ARIC Manuscript Proposal #2121

PC Reviewed: 5/14/11	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Distribution & correlates of the 25-hydroxyvitamin D_3 C-3 epimer in a population-based sample: The Atherosclerosis Risk in Communities Study (ARIC)

b. Abbreviated Title (Length 26 characters): The vitamin D_3 epimer

2. Writing Group: Pamela L Lutsey, Erin D Michos, John Eckfeldt, Myron Gross, Aaron Folsom. Others welcome

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

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3. Timeline: Data analyses will begin immediately. We anticipate completion of the manuscript within 1 year.

4. Rationale:
Although 25(OH)D is not the active form of vitamin D, it is widely viewed as the superior biomarker for assessing vitamin D status, since in the serum the active form, $1,25(OH)_2D$, is not reflective of body stores as it is tightly regulated by serum calcium, phosphate, and PTH[1, 2]. Suboptimal vitamin D is hypothesized to be associated with elevated risk of numerous conditions, including CVD[3]. *If* a causal relationship is present,

refining 25(OH)D measurement may enhance CVD risk prediction and aid in targeting supplementation.

Understanding of the vitamin D epimer [3-epi-25(OH)D3] is in its infancy. Epimers have identical chemical structures except for a single site of molecular asymmetry (in this case C-3α- vs. C-3β-hydroxy)[4]. The presence of the epimer in neonates is established[5, 6], but only recently has its presence been noted in adults[4, 6-8]. It has been estimated that adults have detectible amounts of this epimer with a range of 4-27% of the total 25(OH)D3 [i.e. 25(OH)D3 + 3-epi-25(OH)D3]. However, existing data is largely from clinical populations of Caucasians, and samples sizes have been small (N <250). The physiological importance (if any) of the vitamin D3 epimer is uncertain. Preliminary work *in vitro* and in rodent models has demonstrated that the vitamin D3 epimer is capable of binding to the vitamin D receptor[9-11], and can influence PTH levels[12, 13].

High-performance liquid chromatography tandem mass spectrometry has emerged as the preferred method for measuring 25(OH)D[5, 8]. However, if the epimer is not explicitly measured, actual levels of vitamin D3 will be overestimated, as epimer values will be counted as vitamin D3 given it's similar structure and mass[4, 8, 14].

Work is needed to delineate several characteristics of the vitamin D3 epimer, including its prevalence in the general population, environmental and behavioral factors which contribute to epimer levels, and its biological function in humans.

5. Main Hypothesis/Study Questions:

The aims of this paper are to describe the distribution and correlates of the vitamin D epimer in a population-based sample of African Americans and whites. We hypothesize that the vitamin D epimer will be present in a large proportion of participants and that it will be positively correlated with vitamin D_3 levels and inversely correlated with PTH.

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

Predominantly cross-sectional, using visit 2 data. We do, however, anticipate reporting the correlation between epimer values measured at visit 2 (n = 14,000) repeated epimer values in a subset (n = 2,000) at visit 3.

Inclusion/Exclusion

Those who are not white or African American, African Americans from the Minnesota and Maryland centers.

Variables

Primary exposure:

The serum 3-epi-25(OH)D3 epimer is being measured concurrently with $25(OH)D_2$ and $25(OH)D_3$ using a high sensitivity mass spectrometer (AB Sciex 5500) at

the University of Minnesota Advanced Instrumental Analysis and Research Laboratory. In this manuscript we intend to describe the vitamin D measurement processes, in detail.

Other variables of interest: Age, sex, race, season of blood draw, education, smoking, physical activity, BMI, waist circumference, serum PTH, calcium, phosphorous, dietary and supplemental vitamin D intake (visit 3).

Data analysis

This manuscript will be largely descriptive, and will likely include many figures. Average levels of 25(OH)D are known to vary dramatically by race, however whether this extends to the vitamin D epimer is unknown. Interactions by race will be formally tested. Regardless of whether interactions are present, we will likely show race-specific results, given inherent interest. We anticipate presenting the following:

1. The distribution of both the vitamin D3 and the vitamin D3 epimer. For the vitamin D3 epimer we will be certain to report the proportions with undetectable levels, and below the limit of quantification. Laboratory coefficients of variation, based on blind duplicate samples, will also be reported.
2. Relationship between the vitamin D3 epimer and age, season of blood draw, vitamin D3, vitamin D2, and PTH. (Figures; likely restricted cubic splines.)
3. General linear regression will be used to describe the relation of the other variables of interest (noted above) and vitamin D3 epimer levels. We will likely categorize the vitamin D epimer variable in order to retain participants who had levels that were either undetectable or below the limit of quantification.
4. Relations of vitamin D and the vitamin D3 epimer in samples measured ~3 years apart. (Correlations, and weighted kappa statistics.)

The major limitation of this analysis is that the serum we are using is from 1990-1992. It is possible that contemporaneous epimer levels (or their proportions relative to 25(OH)D3) in the general population (2013) differ from those measured 20 years ago.

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 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 __ 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 __ X __ 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)?

No related proposals exist.

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

2009.17 (Lutsey PI)
- "Serum vitamin D and cardiovascular disease risk in the biethnic ARIC cohort"

2010.01 (Michos PI)
- "The association of 25-hydroxyvitamin D levels with subclinical cerebrovascular disease and cognitive function in the ARIC Brain MRI substudy"

___ 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, 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.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.

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