ARIC Manuscript Proposal #2440

PC Reviewed: 9/9/14  Status: A  Priority: 2
SC Reviewed: ________  Status: ______  Priority: ____

1.a. Full Title: Serum 25-hydroxyvitamin D and inflammatory biomarkers: The Atherosclerosis Risk in Communities (ARIC) Study

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

2. Writing Group:
   Writing group members: Jeffrey R. Misialek, Elizabeth Selvin, Alvaro Alonso, Erin D. Michos, Casey M. Rebholz, Myron Gross, Danni Li, Pamela L. Lutsey

   Other interested investigators are welcome.

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

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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 analysis will begin immediately. We anticipate completion of the manuscript within 1 year.
4. **Rationale:**

Vitamin D is a fat-soluble vitamin generated naturally through cutaneous synthesis stemming from sun exposure, and it can also be acquired through dietary intake of certain foods and supplements. For research of vitamin D and its biological effects within humans, serum 25-hydroxyvitamin D (25(OH)D) is presently considered the biomarker best suited to examine a person’s vitamin D status. Worldwide, it is estimated that 50% of individuals have insufficient 25(OH)D levels,

and suboptimal 25(OH)D has been associated with increased risk of cardiovascular diseases (CVD) and other conditions.1

Overall, low levels of 25(OH)D are believed to affect CVD risk largely through established CVD risk factors, one of which is inflammation.3–5 Prior observational studies have examined the association between 25(OH)D and various inflammatory biomarkers such as high sensitivity C-reactive protein (hs-CRP),6–16 albumin,7,13 and total white blood cell (WBC) count.8,13 However, the results have been mixed with some finding a significant association of low 25(OH)D with higher levels of biomarkers6–12 while others finding no association.13–16 In addition, randomized controlled trials looking at vitamin D supplementation and its effect on inflammatory biomarkers have faced similar inconsistent results and have suffered from limited follow-up to look at changes in inflammation markers over time.17–22 Very few studies have also specifically looked at whether the associations between 25(OH)D and various inflammatory biomarkers varies by race/ethnicity, possibly due to limited power.

Using ARIC data we propose to explore the cross-sectional association between 25(OH)D and select inflammatory biomarkers in both whites and African Americans at visit 2. We will also examine the association between 25(OH)D and change in select inflammatory biomarkers over a six year period (visit 2 to visit 4). Additionally, we will test whether there is an interaction by race. In exploratory analyses, we will examine whether levels of the C-3 vitamin D3 epimer (3-epi-25(OH)D3) are associated with inflammatory biomarkers and their six year change. The clinical significance of the C-3 vitamin D3 epimer is not known currently.23

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

Hypothesis #1.) Low serum 25(OH)D will be associated with higher inflammatory biomarker levels.

Hypothesis #2.) Low serum 25(OH)D will be associated with greater increases in inflammatory biomarker levels over six years.

Hypothesis #3.) This association will be stronger among whites than blacks.

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 study using visit 2 (1990-1992) and visit 4 (1996-1998) to evaluate the association of 25(OH)D at visit 2 and change in inflammatory biomarker levels over six years.

Inclusion/Exclusion
- Exclusions:
  - Missing at visit 2.
  - Non-whites in Minneapolis and Washington County and non-whites non-African Americans in Forsyth County.
  - Missing 25(OH)D at visit 2.
  - Missing covariate information at visit 2.

Variables
- Primary exposures:
  - Seasonally adjusted serum 25(OH)D since 25(OH)D concentrations vary by season.
  - 3-epi-25(OH)D

- Outcomes: Inflammatory biomarkers
  - Cross-sectional Visit 2 analyses: hs-CRP, gamma-glutamyl transferase (GGT), beta-2 microglobulin (B2M), WBC count/differential, albumin, fibrinogen (measured at visit 1)
  - Change between visit 2 & visit 4: hs-CRP, GGT, B2M.

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

- Other confounders and/or mediators: Age, sex, race, ARIC field center, education, physical activity, smoking status, body mass index (BMI), lipid medications (statins in particular), serum parathyroid hormone (PTH), serum fibroblast growth factor 23 (FGF23), serum calcium, serum phosphorus, and eGFR calculated using the 2012 CKD EPI equation, which incorporates both cystatin C and creatinine.

Analysis
Baseline characteristics of participants will be shown using means and proportions stratified by 25(OH)D quintiles. All variables will be checked for normality, and log transformations will be done if necessary. General linear regression will be used to examine the relationship of 25(OH)D with each of the inflammatory biomarkers at visit 2 and change in inflammatory biomarkers over six years when possible, using either
absolute or relative percent change. Cubic splines will be used to visually depict the associations and aid in selecting the most appropriate representation for modeling these associations. Most likely, 25(OH)D will be modelled as quintiles. The linear model assumptions will be checked.

The following models will be used to analyze the association:

- Model 1: adjustment for age, gender, and race/center (where appropriate).
- Model 2: Model 1 + adjustment for education, physical activity, smoking status, BMI.
- Additional models will adjust for each of these covariates separately: statins, eGFR, PTH, serum calcium, serum phosphorous, FGF23.

Effect modification by age, sex, race, eGFR category (<60, 60-90, >90), and DBP SNPs (rs7041 & rs4588) will be evaluated by including multiplicative terms between the potential effect modifier and 25(OH)D in the models. Stratified results will be presented, as appropriate. Given inherent interest, race-stratified results will be presented regardless of whether there is a statistically significant interaction. All analyses will be repeated with 3-epi-25(OH)D$_3$ as the exposure of interest.

Additional sensitivity analyses:

- Excluding those with a hs-CRP value >10 mg/L, as a value this high may indicate an acute infection.\textsuperscript{26}
- Excluding those on statins since statins may lower CRP levels.
- Restricting to those self-reporting good or excellent health at visit 2.
- Adjusting for non-response at visit 4 using inverse probability weighing for the change in biomarker analysis.

Limitations:

- The lack of temporality when using a cross-sectional design.
- This is an observational study, and some evidence from experimental trials does exist. However, six years of follow-up is typically longer than most trials.
- Some inflammatory biomarkers aren’t measured at both visits, making it impossible to do the change analysis for those biomarkers.

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 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.cscu.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 (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. CRP).

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


