ARIC Manuscript Proposal #2569

PC Reviewed: 6/9/15  Status: A  Priority: 2
SC Reviewed: ________  Status: _____  Priority: _____

1.a. Full Title: Association between circulating and tissue biomarkers of n-6 PUFA intake and incidence of cardiovascular disease

b. Abbreviated Title (Length 26 characters): N6 fatty acids and CVD

2. Writing Group
   Writing group members: Matti Marklund, Ulf Riserus, Johan Sundstrom, Del Gobbo Liana C, PhD; Imamura Fumiaki, PhD; Virtanen Jyrki K PhD; Wennberg Maria PhD; YakoobMohommad Y, PhD; Aslibekyan Stella, PhD; Chiuve Stephanie E, ScD; dela Cruz Luicito, PhD; Frazier-Wood Lekki AC, PhD; Fretts Mandy, MPH PhD; Guallar Eliseo, PhD; Matsumoto Chisa, PhD; Prem K, PhD; Tanaka Tosh, PhD; Wu Jason, HY PhD; Zhou Xia PhD; Gaziano John M, MPH MD; Helmer Catharine, MD, PhD; Ingelsson Erik, MD, PhD; Tunstall-Pedoe Hugh, MD; Yuan Jian-Min, PhD; Barberger-Gateau Pascale, PhD; Campos Hannia, PhD; Chaves Paulo HM, MD PhD; Djoussé Luc, MD ScD; Gibson Robert, PhD; Giles Graham G, PhD; Gómez-Aracena Jose, PhD; Hodge Allison, PhD; Hu Frank B, PhD MD MPH; Jansson Jan-Håkan, PhD; Johansson Ingegerd, PhD; Khaw Kay-Tee, MD PhD; Koh Woon-Puay, PhD; Lemaitre Rozenn N, MPH PhD; Lind Lars, PhD; Luben Robert N, PhD; Rimm Eric B, ScD; Samieri Cecilia, PhD; Selberg-Franks Paul, PhD; Siscovick David S, MPH MD; Stampfer Meir, MD; Steffen Lyn M, MPH PhD; Steffen Brian T, PhD; Tsai Michael Y, PhD; van Dam Rob M, PhD; Voutilainen Sari, PhD; Willett Walter C, MD DrPH; Woodward Mark, PhD; Mozaffarian Dariush, MD DrPH, on behalf of the CHARGE Fatty Acids & Outcomes Pooling Project.

Lyn M. Steffen, Xia Zhou, and 22 other investigators collaborating in this project. See attached list of investigators.

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

First author: Matti Marklund, PhD

Address: Department of Public Health and Caring Sciences, Clinical Nutrition and Metabolism, Uppsala University, Sweden

Phone: +46 18 611 79 63  Fax: NA
E-mail: matti.marklund@pubcare.uu.se
**ARIC author** to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name:  Lyn M. Steffen  
Address:  University of Minnesota School of Public Health

Phone:  612 625 9307  
Fax:  612 624 0315  
E-mail:  steffen@umn.edu

3. **Timeline:**
   Now through Dec 2015

4. **Rationale:**
   During the last 10 years, research on dietary intake of polyunsaturated fatty acids (PUFA) from the n-6 family has in general received less interest than the n-3 PUFA. The major PUFA in the diet is however linoleic acid (18:2n-6, LA), that has well-known lipid cholesterol-lowering effects (1), but may also have insulin sensitizing properties, and inverse associations with risk of developing type 2 diabetes have been shown in a number of prospective cohort studies (2). Compared with saturated fats (SFA), n-6 PUFA also reduces liver fat content and certain inflammation markers (3). In addition, there have been a number of RCTs performed several decades ago that investigated the effects of foods mainly rich in n-6 PUFA compared with foods rich in saturated fatty acids (SFA) (4). Those studies are not consistent but overall suggest that higher intake of n-6 PUFA from vegetable sources moderately reduces risk of coronary heart disease (CHD) and CHD death (4). This conclusion is also in line with pooled analyses from cohort studies that measured dietary PUFA intake from food frequency questionnaires (5). In contrast to these studies a recent fairly small RCT was re-analyzed and added to a meta-analysis, and indicated that very high intake of LA (~15%E) were associated with increased CHD mortality (6). Such association may however also be confounded by trans fatty acid (TFA) intake found in the margarines that was partly used in the study to increase LA intake. It is known that high LA intake before year 2000 (in Scandinavian countries most TFA was removed from margarines during the mid-90s) is correlated with TFA intake found in margarines. This suggests that a high intake of LA may have been confounded by high intake of TFA, especially in individuals in which their main source of LA comes from margarines rather than vegetable oils and nuts. Nevertheless, the slight inconsistencies concerning n-6 PUFA and LA in particular, with CHD and CVD risk warrant further investigation given the current recommendations suggest replacement of SFA with PUFA, where the majority is said to come from vegetable oils in the form of LA. New large-scale RCTs with mortality data comparing LA from non-hydrogenated vegetable oils at recommended levels (5-10%E) as compared with a higher intake of saturated fats would be optimal and much welcomed, but is not likely to be performed in any near future. Thus, there is a need for long-term studies with mortality data. A few individual cohorts, e.g. in the ULSAM cohort in Sweden, have reported inverse association between circulating LA levels and various CVD outcomes as well as total CVD risk (7-9).
For the study of dietary n-6 PUFA intake and CVD risk in cohort studies, the use of LA as a biomarker is particularly suitable since LA is an essential fatty acid and is a good validated biomarker of n-6 PUFA intake (10,11). Other n-6 PUFAs (e.g. 18:3n-6 and 20:3n-6) will not be included in the analyses since they do not directly reflect their intakes, but mainly reflect the activities of desaturases and elongases and thus are markers of fatty acid metabolism rather than biomarkers of dietary n-6 PUFA intake. In fact, the proportions of 18:3n-6 and 20:3n-6 in cholesterol esters and phospholipids are either decreased or unchanged after a diet high in LA and PUFA from vegetable oils (and low in SFA) as shown form controlled feeding trials (3,12). Thus, these minor n-6 PUFAs should not be confused or combined with LA since they are not biomarkers of high LA intake, but rather of high SFA and low LA intake, if anything. Part from LA, the second most abundant n-6 PUFA in the diet is arachidonic acid (AA). AA is however not as good biomarker of its intake as LA, but AA could still be of interest to investigate together with LA considering it can be converted from LA, and has been implicated to have possible adverse effects on inflammation and possibly CVD risk (mainly through AA-derived metabolites, e.g. prostaglandins). While AA is found primarily in animal fats, it is also present in numerous types of vegetable oils and nuts. Thus the food sources of LA and AA partly overlap, but are mixed and AA is thus not a specific biomarker of vegetable fat intake alone which is more the case with LA. Although AA is found in significant amounts in the diet, the interpretation of circulating levels of AA is more difficult and complex compared with LA. The relationship between AA and CVD risk will therefore be a secondary analysis.

References listed at the end of the proposal.

5. Main Hypothesis/Study Questions:
We hypothesize that circulating LA, and possibly AA, will be associated with lower CVD risk of. These n-6 PUFAs are the main dietary sources of n-6 PUFA, whereas other minor circulating n-6 PUFAs (e.g. 20:3n-6 and 18:3n-6) are not biomarkers of n-6 PUFA intake.

**Specific Aim 1:** To investigate whether circulating/adipose tissue LA and AA, are associated with risk of incident CVD.
The associations of circulating LA and AA will be done, individually, with the following outcomes:
1) Total CVD
2) CVD mortality
3) Total CHD
4) Ischemic stroke (if available in the database, otherwise use total stroke)

**Specific Aim 2:** To investigate potential effect modification (age, sex, race, ALA biomarker concentration, EPA biomarker concentration, genotype at rs174547, type 2 diabetes status, statin use, aspirin use) in the association of LA and AA with incident CHD. To address this aim, we will pool stratified estimates from each study for each potential effect modifier using meta-analysis
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:** meta-analysis of 21 cohort or nested-case control studies. Each center will conduct their own analysis and send results to Matti.

**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 blood or tissue n6 fatty acids (linoleic and arachidonic acids). ARIC has plasma n6 fatty acids from the Minnesota participants

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 cardiovascular disease (CVD) (fatal or nonfatal myocardial infarction, coronary heart disease (CHD) death, sudden cardiac death (SCD) or ischemic stroke*)
2. CVD mortality (CHD death or SCD, fatal ischemic stroke, a sudden pulse-less condition with a cardiac origin in a previously stable individual, or otherwise as defined by the study)
3. Total CHD (fatal or nonfatal myocardial infarction, CHD death or sudden cardiac death, SCD)
4. Total ischemic stroke*

*if available in the database, otherwise use total stroke

**Covariates:** Please confirm covariate categorization with Dr Matti Marklund before proceeding with analysis: matti.marklund@pubcare.uu.se

Variables will be classified across studies in a standardized fashion. These include BASELINE:
1. Age (continuous)
2. Sex (binary; male/female)
3. Race (not applicable in ARIC, all white)
4. Field center, not applicable for ARIC)
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 Matti Marklund, se e-mail below)

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 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. Alpha-linolenic acid (ALA; 18:3n-3) biomarker concentrations (continuous; % total fatty acids)

15. Eicosapentaenoic acid (EPA; 20:5n-3) biomarker concentrations (continuous; % total fatty acids)

16. Sum of elaidic acid (trans-18:1) biomarker concentrations (trans not available in ARIC)

17. Sum of linoleic acid (trans-18:2 n-6) biomarker concentrations (trans not available in ARIC)

**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 CVD, fatal CVD, total CHD, total ischemic stroke 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.

**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. ALA biomarker concentration (< or ≥ median value in your study)
5. EPA 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)
**Genotype analyses:**
If your study has genetic data, please refer to the file ‘n6.SNP’ to provide information on imputation quality and interactions for the SNP: rs174547 (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 this SNP, 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 term will be constructed by creating a cross-product term of n-6 PUFA (AA and LA, respectively) exposure (continuous) by the SNP (ordinal; 0, 1, or 2 T alleles) and added to the fully adjusted model:

\[ S(x) = \exp(\beta n_3 + \beta \text{SNP} + \beta n_3 x \text{SNP} + \ldots) \]

The $\beta$ coefficient and its robust standard error (SE) will be recorded for the main effect of the n-6 FA exposure and the interaction term.

**Sensitivity analysis:** For prospective studies, a sensitivity analysis will be conducted on the main models only (models without interaction terms):
Participants will be censored at the first 10 years of follow-up to minimize exposure misclassification due to within-person variation over time.
For sensitivity analysis, the $\beta$ 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 _X_ 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? ____ X__ Yes _____ No (to examine interaction by rs174547)

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 The data analysis will be done at UMN SPH.

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.c.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)?

Liana del Gobbo et al., Omega-3 fatty acid biomarkers and coronary heart disease: pooling project of 19 cohort studies

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* 1997.04)

____ 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.

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.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript __X__ Yes _____ No.

References


