ARIC Manuscript Proposal # 1131

1.a. Full Title: Association of nitric oxide synthase Glu298Asp polymorphism with serum levels of inflammation biomarkers and possible effect modification of dietary antioxidants: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): eNOS, inflam, & antioxidants

2. Writing Group:
   Writing group members: Suzette J. Bielinski, Christina L. Wassel Fyr, Lyn M. Steffen, Kari North, James S. Pankow

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:
   Starting Analyses: March 1, 2006
   First Draft: June, 2006
   Submission for Publication: August, 2006

4. Rationale:

Nitric oxide (NO) is an important mediator in human tissues. NO functions as a vasodilator, neurotransmitter, antimicrobial agent, and immunomodulator.¹ NO is produced by nitric oxide synthase (NOS) enzymes. To date, three isofoms of NO have been identified corresponding to three different NOS gene products: neural, endothelial, and inducible. Of
interest to this study is the endothelial derived NO, previously referred to in the literature as endothelial derived relaxation factor (EDRF), which plays a major role in vascular tone and minimizing endothelial damage. The gene responsible for the production of endothelial NO is endothelial nitric oxide synthase (eNOS also called NOS3). ENOS derived NO is continuously produced at low levels and maintains homeostasis within the vessel wall by vasodilatory and anti-inflammatory actions. Anti-inflammatory actions of NO include inhibition of platelet aggregation and activation and reduction of leukocyte adhesion. In addition, NO functions as a free radical scavenger and plays a role in non-specific host response.2

The eNOS gene has been mapped to region 7q35-36 and is composed of 26 exons and 25 introns and contains a highly polymorphic (CA)n dinucleotide repeat within intron 13. The promoter of the eNOS gene contains a shear stress responsive element suggesting that expression levels are controlled in part by hemodynamic forces of shear stress within vessel walls. More than 100 polymorphisms have been identified to date within the eNOS gene.3 One of the most widely studied polymorphisms resides within exon 7, an 894G to T base substitution at codon 298 changes the amino acid from glutamate to aspartate (Glu298Asp). Preliminary evidence indicates that the Asp298 allele reduces vascular NO production.4 Studies have shown that individuals with the Asp298-allele are at greater risk for endothelial dysfunction and consequently diseases of impaired endothelial function such as hypertension, diabetes, and atherosclerosis, although inconsistent results have been reported.5 Lee et al (unpublished ARIC manuscript #950) observed a significant interaction with the Asp298 allele, incident cardiovascular disease, and smoking in whites (HR = 2.07). Casas et al conducted a meta-analysis of 30 studies and reported an odds ratio for ischemic heart disease of 1.33 (95% CI 1.15-1.54) among Asp298 homozygotes.5 More research is required to determine the functionality of the Glu298Asp polymorphism, however, it is clear that decreased production, release, and/or activity of endothelial NO is a characteristic of endothelial dysfunction and a strong risk factor for cardiovascular disease.

One pathogenic mechanism of endothelial dysfunction involves NO and free radicals. Reactions between NO and reactive oxygen species (ROS) can result in formation of deleterious nitrogen and oxygen free radicals that can cause DNA damage, lipid and protein oxidation, mitochondrial damage, cellular injury, and increased expression of inflammatory mediators.2 Laboratory studies have demonstrated that endothelial cells exposed acutely to oxidants or to hyperglycemia produce superoxide anion in addition to NO due to impairment of eNOS function resulting in increased endothelial cell apoptosis and adhesion molecule expression.6,7 This same process was also observed in the kidney and heart of diabetic atherosclerotic mice.8 Human studies have shown an impairment of endothelial dependent vasodilation in diabetic patients caused by decreased NO bioavailability.9,11 Antioxidant therapy for the treatment of endothelial dysfunction has been studied in observation and clinical studies with mixed results.12 The majority of these studies have focused on vitamins C, E, and A. Mechanisms of antioxidant function include the reduction of oxidant formation, elimination of ROS, and repair of oxidant-induced injury. Therefore, decreasing ROS through the use of antioxidant therapy may prevent increases in inflammation mediators that are typically associated with risk of cardiovascular disease and diabetes by enhancing NO activity. We propose to investigate the relationship between the Glu298Asp allele and circulating levels of inflammation mediators in the whole ARIC cohort and investigate antioxidant consumption as a possible effect modifier. Furthermore, we aim to investigate this relationship in two subgroups of diseased individuals known to have endothelial dysfunction, those with incident diabetes and those with incident cardiovascular disease.

5. Main Hypothesis/Study Questions:
1. To estimate the frequency of the eNOS Glu298Asp polymorphism in a population sample of blacks and whites.
2. To describe the association of the eNOS Glu298Asp polymorphism and serum or plasma levels of inflammation markers within levels of dietary antioxidant consumption in the ARIC population. (Total cohort – plasma levels of fibrinogen, factor VIII, and von Willebrand factor antigen)
3. To describe the association of the eNOS Glu298Asp polymorphism and serum or plasma levels of inflammation markers within levels of dietary antioxidant consumption in two subgroups of diseased individuals.
   - Diabetes case sample- serum or plasma levels of adiponectin, complement 3, leptin, sICAM-1, CRP, IL-6, orosomucoid, sialic acid, and oxidized LDL
   - CVD case sample- serum or plasma levels of sICAM-1, sVCAM-1, L-selectin, MCP-1, CRP, E-selectin, P-selectin, and TNF-alpha

6. Data (variables, time window, source, inclusions/exclusions):
   Design: Cross sectional
   Outcome: Serum or plasma levels of inflammation mediators
   Exposure: eNOS Glu298Asp polymorphism
   Effect Modifier: Dietary intake of antioxidants (Vit A, C, and E, fruits and vegetable intake, coffee intake, diet patterns, beta carotene, folate)
   Covariates include, but are not limited to, traditional risk factors including age, sex, race, lipid levels, hypertension, obesity, smoking status, estrogen use, and physical activity.

Analysis Plan
1. Hardy Weinberg equilibrium among genotypes will be calculated using the chi-square test on race-specific datasets.
2. An additive genetic model will be assumed unless indicated otherwise by the results. Therefore, genotypes will be coded as 0 (0 copies of candidate allele), 1 (1 copy), or 2 (2 copies). If appropriate given the results, a dominant model combining homozygotes and heterozygotes will be used.
3. Multivariate linear regression, controlling for potential confounders/covariates, will be used to test the null hypothesis that the mean inflammation mediator serum levels are the same across eNOS Glu298Asp polymorphism genotypes within levels of dietary antioxidant consumption.

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?  ____ X_ 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 ICTDER02 must be used to exclude those with value
RES DNA = “No use/storage DNA”?  __X__ 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)?

Manuscript #950, 1033, and 1001

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

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)* __1995.09__ )

*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.


