ARIC Manuscript Proposal #2695

PC Reviewed: 1/12/16	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Short-term variability of vitamin D-related biomarkers

b. Abbreviated Title (Length 26 characters): Vitamin D marker variability

2. Writing Group:

Writing group members: Pamela L Lutsey, Christina M Parrinello, Jeff Misialek, Andy N Hoofnagle, Clark M Henderson, Thomas J Laha, Erin D Michos, John H Eckfeldt, Elizabeth Selvin, other interested Investigators welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __PL___ **[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: Analyses will begin immediately. Estimated completion Summer 2016.

4. Rationale:

Compelling observational evidence[1-6] that low 25(OH)D is associated with diabetes, cardiovascular disease and cancer has prompted the NIH to make strategic investments in randomized clinical trials to test whether vitamin D3 supplementation reduces the risk of

developing type 2 diabetes (D2D),[7] and heart disease, stroke and cancer (VITAL).[8] Existing research has focused on total 25(OH)D; however, as highlighted in a 2015 U.S. Preventive Services Task Force Recommendation Statement[9], it is possible that constructs of vitamin D other than 25(OH)D may be most relevant to human health.

Other biomarkers on the vitamin D pathway of potential clinical importance include fibroblast growth factor 23 (FGF23), vitamin D binding protein (VDBP), free 25(OH)D, bioavailable 25(OH)D, calcium and phosphorus. While some of these biomarkers are long-established (e.g. 25(OH)D, calcium and phosphorus), others are novel (e.g. VDBP, FGF23, free 25(OH)D, bioavailable 25(OH)D) [10,11] and their variability has not been well-studied. For the more established biomarkers, older evaluations of their variability also require re-examination., due to changes in laboratory methodology. For example, historically most studies measured 25(OH)D using immunoassay methods, while liquid chromatography mass spectrometry (LCMS) is now considered the gold standard.[12] The newer methods typically have less laboratory variability, which would result in lower total variability.

Quantifying the amount of within-person short-term variability inherent in these vitamin D markers will provide insight into the extent of potential misclassification of vitamin D markers in both research and clinical settings. High within-person variability, or random fluctuations around a set point, can lead to false positive results at the individual level, and substantial overestimates of disease prevalence on a population level, especially if the biomarker is only measured once.[13,14] It is also possible that measurements from this study and resulting estimates of short-term variability could be incorporated into regression models, i.e. regression calibration which allows for the simultaneous correction of variation or measurement error in exposure(s) as well as accounts for the relationship of that variation with other risk factors, and could have a substantial effect on estimates of association.[15]

In sum, this study will enable us to quantify the variability of fasting serum 25(OH)D, FGF23, VDBP, free 25(OH)D, bioavailable 25(OH)D, calcium and phosphorus over a mean of 6 weeks. As we await results of randomized clinical trials which will provide insight into whether vitamin D is causally associated with health benefits,[16] it is important to understand the biological variability of these markers. This is especially relevant for 25(OH)D, which has a high prevalence of inadequacy (81% in blacks and 18% in whites according to NHANES), and is frequently measured in clinical settings.[17]

5. Main Hypothesis/Study Questions:

Primary Aim: To quantify the short-term within-person variability of 25(OH)D, FGF23, VDBP, calcium and phosphorus in a sample of approximately 200 ARIC participants who were included in a repeatability study 4-8 weeks after the original visit 5 examination.

Hypotheses: Fibroblast growth factor 23 has the highest within-person variability while 25(OH)D, calcium and phosphorus have the lower within-person variabilities. Within-person variability of VDBP will be intermediate.

Secondary Aim: To quantify the short-term within-person variability of free 25(OH)D and bioavailable 25(OH)D. These biomarkers are calculated from other inputs [i.e. 25(OH)D, VDBP, albumin, rs7041, rs4588], and as such, their variability will be a function of the variabilities of the inputs.

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 and Study Population

We will include the 200 participants (50 from each field center) who attended the visit 5 exam and were asked to return for a repeat visit 4-8 weeks following the initial visit. All laboratory measurements needed for this analysis have been completed.

Statistical Analysis:

We will compare measurements of each marker obtained at the two time points in the ~200 participants.

1) We will examine descriptive statistics and calculate the mean difference between the measures.
2) To visually compare these two measures, we will create scatterplots and Bland-Altman plots.
3) We will compare the total variability of these biomarkers using the within-person coefficient of variation (CV_W), Spearman's rank correlation coefficient (r), intraclass correlation coefficient (ICC), and index of individuality (II) for each biomarker.

To calculate some of the aforementioned measures, we will use one-way analysis of variance to obtain the within-subject and between-subject variances. We will also obtain the inter-assay CV from the lab's internal quality control.

In sensitivity analyses, we will additionally stratify by time between the initial and repeat visit (e.g <4 weeks versus ≥4 weeks) and participant race (black versus white).

Limitations

In stratified analyses we may have limited precision to estimate variability of these biomarkers, depending on the sample size in each stratum. Nonetheless, it will be important to describe potential differences in variability of these markers in subgroups.

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? __X__ Yes (used in the calculation of bioavailable D and free D) **____ 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"? __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)?

2360, "Change in 25-hydroxyvitamin D levels over 3-years and 10-years of follow-up: the ARIC study (Michos senior author; PMID:26509869)
2429 – Short-term variability of markers of hyperglycemia (Parrinello first author; PMID:26503966)
2494 – Vitamin D assay comparison and method development (Hoofnagle/Clark first author; PMID: 26397952; PMID:26453697)
2121 – Distribution and correlates of the vitamin D epimer (Lutsey first author; PMID:25578393)

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* 2006.16)
___ 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.

13. Per Data Use Agreement Addendum for the Use of Linked ARIC CMS Data, approved manuscripts using linked ARIC CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript ____ Yes ____ No.

References

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