ARIC Manuscript Proposal #2376

PC Reviewed: 6/10/14	**Status: <u>A</u>**	**Priority: <u>2</u>**
SC Reviewed: _____	**Status:** _____	**Priority:** ____

1.a. Full Title:

25-hydroxyvitamin D levels, vitamin D binding protein gene polymorphisms, and vitamin D3 epimer with risk of incident fracture-related hospitalization: Twenty-year follow up in a bi-ethnic cohort

  b. Abbreviated Title (Length 26 characters):

Vitamin D and fracture

2. Writing Group:
    Writing group members:

Erin D. Michos	Johns Hopkins	Lead author*
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*may work with a visiting medical student participating in MSTAR geriatric summer research program.
(notes: Myron Gross, U of Minnesota, was invited several times – no reponse)

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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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 for this proposal will take place in later spring of 2014 immediately pending approval of proposal, with goal to submit for publication by the end of summer 2014.

4.   Rationale:

Vitamin D and bone mineral density (BMD):
        25-hydroxyvitamin D [25(OH)D] deficiency (generally defined as <20 ng/ml) has been associated with increased risk of cardiovascular disease and all-cause mortality in observational studies (Michos 2009, Lavie CJ 2011, Wang L 2012). Vitamin D also plays a role in the normal development and maintenance of the skeleton (Christodoulou, 2013). There are well-established associations between low vitamin D levels and both osteomalacia and rickets (Christodoulou, 2013). Low BMD is associated with osteopenia and osteoporosis, and it is an important predictor for increased fracture risk (Stone, 2003).
        The associations between 25(OH)D, low BMD, and fracture risk seen in observational studies however, remain unclear (Reid, 2013). Some studies have found significant associations between 25(OH)D level and BMD (Bischoff-Ferrari, 2004, Adami, 2009, Bischoff-Ferrari, 2009, Zhou, 2013). In contrast, other studies have found no association between 25(OH)D level and BMD (Marwaha, 2011, Sigurdsson, 2000, Hosseinpanah, 2008).

25-hydroxyvitamin D and hip fracture:
      Low 25(OH)D levels also have been associated with incident hip fracture in prior observational studies (Holvik K 2013, Cauley JA 2008, Cauley JA 2010). Associations have typically been stronger in men compared to women; less is known about whether associations between 25(OH)D levels and hip fracture vary by race/ethnicity. In the Women's Health Initiative, higher 25(OH)D was associated with increased facture risk compared to lower 25(OH)D levels among black women, the opposite association of what was seen in white women (Cauley JA 2011). These findings suggest the association of 25(OH)D with fracture may vary by race/ethnicity. Utilizing ARIC, a large bi-racial cohort, will allow us to further explore interactions of 25(OH)D with fracture by race in more depth.
        Finally, previous studies that have evaluated the association of 25(OH)D with fracture were based on a single measure of 25(OH)D which may not reflect

lifetime patterns of vitamin D.   Little is known about change in vitamin D status and risk of fracture. In ARIC, a subset of participants did undergo repeated measures of vitamin D approximately 3 years apart, allowing us the opportunity to evaluate change in categories of vitamin D status with fracture risk.

RCTs of Vitamin D supplements and hip fracture:
Regarding vitamin D supplements, there is uncertainty about whether treatment with vitamin D supplementation can reduce incident hip fracture. In a recent meta-analysis of randomized clinical trials (RCTs) of vitamin D supplementation (12 trials, 27834 participants), vitamin D supplements alone did not reduce hip fracture (pooled RR 1.11, 95% CI 0.97-1.27). There was a reduction in hip fracture seen in trials using combined vitamin D with calcium (pooled RR 0.84, 95% CI 0.74-0.96), but sensitivity analyses suggested this benefit was limited to institutionalized individuals residing in nursing homes. However RCT meta-analyses are limited by heterogeneity of vitamin D supplement types, vitamin D supplement doses, and patient populations. Furthermore, uncertainty exists as: (1) the low dosage used in some trials was unlikely to adequately boost 25(OH)D levels; (2) many studies did not measure 25(OH)D levels or enroll an exclusively deficient population, and vitamin D supplements unlikely to benefit those without a vitamin D deficiency.
Since adequacy of serum stores of vitamin D are likely more biologically important that a fixed vitamin D supplement dose (such as those given in an RCT), understanding the association of 25(OH)D levels and risk of incident hospitalized fracture across a non-institutionalized population would be very helpful.

Genetic polymorphisms in vitamin D binding protein and bioavailable vitamin D
As mentioned above, the association of low vitamin D with fracture outcomes may vary by race. Similarly, prior studies have found low 25(OH)D was associated with increased risk of CHD and stroke in whites but not blacks (Robinson-Cohen C, Michos ED 2012).
The differences in associations of total 25(OH)D and health outcomes by race may in part be explained by racial differences in vitamin D binding. Blacks tend to have lower levels of total 25(OH)D compared to whites (Powe CE 2013). However, recent work has shown that blacks and whites have similar concentrations of estimated bioavailable 25(OH)D, because blacks have lower levels of both total 25(OH)D and vitamin D binding protein (DBP) compared to whites (Powe CE 2013).There are two common single nucleotide polymorphisms (SNPs) on the *DBP* gene, rs7041 and rs4588, which are believed to explain ~80% of the variability in serum DBP levels (Powe CE 2013). Blacks have been shown to be more likely than whites to have a T allele at rs7041 and to have a C allele at rs4588, which both result in lower levels of serum DBP. While we do not have measured DBP levels in ARIC to directly calculate bioavailable vitamin D, ARIC does have genetic data regarding these 2 polymorphisms. It is possible that these DBP SNPs modify the relationship between 25(OH)D levels and fracture risk.

Vitamin D3 epimer and health outcomes – unknown clinical significance

    Epimers have identical chemical structure except for stereochemical configuration. Once believed to be only present in neonates, the C-3 epimer of 25-hydroxyvitamin D_3 [3-epi-25(OH)D3] has been shown to be present among some adults (Lensmeyer 2012). However, the understanding of the functional significance of 3-epi-25(OH)D3 is not known, and it is unclear how this epimer is related to bone related diseases. For 25(OH)D measured by radio-immunoassay, the epimer might affect the vitamin D assay and the reliability of the 25(OH)D3 measurement leading to over-estimation of 25(OH)D levels. This is not the case when 25(OH)D is measured by mass spectroscopy and epimer is quantified separately, as was done in the ARIC cohort.   But whether the epimer is biologically active is unknown (Bailey D 2013). The ARIC study offers the opportunity to explore the associations of the vitamin D3 epimer with fracture outcomes, as hypothesis-generating analyses for design of future studies.

Proposal Significance Summary:
We propose to examine the independent associations of 25(OH)D, DBP SNPs, and 3-epi-25(OH)D3 levels with incident fracture-hospitalization during over 20 years of ARIC follow-up, with focus on the potential interactions by race and by DBP SNP status.

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

Hypotheses:

1.   We anticipate the association of 25(OH)D with fracture risk to be non-linear. Low 25(OH)D levels (at a threshold of approximately <17.2 ng/ml, or bottom quintile of distribution) will be associated with incident hospital-associated fracture independent of traditional risk factors, lifestyle factors, and socioeconomic status. This relationship will remain significant even after adjustment for calcium, phosphate, and parathyroid hormone levels.

2.   We hypothesize that the association between low 25(OH)D and fracture will be modified by race. Low 25(OH)D levels will be more strongly associated with incident fracture among white but not black participants.

3.   We hypothesize that the association between 25(OH)D and fracture will be modified by rs7041 and rs4588 SNP status. We anticipate to see a higher risk among those with low 25(OH)D with rs7041 G versus T allele or rs4588 A versus C allele, (i.e. those genetically predisposed to higher DBP levels and thus those with lower estimated levels of bioavailable vitamin D for a given total 25[OH]D level).

4.   Due to the unknown clinical significance of 3-epi-25(OH)D$_3$, we do not have an *a priori* hypothesis about the association of 3-epi-25(OH)D$_3$ with incident fracture. However given the paucity of data in the literature regarding the vitamin D epimer with clinical outcomes, we think this analysis is still important to perform as exploratory for developing future research hypotheses.

5.   Among the 1700 individuals with repeat vitamin D measures (~3 years apart), compared to those "replete at visit 2 and replete at visit 3", those who are Deficient/Replete, Replete/Deficient, and Deficient/Deficient will have greater risk for incident fracture.

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**:** prospective cohort study. Baseline: ARIC visit 2, 1990-1992. Follow-up through 12/31/2011 (or recent hospitalization data available).

Inclusion/Exclusion
       All ARIC participants who had 25(OH)D measured from stored serum from ARIC visit 2 1990-1992; n=13,753.   Participants with incident fracture-hospitalization prior to visit 2 will be excluded, as will those who are neither African American nor white, and African Americans from the MN and MD centers. For DBP SNP analyses, we will also exclude those who did not consent to genetic research.
       Note than a subset of participants (approximately 1700) from the Forsyth County and Jackson centers had a repeat measure of 25(OH)D and epimer in ARIC visit 3, approximately 3 years later (1993-1994). These participants will be considered in a supplementary analysis regarding change in vitamin D levels.

Variables
  Exposures:
   Primary: Serum 25(OH)D (measured in visit 2 serum). Since serum vitamin D levels vary greatly by season (Shoben AB 2011), we will account 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, performed separately by race. By definition, these residuals will be uncorrelated with month of blood draw. The grand mean will be added to the vitamin D residuals obtained from this model. This new variable "vitamin D adjusted for month of blood draw" will be used as the main exposure variable for all analyses. This estimated annual 25(OH)D value will be divided into quintiles based on the distribution in the overall population (<17.2 ng/ml; 17.2-<21.7 ng/ml; 21.7-<26 ng/ml; 26.0-<31 ng/ml; ≥31.0 ng/ml).

Secondary Vitamin D Analyses:

1. We will also look separately at associations of the vitamin D epimer [3-epi-25(OH)D3] with risk of incident hospitalized-associated fracture. 3-epi-25(OH)D_3 concentration varies with 25(OH)D concentration, but do not vary as much by season, so we will not perform seasonal adjustment, but we do plan to adjust for season (January-March; April-June; July-September; October-December) and 25(OH)D in our 3-epi-25(OH)D_3 regression models.

2. Among the 1700 individuals with repeat vitamin D levels at visit 3, we will consider the association of categories of vitamin D status at both time points (Deficient/Deficient, Deficient/Replete, Replete/Deficient, and Replete/Replete) with fracture risk. The 2010 Institute of Medicine (IOM) considers 25(OH)D <20 ng/ml as deficient, although other studies have used a <15 ng/ml as deficient (Wang T 2008, Giovannucci 2008) For consistency with the main analyses (and based on threshold of risk seen in our prior ARIC analyses of stroke), we likely will consider levels <17.2 ng/ml (the bottom quintile) as deficient and >=17.2 ng/ml as replete for both time points.

Main covariates: Age, race/center, sex, education[&], physical activity[&], smoking status, alcohol use, BMI, diabetes, LDL-C, HDL-C, triglycerides, antihyperlipidemic medication use, CRP, systolic blood pressure, antihypertensive medication, use of thiazide diurectics, use of hormone replacement therapy, and eGFR (modeled as ≥90, 60-89, and 15-59 ml/min/1.73 m^2). eGFR will be estimated using both creatinine and cystatin-C.

Unfortunately, vitamin D supplement use was not well characterized at ARIC visit 2 to be considered as a potential covariate.

&Education and physical activity were measured at ARIC visit 1

 # for change analysis, covariates measured at visit 2 will be carried forward

Potential effect modifiers: Age, race, sex, eGFR, serum magnesium, DBP SNPs

Outcome Ascertainment:
1. Primary outcome: Incident fracture-related hospitalization will be defined as any hospitalization after visit 2 (1990-1992) meeting the International Classification of Diseases, 9th revision, discharge codes of 733.1-733.19, 733.93-733.98, or 800-829.
2. Secondary outcome will be looking at incident hip fracture-related hospitalization is defined as any hospitalization after visit 2 (1990-1992)

meeting the International Classification of Diseases, 9th revision, discharge codes 820.xx.

We will use the ARIC hospitalization ICD9 Clinical Classification System (CCS) coding through 2011. (approximately 1450 fracture hospitalizations identified so far using this coding).

Statistical methods:

Visit 2 will serve as baseline for the current analysis. Baseline characteristics (1990-1992) of the study population will be described using means, medians, and proportions across quintiles of 25(OH)D and by race.

Cox proportional hazards regression will be used to estimate the hazard ratios (95% confidence intervals) for the association of 25(OH)D with incident fracture. Person-years will accrue from the date of the participant's visit 2 exam until the date of the incident event of interest, death, loss-to-follow-up, or the end of ARIC follow-up for hospitalizations (currently 12/31/2011). The competing risk of mortality will be considered in analysis. The proportional hazards assumption will be tested visually by graphing the log(-log(survival)) versus log(time).

Prior studies in the literature and prior analyses in ARIC have found the association of 25(OH)D with health outcomes to be non-linear with greatest risk confined to those with lowest levels ((<20 or <15 ng/ml).Thus seasonally-adjusted 25(OH)D will be modeled in several ways: 1) as a continuous variable in 25(OH)D using restricted cubic splines 2) quintiles of 25(OH)D, and 3) categorized according to existing 25(OH)D cut-points (i.e. deficient: <20 ng/mL; insufficient: 20-29.9 ng/mL; optimal: ≥30 ng/mL).

C-3-epi-25(OH)D3 will be categorized as undetectable, detectable but below the level of quantification, or quantifiable (>1.4 ng/ml).

Primary analysis: For the outcome (incident fracture-related hospitalization), we will run a series of models. The primary model will adjust for demographic factors (age, sex, race/field center [overall models] or center [race-stratified models]) and behavioral/socioeconomic variables (education, income, physical activity, smoking, alcohol use, body-mass index, waist circumference). We will perform two additional models: 1) adding potential mediators (diabetes, systolic and diastolic blood pressure, use of hypertension medication, use of thiazide diurectics, use of hormone therapy, total and HDL cholesterol, hsCRP, and estimated GFR) and 2) adding biomarkers related to vitamin D metabolism (calcium, phosphate and PTH).

Interaction Testing: We will formally test for two-way multiplicative interactions of 25(OH)D by race and *DBP* gene polymorphisms rs7041 and rs4588 using Wald tests and stratified analyses were presented if there was any evidence for

interaction. However, *a priori* we plan to present results overall and stratified by race based on prior studies and inherent interest, regardless if a significant race interaction is present. When testing for interaction, we will compare the lowest quintile of 25(OH)D to the other four higher quintiles to increase statistical power (this cutpoint also corresponds to our anticipated threshold effect seen in other analyses of 25(OH)D and incident stroke).

For DBP genotypes: TT (for rs7041) and CC (for rs4588) will be reference groups [i.e. those predicted to have low DBP and higher bioavailable 25(OH)D for given total 25(OHD)]. However, very few blacks have GG (for rs7041) and AA (for rs4588) genotypes. One approach is to collapse TG/GG and AC/AA groups into two genotypes rather than 3. Another potential approach, as to avoid any assumptions about the genetic model, would be to consider excluding people with GG or AA genotypes from the interaction analysis.

Epimer analysis: We examine whether there is an association of 3-epi-25(OH)D3 with incident fracture. If there is an association, we will further adjust for 25(OH)D levels to see if the association of the 3-epi-25(OH)D3 with fracture is attenuated after considering total 25(OH)D levels.

Change in Vitamin D status analysis: Among the 1700 individuals with repeat vitamin D levels at visit 3, we will consider vitamin D status at both visit 2 and visit 3 time points (categorized as Deficient/Deficient, Deficient/Replete, Replete/Deficient, and Replete/Replete) with CHD risk. Given low power in this subset, we will unlikely be able to perform interactions by race or by SNP. Change in vitamin D levels as a continuous variable (visit 3 levels minus visit 2 levels) will also be considered in exploratory analysis.

Limitations:
The non-CVD outcome data based on hospital discharge diagnoses in ARIC is not adjudicated as it is for CHD, stroke, and heart failure outcomes. It is anticipated to be very specific for the diagnosis of fracture, but may not been a very sensitive measure. Furthermore, it is likely biased to capturing only the most severe cases. Thus, our results will need to be interpreted in this context.

We plan to address these limitations in a couple of ways as follow:
(1) While total fractures is our primary outcome of interest, we will also do analyses with a secondary outcome of incident hospitalization for hip fracture. Hip fracture is a major event, almost always universally hospitalized, and therefore we feel the outcome ascertainment would be more complete as we are less likely to miss cases. Other types of fractures (i.e. wrist, arm, etc) may be treated on an outpatient/urgent care basis and not hospitalized; therefore would be missed. Results related to hospitalized associated fractures maybe biased to only the most severe types of fractures. This may limit generalizability of findings. However, given fewer cases of hip fracture identified in ARIC, statistical power

will be with using just hip fractures alone.

(2) Given concerns about bias regarding the definition of hospitalized-associated facture, we will do a sensitivity analysis where we restrict analysis to only those who have ever been hospitalized. Approximately 75-80% of ARIC participants have been hospitalized at some point and would be eligible for an ICD-9 fracture diagnosis.

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

YES –   Fracture is the outcome.

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?   __X__ Yes ____ No

YES – we are looking for interaction by 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)?

Since 25(OH)D is being newly measured in ARIC, this is the first paper to evaluate the relationship of vitamin D with incident fracture in ARIC.

#1769: Previously Drs. Andrea Christman Schneider (ALCS) and Elizabeth Selvin (ES) have published on the risk of diabetes with incident fracture in the ARIC study. We have invited both to be co-authors on this paper to ensure similar methodology to her previously published ARIC fracture paper.

There are 2 other fracture proposals in development, again there is over-lap with that author group with the current proposal to unsure similar methodology. LA=Lawrence Apple, MG=Morgan Grams

#2371: CKD and fracture (LA, ALCS, MG, ES)

#2329: ABI and fractures (ALCS, ES, MG)

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

Lutsey ARIC Ancillary Study number 2009.17
        -Vitamin D at visit 2
Michos ARIC Ancillary Study Number 2010.01
        -Vitamin D at visit 3
Selvin ARIC Ancillary Study 2009.16
        - CysC, CRP

11.b. If yes, is the proposal
        __X__ A. primarily the result of an ancillary study (list number* __see above_____)
        ___   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 amanuscript 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, 2008which 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.