ARIC Manuscript Proposal #3219

PC Reviewed: 08/14/18  Status: _____  Priority: 2
SC Reviewed: _________  Status: _____  Priority: _____

1.a. Full Title:
Addressing Disparities in Optimal Vitamin D Levels for Functional Outcomes in Older Adults

b. Abbreviated Title (Length 26 characters): Optimal Vitamin D for Function

2. Writing Group:
   Writing group members: Michelle Shardell, Nancy Chiles Shaffer, Michael Griswold, Erin D. Michos, Pamela Lutsey, Gwen Windham; open to other suggestions from the committee

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

First author: Michelle Shardell
Address: National Institute on Aging,
   3001 S Hanover Street Room NM529
   Baltimore, MD 21225

   Phone: 410.350.7370  Fax: 410-350-7304
   E-mail: michelle.shardell@nih.gov

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).
   Name: Michael Griswold
   Address: Guyton Bldg G551-07
   Univ MS Medical Center
   2500 North State Stree
   Jackson, MS 39216
   Phone: (601) 984-4933  Fax:
   E-mail: mgriswold@umc.edu

3. Timeline: Analysis will begin following proposal approval and compilation of the data. A manuscript will be completed within 6 months after receiving necessary data for this proposal and harmonizing with other participating cohorts.
4. **Rationale:**

Vitamin D relates to better health in observational studies of older adults, but controversy remains regarding consensus target serum 25-hydroxyvitamin D [25(OH)D] concentrations. Debate escalated upon the Institute of Medicine’s 2011 recommended vitamin D intakes to achieve 25(OH)D concentration of 20 ng/mL. The Endocrine Society disagreed, endorsing a minimum target concentration of 30 ng/mL. In commentaries, both groups agreed that recommendations are only for bone health and that evidence was insufficient to endorse target 25(OH)D for a variety of extra-skeletal outcomes including mobility, physical performance, and independence—functional outcomes critical for older adults. Given that ~50% of elderly have 25(OH)D levels ranging between 20 and 30 ng/mL, this debate is crucial to resolve. Furthermore, although current 25(OH)D recommendations focus on bone health in old age, it is unknown if achieving recommended values in middle age relates to health outcomes in old age.

A racial/ethnic paradox exists where black older adults have lower 25(OH)D concentrations and more mobility disability, but fracture less than whites; thus one target 25(OH)D may not fit all. 25(OH)D health effects may differ by race; the few vitamin D trials in older African Americans conflict; racial disparities of 25(OH)D and functional outcomes are equivocal; and disparities may have genetic origins. Two single nucleotide polymorphisms in the vitamin D binding protein (VDBP) gene, rs4588 and rs7041, have population stratification whereby genotype prevalence differs by race. An initial theory was that variants more common in blacks may lead to lower VDBP levels with lower affinity to 25(OH)D, producing bioavailable 25(OH)D (available for biological action) at similar levels as whites despite lower total 25(OH)D—the controversial “free 25(OH)D hypothesis.” However, later work disputed this theory by noting that the racial differences, which were due to VDBP assay, disappear when VDBP is measured by mass spectrometry. Even if VDBP does not explain the paradox, it may still be relevant as demonstrated by a recent study on vitamin D and coronary heart disease events. Furthermore, the U.S. Preventive Services Task Force recently questioned if bioavailable 25(OH)D is perhaps a better vitamin D status indicator than total 25(OH)D.

To fill the evidence gap on vitamin D and function, the NIH had issued RFA-AG-14-001 “Dose Response and Efficacy Studies on the Effects of Vitamin D Supplementation on Functional Outcomes in Elderly.” Motivations are: observational studies “do not prove …causality” and “confounding due to…factors such as…skin color and gender.” However, 25(OH)D targets are needed to inform design and analysis of vitamin D trials. Unlike supplementation, 25(OH)D cannot be directly randomized. Older adults differ in ability to convert vitamin D from sunlight exposure and intake into 25(OH)D by sex, race, and genetic variants, thus observational studies are key to identify 25(OH)D targets for functional outcomes. Targets can inform design of vitamin D trials for extra-skeletal outcomes, and hence recommendations, by identifying adults who may benefit from vitamin D supplement. Baseline targets may also serve as useful subgroups in secondary analyses of vitamin D trials. Indeed, a vitamin D trial on prevention of falls in the elderly is currently underway (STURDY, PI, Larry Appel, [https://www.ncbi.nlm.nih.gov/pubmed/28472285](https://www.ncbi.nlm.nih.gov/pubmed/28472285)). Lastly, it is important to empirically test
whether achieving 25(OH)D targets in middle age is predictive of better physical function in old age.

Our preliminary meta-analysis of 4 NIH-supported cohorts (AGES-Reykjavik; Health Aging, and Body Composition [HABC]; InCHIANTI; Study of Osteoporotic Fractures) of older women aged ≥65 y identified a threshold 25(OH)D concentration of 24.5 ng/mL predicting incident slow gait (<0.8 m/sec) over three years. This is proof of concept that 20 ng/mL may be too low for optimal function, but 30 ng/mL may be higher than necessary to benefit from supplements. Only HABC had measured 25(OH)D in non-whites; thus, we aim to pool cohorts of racially diverse older adults to identify sex- and race-specific 25(OH)D targets for function.

Our overarching plan is to pool data from HABC, the Women’s Health and Aging Study (WHAS), Cardiovascular Health Study (CHS), and Osteoporotic Fractures in Men Study (MrOS) to identify and internally cross-validate sex- and race-specific 25(OH)D thresholds in older adults. Next, we plan to externally validate the thresholds using data from ARIC and the Baltimore Longitudinal Study of Aging (BLSA). ARIC and BLSA include middle aged as well as older adults and long follow-up times allowing us to assess whether the 25(OH)D thresholds in middle age are predictive of slow gait and mobility disability in old age. If not, then ARIC and BLSA can be leveraged to identify relevant 25(OH)D thresholds in middle age that may differ from those in older adults. Notably, ARIC and BLSA use the same protocol to assess physical function.

5. Main Hypothesis/Study Questions:

The central hypotheses of this proposed project are, for both older men and women, and by race:

- **H1.** Optimal 25(OH)D targets for physical function exist where poor physical function is less likely in old age (age≥65 y), and these 25(OH)D targets in middle age predict functional status in old age.
- **H2.** The optimal 25(OH)D targets for physical function differ by sex and race.
- **H3.** Differences in 25(OH)D targets for physical function are due, in part, by rs4588 and rs7041.

**Aim 1:** To identify and validate sex- and race-specific 25(OH)D targets for functional outcomes (shown in Table 2 below) and determine whether 225(OH)D targets in middle relate to physical function in old age.

Approach: **Sex- and race-specific** analyses will **identify 25(OH)D target(s)** using 2/3 of data; **internally validate identified 25(OH)D target(s)** in the other 1/3 data (using HABC, WHAS, CHS, and MrOS), and **externally validate identified 25(OH)D targets using ARIC and BLSA.** (See Table 1 for cohorts and their roles and Table 2 for outcomes and other variables).

**Aim 2:** To determine if sex-, race-specific 25(OH)D targets differ by rs7041 and rs4588 variants.

Approach: Assess 25(OH)D-by-genetic variant interaction terms (identify and internally cross-validate using HABC, WHAS, CHS, and MrOS, then externally cross-validate using ARIC and BLSA).
Aim 3: To estimate bioavailable 25(OH)D using genetic variants (rs7041 and rs4588) and vitamin D binding protein to determine if target bioavailable 25(OH)D differs by race.

Approach: Pool data across races and assess bioavailable 25(OH)D by race interaction terms (identify and internally cross-validate using HABC, WHAS, CHS, and MrOS, then externally cross-validate using ARIC and BLSA).

### Table 1. Cohorts and their role

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Sexes</th>
<th>Races</th>
<th>Role in project</th>
</tr>
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<tbody>
<tr>
<td>ARIC</td>
<td>F/M</td>
<td>B, W</td>
<td>External validation</td>
</tr>
<tr>
<td>BLSA</td>
<td>F/M</td>
<td>B, W, O</td>
<td>External validation</td>
</tr>
<tr>
<td>CHS</td>
<td>F/M</td>
<td>B, W</td>
<td>Identification/Internal validation</td>
</tr>
<tr>
<td>HABC</td>
<td>F/M</td>
<td>B, W</td>
<td>Identification/Internal validation</td>
</tr>
<tr>
<td>MrOS</td>
<td>M</td>
<td>B, W, O</td>
<td>Identification/Internal validation</td>
</tr>
<tr>
<td>WHAS</td>
<td>F</td>
<td>B, W</td>
<td>Identification/Internal validation</td>
</tr>
</tbody>
</table>

F=female, M=male, B=Black, W=White, O=Other 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).

Overall approach is to identify/externally cross-validate 25(OH)D targets for functional outcomes in older adults. We identify/validate 25(OH)D targets using modern statistical methods in 4 racially diverse cohorts of community-dwelling older adults: Cardiovascular Health Study (CHS), Health, Aging, & Body Composition (HABC), Osteoporotic Fractures in Men (MrOs), and Women’s Health & Aging Study (WHAS). Then we will externally cross-validate using Atherosclerosis Risk in Communities (ARIC) and Baltimore Longitudinal Studies of Aging (BLSA). Exploratory analysis (scatterplots, frequencies) will find potential problems (outliers, errors, sparsity) and address them (transformations, analysis with/without outliers).

### Table 2. Relevant measures in ARIC

<table>
<thead>
<tr>
<th>Measure</th>
<th>Notes:</th>
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<tbody>
<tr>
<td><strong>FUNCTIONAL OUTCOMES (follow-up) V4 or V5</strong></td>
<td></td>
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<tr>
<td>Gait Speed (m/sec) (V5)</td>
<td>Primary outcome. Measured: 4m or 6m</td>
</tr>
<tr>
<td>Chair Stand (s) (V5)</td>
<td>Secondary outcome. Measured: time to complete 10 chair stands</td>
</tr>
<tr>
<td>Mobility, Stair Climb Difficulty (V4)</td>
<td>Secondary outcome. Self-reported: ¼ mile, climb up 10 steps</td>
</tr>
<tr>
<td>Grip Strength (kg) (V5)</td>
<td>Secondary outcome. Dynamometer: maximum of preferred hand</td>
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<tr>
<td><strong>EXPOSURE (baseline-V2, primary analysis; V3, secondary analysis)</strong></td>
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<tr>
<td>25-hydroxy-vitamin D [25(OH)D]</td>
<td>liquid chromatography-tandem mass spectroscopy (LC-MS/MS) (will be calibrated with other studies that used RIA by applying published equations). (Seasonal calibration will not be used in primary analysis in order to match methods in clinical settings, but will be used in secondary analysis to assess robustness of results. Rather, we will perform a secondary instrumental variable analysis using season as the instrument. We will also perform a secondary subgroup analysis by season to determine whether performance of thresholds depends on season.)</td>
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<tr>
<td><em><em>STRATIFICATION FACTORS (baseline</em>)</em>*</td>
<td></td>
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<tr>
<td>Demographics: Sex, Race</td>
<td>Men &amp; Women; White &amp; Black</td>
</tr>
<tr>
<td>Genetic variants (rs7041 and rs4588)</td>
<td>Variants on the coding region of vitamin D binding protein</td>
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<tr>
<td><em><em>CONFOUNDERS (baseline</em>)</em>*</td>
<td></td>
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</table>
Analytic plan: The analytic plan has three stages: identify, internally cross-validate, and externally validate sex-, race-specific target 25(OH)D. Stage 1: identify 25(OH)D targets in a random 2/3 data sample (“Training” set) using classification and regression trees (CART), a machine-learning method that partitions participants into predictor-defined groups with similar outcomes. Stage 2: validate targets in other 1/3 of data (“Testing” set). Stage 3: validate the targets using participants from different cohort studies and determine whether the 25(OH)D thresholds in middle age predict slow gait and mobility disability in old age.

Stage 1: Identify 25(OH)D Targets (CHS, HABC, MrOS, WHAS). In a random 2/3 pooled sex-, race-stratified data sample (CHS, HABC, MrOS, and WHAS), we will use CART to identify 25(OH)D targets to predict physical function. We adjust for confounders via standardization, by weighting observations by inverse propensity score (probability of 25(OH)D value given covariates). To mitigate model misspecification, we estimate 25(OH)D densities by multiple machine-learning methods26 (including elastic net, random forest, Bayesian GLM, boosted regression, and multivariate adaptive regression splines) and combine using the SuperLearner algorithm in R statistical software. To address missing data, loss to follow-up, and selective survival, we use SuperLearner and covariates to predict missingness and compute the inverse probability of being observed (non-missing). Our previous work published in Biostatistics demonstrated the validity of inverse probability weighting to handle missingness and selective survival.27 To mitigate differing 25(OH)D assays (DiaSorin RIA vs LC-MS/MS), we calibrate RIA measures to LC-MS/MS using: √LC-MS/MS = 0.9542 + 0.8621×√RIA in Chen et al.28 We use calibration equations for differing assessment. Studenski et al29 converted 6m gait speed to 4m speed: 4m speed = -0.0341 + 6m speed×0.9816 (R²=0.93) (primary outcome).

Stage 2: Internally Cross-Validate 25(OH)D Targets (CHS, HABC, MrOS, WHAS) In the remaining 1/3 pooled sex-, race-stratified data sample (CHS, MABC, MrOS, and WHAS), we use meta-analysis to combine study-specific marginal structural models to estimate effects accounting for confounding, missingness, loss to follow-up, and selective survival.27 We use machine-learning to reduce model misspecification. We will estimate random-effects models to address and quantify study heterogeneity (e.g., Higgins’ I²). We successfully used this approach in another project.30 Secondary instrumental variable (IV) meta-analysis31 will address potential unmeasured confounding. We use month of blood draw as an IV because it unlikely directly affects outcomes years later, avoids survivor bias (unlike Mendelian randomization),32 and affects 25(OH)D owing to the seasonality of 25(OH)D. We will additionally validate the thresholds by performing a subgroup analysis, notably by season of 25(OH)D assessment and genetic variants (rs4588 and rs7041), and testing the interaction as estimated by meta-analysis.
Stage 3: Externally Validate 25(OH)D Targets (ARIC, BLSA) In separate cohorts (ARIC and BLSA), we will determine whether the sex- and race-specific 25(OH)D thresholds predict slow gait speed and mobility disability in older age, and whether predictive performance depends on genetic variants (rs4588 and rs7041) and age of vitamin D measure (middle age: < 55 y vs older age: ≥ 55 y). These two cohorts will be analyzed separately; marginal structural models will be used as describe to address confounding, missing data, loss to follow up, and selective survival. In ARIC, we will primarily use 25(OH)D measured at V2 (ages 46-70 y) and physical performance assessed at V5 (ages 66-90 y). As a secondary analysis, we will assess the sub-sample with 25(OH)D measured at V3 (n=1700 from the Brain MRI subset in Jackson and Forsyth County) to increase the maximum age of 25(OH)D assessment. We will account for factors that may influence selection in to the Brain MRA study in this analysis. If the performance of 25(OH)D appears to be age-dependent, then results from this project will be used to motivate a follow-up project focused on identifying 25(OH)D targets for middle-aged adults for favorable function in old age.

Strengths of this external validation include 1) the ability to rule out reverse causality because function is measured much later than 25(OH)D, 2) the large sample size with rich set of measures for confounder control, and 3) availability of novel measures of vitamin D status. A major complication in ARIC is that physical performance measures are assessed ~20 years after 25(OH)D was assessed, and 25(OH)D can change substantially within a year let alone over multiple decades. However, we note that 25(OH)D guidelines that were established to prevent adverse skeletal outcomes in old age are often used to interpret 25(OH)D results for middle-aged adults in clinical settings. ARIC provides a rare opportunity to validate a 25(OH)D guideline for aging-related poor physical function in a cohort of mostly middle-aged adults. Furthermore, as noted in Table 2, secondary analysis will use seasonally adjusted 25(OH)D to assess robustness of results. This will be carried out using the race- and sex-specific residuals approach often applied to ARIC studies.

However, to additionally obtain an external validation using outcomes assessed closer in time to 25(OH)D assessment, we will analyze self-reported function at V4. Specifically, mobility disability (difficulty walking ¼ mile and difficulty climbing 10 steps) at V4 will be used to determine shorter term predictive performance of 25(OH)D thresholds for closer comparison to Stage 1 and Stage 2 analysis.

7.a. Will the data be used for non-CVD analysis in this manuscript? _X_ Yes  ____ 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
Yes – we will look at interactions with vitamin D binding protein 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)?

Aric proposal # 2919 on kidney function and physical function. We control for kidney function (eGFR estimated using creatinine), but kidney function is not one of our aims. Plus, key authors on that proposal are involved in the present proposal.

Aric proposal # 2304 on diabetes and physical function. We control for diabetes, but diabetes is not one of our aims. Plus, key authors on that proposal are involved in the present proposal.

Aric proposal # 2752 on physical activity, vitamin D, and incident cardiovascular disease. We control for physical activity and prevalent cardiovascular disease, but these are not part of our study aims. Plus, key authors on that proposal are involved in the present proposal.

Aric proposal #2502 on vitamin D biomarkers, race, and later life neuropsych testing performance and physical performance. Cognition is not part of our outcomes; however we do consider physical performance. An important differences is that we aim to identify and validate sex- and race-specific candidate optimal 25(OH)D thresholds for favorable physical performance. Furthermore, we additionally assess self-reported mobility disability at V4. We also note that key authors on that proposal are involved in the present proposal.

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, 2010.01__)
B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)*_2009.16 (control variables, eg, CRP), 2008.06 (secondary analysis to assess change in function))

2009.17 (Lutsey PI) - “Serum vitamin D and cardiovascular disease risk in the biethnic ARIC cohort” – measured 25(OH)D in whole ARIC cohort at visit 2

2009.16 (Selvin, PI) - “Short-term markers of glycemia and long-term outcomes” - Biomarkers which may be confounders and/or effect modifiers in the present analysis were measured as part of this grant (e.g. CRP).

2010.01 (Michos, PI) “The association of 25-hydroxyvitamin D levels with subclinical cerebrovascular disease and cognitive function in the ARIC Brain MRI substudy” – measured 25(OH)D in a subset at visit 3

2008.06 (Coresh, PI) “Prediction of cognitive impairment from mid-life vascular risk factors and markers: The ARIC Neurocognitive Study (ARIC-NCS)” – measured functional measures at V6 to allow assessment of change in function.

*ancillary studies are listed by number at [http://www.cscc.unc.edu/aric/forms/](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/](http://publicaccess.nih.gov/) are posted in [http://www.cscc.unc.edu/aric/index.php](http://www.cscc.unc.edu/aric/index.php), under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit_process_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to PubMed central.

References


