ARIC Manuscript Proposal #2019

PC Reviewed: 10/9/12   Status: **A**   Priority: **2**
SC Reviewed: _____   Status: _____   Priority: ____

1.a. Full Title: 25-hydroxyvitamin D levels and incident stroke: Twenty-year followup in a biethnic cohort

  b. Abbreviated Title (Length 26 characters):

2.   Writing Group:
   Writing group members:

Erin D. Michos   Johns Hopkins   First Author
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _EM_ **[please confirm with your initials electronically or in writing]**

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3.   Timeline:

Visit 2 serum samples are currently being processed and anticipated to be finished by February 2013.   Analyses for this proposal will take place in early 2013. Goal is to have analyses and abstract completed in time for submission for AHA 2013 (submission deadline June 2013).

4.   Rationale:

25-hydroxyvitamin D [25(OH)D] deficiency has been associated with increased risk of cardiovascular disease (CVD) and all-cause mortality.[1] Suboptimal vitamin D status is thought to influence CVD risk predominantly by acting on established CVD risk factors, namely hypertension, diabetes, and inflammation. Vitamin D may also prevent vascular injury through lowering blood pressure, inhibiting the renin-angiotensin-aldosterone system, and inhibiting atherogenesis.[1]

Low 25(OH)D has also been associated with incident cerebrovascular disease. Inverse associations between 25(OH)D levels and fatal stroke were also observed among members of the Intermountain Healthcare system[2], among white but not black participants in an analysis of NHANES-III data[3], in a sample of patients referred for coronary angiography[4], and in two small case-control studies[5,6]. Low dietary vitamin D intake predicts 34-year stroke incidence among Japanese-American men[7].

In 6,219 participants in the Mini-Finland Health Survey, individuals with 25(OH)D levels in the highest quintile were at lower risk of fatal stroke over 27 years than were those in the lowest quintile [HR:0.48 (95% CI: 0.31-0.75)].[8] A recent meta-analysis which pooled the results of 7 prospective observational studies found that low 25(OH)D levels were associated with increased risk of incident stroke [RR 1.52 (95% CI 1.20-1.85].[9]

The observational studies mentioned above have their limitations. Most of the studies looked at the relationship of vitamin D status with fatal stroke, an outcome ascertained through National Death Index (or similar national databases) using death certificate coding, which has the limitation of misclassification of events with events not being formally adjudicated. The relationship of vitamin D status with non-fatal stroke, the leading cause of morbidity in the United States, has not been well established. Furthermore, the previously published studies did not discern between hemorrhagic and ischemic stroke. And finally, most of the above studies were exclusively Caucasian populations, yet African Americans have the highest prevalence of 25(OH)D deficiency in the United States, and are at elevated stroke risk relative to whites.

This proposal will, in a biethnic population with long-term followup, provide some of the first information on the relation of 25(OH)D and associated biomarkers to incident stroke (non-fatal and fatal stroke),using the established ARIC stroke outcome adjudication, explore the relationship of 25(OH)D with

subclasses of stroke (hemorrhagic and ischemic), and explore potential race differences in stroke risk by vitamin D status.

5.   Main Hypothesis/Study Questions:

Hypotheses:
1. Low 25(OH)D levels will be associated with incident clinical stroke independent of traditional CVD risk factors, lifestyle factors, and socioeconomic status. This relationship will remain significant even after adjustment for calcium, phosphate, and parathyroid levels.
2. Low 25(OH)D levels will be associated with ischemic thrombotic stroke, but not hemorrhagic stroke or cardioembolic stroke.
3. Low 25(OH)D levels will be associated with incident stroke among both white and black participants. Black participants will have lower 25(OH)D levels than whites, and the relative risk of stroke associated with vitamin D deficiency will differ by race.

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).

Participants: Inclusion: All ARIC participants who have stored serum from ARIC visit 2 1990-1992; n = 13,753.   Exclusions: Participants with a diagnosis of TIA or stroke prior to visit 2 will be excluded.

Biomarkers of interest: This proposal makes use of the newly measured 25(OH)D, PTH, FGF23, calcium, and phosphorous levels measured from ARIC visit 2 serum, as outlined in the Ancillary proposal by Lutsey et al.

Covariates: Demographic factors (age, race, gender), lifestyle factors (smoking, alcohol physical activity, newly created Sun Exposure score, diet/calorie intake), traditional CVD risk factors (BMI, WC, HTN, DM, lipids), SES factors (education), season of blood draw, ARIC stroke risk score, LVH by EKG, fibrinogen

Outcome Ascertainment: Incident stroke using ARIC standard definition. Diagnosis was based on a rapid focal neurologic deficit lasting 24 hours and usuallyconfirmed by neuroimaging. Strokes are subclassified by type (e.g., hemorrhagic, cardioembolic, ischemic non-cardioembolic). A composite stroke analysis (including all types of stroke) will be the primary outcome, and ischemic stroke (ischemic cardioembolic and ischemic thrombotic) will be an alternative outcome. [The number of hemorrhagic strokes is quite low, and likely there will not be enough power to detect an association with this outcome alone but will be included in composite outcome.]

Statistical methods: Cox proportional hazards regression will be used to evaluate the relation of 25(OH)D to incident stroke. Person-years will accrue from the date of the participant's visit 2 exam until the date of the incident event of interest, death, loss-to-

follow-up, or the end of ARIC follow-up. The proportional hazards assumption will be tested by graphing the log(-log(survival)) versus log(time).

25(OH)D will be modeled in several ways: 1) as a continuous variable (log-transformed if right skewed), 2) in quintiles, and 3) categorized according to existing 25(OH)D cut-points (i.e. deficient: <10 ng/mL; optimal: ≥30 ng/mL). Cubic splines will aid in selecting the most appropriate representation. In quintile analyses we will explore associations relative to a referent category by entering the quintiles into the models as a categorical variable, and also linear trends by entering the quintiles into the models as a continuous variable.

The anticipated events and power calculations (for composite stroke outcome) are noted in the table below. Power will be even greater for tests of trend across quintiles, or when 25(OH)D is modeled as a continuous variable.

Number of incident events, and minimum detectable HR's for extreme quintiles of biomarkers at 80% power[¥] for the entire ARIC sample and by race.						
	Blacks		**Whites**		**All ARIC**	
	Events	HR	Events	HR	Events	HR
Expected events through 2010*						
Stroke	454	1.5	625	1.4	1079	1.3
* Estimates based on 2006-2007 incidence rates; [¥] alpha = 0.05, and a two-tailed test						

For each outcome (i.e. incident total stroke, ischemic stroke, hemorrhagic stroke), we will run a series of models. The first will likely adjust for demographics, center (proxy for latitude) and season of blood draw, while further models will additionally adjust for behaviors, diet, physical activity, physiologic characteristics, and, finally, for biomarkers related to 25(OH)D, such as calcium, phosphorous, and PTH. Final decisions to include additional covariates in the multivariate analysis will be related to their role as a possible common cause of the outcome and the exposure of interest, with attention to avoid overadjustment for mediating variables.

Multivariate population attributable risks, which estimate the excess rate of stroke in the total study population that is attributable to the vitamin D deficiency, adjusting for potential confounders, will also be calculated.

To evaluate whether differing vitamin D levels explains racial differences in stroke risk, we will run models with and without vitamin D as a covariate and compare subsequent relative risks (RR) for race/ethnicity. We will calculate the excess risk explained by 25(OH)D levels using the formula: % Excess risk = RR_1-RR_2 / RR_1-1, where RR_1 is the multivariable-adjusted RR of incident stroke for blacks compared to whites without adjustment for 25(OH)D and RR_2 is the RR from a model including adjustment for 25(OH)D. [Part 2]: Additionally to see whether the association of vitamin D with incident stroke differ between blacks and whites, interaction testing by race will be performed by including cross-product terms in the models, and results will be stratified by race if an interaction is found.

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 ICTDER03 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 _X___ 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.cscc.unc.edu/ARIC/search.php

   __X____ Yes _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged tocontact lead authors of these proposals for comments on the new proposal or collaboration)?

Since 25(OH)D is being newly measured in ARIC, this is the first paper to evaluate the relationship of vitamin D with stroke.   There have been numerous other ARIC papers that used stroke as the clinical outcome of interest. Many of those papers have been co-authored by writing panel members Drs. Sharrett, Mosley, Folsom, and Gottesman who are familiar with the stroke data in ARIC.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? _X___ Yes ____ No

Lutsey ARIC Ancillary Study number 2009.17
Michos ARIC Ancillary Study Number 2010.01

11.b. If yes, is the proposal
   _X__ **A. primarily the result of an ancillary study (list number* __see above_____)**

_____   **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 amanuscript 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, 2008which 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 fromhttp://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.

Literature Cited:

1. Michos ED, Reis JP, Melamed ML. Vitamin D status and cardiovascular health: a 2009 update. Open Clinical Chemistry Journal, 2010;3:51-59. Published on-line. http://bentham.org/open/tocchemj/openaccess2.htm

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3. Michos ED, Reis JP, Post WS, Lutsey PL, Gottesman RF, Mosley TH, Sharrett AR, Melamed ML. 25-hydroxyvitamin d deficiency is associated with fatal stroke among whites but not blacks: The nhanes-iii linked mortality files. Nutrition 2012 Apr;28(4):367-71. Epub 2012 Jan 20.

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6. Poole KE, Loveridge N, Barker PJ, Halsall DJ, Rose C, Reeve J, Warburton EA. Reduced vitamin d in acute stroke. Stroke; a journal of cerebral circulation. 2006;37:243-245

7. Kojima G, Bell C, Abbott RD, Launer L, Chen R, Motonaga H, Ross GW, Curb JD, Masaki K. Low dietary vitamin d predicts 34-year incident stroke. Stroke. 2012

8. Kilkkinen A, Knekt P, Aro A, et al. Vitamin d status and the risk of cardiovascular disease death. American Journal of Epidemiology 2009;170(8):1032-1039.

9. Sun Q, Pan A, Hu FB, Manson JE, Rexrode KM. 25-hydroxyvitamin D levels and the risk of stroke: a prospective study and meta-analysis. Stroke 2012;43:00-00 (epub ahead of print).