ARIC Manuscript Proposal # 2533

PC Reviewed: 4/14/15	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title:

Cross-sectional and prospective associations between 25-hydroxyvitamin D levels and subclinical myocardial damage or cardiac wall stress from the ARIC Study

b. Abbreviated Title (Length 26 characters):

Vitamin D, hs-cTnT, NT-proBNP

2. Writing Group:
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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**

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3. Timeline:

Analyses for this proposal will take place in spring of 2015 immediately following approval of this proposal, with goal to submit an abstract for the American Heart Association meeting (deadline June 10) and manuscript to follow.

4. Rationale:

Low levels of vitamin D, as measured by serum 25-hydroxyvitamin D [25(OH)D], have been estimated to affect approximately 1 billion people worldwide[1] and are associated with increased risk of cardiovascular diseases (CVD)[2], as well as specifically for coronary heart disease (CHD).[3,4] Suboptimal vitamin D status is thought to influence CVD risk predominantly by acting on established CVD risk factors, namely hypertension, diabetes, and inflammation.[5] Whether adequate vitamin D supplementation in those that are deficient can prevent CVD events is still unknown, and clinical trials are in progress to test this question.

In the ARIC study, we previously have studied the association of low 25(OH)D with CHD (*manuscript under review)* and heart failure (HF)[6] outcomes as follows:

1) Over a median of 20 years, there were 1230 incident CHD events. Whites in the lowest quintile of 25(OH)D (<17 ng/ml) compared to the upper 4 quintiles had an increased risk of incident CHD (HR 1.28, 95% CI 1.05-1.56), but blacks did not (HR 1.03, 0.82-1.28), after adjustment for demographics and behavioral/socioeconomic factors (p-interaction with race=0.22).
2) During 21 years of follow-up 1,799 incident HF events accrued. The association between 25(OH)D and HF varied by race (p-interaction =0.02). Among whites, risk was 2-fold higher for those in the lowest (≤17 ng/mL) versus highest (≥31 ng/mL) quintile of 25(OH)D. The association was attenuated but remained significant with covariate adjustment. In blacks there was no overall association.

High-sensitivity cardiac troponin T (hs-cTn) and n-terminal pro-Brain Natriuretic Peptide (NT-proBNP) are considered biomarkers of myocardial damage and wall stress, respectively. Subclinical myocardial damage may underlie an intermediate phenotype between low vitamin D and incident CHD and/or HF. Potentially, if the association of vitamin D and CHD and/or HF is causal, early myocardial injury may even represent a stage where intervention such as vitamin supplementation may prevent progression to clinical outcomes.

When cardiac damage occurs (from ischemia or various other causes), cardiomyocytes release cardiac troponin into the blood in proportion to the degree of damage. The most important use of troponin testing is to guide management of patients suspected of having acute coronary syndromes. However, elevated levels may be due to cardiac damage associated with chronic structural heart disease rather than from acute ischemia, especially when levels remain generally consistent over the short term.[7] Troponin assays have evolved over time, becoming ever more sensitive. Novel high sensitivity assays (pre-commercial in U.S.) have detection limits 10 to 100 times lower than currently available commercial troponin assays. These highly-sensitive cardiac troponin assays can detect troponin concentrations in a larger percentage of presumably healthy people with no history of CVD—redefining what is "normal".[8] However, hs-cTn elevations, even among those without known clinical CVD, are associated with increased risk for incident CHD, HF,[9] and stroke[10] events.

B-type natriuretic peptides are secreted from cardiomyocytes in response to increased wall stress and play an important role in cardiovascular remodeling, volume homeostasis, and response to ischemia.[11] Elevated levels of NT-proBNP among individuals free of clinical CVD have also been shown to be associated with increased risk of mortality,[12] HF[13], and stroke[10].

Whether low vitamin D levels are associated with subclinical myocardial damage and wall stress is not well established. In previous cross-sectional analyses, serum 25(OH)D levels were found to be associated with cTn levels in patients with stable CHD[14] and in hemodialysis patients[15] using traditional assays, but were not associated with hs-cTnT in the population based Cardiovascular Health Study.[16] In cross-sectional analyses, 25(OH)D was also not associated with NT-BNP in the Hoorn study (older general population of Caucasian men and women),[17] nor was 25(OH)D associated with BNP in a populations of CHD patients[14,18] or in dialysis patients[15]. In the Cardiovascular Health Study, 25(OH)D was associated with NT-proBNP in unadjusted analyses, but not in analyses adjusted for demographic and CVD risk factors.[16] However to our knowledge, there have been no prospective population based studies evaluating low vitamin D with changes in levels of hs-cTnT or NT-proBNP..

The association of low vitamin D with myocardial damage and CVD risk may also vary by race. In ARIC 25(OH)D has been shown to be a stronger risk factor for incident HF and CHD in whites than blacks. Similar findings were observed in Multi-Ethnic Study of Atherosclerosis (MESA); low 25(OH)D was associated with increased CHD risk among whites and Chinese, but not in blacks or Hispanics.[4] Likewise, a prior NHANES analysis found that low 25(OH)D was associated with fatal stroke in whites but not blacks.[19] Racial differences in genetic variations of the vitamin D binding protein (DBP) may underlie these differences in associations of total 25(OH)D and CVD outcomes by race.[20] There are two common single nucleotide polymorphisms (SNPs) on the *DBP* gene, rs7041 and rs4588. Our prior work in ARIC has found significant interactions between these DBP SNPs and low 25(OH)D with risk incident HF,[6] and suggestive (but not statistically significant) interactions with risk of fracture and stroke, but no interaction with CHD risk (*manuscripts under review*). To our knowledge, no prior studies have investigated the association of DBP genetics with subclinical myocardial damage or wall stress as assessed by cTnT and NT-proBNP.

<u>Proposal Significance Summary:</u>

We propose to examine the independent associations of 25(OH)D with prevalent and incident subclinical myocardial damage and wall stress (as assessed by hs-cTnT and NT-proBNP levels) at baseline (visit 2, 1990-1992) and during follow-up from baseline to visit 4 (1996-1998). These analyses will focus on potential interactions by race and by DBP SNP status. Investigating prospective and race-stratified relationships between vitamin D and these prognostic cardiac biomarkers will add to the existing literature in our understanding of a potential intermediate phenotype between low vitamin D status and clinical CHD and HF outcomes.

5. Main Hypothesis/Study Questions:

Hypotheses:

In persons with no history of clinical CVD:

1. Low 25(OH)D levels (<20 ng/ml) will be associated with prevalent and incident elevated hs-cTnT levels (≥14 ng/L), a marker of subclinical myocardial damage, 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. Low 25(OH)D levels (<20 ng/ml) will be associated with prevalent and incident elevated NT-proBNP (≥100 pg/ml), a marker of subclinical myocardial wall stress, 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.

3. We hypothesize that the association between low 25(OH)D with elevated hs-cTnT and elevated NT-proBNP will be modified by race. Low 25(OH)D levels will be more strongly associated with elevated cardiac biomarker levels among whites than among black participants.

4. Similar to the findings from our prior HF outcome analyses, we hypothesize that there will be an effect modification of the association of 25(OH)D with both cardiac biomarkers by rs7041 and rs4588 SNP status. Specially we hypothesize that in both races those with low 25(OH)D and the rs7041 G allele, which predisposes to high DBP (and thus lower bioavailable vitamin D), will be at greater risk for incident elevated hs-cTnT and elevated NT-proBNP.

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**:**

- Cross-sectional at ARIC visit 2
- Prospective: ARIC visit 2 (1990-1992) through biomarker measurement at ARIC visit 4 (1996-1998).

Inclusion/Exclusion

All ARIC participants who had 25(OH)D, hs-cTnT, and NT-proBNP measured from stored serum at ARIC visit 2 (1990-1992). Participants with prevalent CHD and HF reported at visit 1 or incident CHD or HF at or 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.

Variables

Exposure: Serum 25(OH)D measured at visit 2. Since serum vitamin D levels vary greatly by season,[21] 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. 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. We will consider 25(OH)D levels <20 ng/ml as deficient, per Institute of Medicine guidelines, and levels ≥20 ng/ml as sufficient. In supplemental analyses, we will also model 25(OH)D continuously.

Outcomes: Prevalent and incident subclinical myocardial damage and wall stress as measured by hs-cTnT and NT-proBNP.

- Cardiac troponin T levels were measured from stored serum samples at visit 2 (Roche Elecys 2010 Analyzer) in 2012-2013 at the University of Minnesota as part of Dr. Selvin's ancillary study. Cardiac troponin levels were also measured from visit 4 in 2010 using the same assay as part of Dr. Ballantyne's ancillary study (but in plasma on a different machine).
- NT-ProBNP levels were measured from stored samples at visit 2 on a Roche Elecys 2010 Analyzer in 2012-2013 at University of Minnesota as part of Dr. Selvin's ancillary grant. NT-proBNP was also measured from samples at visit 4 on a Cobas e411 analyzer using the Elecys proBNP II immunoassay (Roche Diagnostics) at the Baylor College of Medicine as part of Dr. Ballantyne's ancillary study. Note visit 2 and visit 4 NT-ProBNP used the same Roche assay but was implemented on different machines. However a calibration study was done, and the differences were small enough that a statistical correction was not recommended.

Main covariates (measured at visit 2): Age, race-center, sex, education†, physical activity†, smoking status, alcohol use, BMI, diabetes, LDL-C, HDL-C, triglycerides, antihyperlipidemic medication use, hsCRP, systolic blood pressure, antihypertensive medication, eGFR (modeled as ≥90, 60-89, and 15-59 ml/min/1.73 m^2). eGFR will be estimated using the CKD-Epi equation.
**Unfortunately, vitamin D supplement use was not sufficiently characterized at ARIC visit 2 to be considered as a potential covariate.*
†*Education and physical activity were measured at ARIC visit 1*

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

Data analysis

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 25(OH)D status (deficient vs. replete) and by race.

Hs-cTnT:

- For the primary analysis, we will consider hs-cTnT in a binary fashion, with levels ≥14 ng/L considered elevated.
- For cross-sectional analyses, we will use multivariable-adjusted logistic regression to assess the association of low 25(OH)D <20 ng/ml with prevalence of subclinical myocardial damage (hs-cTnT ≥14 ng/L)
- For prospective analysis, we will exclude individuals with hs-cTnT ≥14 ng/L at visit 2. We will use multivariable-adjusted Poisson relative risk regression to evaluate the risk of

incident elevated hs-cTnT associated with deficient vitamin D status compared to sufficient.

- In a supplemental model, we will also examine the association of vitamin D levels with change in hs-cTnT modeled as a continuous variable using robust linear regression. For this analysis, hs-cTnT levels that are undetectable will be imputed as 1.5 ng/L, which is half the lower limit of blank detection of the Roche assay.

NT-proBNP

- proBNP will be categorized in quintiles, as has been done in prior ARIC analyses.[22]
- We will also consider NT-proBNP in a binary fashion, with levels ≥100 pg/ml as elevated as has been done in MESA[23]
- For cross-sectional analyses, we will use multivariable-adjusted logistic regression to assess the association of low 25(OH)D <20 ng/ml with prevalence of subclinical myocardial damage (top quintile of NT-proBNP or level ≥100 pg/ml)
- For prospective analysis, we will exclude individuals with elevated NT-proBNP at visit 2. We will use multivariable-adjusted Poisson regression to look at the risk of incident elevated NT-proBNP by vitamin D status.
- In a supplemental model, we will also model vitamin D levels with change in log NT-proBNP continuously using robust linear regression.

For the prospective analyses we will explore the impact of accounting for attrition through inverse probability of attrition weighting (IPAW) and/or MICE.

Our models will be sequentially adjusted. We will perform two primary models: Model 1 will be adjusted for demographic factors (age, sex, race/field center [overall models] or center [race-stratified models]). Model 2 will be adjusted for variables included in Model 1 + behavioral/socioeconomic variables (education, physical activity, smoking, body-mass index). We will perform two additional analyses: Model 3: Model 2 + potential mediators (diabetes, systolic, use of hypertension medication, total and HDL cholesterol, use of cholesterol meds, hsCRP, and eGFR), and Model 4: Model 2 + potential mediators + biomarkers related to vitamin D metabolism (calcium, phosphorous, and PTH)

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. Stratified results will be presented if there is evidence for interaction. However, *a priori* we plan to present results overall and stratified by race based on prior studies and inherent interest, regardless of whether or not a significant race interaction is present.

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? __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)?

This is the first ARIC proposal to evaluate vitamin D and subclinical myocardial injury. There are several other ARIC proposals that are investigating other exposures with hs-cTnT and NT-proBNP such as noted below. We have invited several of these authors to participate in this manuscript proposal. Others are welcome to join as well.

#2269 (Bill McEvoy): Risk factors for progression of subclinical myocardial injury: six-year change in highly-sensitive troponin T in a community-based population study.
#2129 (Elizabeth Selvin): Diabetes and incidence and progression of subclinical myocardial injury
#2307 (Anna Fretz): SES and incidence of subclinical myocardial damage
#2140 (Mariana Lazo): 6-year change in NT-proBNP and metabolic changes
#1759 (J Rubin): Associations of traditional cardiovascular risk factors and hs-cTnT
#2025 (Chiadi Ndumele): Obesity and subclinical myocardial injury

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* 2008.10, 2009.16, 2009.17, 2010.01)
___ 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, 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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