

## ARIC Manuscript Proposal # 1336

PC Reviewed: 01/15/07

Status: A

Priority: 2

SC Reviewed: \_\_\_\_\_

Status: \_\_\_\_\_

Priority: \_\_\_\_\_

**1.a. Full Title:** Cross-sectional associations between dietary patterns and platelet and leukocyte markers assessed by flow cytometry in the Atherosclerosis Risk in Communities Carotid MRI study

**b. Abbreviated Title (Length 26 characters):** diet and flow cytometry markers

**2. Writing Group Lead:** Jennifer A. Nettleton

Writing group members: Eric Boerwinkle, Aaron Folsom, Nena Aleksic

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

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**Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):**

As above

### 3. Timeline:

Data preparation and analysis will begin upon approval, and manuscript drafting will commence once suitable analytical models are finalized. Initial drafts will be circulated among the writing group members within four months of proposal approval.

### 4. Rationale:

Inflammation and vascular endothelial activation reflect the early stages and progression of atherosclerotic processes and are associated with incident CVD<sup>1-4</sup>. Much of the support for these associations stems from large epidemiological studies where systemic biomarkers of inflammation and endothelial activation, such as C-reactive protein (CRP), interleukin-6 (IL-6), homocysteine, fibrinogen, soluble intracellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and e-selectin, have been measured. In addition to strong associations with basic demographic characteristics such as age, sex, and race, lifestyle factors also predict concentrations of these analytes. In particular, several epidemiological studies have shown that dietary factors are associated with circulating biomarkers of systemic inflammation and endothelial activation<sup>5-7</sup>.

Data from four large cohort studies have shown inverse associations between such biomarkers and dietary patterns reflecting high intake of fruits & vegetables, whole grains, nuts, fish, and low-fat dairy foods<sup>8-11</sup>. Conversely, studies also report positive associations between biomarkers of inflammation/endothelial activation and dietary patterns reflecting high intake of red meat, refined grains, high-fat dairy, and fried foods<sup>8-11</sup>. Consistent with these data are associations between inflammatory biomarkers and select nutrients (*inversely associated*: fiber<sup>12, 13</sup>, antioxidant vitamins<sup>14</sup>, polyunsaturated fatty acids<sup>15, 16</sup>, *positively associated*: trans fatty acids<sup>17, 18</sup>) and foods/beverages (*inversely associated*: whole grains<sup>13</sup>, fruits & vegetables<sup>19</sup>, nuts<sup>20</sup>, coffee<sup>21</sup>). These observational data are further supported by intervention studies showing dietary change can influence concentrations of inflammatory biomarkers<sup>22-24</sup>.

The function and activities of platelet and leukocytes are important in inflammatory and atherosclerotic processes<sup>25-27</sup>. Flow cytometry can be used to characterize platelet and leukocyte activation and cellular aggregation in whole blood, as well as quantify both intracellular and membrane-bound platelet- and leukocyte-derived factors<sup>28</sup>. Thus, platelet and leukocyte dynamics measured by flow cytometry are a closer representation of real-time biological processes than are the biomarkers typically measured in large-scale epidemiological studies. However, because the technique is labor-intensive and expensive, few large scale studies have utilized flow cytometry, and no large-scale studies have both measures by flow cytometry and measures of dietary intake.

We propose to study the cross-section relations between cellular activation markers and aggregates measured by flow cytometry and dietary patterns and their constituent food groups in the Atherosclerosis Risk in Communities Carotid MRI Study.

References pages 4-5

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

We hypothesize that dietary patterns high in whole grains, fruits & vegetables, nuts and other food sources of polyunsaturated fatty acids will be inversely associated with markers of platelet and leukocyte activation and cellular aggregation. Conversely, we hypothesize that dietary patterns high in red meat, refined grains, high-fat dairy, and fried foods will be positively associated with these markers. Furthermore, we plan to explore how much of the dietary pattern-marker associations are contributed by individual food groups (e.g., whole grains, total fruits and vegetables, red/processed meat).

## **6. Design and analysis**

### ***Exclusions—***

Participants from Forsyth County, NC and Jackson, MS (no dietary assessment)  
Non-white participants  
Missing flow cytometry data  
Taking chemotherapy or steroids

### ***Dependent Variables—***

#### Leukocyte markers:

lipopolysaccharide receptor (CD14+), toll-like receptor 2/4 (TLR-2/-4), P-selectin glycoprotein ligand-1 (CD162+— in monocytes, granulocytes, & lymphocytes), Pan-leukocyte marker (CD45+), myeloperoxidase (MPO+— in monocytes & granulocytes)

#### Platelet markers:

Platelet glycoprotein IIIa (GPIIIa, CD61), platelet glycoprotein IIb (GPIIb, CD41), P-selectin (CD62P), CD40 ligand (CD154).

#### Cell aggregates:

Platelet-monocyte aggregates, Platelet-granulocyte aggregates, Platelet-lymphocyte aggregates

### ***Independent Variables—***

Dietary patterns derived by principal components analysis—

Preliminary data from the 1102 participants who provided dietary data show two clear dietary patterns:

Factor 1: high factor loadings (>0.30) for the food groups other vegetables, dark yellow vegetables, cruciferous vegetables, green leafy vegetables, fruit, legumes, fish (not fried), tomatoes, whole grains, poultry, nuts

Factor 2: high factor loadings (>0.30) for the food groups processed meat, red meat, French fries, refined grains, high-fat dairy, desserts, sugar-sweetened beverages, candy, white potatoes, eggs, pizza, added animal fat

*We will also explore associations between individual food groups loading highest on these dietary patterns (e.g., vegetables, fruit, fish, whole grain, nuts, processed or red meat, refined grains) and flow cytometry markers (analytical details presented in subsequent sections)*

*Please note that we propose cross-sectional analyses to take advantage of the more comprehensive dietary assessment completed by ARIC MRI participants. Furthermore, we anticipate that proximal dietary exposures are more relevant to the real-time biological processes reflected in flow cytometry markers. Lastly, recent rapid changes in the food supply and in some dietary recommendations between early exam diet assessments and the ARIC MRI study complicate interpretation of study results with an outcome of this nature. This may be especially true in an older population where disease burden is high.*

### **Data Analysis—**

Participant characteristics and means of each dependent variable will be calculated by dietary pattern score quartile (or quintile) separately for each dietary pattern. P-trends across categories will be calculated with the dietary pattern modeled continuously.

\*Model 1 adjusted for energy intake, age, sex, and study center

\*Model 2 adjusted for above + education level, smoking status & cigarette years, physical activity level, ethanol intake, lipid-lowering medication use

\*†Minimal model adjusted for study center, energy intake + variables consistently correlated with flow cytometry markers (p <0.01 for ≥3 markers Folsom, et al. MS#1206): + sex, smoking, ethanol intake, lipid-lowering medication use, and hypertension medication use

†*Note: This list does not include LDL-C and TG which also satisfy the above criteria but are potential pathway intermediates.*

### **Additional analyses—**

Because associations attributed to dietary patterns may be largely accounted for by individual food groups (driving forces), models that simultaneously include a single top-loading food group (e.g., whole grains) and the dietary pattern to which that food groups contributes (in the whole grains example, factor 1) will be explored.

Tests of interaction between dietary patterns and sex, medication use, diabetes, and CHD will also be conducted.

\* All models will be appropriately weighted as necessitated by IMT sampling procedures

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**7.a. Will the data be used for non-CVD analysis in this manuscript?**    ☐ Yes    ☒ 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    ☒ 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.csc.unc.edu/ARIC/search.php>

☒ 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)?**

MS 1206 FOLSOM

“Association of risk factors with blood platelet and monocyte cell-markers and cell aggregates” (ARIC MRI)

**11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?**    ☐ Yes    ☒ 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.csc.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.**

Agreed

**References**

1. Hwang SJ, Ballantyne CM, Sharrett AR, et al. Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases: the Atherosclerosis Risk In Communities (ARIC) study. *Circulation*. Dec 16 1997;96(12):4219-4225.
2. Ridker PM, Rifai N, Stampfer MJ, et al. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. Apr 18 2000;101(15):1767-1772.
3. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. Jan 14 1999;340(2):115-126.
4. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. *J Intern Med*. Oct 2002;252(4):283-294.
5. Esposito K, Giugliano D. Diet and inflammation: a link to metabolic and cardiovascular diseases. *Eur Heart J*. Jan 2006;27(1):15-20.
6. Brown AA, Hu FB. Dietary modulation of endothelial function: implications for cardiovascular disease. *Am J Clin Nutr*. Apr 2001;73(4):673-686.
7. Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. *Arterioscler Thromb Vasc Biol*. May 2006;26(5):995-1001.
8. Fung TT, Rimm EB, Spiegelman D, et al. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. *Am J Clin Nutr*. Jan 2001;73(1):61-67.
9. Lopez-Garcia E, Schulze MB, Fung TT, et al. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr*. Oct 2004;80(4):1029-1035.
10. Nettleton JA, Steffen LM, Mayer-Davis EJ, et al. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr*. Jun 2006;83(6):1369-1379.
11. Esmailzadeh A, Kimiagar M, Mehrabi Y, et al. Dietary patterns and markers of systemic inflammation among Iranian women. *J Nutr*. Apr 2007;137(4):992-998.
12. Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from national health and nutrition examination survey data. *J Nutr*. May 2004;134(5):1181-1185.
13. Qi L, van Dam RM, Liu S, et al. Whole-grain, bran, and cereal fiber intakes and markers of systemic inflammation in diabetic women. *Diabetes Care*. Feb 2006;29(2):207-211.
14. Rowley K, Walker KZ, Cohen J, et al. Inflammation and vascular endothelial activation in an Aboriginal population: relationships to coronary disease risk factors and nutritional markers. *Med J Aust*. May 19 2003;178(10):495-500.
15. Pischon T, Hankinson SE, Hotamisligil GS, et al. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation*. Jul 15 2003;108(2):155-160.
16. Lopez-Garcia E, Schulze MB, Manson JE, et al. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr*. Jul 2004;134(7):1806-1811.
17. Lopez-Garcia E, Schulze MB, Meigs JB, et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr*. Mar 2005;135(3):562-566.
18. Mozaffarian D, Pischon T, Hankinson SE, et al. Dietary intake of trans fatty acids and systemic inflammation in women. *Am J Clin Nutr*. Apr 2004;79(4):606-612.
19. Gao X, Bermudez OI, Tucker KL. Plasma C-reactive protein and homocysteine concentrations are related to frequent fruit and vegetable intake in Hispanic and non-Hispanic white elders. *J Nutr*. Apr 2004;134(4):913-918.
20. Jiang R, Jacobs DR, Jr., Mayer-Davis E, et al. Nut and Seed Consumption and Inflammatory Markers in the Multi-Ethnic Study of Atherosclerosis. *Am J Epidemiol*. Dec 15 2005.
21. Lopez-Garcia E, van Dam RM, Qi L, et al. Coffee consumption and markers of inflammation and endothelial dysfunction in healthy and diabetic women. *Am J Clin Nutr*. Oct 2006;84(4):888-893.

22. Esposito K, Marfella R, Ciotola M, et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *Jama*. Sep 22 2004;292(12):1440-1446.
23. Jenkins DJ, Kendall CW, Marchie A, et al. Direct comparison of dietary portfolio vs statin on C-reactive protein. *Eur J Clin Nutr*. Jul 2005;59(7):851-860.
24. King DE, Egan BM, Woolson RF, et al. Effect of a high-fiber diet vs a fiber-supplemented diet on C-reactive protein level. *Arch Intern Med*. Mar 12 2007;167(5):502-506.
25. Platelet activation and arterial thrombosis. Report of a meeting of Physicians and Scientists, University of Texas Health Science Center at Houston and Texas Heart Institute, Houston. *Lancet*. Oct 8 1994;344(8928):991-995.
26. Freedman JE, Loscalzo J. Platelet-monocyte aggregates: bridging thrombosis and inflammation. *Circulation*. May 7 2002;105(18):2130-2132.
27. Steinhubl SR. Platelets as mediators of inflammation. *Hematol Oncol Clin North Am*. Feb 2007;21(1):115-121.
28. Ault KA. The clinical utility of flow cytometry in the study of platelets. *Semin Hematol*. Apr 2001;38(2):160-168.