1.a. Full Title: Serum 25-hydroxyvitamin D and incident hypertension

b. Abbreviated Title (Length 26 characters): 25(OH)D and hypertension

2. Writing Group: Pamela L Lutsey, Erin D. Michos, James S. Pankow, Elizabeth Selvin, Jared P. Reis, Danni Li, Jeffrey Misialek. Other interested investigators are welcome.

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]

First author: Pamela L Lutsey
Address: 1300 South 2nd St, Suite 300
Minneapolis, MN 55126

Phone: (612) 624-5812 Fax: (612) 624-0315
E-mail: lutsey@umn.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: James Pankow
Address: 1300 South 2nd St, Suite 300
Minneapolis, MN 55126

3. Timeline: Data analyses will begin immediately. Goal completion by Nov 2014.

4. Rationale: Vitamin D is a fat-soluble vitamin obtained through cutaneous synthesis resulting from sun exposure, and through oral intake from food and supplement sources\(^1\). Globally it is estimated that more than 50% of the world population has inadequate concentrations of 25(OH)D\(^2\), which has traditionally been viewed as the superior biomarker for measuring vitamin D status.

Existing evidence suggests suboptimal 25(OH)D may be associated with hypertension development. 25(OH)D is involved in regulation of the renin-angiotensin system, and low 25(OH)D levels are associated with hypertrophy of the vascular smooth muscle cells\(^3\)\(^-\)\(^5\). The majority of observational prospective cohort studies\(^6\)\(^,\)\(^7\), though not
all\textsuperscript{8,9}, have reported inverse associations between 25(OH)D and risk of incident hypertension. Experimentally, several small clinical trials have found that supplementing vitamin D (either by dietary supplements or with UVB radiation) successfully reduced blood pressure\textsuperscript{10-14}.

While there appears to be a role for 25(OH)D in the regulation of blood pressure, prior findings need replication and several aspects of this association require further elucidation. For instance, most existing studies have been conducted in largely white populations, yet relative to whites, blacks are known to have both low 25(OH)D levels\textsuperscript{15} and be at greater risk of prevalent and incident hypertension\textsuperscript{16-18}. However, blacks may require lower levels of 25(OH)D for optimal functioning. Concentrations of the vitamin D binding protein (DBP), which is a carrier protein for vitamin D, differ greatly by race, with whites tending to have higher levels of DBP, relative to blacks\textsuperscript{19}. Recent evidence suggests that levels of bioavailable D are similar in blacks and whites, since although blacks have lower levels of 25(OH)D relative to whites, they also have lower levels of DBP\textsuperscript{19}. Unfortunately, DBP has not been measured in ARIC, thus precluding the calculation of bioavailable D. However, two SNPs (rs7041 and rs4588) are believed to explain 80\% of the variation in DBP levels\textsuperscript{19}. Allele frequencies of these SNPs also vary greatly by race, with whites genetically predisposed to having higher DBP concentrations.

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

We hypothesize that low serum 25(OH)D is associated with incident hypertension, and that the association is (a) stronger among whites than blacks, and (b) stronger among those genetically predisposed to having high levels of DBP.

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**

Prospective cohort from ARIC visit 2, when serum 25(OH)D was measured, to the development of incident hypertension.

**Inclusion/Exclusion**

Participants with prevalent hypertension (diagnosed or undiagnosed according to ARIC criteria) will be excluded at baseline (visit 2). Additionally, individuals who are neither African American nor white, and African Americans from the MN and MD centers will be excluded at baseline.

**Variables**

*Exposures*: Serum 25(OH)D. 25(OH)D will be adjusted for season, as has been done in other ARIC papers\textsuperscript{20}.

*Outcome*: 
- **Primary**: Incident hypertension based on measured blood pressure (SBP $\geq 140$ mmHg and/or DBP $\geq 90$ mmHg) and/or antihypertensive medication use at ARIC visits 3 and 4. This approach captures both undiagnosed and diagnosed cases.

- **Secondary**: Incident self-reported hypertension based on self-report of diagnosis or medication use at visits 3 and 4, and via the ARIC annual follow-up phone calls. This approach captures only diagnosed cases.

Potential effect modifiers: Race, sex, diabetes, and DBP SNPs rs7041 and rs4588.

Other confounders and/or mediators: Age, sex, ARIC field center, education, physical activity, smoking status, BMI, serum parathyroid hormone, serum fibroblast growth factor 23, serum calcium, serum phosphorus, and eGFR.$^{36}$

**Data analysis**

Baseline characteristics of participants will be described using means and proportions stratified by levels of the potential covariates. For the primary analyses, which defines incident HTN according to objective measures at visits 3 and 4, complimentary log-log (cloglog) regression will be used. In secondary analyses, which define incident HTN using self-reported Annual Follow-Up call data as well as some information from visits 3 and 4, Cox proportional hazards regression will be employed. Restricted cubic splines will be used to visually depict the associations, and aid in selecting the most appropriate representation. 25(OH)D will likely be modeled categorically.

Our first model will adjust for age, sex, and race/ARIC field center. Model 2 will additionally adjust for education, physical activity, smoking status, and BMI. Model 3 will further adjust (in separate models) for: eGFR, parathyroid hormone, fibroblast growth factor 23, phosphorus, and calcium. Cross-product terms will be used to evaluate whether race, sex, or key DBP SNPs (i.e. rs7041 and rs4588) modify the relationship between 25(OH)D and risk of incident hypertension. Stratified results will be presented, as appropriate.

Sensitivity analyses will explore, for analyses of self-reported HTN, retaining individuals with HTN identified by blood pressure measurements at visit 2. Additionally, we will conduct analyses stratifying by whether the participant was normotensive or prehypertensive at baseline.

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 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      (Interaction testing only; subgroup analyses if warranted)

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.cscce.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)?

#2019 – 25-hydroxyvitamin D levels and incident stroke: twenty-year follow-up in a biethnic cohort

#2224 - 25-hydroxyvitamin D and risk of incident heart failure: The Atherosclerosis Risk in Communities Study (ARIC)

#2340 – 25-hydroxyvitamin D and incident diabetes: The Atherosclerosis Risk in Communities (ARIC) Study

#2377 – 25-hydroxyvitamin D levels, vitamin D binding protein gene polymorphisms, and vitamin D3 epimer with risk of incident coronary heart disease (CHD) among whites and blacks: the ARIC Study

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

   __X__   A. primarily the result of an ancillary study (list number* 2009.17)

   ____   B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ __________ )
References