EPA, DPA, and DHA with CHD endpoints is sparse, and particularly in light of current controversy on the relation of LC-PUFA with CHD risk (11), requires further investigation. Evidence linking plant-derived omega-3 fatty acid, α-linolenic acid (ALA; 18:3n-3) to incident CHD is promising but very limited. Most studies have estimated ALA exposure using self-reported dietary records, with few published biomarker results. A recent meta-analysis demonstrated that elevated ALA biomarkers are associated with a nonsignificant trend towards lower risk of cardiovascular disease (12), but high, unexplained heterogeneity was observed, with few studies evaluating specific ALA biomarker types (eg. plasma phospholipids, cholesterol esters, or adipose tissue) and CHD endpoints. Given the relative affordability and global accessibility of ALA relative to EPA and DHA, and concerns related to the long-term sustainability of fisheries (13), the question of whether or not ALA exposure, as assessed using objective biomarkers, is associated with CHD is a key public health priority. Examination of potential effect modifiers of the association of omega-3 fatty acids with CHD also requires further study. An important, and yet unanswered question, pertains to the importance of background diet in modifying omega-3 CHD associations, particularly background fish consumption for ALA-CHD associations, and intakes of omega-6 fatty acids, linoleic acid (LA; 18:2n-6) and arachidonic acid (AA; 20:4n-6). In the Health Professionals Follow-Up Study, dietary ALA was inversely associated with CHD risk only when LC-PUFA intake was very low (<100mg/d) (14), but previous studies have not evaluated background fish consumption as a potential effect modifier of an ALA biomarker-CHD association. Recent work in CHARGE (15) has shown that conversion of ALA to longer chain n-3 PUFAs is less effective in people with certain genetic variants. In this meta-analysis (15), the association of plasma phospholipid ALA with EPA and DPA was lower among carriers of genetic variants in FADS desaturase genes (rs1535, rs174546, rs968567) which suggest less conversion of ALA to longer chain n-3 PUFAs among carriers. Evaluation of these effect modifiers could have important implications for people who do not eat fish and individual-based recommendations for consumption of seafood and plant-derived omega-3 fatty acids.

A meta-analysis pooling summary estimates for omega-3 fatty acid-CHD associations, with standardized modeling of the exposure, covariates, and outcome variables for each association, would reduce heterogeneity and improve precision of the currently available estimates of circulating omega-3 fatty acids with CHD endpoints. By obtaining summary estimates for specific CHD endpoints directly through collaboration with cohort investigators, included estimates in the meta-analysis would not depend on prior publication of the findings for each given endpoint. Further, the larger sample sizes of pooled analyses would increase statistical power to investigate associations of circulating omega-3 fatty acids with specific CHD endpoints.
and evaluate interactions, including effect modifiers potentially influencing the association of omega-3 fatty acids with CHD.

REFERENCES:


5. **Main Hypothesis/Study Questions:**

**Specific Aim 1:** To investigate whether separate and total concentrations of EPA, DPA and DHA, as assessed by objective circulating biomarkers, are associated with lower risk of incident CHD. To address this aim, we will examine associations of circulating EPA, DPA and DHA individually, and in combination, with incident total CHD, non-fatal MI, and fatal CHD in all participating cohorts with available exposures of interest.

**Specific Aim 2:** To investigate whether circulating ALA is associated with lower risk of incident CHD. To address this aim, we examine associations of ALA biomarkers in all participating cohorts with available exposures of interest. Variable modeling and model selection will be standardized across cohorts and study-specific estimates for each endpoint will be subsequently pooled using standard meta-analysis approaches.

**Specific Aim 3:** To investigate potential effect modification (age, sex, race, LA biomarker concentration, AA biomarker concentration, genotype at rs1535, rs174546, rs968567, type 2 diabetes status, statin use, aspirin use) in the association of ALA, EPA, DPA, DHA, and EPA+DPA+DHA with incident CHD. To address this aim, we will pool stratified estimates from each study for each potential effect modifier using meta-analysis. To evaluate potential background fish consumption as an effect modifier of ALA-CHD associations, EPA+DHA biomarkers will also be evaluated for effect modification in multivariate ALA-CHD models.

**Hypotheses:** We hypothesize that circulating ALA will be associated with lower risk of incident CHD, with stronger associations observed among those with lower concentrations LC-PUFA biomarkers. Longer-chained omega-3 fatty acids will be associated with a significantly lower risk of incident CHD, particularly fatal CHD.

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).**

**Population:** Included participants should meet the following criteria:

1.) adults (≥ 18yrs)
2.) no prevalent myocardial infarction (MI), angina, coronary revascularization or stroke at time of biomarker sampling (prospective studies only)
**Exposures:** Exposures to be assessed include all available biomarkers [e.g., plasma (total plasma, phospholipid, cholesterol esters, or triglyceride), serum, erythrocytes, adipose, or other, all as % total fatty acids] of the following omega-3 fatty acids:

1.) alpha-linolenic acid (ALA; 18:3n-3)
2.) eicosapentaenoic acid (EPA; 20:5n-3)
3.) docosapentaenoic acid (DPA; 22:5n-3)
4.) docosahexaenoic acid (DHA; 22:6n-3)
5.) EPA + DPA + DHA

Each exposure will be analyzed in two ways:
1.) as a continuous variable (% total fatty acids, per 1 SD increment);
2.) in study-specific quintiles

**Outcomes:**

1.) Total coronary heart disease (CHD) (fatal or nonfatal myocardial infarction, CHD death or sudden cardiac death, SCD)
2.) Non-fatal MI (chest pain with abnormal cardiac enzyme concentrations or serial electrocardiogram changes, or otherwise as defined by the study)
3.) Fatal CHD (CHD death or SCD, a sudden pulse-less condition with a cardiac origin in a previously stable individual, or otherwise as defined by the study)

**Covariates:** Please confirm covariate categorization with Liana ldelgobb@hsph.harvard.edu before proceeding with analysis. Variables will be classified across studies in a standardized fashion. These include:

1.) age (continuous)
2.) sex (binary; male/female)
3.) race (binary; Caucasian/non-Caucasian, or study-specific )
4.) field or clinical center, if applicable (study-specific categories)
5.) BMI (continuous)
6.) education [4 categories; some high school (or less), high school graduate, some college or vocational school, college graduate]
7.) smoking (3 categories; current, former, never. If only two categories are available, use binary: current, not current).
8.) physical activity (4 categories; first preference is quartiles of METs. If METs are not available, use four categories of physical or leisure activity as defined in your study. For any questions, contact Liana)
9.) alcohol intake (4 categories; none, 1-6 drinks/week, 1-2 drink/day, >2 drink/day. If your study’s alcohol unit is grams, please convert to drinks using the conversion 14 grams alcohol=1 standard drink)
10.) diabetes mellitus status (binary; yes=treatment with oral hypoglycemic agents, insulin, fasting glucose >126mg/dL, or no. If this information is not available, use study-specific definition)
11.) treated hypertension (binary; yes= hypertension drug use, or no. If this information is not available, use in the following order: a.) diagnosed/history of hypertension, or b.) study-specific definition)
12.) treated hypercholesterolemia (binary; yes=lipid-lowering drug use, or no. If this information is not available, use in the following order: a.) diagnosed/history of hypercholesterolemia, or b.) study-specific definition)
13. aspirin use [binary; yes=regular aspirin use (for example, 3+ times per week), or no]
14. linoleic acid (LA; 19:2n-6) biomarker concentrations (continuous; % total fatty acids)
15. arachidonic acid (AA; 20:4n-6) biomarker concentrations (continuous; % total fatty acids)
16. trans fatty acids biomarker concentrations (continuous; % total fatty acids)

**Missing data:** To retain study power, missing indicator categories will be used for missing covariates.

**Survival Analysis:** For prospective cohort studies, Cox proportional hazards models, with robust variance, will be used to estimate the hazard ratio for incident total CHD, nonfatal MI, and fatal CHD endpoints. Follow-up time will be calculated from baseline (biomarker measurement) to date of failure, end of follow-up, loss to follow-up, or death, whichever occurred first. For nested-case control studies, conditional logistic regression analyses will be used to estimate odds ratios as proxies of relative risks or rate ratios, after confirming a sampling strategy in each study. For retrospective studies, unconditional log-linear models will be used. For each exposure-outcome analysis, the β interaction coefficient and its robust standard error for each exposure will be recorded.

**Heterogeneity:** To examine heterogeneity, stratified analyses will be conducted. For the following variables listed below, the β coefficient and its robust standard error (SE) will be recorded for each specified strata:
1. age (< 60 years, ≥ 60 years)
2. sex (males, females)
3. race (Caucasian, race #2, race #3, etc. Please provide β + SE for each ethnic group in your study)
4. LA biomarker concentration (< or ≥ median value in your study)
5. AA biomarker concentration (< or ≥ median value in your study)
6. type 2 diabetes status (yes, no)
7. statin use (yes, no)
8. regular aspirin use (yes, no)
9. EPA+DHA biomarkers (for ALA exposure only) (< or ≥ median value in your study)

If your study has genetic data, please refer to ‘n3 SNPs’ to provide information on imputation quality and interactions for the following SNPs:
1. genotype at rs174546 (FADS1 gene) (2 copies of T allele, 1 copy of T allele, 0 copies of T allele)
2. genotype at rs968567 (FADS2 gene) (2 copies of T allele, 1 copy of T allele, 0 copies of T allele)

Please exclude individuals missing genotypes before coding the genotypes outlined above. To assess interactions for these SNPs, linear regression analysis using an additive genetic model, i.e. regression of phenotype on the number of reference alleles, or equivalently the imputed dosage for imputed genotypes, will be conducted. Interaction terms for each SNP will be constructed by creating a cross-product term of the omega-3 FA exposure of interest (continuous) by the SNP (ordinal; 0, 1, or 2 T alleles) and added to the fully adjusted model:
\[ S(x) = \exp(\beta_{n3} + \beta_{SNP} + \beta_{n3 \times SNP} + \ldots) \]
For each SNP, the β coefficient and its robust standard error (SE) will be recorded for the main effect of the omega-3 FA exposure, the interaction term, and the covariance matrix (In STATA, the variance-covariance matrix is stored in e(V) and can be obtained using the lincom command).

If rs174546 has not been genotyped or imputed in your study, one high-LD SNPs indicated below may be used as a proxy:

- rs174545
- rs174547
- rs174550
- rs102275
- rs174537
- rs174535
- rs1535
- rs174574
- rs174576
- rs174577
- rs174578
- rs174583

**Sensitivity analyses:** For prospective studies, two separate sensitivity analyses will be conducted on the main models only (models without interaction terms):

1.) Cases identified in first 2 years after biomarker sampling will be excluded to minimize effect of reverse causation due to pre-existing health condition

2.) Participants will be censored at the first 6 years of follow-up to minimize exposure misclassification due to within-person variation over time

For each sensitivity analysis, the β interaction coefficient, its robust standard error (SE), and the Wald 2-sided p-value (using the robust SE) will be recorded.

7.a. Will the data be used for non-CVD analysis in this manuscript? __ 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? __ 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 ___ 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.csc.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)?

#890 Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: The Atherosclerosis Risk in Communities (ARIC) Study
Lead author: Lu Wang

#1600: Genome-wide Association Study of Plasma Phospholipid Fatty Acids within the CHARGE Consortium. Lead author: Rozenn Lemaitre

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?

___ X ___ Yes ______ No

GWAS via STAMPEDE & GENEVA, #2006.03

11.b. If yes, is the proposal

___ X ___ A. primarily the result of an ancillary study (list number* __________)

GWAS via STAMPEDE & GENEVA, #2006.03

___ 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.cscc.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. We understand this policy.

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.