

**ARIC Manuscript Proposal #2340**

**PC Reviewed:** 4/8/14  
**SC Reviewed:** \_\_\_\_\_

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

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

**1.a. Full Title:** 25-hydroxyvitamin D and incident diabetes: The Atherosclerosis Risk in Communities (ARIC) Study

**b. Abbreviated Title (Length 26 characters):** Vitamin D & incident diabetes

**2. Writing Group:** Jared P. Reis, Pamela L. Lutsey, James S. Pankow, Erin D. Michos, Elizabeth Selvin. Others welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. X

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**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).

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**3. Timeline:** Data analyses will begin immediately upon receiving data. Goal completion is Sept 2014.

**4. Rationale:**

Vitamin D, a fat-soluble vitamin, plays a critical role in regulating plasma calcium concentration through effects on intestinal calcium absorption and bone metabolism [1]. Vitamin D is produced in the skin from 7-dehydrocholesterol during exposure to solar ultraviolet B radiation. Although vitamin D can also be derived from diet, only a few foods naturally contain vitamin D (e.g., oily fish). Fortified foods include milk, some cereals, bread, and orange juice.

The relation between vitamin D status and bone health is well established, but there is emerging evidence from both in vivo and in vitro studies that has suggested extraskeletal effects of vitamin D on insulin secretion and insulin action [2]. Clinical studies have provided further evidence to support the hypothesis that low vitamin D status is associated with impaired beta cell function, insulin resistance, and glucose intolerance [3-5]. In a recent meta-analysis of 21 prospective studies of serum 25-hydroxyvitamin D [25(OH)D] and incident type 2 diabetes, the summary relative risk comparing the highest to the lowest category of 25(OH)D was 0.62 (95% CI: 0.54, 0.70) [6]. The inverse dose-response relationship did not vary substantially by a number of factors, including sex, duration of follow-up, study sample size, diabetes diagnostic criteria, or 25(OH)D assay method [6]. However, it is unknown whether the association between vitamin D status and diabetes varies by race, since few available studies (only 2 of the 21 studies included in the meta-analysis) have included racially/ethnically diverse samples of individuals.

Relative to whites, blacks have low vitamin D levels, but paradoxically higher bone mineral density and lower fracture risk [7]. Low levels among blacks are suspected to be due, at least in part, to greater cutaneous melanin content, which blocks the initial conversion of 7-dihydrocholesterol to previtamin D<sub>3</sub> in the skin [8, 9] and a dietary intake that includes a lower consumption of dairy products and other foods fortified with vitamin D [10, 11]. However, recent evidence suggests that although 25(OH)D levels differ between the races, levels of bioavailable vitamin D, the non-vitamin D-binding protein fraction, are similar [12]. Common genetic polymorphisms in the vitamin D-binding protein gene (i.e., rs7041 and rs4588), the frequency of which varies between racial groups [12], produce variant proteins that differ in their affinity for vitamin D [13]. Alternate mechanisms that may explain the racial differences in the association of vitamin D with outcomes include findings which suggest black individuals have higher circulating concentrations of 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] and parathyroid hormone at a given level of 25(OH)D [14, 15], and vitamin D binding protein serum levels and vitamin D receptor gene affinity and polymorphism frequencies vary by race [12, 16].

## **5. Main Hypotheses:**

1. Serum vitamin D will be inversely associated in a linear dose-response fashion with risk of diabetes.
2. The association between vitamin D and diabetes will be stronger among whites than among African Americans.
3. In race-stratified analyses, there will be an interaction by vitamin D binding protein gene polymorphisms. The association of 25(OH)D levels with risk of diabetes will be stronger among those genetically predisposed to having higher levels of vitamin D binding protein, and thus lower bioavailable vitamin D levels.

**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 study from visit 2, when serum 25(OH)D was measured, to the development of incident diabetes.

## Inclusion/Exclusion

Participants with prevalent diabetes and those missing data on vitamin D and diabetes status at visit 2 will be excluded, as will those who are neither African American nor white, and African Americans from the MN and MD centers. We will further exclude those with no follow-up or incomplete incident diabetes information. For genetic analyses we will also exclude those who did not consent to genetic research.

## Variables

### *Exposures:*

**Primary:** Serum 25(OH)D (measured in visit 2 serum; sum of 25(OH)D<sub>2</sub> + 25(OH)D<sub>3</sub>).

Since serum vitamin D levels vary greatly by sun exposure, which is seasonal, we will account for seasonal variation by computing the residuals from a linear regression model with vitamin D as the dependent variable and month of blood draw as the independent variable. By definition, these residuals will be uncorrelated with month of blood draw. The grand mean will be added to the vitamin D residuals obtained from this model. This new variable “vitamin D adjusted for month of blood draw” will be used as the main exposure variable for all analyses.

**Secondary:** We will also look, separately, at associations of the vitamin D epimer [3-epi-25(OH)D<sub>3</sub>], vitamin D<sub>2</sub> [25(OH)D<sub>2</sub>], and vitamin D<sub>3</sub> [25(OH)D<sub>3</sub>] with risk of incident diabetes.

### *Outcome:*

- *Primary:* Incident type 2 diabetes will be identified at ARIC visits 3 and 4 by meeting any of the following four criteria: 1) fasting glucose level of at least 7.0 mmol/L (126 mg/dL); 2) nonfasting glucose of at least 11.1 mmol/L (200 mg/dL); 3) current use of diabetes medication; or 4) a positive self-reported physician diagnosis. Date of occurrence of diabetes will be assigned according to the method of Duncan and colleagues [17].
- *Secondary:* Incident self-reported physician diagnosis or use of diabetes medications from the annual telephone calls after visit 2.

If results are consistent across definitions, we will consider combining the two definitions to form a single diabetes outcome variable.

*Main covariates:* Age, race, center, sex, education, physical activity, smoking status, alcohol use, family history of diabetes, BMI, CRP, LDL-C, HDL-C, triglycerides, antihyperlipidemic medication use, mean systolic blood pressure, antihypertensive medication, eGFR (modeled as  $\geq 90$ , 60-89, and 15-59 ml/min/1.73 m<sup>2</sup>). eGFR will be calculated using both creatinine and cystatin-C [18].

*Potential effect modifiers:* Age, race, sex, eGFR, vitamin D binding protein SNPs (rs7041 and rs4588).

Data analysis

Visit 2 will serve as baseline for the current analysis. Visit 2 participant characteristics will be described using means and proportions stratified by levels of vitamin D.

Cox proportional hazards regression will be used to examine the association between vitamin D and risk of diabetes. We will evaluate the proportional hazards assumption quantitatively by testing the interaction between 25(OH)D and ln(time), and qualitatively by inspection of ln(-ln) survival curves. We will use restricted cubic splines to characterize the continuous association of vitamin D with diabetes, evaluate the potential for a threshold effect, and aid in selecting the most appropriate exposure representation [19]. Our first model will adjust for age, sex, race-center, education, physical activity, smoking status, alcohol use, and family history of diabetes. Model 2 will additionally adjust for BMI and waist circumference. Model 3 will further adjust for CRP, LDL-C, HDL-C, triglycerides, antihyperlipidemic medication use, mean systolic blood pressure, antihypertensive medication use, and eGFR.

Multiplicative interaction terms will be used to evaluate whether age, race, sex, eGFR, and/or vitamin D binding protein SNPs modify associations between vitamin D and risk of incident diabetes. Given inherent interest due to few available large studies of racially diverse populations, we will report race-stratified results, regardless of whether a significant race-interaction is present.

In sensitivity analyses, we will restrict our analysis to participants whose self-reported health was good, very good, or excellent at visit 2. The rationale for restriction based on self-reported health is that participants who are ill may be less likely to go outside and be exposed to sunlight, and thus have lower vitamin D levels.

**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?**  
 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)?

#2224: 25-hydroxyvitamin D and risk of incident heart failure: The Atherosclerosis Risk in Communities Study. *Pamela L Lutsey, Erin D. Michos, Jeffrey R. Misialek, Jim Pankow, Laura Loehr, Elizabeth Selvin, Jared P. Reis, Myron Gross, John Eckfeldt, Aaron R. Folsom*

#2019: 25-hydroxyvitamin D levels and incident stroke: Twenty-year followup in a biethnic cohort. *Erin D Michos, Pamela Lutsey, Tom Mosley, Richey Sharrett, Kathryn Carson, Wendy Post, Rebecca Gottesman, Aaron Folsom, Jim Pankow*

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

2009.17 (Lutsey PI)

- "Serum vitamin D and cardiovascular disease risk in the biethnic ARIC cohort"

2009.16 (Selvin PI)

- "Short-term markers of glycemia and long-term outcomes"

- Numerous biomarkers which may be confounders and/or effect modifiers in the present analysis were measured as part of this grant (e.g. CysC, CRP).

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/>

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.csc.unc.edu/eric/index.php>, under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit\\_process\\_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to Pubmed central.

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