1.a. **Full Title:** Association of an Endothelial Nitric Oxide Synthase 3 (NOS3) Polymorphism (Glu298Asp) with Pulse Wave Velocity and Blood Pressure, and Possible Effect Measure Modification by Fruit and Green Leafy Vegetables Intake.

b. **Abbreviated Title (Length 26 characters):** NOS3, PWV, BP and vegetables

2. **Writing Group:**

Writing group members: Jingkai Wei, Misa Graff, Lyn M. Steffen, Yan Zheng, Kari E. North, Gerardo Heiss, others welcome

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

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3. **Timeline:**  
Analyses to start upon approval of proposal. Submit for publication within 9 months from proposal approval.

4. **Rationale:**  
Nitric oxide (NO) is known to influence blood flow. There are two pathways for NO synthesis: NO synthase (NOS) dependent and NOS independent. (1) The NOS dependent pathway is relevant in examining the long-term associations between NO and cardiovascular health effects. Previous studies have shown that NOS gene polymorphism influences arterial stiffness and hypertension in adults. (2-6) Seidlerová et al. and Chen et al found that the T allele of rs3918226 in NOS3 was associated with central arterial stiffness and lower arterial wall stiffness, respectively. Wrzosek et al. found that NOS3 894T allele was associated with 1.37 fold higher risk of hypertension among obese adults.

Pulse wave velocity (PWV) is considered the gold standard for measurement of arterial stiffness, (7) and it is a good choice to examine the association between NOS and PWV. There are several coding polymorphisms that have demonstrated importance and function in reducing arterial...
stiffness and blood pressure. One of the polymorphisms is NOS3 Glu298Asp single nucleotide polymorphism (SNP), reported to be associated with a lower level of arterial stiffness and a lower risk of hypertension.(6, 8)

Inorganic nitrate, which can be converted to nitric oxide, is abundant in several foods, particularly in green leafy vegetables.(9) Studies have shown that dietary nitric oxide has short-term effects in reducing arterial stiffness and blood pressure.(10, 11) Due to its short half-life, NO from the NOS independent pathway cannot be assayed in population studies, while a previous study suggested the Glu298Asp SNP in the endothelial nitric oxide synthase gene differentially affects the vascular response to acute consumption of fruit and vegetable puree based drinks.(12) It is desirable to examine whether habitual intake of green leafy vegetables is a potential effect modifier of the association between NOS3 (Glu298Asp) with arterial stiffness and blood pressure in a population based setting.

Because of its large sample size, the ARIC study offers an opportunity to better examine the association between NOS3 Glu298Asp SNP (rs1799983) with PWV and blood pressure. Furthermore, we propose to analyze dietary data from exam visits 1 and 3 to examine green leafy vegetables as potential effect measure modifier between NOS3 Glu298Asp SNP with PWV and blood pressure.

5. Main Hypothesis/Study Questions:
1. There is a race-specific, cross-sectional association between NOS3 (Glu298Asp) with a lower level of arterial stiffness.
2. There is a race-specific longitudinal association between a higher level of NOS3 (Glu298Asp) with a reduced level of blood pressure.
3. Intake of green leafy vegetables is an effect measure modifier between NOS3 with arterial stiffness and blood pressure.

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).

Study Design: Cross-sectional study of NOS3 (Glu298Asp SNP (rs1799983)) with arterial stiffness (visit 5); longitudinal study of NOS3 (Glu298Asp SNP) with blood pressure (visit 1 to 5)

Inclusions: participants with pulse wave velocity at visit 5, and blood pressure measured from visit 1 to 5 with dietary information from visit 1 and 3 available.

Exposures: NOS3 (Glu298Asp SNP (rs1799983))

Outcome: Carotid-femoral pulse wave velocity (visit 5): measured by the VP-1000 plus system (Omron Co., Ltd., Kyoto, Japan) and the path length was calculated using the following formula: path
length (cm) = carotid-femoral distance (cm) – (suprasternal notch – carotid distance (cm)). A minimum of two measurements were taken per participant and the last two usable measurements (i.e. non-zero values) were averaged.

Change of sitting systolic blood pressure from visit 1 to 5

**Statistical Analysis**

Hardy Weinberg equilibrium among genotypes will be calculated using the chi-square test on race-specific datasets. An additive genetic model will be assumed unless indicated otherwise by the results. Genotypes will be coded as 0 (0 copies of candidate allele), 1 (1 copy), or 2 (2 copies).

Hypothesis 1: There is race-specific cross-sectional association between NOS3 (Glu298Asp SNP) with arterial stiffness.

  - Linear regression models will be conducted between NOS3 (Glu298Asp SNP) and pulse wave velocity.
  - Analysis will be conducted in a race-specific manner.
  - Covariates (at visit 5): age, sex, genotypes, BMI, heart rate, smoking status, diabetes, hypertension and physical activity.
  - The analysis will be race-specific.

Hypothesis 2: There is race-specific association between NOS3 (Glu298Asp SNP) with change of systolic blood pressure change from visit 1 to visit 5.

  - Mixed models will be conducted to examine the associations between NOS3 (Glu298Asp SNP) and systolic blood pressure.
  - Covariates (at visit 5): age, sex, genotypes, BMI, heart rate, smoking status, diabetes and physical activity.
  - The analysis will be race-specific.

Hypothesis 3: Intake of green leafy vegetables is an effect measure modifier between NOS3 with arterial stiffness and blood pressure.

  - Average intake of green leafy vegetables and fruit will be calculated from food frequency questionnaires at visit 1 and 3 and grouped as quartiles. Diet score without fruit and vegetables intake will be created using principal component analysis and be adjusted for. The analysis above will compare the 4th quartile to the other quartiles.
  - The analysis will be race-specific.

Methodological limitations: The dietary data are not available at visit 4 or visit 5, and likely subject to some change.

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 ICTDER 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 ICTDER03 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)?
MS#1228---Bressler et al. Association of an Endothelial Nitric Oxide Synthase 3 (NOS3) Polymorphism (Glu298Asp) with Diabetes and Possible Effect Modification of Obesity
MS#1371---Kingah et al. Association of the NOS3 Glu298Asp SNP with hypertension and possible effect modification of dietary fat intake.
MS#1131---Bielinski et al. 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

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/

12a. 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.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms.
http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes ____ No.

REFERENCES