ARIC Manuscript Proposal # 1743

PC Reviewed: 1/5/11	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: **Circulating l**ong-chain monounsaturated fatty acids and incident heart failure: the Atherosclerosis Risk in Communities Study**

b. Abbreviated Title (Length 26 characters): Long-chain MUFA and HF risk

2. Writing Group:

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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: Jan. 2011 to Aug. 2011

4. Rationale:

Animal experiments in 1970's and a single epidemiologic study (the Cardiovascular Health Study) suggested exposure to long-chain monounsaturated fatty acid (LCMUFA) increases risk of congestive heart failure (HF). More studies are needed to confirm the evidence. Details are appended to this form.

5. Main Hypothesis/Study Questions:

Circulating LCMUFA is prospectively associated with risk of HF

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).

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 ICTDER03 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/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)?

Lead Author Citation	Yamagishi K, Nettleton JA, Folsom AR, ARIC Study Investigators. Plasma fatty acid composition and incident heart failure in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am Heart J. 2008 Nov;156(5):965-74.
MS#	890B

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes __X_ No

11.b. If yes, is the proposal
___ A. primarily the result of an ancillary study (list number* _____)
___ 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/

12. 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.

Appendix: Study proposal

Title: Long-chain monounsaturated fatty acids and incident heart failure: the Atherosclerosis Risk in Communities Study

Background and Rationales

Very long-chain mono-unsaturated fatty acids (LCMUFA, cis-20:1, 22:1 and 24:1 fatty acids) are known to constitute lipids of meat products, fish products and vegetable oils such as rapeseed and mustard oil.[1-6] Even though decades-old animal experiments suggest cardiotoxicity of LCMUFA,[7-13] no prior human studies reported the biological roles of LCMUFA.

Animal evidence for the cardiotoxicity lead development of CANadian Oil Low in erucic Acid – CANOLA – oil to replace repeseed oil containing high amount of erucic acid (22:1n9).[14, 15] However, populations in many developing countries still consume rapeseed oil high in the LCMUFA.[16-19] In the U.S., many dietary sources of LCMUFA, including meats, fish and poultry, are present, according to our recent analysis of the national survey (Figure 1) and of the Cardiovascular Health Study (CHS).

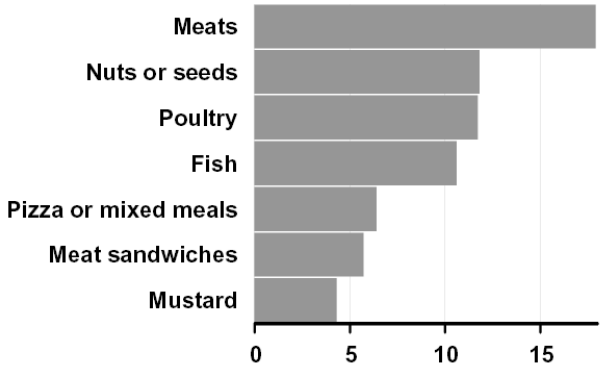

Figure 1. Values represent the proportional contribution (percent) of different food groups to US consumption of total LCMUFA (180 mg/day on average) according to the U.S. national survey (NHANES, 2005-2006)

Studies of rats, rabbits, pigs and non-human primates demonstrated that LUMUFA intake caused cardiac lipid accumulation (cardiac steatosis) and further cardiac necrosis or ischemia.[7-13] Biochemical experiments have been exploring the underlying mechanism of lipid accumulation and potential cardiac ischemia. Briefly, LCMUFA undergoes peroxisomal β-oxidation because mitochondrial enzymes cannot oxidize LCMUFA.[20] Peroxisomal β-oxidation releases acyl-CoA derivatives in myocardium.[21] Elevated cytosolic malonyl-CoA inhibits enzymatic transport of fatty acids to mitochondria and induces suppression of fatty acids oxidation and lipid accumulation in cytosols. Due to accumulated cytosolic fatty acids, PPARα and/or PPARγ are activated to result in suppression of glycolysis.[22-24] Consequently, the condition leads cardiac dysfunction and may elevate heart failure (HF) risk.[25-30]

Recently, human studies have elucidated detrimental effects of cardiac steatosis on risk factors of HF. From myocardial biopsies and non-invasive cardiac imaging, cardiac steatosis were found associated with reduced ejection fraction[30], diastolic dysfunction[31] and increased left ventricular mass[29].

Our recent analysis of plasma phospholipid (PL) LCMUFA from the older adults (65 years or older) in the CHS supported potential adverse effects of LCMUFA on incident HF. We identified prospective positive associations of 24:1, but not 20:1 and 22:1, with incident HF, with multivariable-adjusted hazard ratio (95% confidence interval) for interquintile range of 24:1 of 3.54 (1.79-6.99) (Figure 2). The myocardium-specific association was further supported by a negative-control analysis[32] that detected no association of LCMUFA with incident ischemic stroke (p>0.05), which is not myocardium-specific, but shares many risk factors with CHF (e.g. blood pressure)

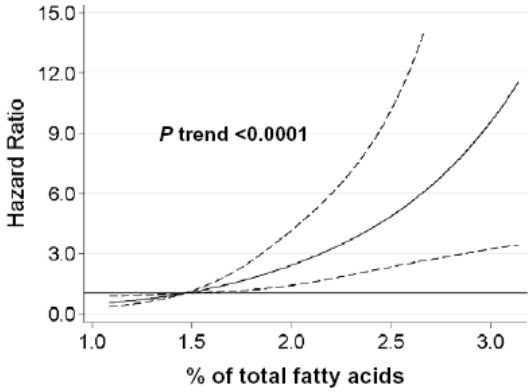

Figure 2. Multivariable-adjusted relationship of plasma phospholipid 24:1 with incident HF over 14 years of follow-up in CHS. Solid line represents the best estimate of hazard ratio, and dotted lines represent 95% confidence limits. The reference level is 10th percentile of 24:1 fatty acid.level.

The etiological evidence is still limited to the single observation in the CHS that recruited older subjects. Plasma PL fatty acids reflect long-term dietary intake and fatty acids constitutes of cellular membranes that are tightly regulated and related to intracellular signaling.[33, 34] On the contrary, fatty acids of circulating cholesteryl esters (CE) and triglycerides reflect short-term dietary intake and fatty acids secreted from liver as components of very large-density lipoprotein (VLDL) and LDL.[33, 34] Levels of LCMUFA of CE are known to be less than those of PL[2, 35, 36] but the potential cardiotoxicity and dietary predictors of LCMUFA of CE remains unknown. A further characterization of cardiotoxicity and potential dietary sources of LCMUFA in an independent cohort using multiple lipid subfractions will provide profound insights into the knowledge of LCMUFA.

Research Hypothesis

Our primary goal is to characterize prospective association of LCMUFA with incident HF, based on fatty acids of PL and CE in the middle-aged adults enrolled to the Atherosclerosis Risk in Communities (ARIC) Study. The hypotheses are:

1. Circulating 24:1 of PL and CE are prospectively associated with elevated incident HF.
    - As the secondary hypothesis, we will examine whether each of circulating 20:1 and 22:1 of PL and CE is associated with incident HF, where the prior analyses in CHS yielded no evidence of associations.
    - We also hypothesize that LCMUFA is unassociated with incident ischemic stroke, to be examined as a negative control outcome.[32]

2. Consumptions of fish, meats, poultry and nuts are associated with circulating LCMUFA

Data and Analysis plan

Design and Population: For the first hypothesis, we will perform prospective analyses of the participants in the ARIC study free from HF with available data of fatty acids at baseline (N=3,800, 1987-1989) and with outcome data based on the most recent adjudication (2002 or later year).[33, 34] When evaluating stroke as the outcome, we will exclude individuals with stroke at baseline. Assuming 240 participants develop incident HF based on the prior publication[33, 34], statistical power is 0.80 to detect 21% increase of HF risk among top quartile group compared to the bottom quartile group of participants ranked by levels of LCMUFA. Based on the previous CHS, more than 50% of elevated risk is expected, for which statistical power is >0.99. For the second hypothesis, we will perform cross-sectional analyses of the participants free from HF, investigating baseline observations of PL and CE fatty acids and habitual diet.[35]

Main Variables: Using the stored fasting blood from the participants in the Minneapolis field center, PL and CE fatty acids were measured in the University of Minnesota Hospital and Clinic Laboratory[36]. From the previous CHS study and others,[37, 38] we anticipate that 24:1 was the major LCMUFA (>90%) in the both fractions. We will consider 24:1 of PL and CE as the main exposure variable and each of 20:1 and 22:1 as the exposure of the secondary analyses. We will assess laboratory error of the LCMUFA assessments, as previously performed in the ARIC study for major fatty acids.[36] For the second hypothesis, dietary variables will be 30 to 40 food groups created based on responses to dietary questionnaires with 66 food items.[39, 40]

Outcomes variables. We will evaluate HF as a primary outcome, and stroke incidence as the secondary outcome (a negative control).[34, 41] The incident cases were identified from annual telephone calls to participants to ascertain the hospitalizations, review of local hospitalization records, and death certificates. Stroke subtypes were classified by professional reviews of medical records. We will consider ischemic stroke as a negative-control outcome. Time at risk will be calculated from the baseline assessments in 1987-1989 until first ascertainment of HF (or stroke), death, loss to follow-up, or the last date of event ascertainment (2008).

Analysis Plan: To test the first hypothesis, we will include each of LCMUFA variables as the main independent variable (categorical or continuous) in the multivariable-adjusted Cox regression models. Proportionality assumption will be tested by assessing whether the association of each LCMUFA with HF varies over time.

` To test the second hypothesis, multivariable-adjusted linear regression analyses will be performed, in which each of LCMUFA will be a dependent variable and dietary variables will be dependent variables. We will primarily assess whether consumptions of fish, meat products, poultry and nuts were independently associated with circulating LCMUFA. Secondary, we will identify dietary predictors of circulating LCMUFA from 30-40 food groups by stepwise backward regression analysis ($p<0.05$ to retain and $p>0.1$ to remove), as previously conducted in CHS.[42] If LCMUFA intake was estimated, we will calculate bivariate and multivariable-adjusted correlation between PL and CE LCMUFA and LCMUFA intake.

Regression models will include covariates to control for potential confounders: sociodemographic factors, smoking, physical activity, dietary factors, medication use, disease

conditions and other factors associated with exposures and outcomes in the current cohort. Individual fatty acids of PL and CE rather than LCMUFA will be carefully treated as covariates. Physiological factors will be considered as potential confounders or mediators, including body-mass index, waist circumference, inflammatory markers (C-reactive protein, fibrinogen) and intima-media thickness. For the longitudinal analysis to address the first hypothesis, we will test whether incident CHD mediates the association of LCMUFA with HF risk, treating CHD incidence as a time-varying covariate. Missing covariates will be imputed by best-subset regression using sociodemographic factors, smoking status, alcohol use, physical activity, body mass index and prevalent diseases of CHD, stroke and diabetes. Potential effect modification will be evaluated for age, sex and prevalent CHD. Furthermore, multivariable measurement error correction for within-person variability of fatty acids assessments will be performed[43], using duplicate measures of PL and CE fatty acids.[36]

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