ARIC Manuscript Proposal #2587

PC Reviewed: 8/11/15	**Status: <u>A</u>**	**Priority: <u>2</u>**
SC Reviewed: _____	**Status:** _____	**Priority:** ____

1.a. Full Title: The Association of Vitamin D with Change in Lipids and Incident Dyslipidemia in the ARIC study

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

2.   Writing Group:

Kamil Faridi	Johns Hopkins	First Author
Di Zhao	Johns Hopkins	Analyst, Second Author
Seth S. Martin	Johns Hopkins	Third Author
Christie Ballantyne	Baylor College of Medicine	Co-author
Eliseo Guallar	Johns Hopkins	Co-Author
Pamela L. Lutsey	University of Minnesota	Co-Author
Erin D. Michos	Johns Hopkins	Senior Author

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

First author: **Kamil Faridi**
Address: 601 North Caroline Street, Baltimore MD 21287
Phone: 704-284-2593   Fax: 410-955-1545
E-mail: kfaridi1@jhmi.edu

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: **Erin D. Michos, MD, MHS**
    Address: Division of Cardiology, 600 N. Wolfe Street, Baltimore, MD 21287
           Phone: 410-502-6813   Fax: 410-502-0231
           E-mail: edonnell@jhmi.edu

3.   Timeline:

Analyses will begin later this summer with plans to target an abstract for either the AHA Epi/Lifestyle Prevention Meeting (submission deadline Oct 14[th,] 2015) or American College of Cardiology (ACC) meeting (submission deadline Oct 27th, 2015) with full manuscript by 2016.

4.   Rationale:

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of death and disability-adjusted life years lost worldwide. Lower serum levels of 25-hydroxyvitamin D [25(OH)D] have been shown to be independently associated with ASCVD events and mortality, even after adjusting for traditional risk factors including hyperlipidemia, diabetes, hypertension, smoking, and body mass index (BMI).[1] Although calcitriol is the active vitamin D metabolite that binds to the vitamin D receptor, serum 25(OH)D is considered the best indicator for vitamin D status.[2] The impact of vitamin D supplementation on ASCVD risk reduction remains inconclusive and is a subject of much investigation and debate.[3]

Elevated serum concentrations of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) and low concentrations of high-density lipoprotein cholesterol (HDL-C) are known to be major risk factors for developing CVD. A growing body of cross-sectional evidence indicates that blood levels of 25(OH)D are inversely associated with an atherogenic lipid profile.[4,5,6,7] These studies have found that individuals with low 25(OH)D (defined as either <20 ng/mL,[4] <30 ng/mL,[5] or in the lowest quartile [6]) have higher LDL-C, higher TG, and lower HDL-C compared to those with higher levels of 25(OH)D (defined as ≥30 ng/mL[4,5] or higher quartiles[6]).

We previously evaluated the association of serum 25(OH)D levels with an extended lipid panel in a large cross-sectional study of over 20,000 adults. We found that deficient serum 25(OH)D <20 ng/ml was associated with significantly lower HDL-C and higher directly-measured LDL-C, intermediate-density lipoprotein cholesterol (IDL-C), very low density lipoprotein cholesterol (VLDL-C), remnant lipoprotein cholesterol (RLP-C), and TGs compared to the optimal vitamin D group (≥30 ng/ml) [submitted work].

Our prior study, as well as almost all previous work that evaluated the associations of 25(OH)D levels with lipids, have been cross-sectional. Therefore direction of the association of vitamin D deficiency with dyslipidemia is uncertain. To our knowledge, there is only one observational (i.e. non-interventional study) that looked at serial changes in lipids associated with 25(OH)D levels. This study by Ponda et al[8] used data from the Quest Diagnostic laboratory of 8592 de-identified patients who had undergone two or more 25(OH)D levels and lipid panels within 4-26 weeks, and who had LDL cholesterol within the 2nd and 3rd quartiles of the LDL change distribution (this restriction on LDL-C was to exclude patients with large LDL-C changes that might have occurred due to initiation of lipid lowering therapy). These authors found that for the patients with deficient 25(OH)D levels <20 ng/ml on the first lab but improved to >30 ng/ml on the repeat test did NOT have a corresponding improvement in LDL-C or TG, but had small increases in total cholesterol and HDL-C compared to vitamin D deficient patients with levels <20 ng/ml at both time points.

One major limitation of the study by Ponda et al[8] was their data were extracted from a clinical diagnostic laboratory dataset and as such did not have information regarding important clinical characteristics of the patients that might have accounted for changes in vitamin D or changes in lipids (such as lipid lowering medication usage, BMI, physical activity, and other ASCVD risk factors). Therefore to confirm the purported lack of improvement in lipids with improvement in vitamin D status, it is needed to replicate these findings in a well-characterized prospective cohort study such as ARIC and consider important clinical characteristics that might either confound or mediate this association.

One important note is that the causal role in vitamin D and lipids has been recently challenged. There was one small interventional study of 151 individuals treated with vitamin D supplementation for 8 weeks that did not show that vitamin treatment improved the lipid profile.[9] Furthermore, Mendelian randomization studies have used single nucleotide polymorphisms (SNPs) leading to variations in 25(OH)D to examine vitamin D's role in the development of an atherogenic lipid profile and ASCVD[10,11,12].These studies have shown evidence that SNPs causing a genetically increased RLP-C[10] and BMI[11] are associated with reduced 25(OH)D. This supports the notion that 25(OH)D may be a marker for overall health rather than an independent risk factor for CVD.

However the well-characterized ARIC study is reasonably suited to illuminate these potential confounders in a prospective fashion. ARIC has lipids, BMI, waist circumference (WC), and diabetes status measured at each visit, including visit 1 (which is ~3 years prior to vitamin D measurement). Therefore in ARIC, we can examine time-varying changes of potential confounders (i.e. BMI and WC) or mediators (incident diabetes) of the association of vitamin D with change in lipids and incident dyslipidemia during ARIC follow-up.

Our goals are to determine (1) whether baseline vitamin D levels are associated with change in lipids over time and incident dyslipidemia and (2) whether favorable changes in vitamin D status are associated with improvements in the lipid profile.

5. Main Hypothesis/Study Questions:

Hypotheses:
1. In multivariable-adjusted <u>cross-sectional</u> analysis, we hypothesize that deficient 25(OH)D levels (<20 ng/ml) compared to optimal (≥30 ng/ml) will be associated with a more adverse lipid profile including higher levels of total cholesterol, non-HDL-C, LDL-C (estimated by Friedewald), LDL-C (estimated by novel method[13]), and triglycerides and lower levels of HDL-C at ARIC visit 2. Deficient vitamin D status will also be associated with prevalent dyslipidemia at baseline.

2. In multivariable-adjusted <u>prospective</u> analysis, we hypothesize that deficient 25(OH)D levels compared to optimal will be independently associated with a worsening lipid profile over ARIC follow-up with increases in total cholesterol, non-HDL-C, LDL-C (estimated by Friedewald), LDL-C (estimated by novel method[12]), and triglycerides and decreases in HDL-C. Deficient 25(OH)D levels will also be independently associated with incident dyslipidemia (for those not dyslipidemic at baseline)

3. In a subpopulation of ARIC participants who had vitamin D levels measured at both ARIC visit 2 and visit 3, we hypothesize that improvements in vitamin D status from deficient to non-deficient will be associated with more favorable improvements in lipids over follow-up and reduced incidence of dyslipidemia compared to those deficient at both visits. Conversely, individuals with worsening vitamin D status from non-deficient to deficient will have unfavorable changes in their lipids over time.

4. We hypothesize that there will be a significant racial interaction, with associations of vitamin D and adverse lipids seen in Whites but not 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).

Participants:

ARIC VISIT 2 PARTICIPANTS (1990-1992): Serum 25(OH)D was measured in samples collected at ARIC visit 2 (1990-1992), which was attended by 14,348 white and black participants. Thus, visit 2 is 'baseline' for the present analysis. Excluded from the analysis are participants self-identified as neither black nor white (n=42), blacks from the Minnesota and Maryland centers (n=49), missing 25(OH)D data (n=1,097), and those with missing lipid data at ARIC visit 2 (n=XX). For the primary analysis our final analytic sample for visit 2 is anticipated to include approximately 12,000 participants.

ARIC VISIT 3 PARTICIPANTS (1993-1994): The ARIC Brain MRI ancillary study contains 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). 25(OH)D was measured in serum samples from ARIC Brain MRI Ancillary Study participants who attended visit 3. Of the 1,934 participants with available MRI data included in the visit 3 Brain MRI Ancillary Study, we excluded those missing stored serum, insufficient serum for 25(OH)D measurement, or samples that did not pass internal quality control (n=165) for a total sample at visit 3 with measured 25(OH)D of 1769. Of these, 1573 individuals have vitamin D levels at both visit 2 and visit 3.

For our main analysis, we will include all participants who have vitamin D levels and measured lipids at visit 2, but will adjust for use of lipid lowering therapy (as a repeated measure updated at each visit) in the analysis. However in a sensitivity analysis, we will exclude participants who were taking lipid-lowering therapy at any time during followup at either visit 2, visit 3, or visit 4. This is because while we may know whether a participant is taking lipid-lowering therapy or not, we do not know dosage of that therapy, or whether the intensity of the dosing of their lipid-lowering therapy was increased or decreased during followup. Therefore simply adjusting for use of lipid lowering therapy in analyses may not be adequate enough to account for the impact lipid lowering therapy has on change in lipid values.

Exposure:

25(OH)D measured at ARIC visit 2 for hypothesis 1 & 2 (n=~12,000), and change in 25(OH)D from ARIC visit 2 to ARIC visit 3 (n=1573) for hypothesis 3.

25(OH)D concentrations vary by season. Therefore we adjusted 25(OH)D [at visit 2 and visit 3] for seasonal variation by computing the residuals from a linear regression model with vitamin D as the dependent variable and month of blood draw as the independent variable. By definition, these residuals are uncorrelated with month of blood draw. The grand mean was then added to the vitamin D residuals obtained from this model. We performed this adjustment separately for whites and for blacks, as seasonal variation in 25(OH)D concentrations also varies by race. This new variable "vitamin D adjusted for month of blood draw" is an estimate of average annual 25(OH)D levels, and will be used as the exposure variable in all analyses.

For analyses evaluating baseline vitamin D levels at ARIC visit 2, 25(OH)D will be examined continuously per 1 SD increase, as well by the clinical classifications of deficient (<20 ng/ml), intermediate (20-29 ng/ml), and optimal (≥30 ng/ml).

For the subset with vitamin D measured at both visit 2 and visit 3, we will consider change in 25(OH)D levels as a continuous measure (visit 3 minus visit 2).

We will also create 4 categories of people based on their vitamin D status at both visits dichotomized at above and below 20 ng/ml with <20 ng/ml being "deficient" and ≥20 ng/ml being "not deficient". This latter group includes both the intermediate and the optimal groups; however the Institute of Medicine considers a 25(OH)D level ≥20 ng/ml as adequate for health.

Deficient/Deficient   Not Deficient/ Not Deficient
Deficient/ Not-Deficient   Not Deficient/Deficient

Given small numbers of people who were optimal at visit 2 and became deficient at visit 3 (n=20) or were deficient and went to optimal (n=10) [Table], we are unable to consider 3 groups (9 categories of change) with reasonable power.

```
 25(OH)D in |      25(OH)D in visit 3
   visit 2 | <20 ng/ml  20-<30 ng  >=30 ng/m |    Total
-------------+-------------------------------+----------
  <20 ng/ml |     481       88        10 |     579
20-<30 ng/ml |    255      361        46 |     662
  >=30 ng/ml |     20      131       181 |     332
-------------+-------------------------------+----------
    Total |    756       580       237 |   1,573
```

Outcomes:

The separate lipid parameters of total cholesterol, LDL-C (by Friedewald and by novel methods), HDL-C, TG, non-HDL-C will be assessed at visits 2, 3, and 4. We will adjust for use of lipid-lowering medication (updated each visit).

The presence of "Dyslipidemia" at visit 2, 3, and 4 will be defined as having an LDL-C ≥ 130, HDL-C <40 (men) or <50 (women), TG ≥150 mg/dl, and/or use of lipid lowering therapy. We will also consider alternate definitions of dyslipidemia such as non-HDL-C ≥160 mg/dl or TC/HDL ratio of 4.

Cholesterol values from ARIC visit 5 will not be evaluated given the long time lag (>12 years) between ARIC visit 4 and visit 5

In a sensitivity analysis, participants who used lipid lowering therapy at any visit - visit 2, 3, and/or 4 will be excluded.

Covariates:

From visit 2: Age, sex, race, center, education, BMI, WC, smoking status, current alcohol use, diabetes status (self-report, medication use, or fasting blood glucose ≥126 mg/dl), systolic blood pressure, diastolic blood pressure, use of antihypertensive medications, use of lipid lowering medication, and eGFR. Education and physical activity were obtained at visit 1 and will be carried forward.

From visit 3 and 4: Will update covariate status at each visit.

Analyses:

Visit 2 (the visit where vitamin D was measured) will be considered the baseline visit for all analyses.

1. We will tabulate the baseline clinical characteristics of the population by vitamin D categories (deficient, intermediate, optimal) using means and proportions [Table 1].

2. In a supplemental table, we will also tabulate the clinical characteristics of the subset of participants who had vitamin D measured at both ARIC visit 2 and visit 3 (as these were participants from Forsythe County and Jackson sites only and may differ in characteristics from the overall ARIC population at visit 2).

3. For cross-sectional analyses, we will use multivariable-adjusted linear regression using the deficient and optimal 25(OH)D groups as an independent variable (binary) with the separate outcomes of total cholesterol, non-HDL-C, LDL-C (Friedewald), LDL-C (Novel), HDL-C, and log-transformed TG as continuous dependent variables. We will also repeat the analyses using 25(OH)D as a continuous measure.

4. We will assess the longitudinal association between change in each separate lipid parameter by baseline vitamin D status (deficient vs. optimal) across the 3 time points (ARIC visits 2, 3, and 4) using a random-intercept linear mixed model for longitudinal data. Repeated measurements over time in the same participant would be accounted for, while allowing for random variations in baseline lipid levels across participants. We would update covariates such as BMI, WC, hypertension, and diabetes status at each visit in the analyses.

5. We will use multivariable-adjusted Poisson relative risk regression to look at incidence of dyslipidemia at either visit 3 or 4 by deficient baseline vitamin D status at visit 2 compared to optimal, among those not dyslipidemic at baseline.

6. All models will be sequentially adjusted as follows:

   Model 1 will consider basic demographic factors age, sex and center
   Model 2 will include lifestyle factors (education, BMI, WC, physical activity, smoking, current alcohol)
   Model 3 will include other CVD risk factors (hypertension, diabetes, eGFR, C-reactive protein)
   We will adjust for use of lipid lowering therapy (updated each visit) in all models that evaluate change in lipids.

7. We will test for interactions by sex, race, and baseline BMI categories.

8. We will perform 3 sensitivity analyses as follows:

    (1) Since any interval changes in the intensity of lipid-lowering therapy will not be adequately accounted for by simply adjusting for "use of lipid lowering therapy", we will perform a sensitivity analysis where we exclude participants taking lipid lowering therapy at visit 2, visit 3, and/or visit 4.

    (2) In the primary analysis, instead of adjusting for lipid lowering medication use, we will also consider using a constant to estimate among medication users what lipid values might have been had the participants not been taking a medication. This approach, which has been done previously,[14] may be preferable to other approaches, such as including medication use as an indicator variable in multivariate models or excluding antihyperlipidemic medication users, which may introduce bias. The constant we will use will depend on the specific type of medications used.

    (3) We will include only the participants with self-reported health status of Excellent, Very Good, or Good (i.e. excluding those reporting Fair or Poor health status).

9. Since both low vitamin D and dyslipidemia contribute to CVD and mortality, there might be informative loss to follow-up between visits 2-4. For the prospective analyses we will explore the impact of accounting for attrition through inverse probability of attrition weighting (IPAW) and/or MICE.

10. For the subset with vitamin D at both visit 2 and visit 3, we will use multivariable linear regression to look at change in lipid parameters between visit 2 and visit 3 by categories of vitamin D status at each visit (with those deficient at both visits as the reference group) as well as by change in vitamin D levels as a continuous measure. We will use similar progressively adjusted models as noted above. We will also use multivariable adjusted logistic regression to look at incident dyslipidemia between visit 2 and visit 3 by vitamin D categories at both visits and by change in vitamin D levels between the two visits (among those not categorized as dyslipidemic at visit 2 baseline).

7.a. Will the data be used for non-CVD analysis in this manuscript? _____ Yes   __X__ No

We are not studying CVD outcomes per se. But we are considered lipids and dyslipidemia which are strong risk factors for associated with CVD.

   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 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"? _____ 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)?**

No prior study has evaluated vitamin D and lipids in ARIC.

The following other proposals did study change in lipids in ARIC

MP089 Brown S et al. Repeatability of lipid data from visit 1 to visit 2
MP149 Ekelund LG et al. Change in lipids v2-v1 to changes in resting BP.
MP1139 Gramenz A et al. Interaction of lipid gene polymorphisms and menopausal transition on LDL, HDL, TG, and total cholesterol levels
MP1482 Lutsey PL et al. Relation of lipid gene score to longitudinal trends in lipid levels and to statin therapy response in Caucasians.

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*)
   ___ 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.

References:

[1] Michos ED, Reis JP, Melamed ML. Vitamin D status and cardiovascular health: a 2009 update. Open Clinical Chemistry Journal, 2010;3:51-59. Published on-line. http://bentham.org/open/tocchemj/openaccess2.htm

[2] Zerwekh JE. Blood biomarkers of vitamin D status. *Am J Clin Nutr*. 2008;87(4):1087S-91S.

[3] Manson JE, Bassuk SS. Vitamin D research and clinical practice: At a crossroads. *JAMA*. 2015.

[4] Ponda MP, Huang X, Odeh MA, Breslow JL, Kaufman HW. Vitamin D may not improve lipid levels: A serial clinical laboratory data study. *Circulation*. 2012;126(3):270-277.

[5] Vacek JL, Vanga SR, Good M, Lai SM, Lakkireddy D, Howard PA. Vitamin D deficiency and supplementation and relation to cardiovascular health. *Am J Cardiol*. 2012;109(3):359-363.

[6] Jorde R, Figenschau Y, Hutchinson M, Emaus N, Grimnes G. High serum 25-hydroxyvitamin D concentrations are associated with a favorable serum lipid profile. *Eur J Clin Nutr*. 2010;64(12):1457-1464.

[7] Skaaby T, Husemoen LL, Pisinger C, et al. Vitamin D status and changes in cardiovascular risk factors: A prospective study of a general population. *Cardiology*. 2012;123(1):62-70.

[8] Ponda MP, Huang X, Odeh MA, Breslow JL, Kaufman HW. Vitamin D may not improve lipid levels: a serial clinical laboratory data study. Circulation. 2012 Jul 17;126(3):270-7.

[9] Ponda MP, Dowd K, Finkielstein D, Holt PR, Breslow JL. The short-term effects of vitamin D repletion on cholesterol: A randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol*. 2012;32(10):2510-2515.

[10] Ooi EM, Afzal S, Nordestgaard BG. Elevated remnant cholesterol in 25-hydroxyvitamin D deficiency in the general population: Mendelian randomization study. *Circ Cardiovasc Genet*. 2014;7(5):650-658.

[11] Vimaleswaran KS, Berry DJ, Lu C, et al. Causal relationship between obesity and vitamin D status: Bi-directional mendelian randomization analysis of multiple cohorts. *PLoS Med*. 2013;10(2):e1001383.

[12] Afzal S, Brondum-Jacobsen P, Bojesen SE, Nordestgaard BG. Genetically low vitamin D concentrations and increased mortality: Mendelian randomisation analysis in three large cohorts. *BMJ*. 2014;349:g6330.

[13] Martin SS, Blaha MJ, Elshazly MB, et al. Comparison of a novel method vs the friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA*. 2013;310:2061-2068

14. Lutsey PL, Rasmussen-Torvik LJ, Pankow JS, Alonso A, Smolenski DJ, Tang W, Coresh J, Volcik KA, Ballantyne CM, Boerwinkle E, Folsom AR. Relation of lipid gene scores to longitudinal trends in lipid levels and incidence of abnormal lipid levels among individuals of European ancestry: the Atherosclerosis Risk in Communities (ARIC) study. Circ Cardiovasc Genet. 2012 Feb 1;5(1):73-80.