ARIC Manuscript Proposal #2377

PC Reviewed: 6/10/14	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title:

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

b. Abbreviated Title (Length 26 characters):

Vitamin D and CHD

2. Writing Group:
Writing group members:

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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:

Analyses for this proposal will take place in spring of 2014 immediately following approval of this proposal, with goal to submit for publication by the end of the summer 2014.

4. Rationale:

Low levels of vitamin D, as measured by serum 25-hydroxyvitamin D [25(OH)D], have been estimated to affect approximately 1 billion people worldwide[1] and are associated with increased risk of cardiovascular diseases (CVD)[2] and well as specifically for coronary heart disease (CHD)[3,4]. Suboptimal vitamin D status is thought to influence CVD risk predominantly by acting on established CVD risk factors, namely hypertension, diabetes, and inflammation.[5] Whether adequate vitamin D supplementation in those that are deficient can prevent CVD events is still unknown, and clinical trials are in progress to test this question.

The association of low vitamin D with CVD outcomes may vary by race. In a recent analysis from the Multi-Ethnic Study of Atherosclerosis (MESA), low 25(OH)D was associated with increased CHD risk among Whites and Chinese, but not in Blacks or Hispanics.[4] Similarly, a prior NHANES analysis found that low 25(OH)D was associated with fatal stroke in Whites but not Blacks.[6]

The differences in associations of total 25(OH)D and CVD outcomes by race may in part be explained by racial differences in bioavailable 25(OH)D. Blacks tend to have lower levels of 25(OH)D compared to whites.[7] However, recent work has shown that blacks and whites have similar concentrations of estimated bioavailable 25(OH)D, because blacks have lower levels of both total 25(OH)D and vitamin D binding protein (DBP) compared to whites.[7] There are two common single nucleotide polymorphisms (SNPs) on the *DBP* gene, rs7041 and rs4588, which are believed to explain ~80% of the variability in serum DBP levels.[7] Blacks have been shown to be more likely than whites to have a T allele at rs7041 and to have a C allele at rs4588, which both result in lower levels of serum DBP. While we do not have measured DBP levels in ARIC to directly calculate bioavailable vitamin D, ARIC does have genetic data regarding these 2 polymorphisms. It is possible that these DBP SNPs modify the relationship between 25(OH)D levels and CHD risk.

Additionally, newer 25(OH)D assays using mass spectroscopy have allowed for measurement of C-3 vitamin D_3 epimer [3-epi-25(OH)D_3] (identical molecular structure but different stereochemical configuration), but the clinical significance of 3-epi-25(OH)D_3 is not known.[8]

Finally, previous studies that have evaluated the association of 25(OH)D with CHD are based on a single measure of 25(OH)D which may not reflect lifetime patterns of vitamin D. Little is known about change in vitamin D status

and risk of CHD. In ARIC, a subset of participants did undergo repeated measures of vitamin D approximately 3 years apart.

Proposal Significance Summary:

We propose to examine the independent associations of 25(OH)D, DBP SNPs, and 3-epi-25(OH)D3 levels with incident CHD over ARIC followup, with focus on the potential interactions by race and by DBP SNP status.

5. Main Hypothesis/Study Questions:

Hypotheses:

1. Low 25(OH)D levels (at a threshold of <17.2 ng/ml, or bottom quintile of distribution) will be associated with incident CHD independent of traditional risk factors, lifestyle factors, and socioeconomic status. This relationship will remain significant even after adjustment for calcium, phosphate, and parathyroid hormone levels.

2. We hypothesize that the association between low 25(OH)D and CHD will be modified by race. Low 25(OH)D levels will be more strongly associated with incident CHD among Whites but not Black participants.

3. We hypothesize that the association between 25(OH)D and CHD will be modified by rs7041 and rs4588 SNP status. We anticipate to see a higher risk of low 25(OH)D with rs7041 G versus T allele and rs4588 A versus C allele, (i.e. those genetically predisposed to higher DBP levels and thus those with lower levels of bioavailable vitamin D for a given total 25[OH]D level).

4. Due to the unknown clinical significance of 3-epi-25(OH)D_3, we do not have an a priori hypothesis about the association of 3-epi-25(OH)D_3 with incident CHD. However given the paucity of data in the literature regarding the vitamin D epimer with clinical outcomes, we think this analysis is still important to perform as exploratory for developing future research hypotheses.

5. Among the 1700 individuals with repeat vitamin D measures (~3 years apart), compared to those “replete at visit 2 and replete at visit 3”, those who are Deficient/Replete, Replete/Deficient, and Deficient/Deficient will have greater risk for CHD.

6. 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. Baseline: ARIC visit 2, 1990-1992. Follow-up using most recent follow-up datafiles available (currently through 2011).

Inclusion/Exclusion

All ARIC participants who had 25(OH)D measured from stored serum from ARIC visit 2 1990-1992; n=13,753. Participants with prevalent CHD reported at visit 1 and incident CHD prior to visit 2 will be excluded, as will those who are neither African American nor white, and African Americans from the MN and MD centers. For DBP SNP analyses, we will also exclude those who did not consent to genetic research.

Note than a subset of participants (approximately 1700) from the Forsyth County and Jackson field centers had a repeat measure of 25(OH)D and epimer in ARIC visit 3, approximately 3 years later (1993-1994). These participants will be considered in a supplementary analysis regarding change in vitamin D levels.

Variables

Exposures:

Primary: Serum 25(OH)D (measured in visit 2 serum). Since serum vitamin D levels vary greatly by season,[9] 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. This estimated annual 25(OH)D value will be divided into quintiles based on the distribution in the overall population (<17.2 ng/ml; 17.2-<21.7 ng/ml; 21.7-<26 ng/ml; 26.0-<31 ng/ml; ≥31.0 ng/ml).

Secondary Analyses:

1. We will also look separately at associations of the vitamin D epimer [3-epi-25(OH)D3] with risk of incident CHD. 3-epi-25(OH)D_3 concentration varies with 25(OH)D concentration, but does not vary as much by season, so we will not perform seasonal adjustment. But given its association with 25(OH)D, we do plan to adjust for season (January-March; April-June; July-September; October-December) and 25(OH)D in our 3-epi-25(OH)D_3 regression models.

2. Among the 1700 individuals with repeat vitamin D levels at visit 3, we will consider the association of categories of vitamin D status at both time points (Deficient/Deficient, Deficient/Replete, Replete/Deficient, and Replete/Replete) with CHD risk. The Institute of Medicine (IOM) considers 25(OH)D <20 ng/ml as deficient, although other studies have used a <15 ng/ml as deficient.[3,6] For consistency with the main analyses (and based

on threshold of risk seen in our prior ARIC analyses of stroke), we likely will consider levels <17.2 ng/ml (the bottom quintile) as deficient and >=17.2 ng/ml as replete for both time points.

Outcome: Incident CHD per ARIC adjudication, events through 2011 followup. We will use the variable includes MI hospitalizations & fatal CHD.

Main covariates (measured at visit 2): Age, race, center, sex, education[&], physical activity[&], smoking status, BMI, diabetes, LDL-C, HDL-C, triglycerides, antihyperlipidemic medication use, hsCRP, systolic blood pressure, antihypertensive medication, eGFR (modeled as ≥90, 60-89, and 15-59 ml/min/1.73 m^2). eGFR will be estimated using both creatinine and cystatin-C.

**Unfortunately, vitamin D supplement use was not well characterized at ARIC visit 2 to be considered as a potential covariate.*

&Education and physical activity were measured at ARIC visit 1

 # for change analysis, covariates measured at visit 2 will be carried forward

Potential effect modifiers: Age, race, sex, eGFR, serum magnesium, DBP SNPs

Data analysis

Visit 2 will serve as baseline for the current analysis. Baseline characteristics (1990-1992) of the study population will be described using means, medians, and proportions across quintiles of 25(OH)D and by race.

Cox proportional hazards models will be used to estimate the hazard ratios (95% confidence intervals) for the association of 25(OH)D and 3-epi-25(OH)D_3 categories with incident CHD. The proportional-hazards assumption will be checked using Schoenfeld residuals and graphic methods (ln[-ln] survival plots). Cox proportional hazards regression will be used to explore associations between vitamin D and risk of incident CHD.

We will use restricted cubic splines to visually depict the associations, and aid in selecting the most appropriate exposure representation. But based on the literature and well as previous ARIC analyses (i.e. vitamin D with risk of incident heart failure and incident stroke), we anticipate the association of vitamin D and CHD to be non-linear with risk only associated with low 25(OH)D at a cutpoint that correlates approximately with the bottom quintile of the distribution [25(OH)D levels <17.2 ng/ml). Therefore our primary analysis will be comparing decreasing quintiles of 25(OH)D, with the highest quintile as reference, with risk for incident CHD.

We will perform two primary models: Model 1 will be adjusted for demographic factors (age, sex, race/field center [overall models] or center [race-stratified models]). Model 2 will be adjusted for variables included in Model 1 + behavioral/socioeconomic variables (education, physical activity, smoking, body-mass index). We will perform two additional analyses: 1) Model 2 + potential

mediators (diabetes, systolic and diastolic blood pressure, use of hypertension medication, total and HDL cholesterol, CRP, and eGFR), and 2) Model 2 + potential mediators + biomarkers related to vitamin D metabolism (calcium, phosphorous, PTH, and magnesium). For the 3-epi-25(OH)D_3 analysis, we will also adjust for season and 25(OH)D.

We will formally test for two-way multiplicative interactions of 25(OH)D by race and *DBP* gene polymorphisms rs7041 and rs4588 using Wald tests and stratified analyses will be presented if there is any evidence for interaction. However, *a priori* we plan to present results overall and stratified by race based on prior studies and inherent interest, regardless if a significant race interaction is present. When testing for interaction, we will compare the lowest quintile of 25(OH)D to the other four higher quintiles to increase statistical power (this cutpoint also corresponds to our anticipated threshold effect seen in other analyses of 25(OH)D and incident stroke).

If there is an interaction present by DBP genotype, we will perform stratified analyses as follows: TT (for rs7041) and CC (for rs4588) will be reference groups [i.e. those predicted to have low DBP and higher bioavailable 25(OH)D for given total 25(OHD)]. However, very few blacks have GG (for rs7041) and AA (for rs4588) genotypes. One approach is to collapse TG/GG and AC/AA groups into two genotypes rather than 3. Another potential approach, as to avoid any assumptions about the genetic model, would be to consider excluding people with GG or AA genotypes from the interaction analysis.

In sensitivity analyses, we will restrict our primary analyses to participants whose self-reported health was good, very good, or excellent at visit 2, and separately, those with normal kidney function.

Furthermore, among the 1700 individuals with repeat vitamin D levels at visit 3, we will consider vitamin D status at both visit 2 and visit 3 time points (categorized as Deficient/Deficient, Deficient/Replete, Replete/Deficient, and Replete/Replete) with CHD risk. Given low power in this subset, we will unlikely be able to perform interactions by race or by SNP. Change in vitamin D levels as a continuous variable (visit 3 levels minus visit 2 levels) will also be considered in exploratory analysis.

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? __X__ 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? __X__ Yes ____ No

YES – we are looking for interaction by polymorphisms rs7041 and rs4588

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)?

Our vitamin D working group also has separate manuscripts for other outcomes as listed below. The author list significantly overlaps author list with these other related proposals for collaboration among our working group and to ensure consistency with the methodology (particularly with DBP SNP analysis), although new authors are included on this proposal as well. This is the first ARIC proposal to evaluate vitamin D and CHD.

#2224: vitamin D and incident heart failure
#2019: vitamin D and incident stroke
#2340 vitamin D and incident diabetes

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
-Vitamin D at visit 2
Michos ARIC Ancillary Study Number 2010.01
-Vitamin D at visit 3
Selvin ARIC Ancillary Study 2009.16
- CysC, CRP

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 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, 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 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.

Reference List

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