

**ARIC Manuscript Proposal # 1228**

**PC Reviewed:** 2 / 13/06  
**SC Reviewed:** \_\_\_\_\_

**Status:** A  
**Status:** \_\_\_\_\_

**Priority:** 2  
**Priority:** \_\_\_\_\_

**1.a. Full Title:** Association of an Endothelial Nitric Oxide Synthase 3 (*NOS3*) Polymorphism (Glu298Asp) with Diabetes and Possible Effect Modification of Obesity

**b. Abbreviated Title (Length 26 characters):** *NOS3, Obesity, and Diabetes*

**2. Writing Group:**

Writing group members: Jan Bressler  
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. x **[please confirm with your initials electronically or in writing]** JB

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- 3. Timeline:** Statistical analyses: February 2007 – May 2007  
Manuscript preparation: May 2007 – July 2007  
Manuscript revision: August 2007  
Manuscript submission: September 2007

**4. Rationale:**

Diabetes is an important and treatable risk factor for cardiovascular disease (CVD) (Haffner SM et al. NEJM 339:229-234, 1998). Obesity is an independent risk factor for diabetes (Stern MP and Haffner SM Arteriosclerosis 6:123-130, 1986) and either body mass index (BMI) or waist-to-hip ratio (WHR) has been commonly used as an index of adiposity. Nitric oxide (NO) is produced by endothelial cells and has been implicated in vascular relaxation in response to increased blood flow as well as multiple agents including acetylcholine, an activity originally attributed to endothelium-derived relaxing factor (EDRF) (Furchgott RF and Zawadzki JV Nature 288:373-376, 1980). NO has also been shown to have a role in the regulation of blood pressure (Rees DD Proc. Natl. Acad. Sci. (USA) 86:3375-3378, 1989) and vascular tone. The production of NO from L-arginine is mediated by a constitutive form of NO synthase (eNOS) encoded on chromosome 7q35-36 by the *NOS3* gene (Marsden PA et al. J. Biol. Chem. 268:17478-17488, 1993).

An association between a *NOS3* Glu298Asp single nucleotide polymorphism (SNP) (rs1799983) (Yoshimura M et al. Hum. Genet. 103:65-69, 1998) and myocardial infarction (Hibi K et al. Hypertension 32:521-526, 1998; Casas JP et al. Circulation 109:1359-1365, 2004), coronary artery disease (Cai et al. J. Mol. Med. 77:511-514, 1999; Hingorani AD et al. Circulation 100:1515-1520, 1999) and hypertension (Miyamoto Y et al. Hypertension 32:3-8, 1998) has been previously reported. In addition, Monti et al. have shown that the *NOS3* 298Asp allele was more frequent in patients with type 2 diabetes than in normal controls (Monti et al. Diabetes 52:1270-1275, 2003).

Defects in endothelial cell function and NO production have been described for subjects with atherosclerosis, hypertension, diabetes, as well as obesity (Cai H and Harrison DG Circulation Res 87:840-844, 2000) and are measured by determining forearm blood flow responses by ultrasound. Since it is possible that the metabolic abnormalities seen in both diabetes and obesity such as insulin resistance are implicated in vascular changes (Ritchie SA et al. Clin Sci (Lond) 107:519-532, 2004) conferring some part of the increased risk for CVD, and that these changes could be dependent on *NOS3* genotype, we propose to study the association of the *NOS3* Glu298Asp polymorphism with both obesity and diabetes in the biracial prospective ARIC study. The possibility that the risk for diabetes is influenced by an individual's *NOS3* genotype and that disease susceptibility may be modified by obesity will also be addressed. The rs1799983 *NOS3* SNP has been genotyped on the entire ARIC cohort.

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

1. To estimate the frequency distribution of *NOS3* gene variation in a population-based sample of whites and African-Americans
2. To evaluate the independent effect of *NOS3* gene variation on measures of body size including body mass index (BMI), weight, waist circumference, and waist-to-hip ratio in a race-specific manner. Age, gender, and field center will be included as covariates.

3. To evaluate the independent effect of *NOS3* gene variation on prevalent diabetes case status in a race-specific manner. Age, gender, and field center will be included as covariates. The relation between *NOS3* gene variation and incident diabetes will also be examined using data obtained during exams 1-4.

4. To evaluate whether obesity as assessed by various measures of body size including BMI, weight, waist circumference, and waist-to-hip ratio modulates the independent effect of *NOS3* gene variation on diabetes susceptibility at visit 1 (ie. gene x obesity interaction). These analyses will be carried out using age, gender, and field center as covariates.

**6. Data (variables, time window, source, inclusions/exclusions):**

Caucasian and African-American participants will be evaluated separately for this analysis. The usual DNA restriction, ethnic group, and missing data exclusion criteria will be used. In analysis models, BMI will be used as both a categorical and a continuous variable. Division into categories of BMI will be carried out based on standard criteria where an individual with a BMI  $\geq 25$  kg/m<sup>2</sup> is considered overweight, a BMI  $\geq 30$  kg/m<sup>2</sup> is considered as a measure of obesity, while those individuals with a BMI  $\geq 40$  kg/m<sup>2</sup> are considered morbidly obese. Waist-to-hip ratio will be analyzed separately for males and females after division into quartiles in controls by gender. Logistic regression will be used to predict prevalent diabetes case status as well as incident diabetes cases. Cases of incident diabetes will be defined as those individuals who developed diabetes in the nine years of follow-up between visit 1 (1987-1989) and visit 4 (1996-1998).

**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 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  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"?  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)?**

#950 Association of a common variant in Endothelial Nitric Oxide Synthase (Glu298Asp) with non-invasively measured atherosclerotic burden and/or risk of adverse cardiovascular events. (Lead author: Kari North, University of North Carolina, Chapel Hill)

#1131 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 Study. (Lead author: Suzette J. Bielinski, University of Minnesota)

#1033 Association of polymorphisms in endothelial genes involved in arachidonic acid metabolism and nitric oxide synthesis with non-invasively measured atherosclerotic burden and risk of adverse cardiovascular events. (Lead author: Craig R. Lee, University of North Carolina, Chapel Hill)

Note: Although all of the above studies describe the analysis of polymorphisms in genes involved in nitric oxide synthesis, none of these manuscript proposals mention body size, insulin resistance, or diabetes as a dependent variable.

**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\* AS#1995.07)**

**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.**