ARIC Manuscript Proposal #3450

PC Reviewed: 8/13/19  Status: _____  Priority: 2
SC Reviewed: _________  Status: _____  Priority: ____

1.a. Full Title: Biomarkers of very long-chain monounsaturated fatty acids and risk of incident congestive heart failure in the Fatty Acids and Outcomes Research Consortium (FORCE)

b. Abbreviated Title (Length 26 characters): LCMUFA and CHF

2. Writing Group:
   Writing group members: Dr Xiongfei Pan, Dr Matti Marklund, Dr Fumiaki Imamura, Prof David Siscovick, A/Prof Rozenn Lemaitre, A/Prof Renata Micha, Prof Dariush Mozaffarian, and A/Prof Jason Wu and co-investigators from each of the participating cohorts.

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

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3. Timeline: 1 year

4. Rationale:
Animal experiments suggest that exposure to very long chain monounsaturated fatty acids (VLCMUFA), including gondoic acid (20:1 n-9), erucic acid (22:1 n-9), and nervonic acid (24:1 n-9) may cause cardiotoxicity 1-5. VLCMUFA are oxidized in peroxisomes instead of mitochondria, resulting in reactive oxygen species and cytosolic lipid metabolites 6 that can activate signaling pathways for mitochondrial damage, synthesis of cardiac lipid droplets, inhibition of glycolysis, and cardiac apoptosis 7-9. Myocardial lipidosis and apoptosis reduce the contractile force of the heart muscle that can contribute to heart failure. Based on observed cardiotoxicity in animal experiments, regulations in the US, EU, and Canada have set limits to on
the contents of 22:1 n-9 in rapeseed-derived oils or daily intake of 22:1 n-9, traditionally a major source of VLCMUFA 10-12.

The discovery of cardiotoxic effects of VLCMUFA from animal experiments have also stimulated epidemiological studies on the impact of VLCMUFA on specific cardiovascular outcome 13. In addition to food-grade rapeseed oil (22:1 n-9: 1.3-5.2 mg/g) and baked goods made from it (0.27-0.39 mg/g for biscuits; 0.24-0.29 mg/g for pastries and cakes) 10, other food sources of VLCMUFA include seafood and nuts or seeds, with lower concentrations also found in poultry and meat products 13. Interestingly, recent studies indicate positive correlation between consumption of these food sources of VLCMUFA with circulating VLCMUFA levels 13, suggesting utility of such VLCMUFA biomarkers to assess their exposure. A limited number of cohort studies have reported associations between higher levels of plasma phospholipid 22:1 n-9 and 24:1 n-9, but not 20:1 n-9, and higher risk of incident congestive heart failure (CHF). None of the VLCMUFA were associated with risk of stroke 13, which indicates a specific link between cardiotoxic effect of VLCMUFA and CHF. However, evidence to date has been limited to studies in two cohorts in North America, and whether such findings are consistent and generalizable to other populations remain unclear.

The primary aim of the present study is to assess the prospective association of VLCMUFA biomarkers with incident CHF in cohorts in the Fatty Acids and Outcomes Research Consortium (FORCE). By collaborating with participating cohorts and using a standardized approach to define exposures, covariates, and outcome variables, our analyses will have substantially increased statistical power to more precisely quantify the association of VLCMUFA with CHF. Conducting analyses across cohorts from diverse demographic and dietary backgrounds will also enhance the generalizability of our findings. The large sample size will also enable detailed evaluation of potential effect modifiers of the relation between VLCMUFA and CHF. To confirm the specificity of the negative effects of VLCMUFA on CHF, we will conduct sensitivity analyses using incident total stroke as the outcome rather than CHF (i.e., total stroke as a negative control for CHF). Stroke shares many risk factors with CHF (e.g., hypertension) but should be unaffected by any causal processes specific to cardiac steatosis, i.e. the hypothesized cardiotoxicity of LCMUFA 13.

Objectives: To examine the association between VLCMUFA biomarkers with incident CHF:

Specific Aim 1: To investigate whether biomarkers of 20:1 n-9, 22:1 n-9, and 24:1 n-9, are associated with risk of incident CHF in participating cohorts with available exposures of interest.

Specific Aim 2: To investigate potential effect modification (sex, BMI, race, hypertension, and CHD) in the association of VLCMUFA biomarkers with incident CHF.

5. Main Hypothesis/Study Questions:

1) Higher 22:1 n-9 and 24:1 n-9 biomarkers are associated with higher risk of CHF.
2) Higher 20:1 n-9 are not associated with higher risk of CHF.
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: A literature search will be conducted to identify all eligible populations (adults ≥18 years) for analysis of the association of VLCMUFA biomarkers with CHF and/or stroke, irrespective of whether or not associations have been published in a given population. We will also contact all cohorts that have participated in prior FORCE analyses to inquire about their eligibility and interest to participate in this study. All prospective studies (cohort studies, nested case-control studies, and case cohort studies) with 20:1 n-9, 22:1 n-9, or 24:1 n-9, measured in blood fractions or adipose tissue are eligible. Cohorts with relevant fatty acid data identified in preliminary literature searches include: the Atherosclerosis Risk in Communities Study Minnesota subcohort (ARIC), Cardiovascular Health Study (CHS), EPIC-Norfolk Prospective Study, and Physicians’ Health Study (PHS). Included participants should meet the following criteria:

1) Adults (≥ 18yrs)
2) No prevalent CHF

In analyses with stroke as an outcome, participants with prevalent stroke at baseline (i.e., the time of biomarker measurement) will be excluded.

Exposures: Exposures to be assessed include VLCMUFA biomarkers measured in any of the following lipid compartments: plasma/serum (total, phospholipid, cholesterol esters, or triglyceride), erythrocyte (total or phospholipids), and adipose tissue; all should be expressed as percentage of total fatty acids.

Specific fatty acids include the following VLCMUFA:

1) Gondoic acid (20:1 n-9)
2) Erucic acid (22:1 n-9)
3) Nervonic acid (24:1 n-9)

Each exposure will be analyzed in two ways:

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

Outcome definition: The primary outcome will be CHF as defined by 1) diagnosis of CHF by a treating physician based on clinical guidelines; or 2) ICD codes for heart failure (ICD-9, 428; ICD-10, I50) or hypertensive heart disease with heart failure (ICD-10, I11.0); or 3) other study-specific criteria. In a sensitivity analysis, where associations of VLCMUFA and incident total stroke (including ischemic and hemorrhagic stroke) will be evaluated, total stroke will be defined by 1) ICD-9 430-438 and ICD-10 I60-I69 or 2) other study-specific criteria.

Covariates: Variables will be classified across studies in a standardized fashion based on data available in each cohort, please confirm with Xiongfei xpan1@georgeinstitute.org.au before proceeding with the analysis. We will examine the association of each VLCMUFA of interest with incident CHF and stroke after adjustment for the following covariates:
1) Age (continuous, years)
2) Sex (binary; male/female)
3) Race (binary; Caucasian/non-Caucasian, or study-specific)
4) Clinical centre / field site, if applicable (study-specific categories)
5) Education (3 categories; <high school, high school graduate, college or higher, if unavailable, cohort-specific categories as dummy variables)
6) Occupation (using cohort specific categories, as dummy variables)
7) BMI (continuous, kg/m^2)
8) Waist circumference (continuous, cm)
9) Smoking (categorical; current, former, never, if only 2 categories available, use binary: current, not current)
10) Alcohol intake (4 categories; none, 1-6 drinks/week, 1-2 drink/day, or >2 drink/day. If your study’s alcohol unit is grams, please convert to drinks using the conversion 14 grams alcohol=1 standard drink)
11) 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 individual study)
12) Prevalent diabetes status (yes/no, defined as treatment with oral hypoglycemic agents or insulin, fasting glucose ≥ 126mg/dL, or cohort-specific definitions)
13) Prevalent coronary heart disease (yes/no, defined by ICD-9 410-414, or ICD-10 I20-I25, or cohort-specific definitions)
14) Prevalent hypertension (yes/no, defined as treatment with blood pressure-lowering medication, systolic blood pressure ≥140 mmHg, diastolic ≥90 mmHg, or study-specific definitions)
15) Alpa-linolenic acid (18:3n-3, ALA) biomarker concentrations (continuous, % total fatty acids)
16) Sum of very long chain n-3 fatty acid (EPA + DPA + DHA) biomarker concentrations (continuous, % total fatty acids)

Missing data: Participants with missing data on VLCMUFA exposure should be excluded. 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:
1. Individual cohort analysis
   We will apply standardized study-specific analyses for each study using individual-level data. For prospective cohort studies, Cox proportional hazards models will be used to estimate hazard ratios (HRs) for incident CHF and stroke. For studies that used a case-cohort design, weighted Cox regression models will be used for estimating HRs. 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. For prospective (nested) case-control studies, conditional logistic regression analyses will be used to estimate odds ratios (ORs) as proxies of relative risks or rate ratios. For each exposure-outcome analysis, the regression β coefficient and its robust standard error (SE) for each exposure will be recorded.
2. Data pooling and meta-analysis
We will use HRs and ORs to approximate relative risks to generate summary results using inverse-variance weighted meta-analysis. Because fatty acids can be measured in different lipid compartments using differing methods, 20:1 n-9, 22:1 n-9, and 24:1 n-9 will be evaluated continuously per study-specific IQR (the distance between the midpoint of the first and fifth quintiles) to facilitate pooling.

We will pool results across all studies regardless of lipid compartments. For studies with multiple measures, we will prioritise the overall pooled analysis as specified in the following ordered list: adipose tissue, erythrocyte phospholipids, plasma phospholipids, cholesterol esters, total plasma or serum, and triglycerides. In case a substantial number of original studies are identified and participate in the analysis with FA measured in different lipid compartments, we will pool results separately for each lipid compartment.

We will assess potential non-linear relationships by pooling the HR or OR for each study-specific quintile, established as an indicator variable against the lowest quintile as the reference. To statistically test the non-linear relationships, we will conduct multivariate inverse-variance weighted meta-regression, modelling the quintile results using restricted cubic splines.

3. Heterogeneity
The following factors will be examined as potential sources of heterogeneity. They were selected in considerations of their potential relevance for biological heterogeneity as demographic factors or because the conditions shared a cluster of known or unknown risk factors with heart failure, which may modify the effect of VLCMUFA on heart failure.
1) Sex (male/female)
2) BMI (<30kg/m2/ ≥30kg/m2)
3) Race (Caucasian, race #2, race #3 etc)
4) Prevalent hypertension (yes/no; defined as as treatment with blood pressure-lowering medication, systolic blood pressure ≥140 mmHg, diastolic ≥90 mmHg, or study-specific definitions)
5) Prevalent CHD (yes/no; defined by ICD-9 410-414, or ICD-10 I20-I25, or cohort-specific definitions)

In each individual study, interaction terms will be constructed as a cross-product term of the VLCMUFA biomarker (continuous) and each possible effect modifier, including the main effects in the model. In addition, the β coefficient for VLCMUFA and robust SE will be recorded for each specified stratum from stratified analyses of potential effect modifiers. Stratum-specific estimates from each study will be pooled for each potential effect modifier, and statistical significance of differences between prespecified subgroups will be assessed using inverse-variance weighted meta-regression.

4. Sensitivity analyses
Four separate sensitivity analyses will be conducted on the main models only (models without interaction terms).
1) VLCMUFA will be assessed for their relationship with incident stroke to confirm whether VLCMUFA are specifically associated with CHF.
2) Cases identified in the first 2 years after biomarker sampling will be excluded to minimize effect of reverse causation due to pre-existing health condition.
3) Participants will be censored at the first 10 years of follow-up to minimize exposure misclassification due to within-person variation over time.
4) Separate analyses for associations between VLCMUFA and CHF will be conducted based on different definitions of CHF.

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? ___ 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 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.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)?
Fumiaki Imamura’s paper about LCMUFA and CHF in ARIC. He is a coauthor on this manuscript.

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* Folsom’s fatty acid study; Mpls cohort at baseline)
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ __________)
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.cscce.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.