ARIC Manuscript Proposal #2752

PC Reviewed: 5/10/16   Status: **A**   Priority: **2**

SC Reviewed: _____   Status: _____   Priority: ____

1. **Full Title: Physical activity, Vitamin D, and Incident Cardiovascular Disease in the Atherosclerosis Risk in Communities (ARIC) Study**

   Abbreviated Title (Length 26 characters):   Vitamin D, PA, and ASCVD

2. **Writing Group:**

   Kathleen (Katie) Chin (1st author)   Johns Hopkins
   Di Zhao (second author, analyst)   Johns Hopkins
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   B. Gwen Windham   University of Mississippi
   Eliseo Guallar   Johns Hopkins
   Erin D. Michos (senior author)   Johns Hopkins
   Other non-Hopkins authors welcome

A note about the multiple Hopkins authors: Erin Michos designed the concept and will guide project as senior author. Katie Chin, a medical student trainee, wrote the proposal and will take the lead as first author in writing up the manuscript. Di Zhao is the analyst. Eliseo Guallar is Di Zhao's mentor and will oversee the statistical analyses. Seth Martin is an expert in physical activity and activity trackers, who agreed to co-mentor the student Katie Chin. Roberta Florido is the lead author of a similar approved ARIC proposal regarding family history and physical activity with incident ASCVD and will contribute her expertise given similar methodology to her prior proposal.

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

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3. **Timeline:** Hopkins medical student Katie Chin will be working on this project during the summer of 2016 as part of the Hopkins Scholarly Concentration Program, beginning June 10th. We hope to have a manuscript completed by the end of the summer.

4. **Rationale:**

Low levels of vitamin D are a widespread problem, with an estimated 1 billion people worldwide having either vitamin D deficiency or insufficiency.[1] In the United States alone, the National Health and Nutrition Examination Survey found that over 40% of the American population were deficient in serum 25-hydroxyvitamin D [25(OH)D]; this was even more prevalent among certain racial/ethnic groups, including blacks and Hispanics.[2] While there has been debate as to the optimal levels of 25(OH)D, vitamin D deficiency is generally defined as having 25(OH)D levels of less than 20 ng/ml.[1,3]

Although vitamin D is most well-known for its role in calcium homeostasis, there is growing epidemiological evidence linking vitamin D deficiency with increased atherosclerotic cardiovascular disease (ASCVD) risk [which includes both coronary heart disease (CHD) and stroke],[4-6] Heart Failure (HF),[7] and mortality.[8] Risk may vary across racial groups, with several studies reporting a lack of association with vitamin D deficiency and incident ASCVD and HF events in blacks.[4-7] While the mechanisms are not fully elucidated, it is thought that vitamin D primarily affects known risk factors for cardiovascular disease, such as hypertension and diabetes. In fact, biological studies have supported this stance by showing that vitamin D negatively regulates the renin-aldosterone-angiotensin pathway, affects insulin secretion, and is also involved in several inflammatory processes.[9,10]

Associations of physical activity (PA) with improved cardiovascular outcomes are well established. A systematic review of over 30 prospective cohort studies found that high levels of PA were associated with a 30–35% lower risk for developing CHD compared to low levels of activity across age, sex, and racial/ethnic groups.[11] Increasing PA is one of the most commonly recommended methods to prevent ASCVD, and the American Heart Association/American College of Cardiology guidelines recommend at least 150 minutes a week of moderate to vigorous physical activity.[12]

While vitamin D deficiency is thought to be associated with low PA,[13-17] this relationship has not been fully explored. Evaluating the association between vitamin D and PA is complicated by confounding factors, including the amount of time spent outdoors and sunlight exposure.[13] However, certain studies have found this relationship to persist even when the majority of PA is reported to be indoors or even after accounting for amount of sunlight exposure.[16,17] Furthermore, evidence suggests that vitamin D may have a modifying effect on physical performance and activity itself, with biological and clinical studies suggesting that vitamin D plays a role in muscle function and balance, in addition to its known role in bone metabolism.[18-20] There is evidence for a positive dose-response relationship between PA and vitamin D, but further studies need to be done with larger and more diverse patient populations.[15] Additionally, despite the strong evidence that PA and vitamin D each individually affect cardiovascular outcomes, little research has been conducted simultaneously examining these variables in regards

to ASCVD and HF outcomes. As both vitamin D and PA levels are potentially modifiable in individuals, this information can help with future prevention strategies regarding ASCVD risk.

In this proposal, we will examine the interrelation between PA and 25(OH)D, and also evaluate low vitamin D as a potential effect modifier of the relation between PA and cardiovascular outcomes.

5. Main Hypothesis/Study Questions:
a. Aims:

Aim 1: The association of PA with 25(OH)D levels

1a: We will first analyze the cross-sectional relationship between PA levels (measured at ARIC visit 1) with serum 25(OH)D levels (measured at ARIC visit 2), to determine if individuals with certain levels of PA are most at risk for vitamin D deficiency. Our models will model PA and vitamin D in several ways, in order to document the pattern of association (i.e. continuous levels, according to clinical cutpoints, and using restricted cubic splines to identify threshold effects).
- We hypothesize the individuals with poor or intermediate PA levels (compared to those with AHA recommended levels) will have lower 25(OH)D levels and greater prevalence of vitamin D deficiency even after adjustment for BMI and confounding lifestyle factors.
- We hypothesize that the relationship between PA and 25(OH)D levels will be roughly linear for PA levels up to the recommended PA levels (≥150 min/week), after which 25(OH)D levels will plateau with further increases in PA levels.

1b: We will evaluate whether changes in PA level over time (between ARIC visits 1 and 3) influence 25(OH)D levels at ARIC visit 2.
- We hypothesize that those who increase their PA levels over time (from poor/intermediate levels at visit 1 to recommended levels at visit 3) will have higher 25(OH)D levels and reduced prevalence of vitamin D deficiency at visit 2 than those who remain with either poor or intermediate PA levels at both visits

Aim 2: Effect modification by 25(OH)D status of the association of PA levels and ASCVD/HF risk
We will evaluate whether lower levels of PA (compared to higher levels) are associated with a relatively higher risk of ASCVD and HF outcomes among individuals with vitamin D deficiency than among individuals with optimal 25(OH)D levels (i.e. effect modification by vitamin D status of the association of PA with ASCVD/HF risk).
- We hypothesize that individuals with low levels of both vitamin D and PA will have higher rates of ASCVD/HF events compared to those with low levels of only one risk marker and compared to those with adequate levels of both.

Aim 3) We will evaluate whether the above associations differ across race, sex, and age.
- We hypothesize that associations between PA and 25(OH)D levels will be qualitatively stronger among whites compared to 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)

a. **Study Design:** This study design is two-fold: We will evaluate (1) the non-concurrent cross-sectional distribution of 25(OH)D concentrations by PA levels and (2) the prospective association of PA with ASCVD/HF events among individuals with and without vitamin D deficiency. We will assess these relationships using PA data (obtained at visit 1 and 3), vitamin D data (obtained at visit 2), and incident ASCVD/HF events occurring after visit 2.

b. **Study Population:** We will include all ARIC participants with complete data on PA at visit 1, complete data on 25(OH)D levels at visit 2, and not missing any of the key covariates of interest found in our primary model 2. We will exclude participants who were non-black or non-white, as well as blacks from MN and MD sites due to small numbers. We will exclude participants with prevalent CHD, stroke, HF at (or prior to) visit 2.

c. **Exposures:**

1) Physical Activity:

   PA was assessed through a modified Baecke questionnaire for sports (intentional exercise) at ARIC visits 1 and 3. Similar to prior ARIC studies, we will convert the Baecke sports indices into minutes per week of moderate or vigorous exercise. We will define moderate and vigorous activities according to the metabolic equivalent of tasks (MET) based on the Compendium of Physical Activities, with moderate activities being defined as those involving 3-6 METS and vigorous activities involving >6 METS. We will then categorize PA into 3 groups according to the AHA guidelines: "recommended" (≥150 min/wk of moderate intensity or ≥75 min/wk of vigorous intensity or ≥150 min/wk of moderate + vigorous intensity), "intermediate" (1–149 min/wk of moderate intensity or 1–74 min/wk of vigorous intensity or 1–149 min/wk moderate + vigorous intensity), or "poor" (0 min/wk of moderate or vigorous exercise). We will also calculate overall PA in MET*min/week and categorize PA into quartiles. For our primary analysis, we will focus on PA assessed at ARIC visit 1, but we will also assess changes in PA from visit 1 to visit 3. In a sensitivity analysis, we will consider an average PA measure (average of visit 1 and 3 PA).

2) Vitamin D:

   Using frozen serum samples obtained from visit 2, 25(OH)D levels were measured using liquid chromatography-tandem high-sensitivity mass spectrometry in 2012–2013 (Waters Alliance e2795, Milford, Massachusetts). Total 25(OH)D concentration was determined by adding $25(OH)D_2$ and $25(OH)D_3$ levels. Because there is variability in 25(OH)D levels by season, we will adjust for seasonal changes by computing residuals from a linear regression model with 25(OH)D as the dependent variable and month of visit as the independent variable. The residuals will be added back to the overall mean to determine an estimated annual 25(OH)D value.

25(OH)D will be modeled as a continuous variable and also as a dichotomous variable (deficient vs. optimal). For our primary analyses, we will consider 3 categories of 25(OH)D recommended by the Endocrine Society of <20 ng/ml (deficient), 20-30 ng/ml (intermediate), and ≥30 ng/ml (optimal) and we will compare the deficient group (<20 ng/ml) to the optimal group (≥30 ng/ml). However there is some controversy about what an optimal 25(OH)D levels should be. Based on Institute of Medicine criteria, we will also define vitamin D deficiency as having total 25(OH)D levels < 20ng/ml and adequate 25(OH)D as ≥20 ng/ml for a sensitivity analysis.

d. Outcomes:
1. In the first cross-sectional aim, PA levels are the independent variable of interest and 25(OH)D levels are the dependent outcome. We will explore the distribution of 25(OH)D levels by PA levels and by change in PA levels. We will examine whether there is a higher prevalence of vitamin D deficiency among those with poor and intermediate PA levels than those with recommended PA levels, after adjustment for key confounding variables like age, race, sex, and BMI.

2. In the second aim, the outcome will be incident ASCVD, defined as definite or probable myocardial infarction, definite coronary death, and definite or probable stroke (defined as sudden or rapid onset of neurological symptoms that lasted for 24 hours or led to death in the absence of another cause), occurring after Visit 2 through 12/31/2012 or the most recent follow-up available. In this analysis, PA is the exposure and 25(OH)D status is the effect modifier of the association of PA with ASCVD. We will also consider incident HF as a secondary outcome of interest.

e. Covariates: Covariates will be from the visit 1 baseline exam (the same visit as the 1st PA assessment) and include: age, sex, race/center, educational level, alcohol use, smoking status, BMI, systolic blood pressure, diabetes, total cholesterol and HDL-cholesterol, use of lipid-lowering medications, and use of anti-hypertensive medications. We will also consider eGFR, assessment at visit 2.

6. Main Analyses:

Part 1: Non-concurrrent Cross-sectional analyses of the association of PA with 25(OH)D levels

1) We will describe baseline characteristics of the study population at ARIC baseline (visit 1) using means, medians, and proportions by AHA-defined PA groups (poor, intermediate, recommended)
2) We will assess and compare average 25(OH)D levels and prevalence of vitamin D deficiency (assessed at visit 2) by PA levels and by PA categories (poor, intermediate and recommended) assessed at visit 1.
3) We will also evaluate 25(OH)D levels and prevalence of vitamin D deficiency by change in PA levels as well as change in AHA-defined categories (i.e. poor, intermediate, and recommended).
4) We will use multivariable-adjusted linear regression to determine the average differences (95% CI) in 25(OH)D concentrations by PA levels and PA categories. We will use

multivariable-adjusted logistic regression to determine the prevalence (95% CI) of vitamin D deficiency by PA levels (continuous) and PA categories after adjusting for demographics and confounding lifestyle factors.

5) We will create an adjusted flexible spline model for the association of continuous PA (METs*min/week) with 25(OH)D levels (ng/ml) to determine if there is a linear dose-response between PA and vitamin D.

Part 2: Prospective analyses: Interaction of PA and vitamin D status with ASCVD/HF Risk

1) Using progressively adjusted Cox proportional hazard models, we will assess the association of higher PA, relative to lower PA, with incident ASCVD risk among individuals with and without vitamin D deficiency. We will test for an interaction between PA and vitamin D status to assess whether any risk reduction associated with PA is different among individuals with and without vitamin D deficiency.

2) Models will be progressively adjusted as follows
- 1. Model 1: Age, sex, race/center
- 2. Model 2: Model 1+ education, BMI, smoking status, and alcohol intake
- 3. Model 3: Model 2+ additional potential mediating variables (of the association between PA or vitamin D and ASCVD risk) – systolic blood pressure, anti-hypertensive medication use, diabetes, total and HDL-cholesterol, use of lipid lowering medications, and estimated GFR.

3) Among individuals with vitamin D deficiency, Wald tests will be used to formally test for two-way multiplicative interactions of PA with race, sex, age, in relation to incident ASCVD, by including cross-product terms in the model.

 g. Sensitivity analyses:
1) Since vitamin D was assessed at visit 2 and PA was assessed at different times (visit 1 and 3), we will also consider using for our primary exposure a PA variable that is an average of visit 1 and 3 to be a "proxy" for PA at visit 2.

2) We will also consider HF as an alternative outcome in addition to ASCVD, since both low PA and low 25(OH)D are each associated with HF as well, although their combined influences have not been evaluated.

h. Limitations: The biggest limitations of this analysis are the observational design, and lack of information on sun exposure. Individuals with higher levels of PA may spend more time outdoors, which result in greater sun exposure and higher vitamin D levels. There are several other limitations of this analysis, as well: Physical activity was self-reported, not directly measured. The Baecke questionnaire, while validated, has known limitations. Physical activity was measured at Visits 1 and 3 while vitamin D was measured at Visit 2 (2.5 years after visit 1), but we will explore a sensitivity analysis where we consider a PA variable that is the average of 1 and 3 to account for possible changes.   We have only a single measurement of 25(OH)D, whereas 25(OH)D levels can vary over time; however, we can adjust 25(OH)D concentrations for seasonal variation. We also do not have information regarding vitamin D supplement use at either ARIC visit 1 or visit 2.

The strengths of performing this analysis in ARIC are the large number of events, the availability of a large, biracial cohort of men and women to examine for demographic differences in the associations of interest.

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.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)?

There are other ARIC proposals evaluating vitamin D levels with ASCVD and HF outcomes (both Drs. Lutsey and Michos are co-authors on them).

There are other ARIC proposals evaluation PA with ASCVD and HF outcomes.

However, this is the first ARIC proposal to evaluate the associations of PA with 25(OH)D, and the combined influence of vitamin D and PA with ASCVD and HF outcomes.

Dr. Lutsey and Dr. Michos have led vitamin D ancillary studies in ARIC and will ensure no overlap with prior vitamin D work in ARIC.

Similar proposals include
Florido: #2631: "Physical activity, family history of premature coronary heart disease (CHD), and incident CHD in the Atherosclerosis Risk in Communities (ARIC) study"
    -R. Florido is a co-author on this paper

Michos: #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"
        -Michos is the senior author on this proposal and will ensure no overlap.

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* see below)
    ___   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/

Lutsey ARIC Ancillary Study number 2009.17
    -Vitamin D at visit 2
Michos ARIC Ancillary Study Number 2010.01
    -Vitamin D at visit 3

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 fromhttp://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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8.   Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med 2008;168:1629-37.
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11.   Shiroma EJ, Lee IM. Physical activity and cardiovascular health: lessons learned from epidemiological studies across age, gender, and race/ethnicity. Circulation 2010;122:743-52.
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