ARIC Manuscript Proposal # 1736

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

1.a. Full Title:
Does fatty acid intake modify the relation of hemostatic and inflammatory biomarkers with incident ischemic stroke and CHD? The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title:
Fat modifies inflammation -CVD relation

2. Writing Group: Huifen Wang, Lyn M. Steffen, Peter J. Hannan, Aaron R. Folsom

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

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3. Timeline:
Literature review: 3 months
Data analysis: 3 months
Draft manuscript: 6 months
Coauthor and P&P committee review: 3 months
4. **Rationale:**

Lesions on the vessel wall may be caused by excessive deposit of oxidized fat components, virus/bacterial infection or toxins (e.g. from smoking). In response to the vessel injury, the process of hemostasis (including the blood coagulation cascade) is stimulated, which may further trigger and propagate inflammation. Hemostasis and inflammation are closely intertwined. Appropriate levels of hemostasis and inflammation are an initial immunological attempt to protect vascular tissues from damage. However, elevated hemostatic factors and prolonged (i.e. chronic) inflammation may promote the development of atherosclerosis. The underlying mechanism for atherosclerosis includes the recruitment of macrophages to the lesion site, expression and secretion of proinflammatory cytokines, and the formation of plaque on vascular walls \(^1\-^6\). The thickening of arterial walls may eventually lead to incident coronary heart disease (CHD), as well as ischemic stroke (IS) \(^2\,^7\,^8\). Circulating biomarkers of hemostasis and inflammation have been associated with cardiovascular events \(^1\,^8\). For example, several published reports using ARIC data have shown significant relations between cardiovascular disease (CVD) outcomes and levels of hemostatic and inflammatory biomarkers (including VWF, factor VIIIc, fibrinogen, white blood cell counts, albumin, CRP, lipoprotein-associated phospholipase A2) \(^4\,^6\,^{10-14}\).

Dietary fatty acids are actively involved in the progression of inflammation and cardiovascular diseases (CVD), which have been examined in a few studies \(^15-^20\). Fatty acids may exert their health effects by modulating the pro-/anti-inflammatory markers \(^21-^24\). Generally, saturated fatty acids (SFA) are pro-inflammatory, resulting in increased risk of CHD and IS \(^25-^27\). However, omega-3 polyunsaturated fatty acids (PUFA) are anti-inflammatory and cardioprotective, as reported in ARIC \(^28\,^29\) and other studies \(^30-^35\). Furthermore, individual fatty acids may have their specific effects. For example, stearic acid (18:0) may not be as atherogenic as palmitic acid (16:0) \(^36\).

Although the effects of different types of fatty acids on CHD have been relatively well established, evidence on the associations of fatty acids with IS is limited and inconsistent \(^37-^39\). Among 79,839 women who were followed up for 14 years, higher consumption of fish and omega-3 PUFA was associated with a reduced risk of thrombotic infarction (i.e. a subtype of IS) \(^40\). Nevertheless, in a six-month randomized clinical trial conducted in 258 adults ages
In 45-70 years, Sanders et al. did not observe any difference in levels of IS-related hemostatic factors among study participants randomized to one of four diets with varying n-6/n-3 fatty acid ratios. In the Framingham Heart Study, higher intakes of total fat and saturated fatty acid (%kcal) were found to be associated with lower risk of IS in males. Assuming that IS shares similar pathological mechanisms with CHD (e.g. inflammation and hemostasis), and since dietary intake is an important modifiable risk factor for CVD, a better understanding of the relations between dietary fatty acids, hemostatic and inflammatory biomarkers, and CHD/IS is warranted.

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

**All the study questions, hypothesis and analyses are separate for CHD and IS.**

- **Study question:**
  Does dietary fatty acid intake modify the relationship between levels of hemostatic/inflammatory biomarkers and risk of incident IS/CHD?

- **Study hypothesis:**
  Higher SFAs (especially palmitic acid (16:0)) intake will interact synergistically, while increased intake of dietary ω-3 PUFAs (e.g. EPA, DHA) will interact antagonistically, with hemostatic/inflammatory biomarkers in relation to CHD incidence (or IS incidence).

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).**

**Exclusions:**

- Participants with prevalent CHD or stroke at baseline
- Participants with extremely low or high values of energy intake derived from the FFQ (less than or greater than 1% of the distribution for energy intake)
- Participants with missing data for white blood cell count, albumin, fibrinogen, vWF or VIIIc
- Participants with missing data for dietary fatty acids
**Exposures Variables:**

- The fatty acid intake at visit 1.
- Hemostatic/inflammatory biomarkers at visit 1 included the plasma levels of vWF, factor VIIIc, white blood cell count and fibrinogen and the serum level of albumin.

**Outcome Variables:**

- Incident CHD will be defined by ARIC criteria as a definite or probable MI, a silent MI between examinations by ELECTROCARDIOGRAM, a definite CHD death.
- Incident IS will be defined according to ARIC criteria.

Note: Because fatty acids and inflammatory biomarkers were only measured at baseline, we will use incident CHD and IS events only through year 1999 (i.e. shorter time interval from the baseline). However, CHD and IS incidence tracked though year 2007 will also be examined for the sensitivity analysis.

**Model Covariates:**

- Potential confounding factors* at baseline such as age, gender, cigarette smoking years, smoking status, physical activity, total energy intake, education level, vitamin supplementation intake, medication use, hormone replacement therapy, alcohol intake, BMI, ECG left ventricular hypertrophy, diabetes, blood pressure, and blood lipid levels, etc.

*Note: confounding factors may be somewhat different for CHD and IS.

**Statistical Analysis:**

- A new variable ‘OMEGA’ will be calculated by summing up dietary EPA and DHA intakes to represent the dietary intake of omega-3 polyunsaturated fatty acid intake.
- An inflammatory biomarker risk score (IBRS) will be created to combine information on levels of factor VIIIc, white blood cell count and fibrinogen and the serum level of albumin.

For each participant, the values for each inflammation marker were expressed as a Z-score and the IBRS was calculated by summing up the five Z-scores. However, analyses will also be conducted for each biomarker separately. Levels of inflammatory markers will be presented as continuous variables and categorical (tertiles) variables.

*Note: factor VIIIc is released from vWF by the action of thrombin when coagulation is stimulated. Therefore, levels of vWF and factor VIIIc are highly correlated and we decided to include only factor VIIIc in creating IBRS.
Fatty acids will be represented as continuous and categorical variables (quartiles). Dietary fatty acids will be presented as % of total energy intake.

Baseline characteristics will be described as percentages or means.

Skewed data will be log-transformed before the analysis.

Effect modification by dietary fatty acids will be tested by including a multiplicative interaction term (biomarker x dietary fatty acids) in the model adjusting for potential confounding factors. If significant modification effects of dietary fatty acids were found, the analyses will be conducted by category of dietary fatty acids intake.

*Note: We examine if the Cox parameter for inflammatory biomarkers differs by levels of dietary fatty acids. This implies a multiplicative effect on the log hazard scale.

Cox proportional hazards regression models will be used to test the main effect of fatty acids intake and inflammatory markers on CHD incidence (or IS incidence), adjusting for potential confounding factors.

Additional sensitivity analysis will be conducted using a longer time interval, i.e. through year 2007, because there will be more incident CHD (or IS) events and potentially increase the statistical power. However, there may also be a trade-off that the baseline exposures may not accurately predict the CVD outcomes, due to the long time interval between the exposure and the outcome.

**Power calculation**: 

For the following calculations, white blood cell count (continuous), dietary palmitic acid (16:0) and incident CHD/IS tracked through year 1999 is used as an example:

Note: The basic statistics of white blood cell count in this population are: min=1.8x10^9/L, mean= 6.0x10^9/L, median=5.7x10^9/L, max= 42.0x10^9/L, SD=1.9x10^9/L

The basic statistics of dietary palmitic acids (%kcal) in this population are: min=0.9, mean=median=6.6, max=14.6. SD=1.6

For incident CHD: based on 1187 cases/13818 participants

At 80% power using a 2-sided type I error rate of 5%, we estimated that we can detect an interaction between white blood cell count and palmitic acid quantified as a relative CHD HR=1.42 if white blood count differs by 1.9x10^9/L (i.e. 1 SD) and palmitic acid intake differs by 1% of total energy intake.
For incident IS: based on 327 cases/13818 participants

At 80% power using a 2-sided type I error rate of 5%, we estimated that we can detect an interaction between white blood cell count and palmitic acid quantified as a relative IS HR=2.01 if white blood count differs by 1.9x10^9/L (i.e. 1 SD) and palmitic acid intake differs by 1% of total energy intake.

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 ICTDER02 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 ICTDER02 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 ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”?

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

   ____ Yes    __x__ (No overlap)

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)?


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. I am aware of this policy.
References


41. Sanders TA, Lewis F, Slaughter S, Griffin BA, Griffin M, Davies I, Millward DJ, Cooper JA, Miller GJ. Effect of varying the ratio of n-6 to n-3 fatty acids by increasing the dietary intake of alpha-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45-70 y: The OPTILIP study. Am J Clin Nutr 2006 Sep;84(3):513-22.


