1.a. Full Title:
Vitamin D and Neurocognitive Decline: the ARIC Brain ancillary study

b. Abbreviated Title (Length 26 characters):
Vitamin D and cognition

2. Writing Group:
Writing group members:

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Alvaro Alonso         University of Minnesota
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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]

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3. **Timeline:**

The Vitamin D lab assays are currently being run from visit 3 serum. Anticipate lab work to be completed in early fall 2012 (estimated Nov 1). Plan to work on this manuscript late fall/early winter 2012/2013.

4. **Rationale:**

Low serum 25-hydroxyvitamin D [25(OH)D] levels are associated with cardiovascular disease and its risk factors such as hypertension and diabetes. Emerging data suggest that vitamin D may be important for cognitive functioning and protective against neurovascular injury. In addition, low 25(OH)D levels have been linked to neurodegenerative disorders such as Alzheimer’s Disease. Vitamin D Receptors (VDR) located in the human cortex and hippocampus are important for cognitive functioning, and VDR down regulation in these key areas has been associated with Alzheimer’s.¹

The literature regarding the cross-sectional association of 25(OH)D levels and performance on neurocognitive testing has been conflicting. Most, but not all, cross-sectional studies have found an association of 25(OH)D levels with at least one marker of cognitive function.²⁻¹¹ These studies were limited by their cross-sectional design, and direction of association cannot be determined.

There have only been a few published prospective studies of vitamin D and cognitive decline.¹²⁻¹⁵ These studies were limited to only elderly white populations, but still give hope for the potential of vitamin D treatment for the prevention of dementia.

Our proposed paper would replicate this finding in relation to vitamin D levels measured in a younger (middle-aged) population, of which approximately half are of black race and half are women, using more extensive cognitive and neurologic testing, and over much longer (12-14 years) followup.

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

**General hypothesis:** Low 25(OH)D levels will be associated with poorer cognitive performance, both with tests such as word fluency (WF) and Digit Symbol Substitution (DSS), reflective primarily of vascular disease, and with tests of memory such as Delayed Word Recall (DWR), more reflective of Alzheimer’s disease. Specific study questions:

1) To determine whether 25(OH)D levels are independently associated with cognitive status (assessed cross-sectionally at visit 3) and with decline in cognitive function over ~12 years of followup (measured at 3 time points between 1992 and 2004 using visit 3 as the baseline) in 3 key cognitive domains (memory, executive function, and processing speed).

2) To determine the association of 25(OH)D with specific neurocognitive domains (comprehensively assessed at the 2004-2006 ARIC Brain visit including global mental status, memory, language, psychomotor speed, executive function, mood, fine and gross motor skills)
3): To determine whether the association of vitamin D with WMH progression and incident infarcts partially explains cognitive decline over followup.

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).

Participants: The ARIC Brain MRI ancillary study (R01 HL70825; PI Mosley) includes a subset of ARIC participants age ≥55 years from the Forsyth County and Jackson sites that were invited for a cerebral MRI and cognitive testing during the first two years of ARIC visit 3 (1993-1994) (n=1949, 60% women and 50% blacks). Inclusion/exclusion criteria for that ancillary study have previously been published. 

A subsequent ancillary grant (1R01NS072243-01; PI Michos) newly measured 25(OH)D, PTH, calcium, and phosphate levels from visit 3 stored blood from [n=1949] participants of ARIC Brain MRI ancillary study.

<table>
<thead>
<tr>
<th>ARIC Study Timeline</th>
<th>ARIC Brain</th>
<th>ARIC Brain</th>
</tr>
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<tbody>
<tr>
<td>Exam</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Followup year</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Number, N</td>
<td>15792</td>
<td>14348</td>
</tr>
<tr>
<td>Age range, y</td>
<td>45-64</td>
<td>48-67</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Incident clinical events (i.e. stroke)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Cognitive Testing</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Brain MRI, n</td>
<td>1949 (1878 free of stroke/TIA)</td>
<td>1134</td>
</tr>
</tbody>
</table>

Cognitive performance was measured by DWR, DSS, and WF at ARIC visits 2, 3 (in a subset) and 4, with an average of 3 year interval separation, and repeated again in 2004-2006 (approximately 14 years after the first cognitive test) in the ARIC Brain ancillary study. At the 2004-2006 ARIC Brain visit, participants also underwent a more comprehensive neuropsychological battery assessing 7 domains. The above timeline outlines when the cognitive testing visits took place.

Analysis:

(1) For each analysis, 25(OH)D levels will be analyzed both as a continuous variable as well as in categorical thresholds of deficient (<15 ng/ml), insufficient (15-30
ng/ml), and sufficient (>30 ng/ml). Prior studies have suggested a non-linear relationship of 25(OH)D with CVD risk, with increased risk primarily limited to the levels below <15 ng/ml). A non-linear spline will also be explored.

(2) The cross-sectional association of 25(OH)D levels measured at visit 3 with cognitive test scores obtained at ARIC visit 3 will be performed using multivariable linear regression. Three measures of cognition will be evaluated: the digit symbol substitution test (DSS); the delayed word recall test (DWR); and the word fluency test (WF).

Cognitive test scores will also be dichotomized at the 10th percentile of the sample as “low” and “not low performance”, as done previously. Multivariate logistic regression will be performed to determine the cross-sectional association of vitamin D deficiency with low cognitive performance.

(3) The association of 25(OH)D with cognitive decline using trends in 3 cognitive test scores (DSS, DWR, WF) over ARIC followup will be studied. There are already several published ARIC papers evaluating the association of CVD risk factors, biomarkers, and clinical factors with cognitive change over 14 years of followup. We intend to use similar methods to test the association of the interaction of vitamin D over time in evaluating cognitive change using a random-effects linear model. We will exclude for confounding neurological conditions (including medication use that might greatly impact cognition such as benzos or antipsychotics), and multivariate models will have adjustments for demographics and potentially confounding covariates.

For the longitudinal analysis, we will use the cognitive assessment at visit 3 (rather than visit 2) as our baseline, since this is the visit where the exposure variable, 25(OH)D levels, is measured. This gives a slightly shorter followup time for the cognitive decline assessment (~12 years rather than the 14 years in the other ARIC papers).

(4) The association of 25(OH)D, measured at visit 3, with more comprehensive neuropsychological cognitive tests at ARIC Brain visit 2004-2006 will also be studied. This testing involves 7 domains and symptoms/function described in the supplemental table below (see page 10). Linear regression will also be used for these analyses, using cognitive scores as well as Z-scores per domain as dependent variables in separate models.

(5) Regression models will include both limited covariate adjustment for age, race-center, gender and comprehensive multivariate adjustment including traditional CVD risk factors, lifestyle factors, SES factors including education, and season of lab draw. Comprehensive adjustment is more important for item #2 above, where a cognitive performance is assessed at a single point in time than for item #3, where change in performance is the outcome.

(6) Additional models will also include PTH, Calcium, Phosphate in the model to see if the association of 25(OH)D with neurocognitive decline is still significant after taking into account these related biomarkers.
Additional models will also include adjustment for white matter hyperintensities (scored from Brain MRIs) to see if the association of vitamin D with cognitive decline is still significant after considering subclinical cerebrovascular disease. Note that cerebrovascular pathology may be a mediator in the causal pathway between low vitamin D status and neurocognitive decline.

Interaction testing by race/ethnicity will be performed. Stratified analyses will be conducted by race group.

[Note that while ARIC-NCS will repeat these cognitive measures, ARIC-NCS is not anticipated to be completed in time for this paper proposal. This paper proposal will assess cognitive trends through the year 2006. The association of vitamin D with brain/cognitive markers measured in ARIC-NCS including brain MRI pathology will be the subject of a separate paper proposal.]

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?  ____ 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”?  ____ 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.csc.unc.edu/ARIC/search.php  
   ____ X____ Yes  _______ No

As 25(OH)D levels are newly being measured as part of my funded ancillary grant (and not previously available in ARIC), there is no overlap.
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)?

The most closely related manuscript studying cognitive decline in only the ARIC Brain MRI subset16 was led by Dr. Mosley, who is a key investigator on my grant and a co-author.

Using the larger ARIC cohort, there has been various papers17-23 studying various biomarkers and clinical markers with cognitive decline over 14-yr followup. We will use similar methodology in these analyses for consistency. Writing panel members Drs. Knopman, Mosley, Gottesman, and Sharrett have been authors/co-authors on some of these prior ARIC cognitive papers.

There is another funded ARIC ancillary grant (PI Lutsey) that will measure 25(OH)D in the entire ARIC cohort to study the role of vitamin D with CVD outcomes. Dr Lutsey is a co-author on this proposal to ensure no overlap.

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? X Yes __ No

(1) Study PI: Erin Michos, Johns Hopkins, Funded grant NIH/NINDS: 1R01NS072243-01
ARIC ancillary #: 2010.01
Vitamin D, cerebrovascular risk, and neurocognitive decline: ARIC Brain MRI study

(2) Study PI: Thomas Mosley, University of Mississippi, Funded grant NIH/NINDS R01 HL70825; Mosley PI)
ARIC ancillary 1999.01

11.b. If yes, is the proposal
X A. primarily the result of an ancillary study (list number* 2010.0________)

___ 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
Literature Cited


<table>
<thead>
<tr>
<th>Domain</th>
<th>Measure</th>
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<tbody>
<tr>
<td>Verbal memory</td>
<td>Delayed Word Recall Test (DWR)(^{a,b,c})</td>
</tr>
<tr>
<td></td>
<td>Short and Long Delay Recognition</td>
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<tr>
<td></td>
<td>Wechsler Memory Scale-Revised(^{b,c})</td>
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<tr>
<td></td>
<td>Logical Memory I &amp; II</td>
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<tr>
<td>Non-verbal Memory</td>
<td>Wechsler Memory Scale-III(^{b,c})</td>
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<tr>
<td></td>
<td>Incidental Learning</td>
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<tr>
<td>Language Function</td>
<td>Category Fluency (animals)(^{b,c})</td>
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<tr>
<td></td>
<td>Boston Naming Test (15 item)(^{c})</td>
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<tr>
<td>Visuospatial</td>
<td>Clock Reading(^{c})</td>
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<tr>
<td></td>
<td>Intersecting Pentagons(^{c})</td>
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<tr>
<td>Attention</td>
<td>Trails A(^{b,c})</td>
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<tr>
<td></td>
<td>Digit Span Backward(^{c})</td>
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<tr>
<td>Executive Function</td>
<td>Digit Symbol Substitution Test (DSS)(^{a,b,c})</td>
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<tr>
<td></td>
<td>Word Fluency (WF)(^{a,b,c})</td>
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<tr>
<td></td>
<td>Trails B(^{b,c})</td>
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<tr>
<td>Premorbid IQ</td>
<td>Wide Range Achievement Test 4(^{c})</td>
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<tr>
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<td>Word Reading</td>
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<tr>
<td>Motor</td>
<td>Finger Tapping(^{b,c})</td>
</tr>
<tr>
<td>Depression</td>
<td>CES-D (11 item)(^{b,c})</td>
</tr>
</tbody>
</table>

**Stage II Assessment**

- Clinical Dementia Rating Scale\(^{c}\)
- Neurological Exam\(^{c}\)
- Folstein Mini-Mental State Exam\(^{b,c}\)
- Timed Gait\(^{b,c}\)
- Neuropsychiatric Inventory Questionnaire\(^{c}\)
- Hachinski Ischemic Scale\(^{c}\)
- NIH Stroke Scale\(^{b,c}\)
- Uniform Parkinson's Disease Rating Scale\(^{b,c}\)

a = collected in all participants at Visits 2 & 4, b = measured in ARIC Brain MRI Study, c = to be measured in ARIC-NCS