ARIC Manuscript Proposal # 1743

PC Reviewed: 1/11/11	**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. **_FI_ [please confirm with your initials electronically or in writing]**

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3. Timeline: **Jan. 2011 to Apr. 2011**

4. Rationale: **(**References are appended to this form)

In the 1970's, experimental models in rodents, rabbits, pigs, and non-human primates demonstrated that consumption of long-chain monounsaturated fatty acids (LCMUFA), such as erucic acid (22:1n9), induced myocardial lipid accumulation (cardiac steatosis) and cardiac necrosis.[1-4] This experimental evidence, although not evaluated in humans, led to concerns that dietary consumption of LCMUFA could be cardiotoxic. For example, Canadian farmers responded by developing a new breed of rapeseed oil to reduce its naturally high content of LCMUFA (2-40% of fatty acids), calling it CANadian Oil Low in Erucic Acid – CANOLA –

oil.[5, 6] With this exception, potential health effects of LCMUFA were largely forgotten. Importantly, few prior human studies have evaluated the effects of LCMUFA, although several potential dietary sources of LCMUFA remain (Figure 1).

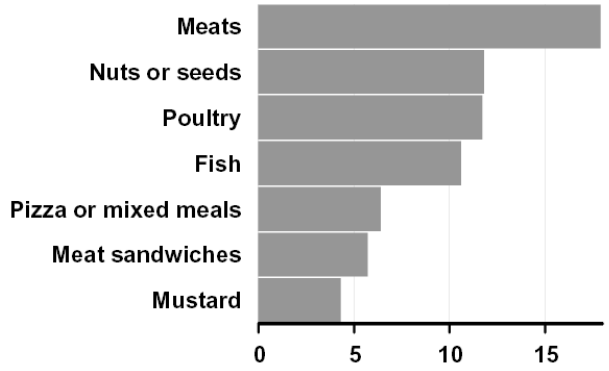

Figure 1. Values represent the proportional contribution (percent) of different food groups to US consumption of total LCMUFA (180 mg/day on average) according to the U.S. national survey (NHANES, 2005-2006)

Experimental studies have been elucidating mechanisms of lipid accumulation in cardiomyocytes and further development of cardiac dysfunction. LCMUFA cannot be oxidized in mitochondria, because LCMUFA cannot enter into mitochondria due to lack of transport enzyme for LCMUFA.[7] Instead, peroxisome oxidizes LCMUFA and releases fatty acid metabolites in cytosol.[7] Accumulated fatty acid metabolites, including triglycerides, malonyl-CoA and ceramides, activate or inhibit number of signaling pathways.[8, 9] The details are not fully understood, but important outcomes include inhibition of fatty acid oxidation by accumulated malonyl-CoA and apoptosis triggered by elevated ceramides.[8, 9] Correlated with lipid accumulation, these consequences contribute contractile dysfunction and cardiomyopathy.[8]

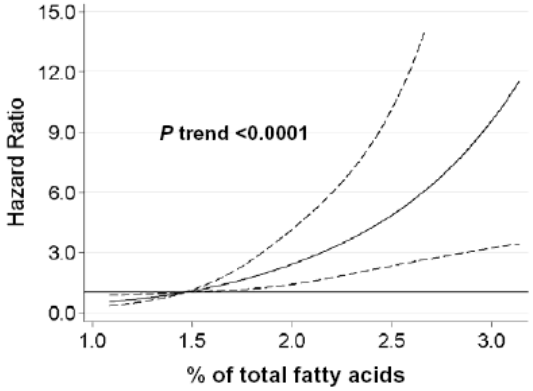

Figure 2. Multivariable-adjusted relationship of plasma phospholipid 24:1 with incident CHF over 14 years of follow-up in the Cardiovascular Health Study. Solid line represents the best estimate of adjusted hazard ratio, and dotted lines represents 95% confidence limits. The reference level is 10th percentile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

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[10], diastolic dysfunction[11] and increased left ventricular mass[12].

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 etiological evidence is still limited to the single observation from the CHS that recruited older subjects and evaluated fatty acids of plasma PL. 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.[13, 14] 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.[13, 14] Levels of LCMUFA of CE are known to be less than those of PL[15-17] 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.

5. Main Hypothesis/Study Questions:

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 in the Minneapolis center of the Atherosclerosis Risk in Communities (ARIC) Study. We will address the following hypotheses:

1. Circulating 24:1 of PL and CE are prospectively positively 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.
2. Consumptions of fish, meats, poultry and nuts are associated with circulating LCMUFA

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).

Design and Population: 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 followed up through 2008, including prevalent CHD or stroke patients at risk of HF. We will exclude those without available data of fatty acids and baseline HF status. Assuming 240 participants develop incident based on the prior publication[18, 19], 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 with available data from PL and CE fatty acids and habitual diet.[20]

Main Variables: Using the stored fasting blood from the participants in the Minneapolis field center, PL and CE fatty acids were measured as a percentage of total fatty acids of each fraction in the University of Minnesota Hospital and Clinic Laboratory[21]. From the previous CHS study and others,[22, 23] 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.[21] For the second hypothesis, dietary variables will be 30 to 40 foods after aggregating similar foods from responses to dietary questionnaires with 66 food items.[24, 25]

Outcomes variables. We will consider HF incidence as previously described.[19] HF cases were identified from annual telephone calls to participants to ascertain HF hospitalizations, ICD codes of local hospitalization records, and death certificates. Time at risk will be calculated from the baseline asssessments in 1987-1989 until first ascertainment of HF, death or administrative censoring of loss to follow-up or at Dec. 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.[26] 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 measured at baseline to control for potential confounders: sociodemographic factors, smoking, physical activity, dietary factors, medication use, disease conditions (diabetes, hypertension, CHD and stroke) 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 measured at baseline will be considered as potential confounders or mediators, including body-mass index, waist circumference, inflammatory markers 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, body-mass index, prevalent diabetes and prevalent CHD. Furthermore, multivariable measurement error correction for within-person variability of fatty acids assessments will be performed[27], using duplicate measures of PL and CE fatty acids.[21]

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	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.

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