25-Hydroxyvitamin D Levels and Markers of Subclinical Myocardial Damage and Wall Stress: The Atherosclerosis Risk in Communities Study

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Background—Low 25-hydroxyvitamin D (25[OH]D) is associated with increased cardiovascular disease risk. Less known is whether 25(OH)D deficiency contributes to subclinical myocardial damage and wall stress (high-sensitivity cardiac troponin T [hs-cTnT] and N-terminal pro−brain natriuretic peptide [NT-proBNP]) or whether associations vary among subgroups.

Methods and Results—Overall, 11 311 Atherosclerosis Risk in Communities participants without prevalent cardiovascular disease had 25(OH)D, hs-cTnT, and NT-proBNP measured at baseline (1990−1992), and 8990 had measurements of hs-cTnT and NT-proBNP repeated 6 years later. We examined associations of deficient 25(OH)D (<20 ng/mL) with prevalent elevated hs-cTnT (≥14 ng/L) and NT-proBNP (≥100 pg/mL), change in hs-cTnT and NT-proBNP, and incident elevated hs-cTnT and NT-proBNP. We tested for interactions by age (<56 and ≥56 years), sex, and race. In fully adjusted models, 25(OH)D was not associated with prevalent elevated hs-cTnT and NT-proBNP. Deficient 25(OH)D, however, was associated with increased 6-year change in hs-cTnT (β=0.54 ng/L [95% CI 0.08−1.01]) but not change in NT-proBNP. Deficiency in 25(OH)D was not associated with incident elevated hs-cTnT in the overall cohort but was associated with incident elevated hs-cTnT in younger but not older adults (relative risk 2.18 [95% CI 1.21−3.94] versus 0.78 [95% CI 0.56−1.08], respectively; *P*=0.01 for interaction by age). Deficient 25(OH)D was also associated with incident elevated NT-proBNP in men but not women (*P*=0.01 for interaction by sex).

Conclusions—Vitamin D deficiency was associated with increased 6-year change in hs-cTnT levels. Hypothesis-generating differences in associations by age and sex, but not race, were observed. If these associations are causal, further research is needed to understand mechanisms by which low 25(OH)D confers increased risk in these subgroups and whether treating deficient 25(OH)D can prevent myocardial damage and wall stress. (*J Am Heart Assoc*. 2016;5:e003575 doi: 10.1161/JAHA.116.003575)

Key Words: brain natriuretic peptide • epidemiology • prevention • troponin T • vitamin D

L̲ow levels of vitamin D, as measured by serum 25-hydroxyvitamin D (25[OH]D), have been estimated to affect ≈1 billion people worldwide[1] and are associated with

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increased risk of cardiovascular disease (CVD),[2] including coronary heart disease (CHD)[3,4] and heart failure (HF).[5] Suboptimal vitamin D status is thought to influence CVD risk predominantly by acting on established CVD risk factors, namely, hypertension, diabetes mellitus, and inflammation.[6] Whether adequate vitamin D supplementation in those who are deficient can prevent CVD events is still unknown, and clinical trials are in progress to test this question.[7]

High-sensitivity cardiac troponin T (hs-cTnT) and N-terminal pro−brain natriuretic peptide (NT-proBNP) are considered biomarkers of myocardial damage and wall stress, respectively. Cardiac troponin is a well-established biomarker of myocardial injury and is used clinically to guide diagnosis and management of patients suspected of having acute coronary syndromes; however, elevated levels may also be caused by cardiac damage associated with chronic structural heart disease rather than acute ischemia, especially when levels remain generally consistent over the short term in asymptomatic populations.[8] New high-sensitivity assays have

expanded the role of cardiac troponin as a prognostic marker even among asymptomatic persons without suspected acute coronary syndromes. Elevations of hs-cTnT, even among those without known clinical CVD, are associated with increased risk of incident CHD and HF events.[9] BNPs are secreted from cardiomyocytes in response to increased wall stress and play an important role in cardiovascular remodeling, volume homeostasis, and response to ischemia.[10] Elevated levels of NT-proBNP among patients free of clinical CVD are also associated with increased risk of CVD[11] and HF.[12]

Subclinical myocardial damage and wall stress may underlie an intermediate phenotype between low vitamin D and incident CHD and/or HF. If the association of vitamin D and CHD or HF is causal, early markers of myocardial injury and stress may represent a stage at which interventions such as vitamin supplementation may prevent progression to clinical outcomes.

Whether low vitamin D levels are associated with subclinical myocardial damage and wall stress is not well established. Prior cross-sectional studies have shown conflicting results.[13–17] To our knowledge, however, there have been no prospective population-based studies evaluating the association of low vitamin D with changes in levels of hs-cTnT or NT-proBNP. It is unknown whether low 25(OH)D is associated with incident subclinical myocardial damage and wall stress and whether this association varies by age, sex, or race subgroups. We hypothesized that low 25(OH)D levels (<20 ng/mL) would be associated with prevalent and incident elevated hs-cTnT (≥14 ng/L) and NT-proBNP (≥100 pg/mL) levels independent of traditional CVD risk factors, lifestyle factors, and socioeconomic status. We further hypothesized that this relationship would remain significant even after adjustment for related markers of mineral metabolism (ie, calcium, phosphate, and parathyroid hormone levels). Based on prior studies, we also hypothesized that associations would be stronger in white compared with black patients,[4,5,18] in younger compared with older patients,[19] and among women relative to men.[20,21]

Methods

Participants

The Atherosclerosis Risk in Communities (ARIC) study is an ongoing community-based prospective cohort of 15 792 middle-aged adults recruited from 4 locations: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland.[22] Participants have taken part in 5 main visits: 1987–1989 (visit 1), 1990–1992 (visit 2), 1993–1995 (visit 3), 1996–1998 (visit 4), and 2011–2013 (visit 5). Participants provided written consent for their involvement in the study. The institutional

review boards from all 4 locations and the coordinating center approved the ARIC study.

The sample for this study comprised participants attending visit 2 (baseline for the present analysis, n=14 348). Exclusion criteria were races other than black or white (n=42) and black participants from the Minneapolis or Washington County sites (n=49), given small numbers; prevalent CHD or HF at visit 2 or missing information on CHD or HF status (n=1519); missing 25(OH)D levels (n=387); missing hs-cTnT or NT-proBNP levels at visit 2 (n=949); estimated glomerular filtration rate <15 mL/min per 1.73 m^2 (n=9); and any missing covariate information (n=82). After all exclusions, the final sample size was 11 311 participants who were available for cross-sectional analysis at ARIC visit 2 (Figure 1).

Of the 11 311 with available 25(OH)D, hs-cTnT, and NT-proBNP levels at visit 2, there were 2321 participants who did not attend ARIC visit 4 or who were missing visit 4 hs-cTnT and NT-proBNP levels, leaving 8990 participants potentially eligible for the evaluation of prospective change in the levels of these biomarkers between ARIC visits 2 and 4. Among these 8990 participants, 257 had elevated hs-cTnT levels (≥14 ng/L) at visit 2 and were excluded from the analyses that evaluated for incident elevated hs-cTnT at visit 4. Also among the 8990 participants, 1767 had elevated NT-proBNP (≥100 pg/mL) at visit 2 and were excluded from the analyses that evaluated for incident elevated NT-proBNP at visit 4 (Figure 2).

Laboratory Analyses

Serum samples used for measurement of 25(OH)D_2 and 25 (OH)D_3 were collected at visit 2 (1990–1992) and stored at −70°C until 2012–2013, when measurement took place using liquid chromatography–tandem high-sensitivity mass spectrometry (Alliance e2795; Waters) at the University of Minnesota Molecular Epidemiology and Biomarker Research Laboratory. Using samples collected in duplicate tubes and stored, the coefficient of variation (processing plus assay variation) was 20.8% for 25(OH)D_2 and 6.9% for 25(OH)D_3. The Pearson correlations from the blind duplicate samples at visit 2 were 0.98 (mean difference −0.04) for 25(OH)D_2 and 0.97 (mean difference −0.38) for 25(OH)D_3. The intraclass correlation coefficients at visit 2 from the blind duplicate samples, calculated using the function *icc* in the R package *irr*, were as follows: 0.96 (95% CI 0.95–0.96) for 25(OH)D_2 and 0.91 (95% CI 0.86–0.92) for 25(OH)D_3. For total 25(OH)D concentration, 25(OH)D_2 and 25(OH)D_3 were added together. To convert 25(OH)D levels from nanograms per milliliter to nanomoles per liter, multiply by 2.496.

In 2012–2013, using stored samples from visit 2, we also measured calcium and phosphorus (Roche Modular P-Chemistry Analyzer; Roche Diagnostics) and parathyroid
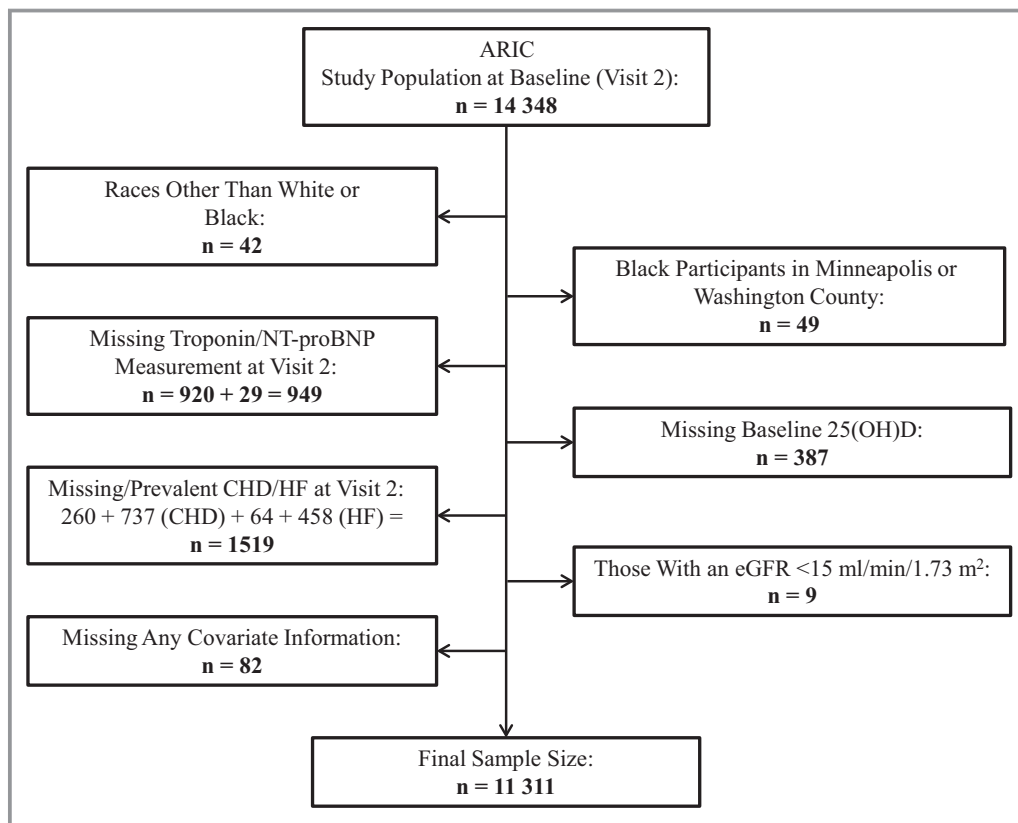
Figure 1. Inclusion and exclusion criteria for participants for cross-sectional analyses at ARIC visit 2 (1990–1992). 25(OH)D indicates 25-hydroxyvitamin D; ARIC, Atherosclerosis Risk in Communities; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; HF, heart failure; NT-proBNP, N-terminal pro–brain natriuretic peptide.

hormone (Elecsys 2010; Roche Diagnostics). High-sensitivity C-reactive protein (hsCRP) was measured from visit 2 samples using a latex-particle–enhanced immunoturbidimetric assay kit (Roche Diagnostics) and read on the Roche Modular P Chemistry analyzer (coefficient of variation 7%). Cystatin C was also measured in 2012–2013 from stored samples collected at visit 2 using the Gentian cystatin C assay on the Roche Modular P Chemistry analyzer. Serum creatinine was measured at visit 2 using a modified kinetic Jaffé reaction. Glomerular filtration rate was estimated using the 2012 Chronic Kidney Disease Epidemiology Collaboration equation, which incorporates both cystatin C and creatinine.[23] Fasting lipids were measured at the time of visit 2. Plasma total cholesterol was determined by enzymatic methods, and high-density lipoprotein cholesterol was measured after dextran–magnesium precipitation.

NT-proBNP and hs-cTnT were measured at 2 time points (ARIC visits 2 and 4, which were 6 years apart) from samples that had been stored at −70°C since collection. For samples collected at ARIC visit 2, hs-cTnT was measured using a Roche Elecsys 2010 Analyzer (Roche Diagnostics) at the University of Minnesota in 2012–2013. The CVs were 6.0% at a mean

hs-cTnT concentration of 25 ng/L and 3.7% at a concentration of 1940 ng/L.[24] For samples from ARIC visit 4, hs-cTnT was measured from stored plasma using the same assay implemented on a Cobas e411 analyzer (Roche Diagnostics) at Baylor College of Medicine in 2010. The coefficients of variation were 6.9% and 2.6% at mean hs-cTnT concentrations of 29 and 2378 ng/L, respectively.[24] The limit of measurement for hs-cTnT was 3.0 ng/L. Samples with levels below the limit of measurement were set to half of the lowest level of measurement (1.5 ng/L). For hs-cTnT at visit 2, 5903 (52.2%) were <3 ng/L and were set to 1.5 ng/L, whereas 5408 (47.8%) were >3 ng/L and measurable.

NT-proBNP levels were measured from stored serum samples at visit 2 on a Roche Elecsys 2010 Analyzer in 2012–2013 at the University of Minnesota and measured from stored plasma samples at visit 4 on a Cobas e411 analyzer using the Elecys proBNP II immunoassay (Roche Diagnostics) at the Baylor College of Medicine in 2010, as described previously.[12] The measurement range was 5 to 35 000 pg/mL, with a value of 2.5 pg/mL assigned to participants with levels below the limit of detection. The coefficient of variation was 3.5% to 4.7%.
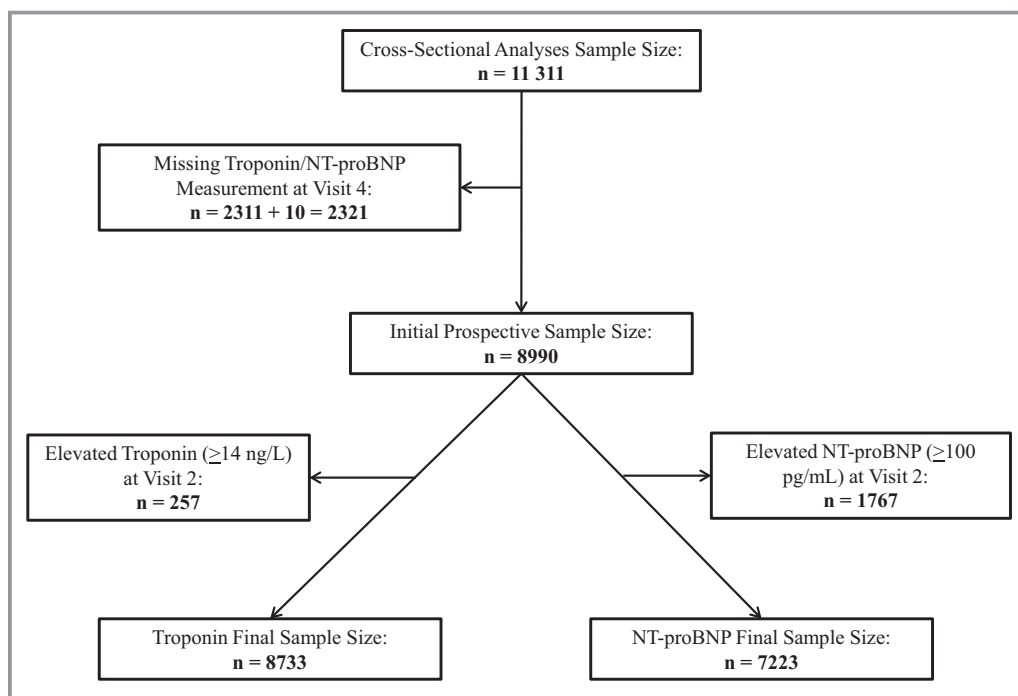
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              ┌─────────────────────────────────────┐
              │ Cross-Sectional Analyses Sample Size:│
              │            n = 11 311                │
              └─────────────────────────────────────┘
                                │
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   │ Missing Troponin/NT-proBNP   │←──────────┤
   │ Measurement at Visit 4:      │
   │ n = 2311 + 10 = 2321         │
   └──────────────────────────────┘
                                │
              ┌─────────────────────────────────────┐
              │   Initial Prospective Sample Size:   │
              │              n = 8990               │
              └─────────────────────────────────────┘
             ╱                                      ╲
┌──────────────────────────┐           ┌──────────────────────────┐
│ Elevated Troponin (≥14 ng/L)         │ Elevated NT-proBNP (≥100 │
│ at Visit 2:              │           │ pg/mL) at Visit 2:       │
│ n = 257                  │           │ n = 1767                 │
└──────────────────────────┘           └──────────────────────────┘
             ╲                                      ╱
┌──────────────────────────┐           ┌──────────────────────────┐
│ Troponin Final Sample Size:          │ NT-proBNP Final Sample Size:│
│ n = 8733                 │           │ n = 7223                 │
└──────────────────────────┘           └──────────────────────────┘
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Figure 2. Inclusion and exclusion criteria for participants for prospective analyses from Atherosclerosis Risk in Communities visit 2 (1990–1992) to visit 4 (1996–1998). NT-proBNP indicates N-terminal pro–brain natriuretic peptide.

The intraindividual reliability of hs-cTnT values has been demonstrated to be high among ARIC study participants.[25] A formal calibration study evaluating heterogeneity across specimen type and laboratory was conducted, and no significant differences were observed (N=200 paired samples).[26]

Covariates

Covariates were measured at visit 2 except for education and physical activity, which were assessed at visit 1. Medication usage, demographic, and behavioral variables were obtained through standard ARIC questionnaires and interviews administered by trained staff.

The main covariates included age, race by center (Minneapolis–white; Washington County–white; Forsyth County–white; Forsyth County–black; Jackson County–black), sex, education, exercise physical activity (measured on a scale of 1–5 based on a modified Baecke Physical Activity questionnaire[27]), smoking status, body mass index (modeled continuously), diabetes mellitus (yes/no; defined as a self-reported physician diagnosis, current diabetes mellitus medication use, fasting serum glucose ≥126 mg/dL, or nonfasting glucose ≥200 mg/dL), sitting systolic blood pressure (continuous; measured in triplicate with a random-zero sphygmomanometer; the mean of the second and third measurements was used in analysis), antihypertensive medications, and total cholesterol, high-density lipoprotein cholesterol, hsCRP (modeled as continuous variables), and estimated glomerular filtration rate (modeled as categories of ≤59.9, 60–89.9, and ≥90 mL/min per 1.73 m^2). In an additional model, we adjusted for the vitamin D–related biomarkers of parathyroid hormone, calcium, and phosphate (modeled continuously).

Statistical Analysis

We adjusted serum 25(OH)D concentrations for seasonal variation because it is well established that 25(OH)D levels are affected by season.[28] To adjust for seasonal variation, we calculated residuals using a linear regression model such that 25(OH)D was the dependent variable, and the month of blood draw was the independent variable. By definition, these residuals are not correlated with month of blood draw. The grand mean was then added to the vitamin D residuals obtained from this model. Residuals were calculated separately for black and white participants. This new variable, "vitamin D adjusted for month of blood draw," is an estimate of average annual 25(OH)D levels and was used as the outcome variable in the analyses. Vitamin D levels were grouped into clinical categories of <20 ng/mL (deficient), 20 to 29 ng/mL (intermediate), and ≥30 ng/mL (optimal).[29]

Elevated hs-cTnT was defined as a level ≥14 ng/L,[30,31] and elevated NT-proBNP was defined as a level ≥100 pg/mL,[32]

consistent with prior studies. Baseline characteristics of the study participants were described using means with their standard deviations or proportions stratified by 25(OH)D groups. To investigate the shape of association between 25(OH)D with hs-cTnT and NT-proBNP, we used restricted cubic splines and adjustment for age, race by center, and sex. The *P* values for nonlinearity for both biomarkers were nonsignificant. The hs-cTnT nonlinear *P* value was 0.23, whereas the NT-proBNP nonlinear *P* value was 0.13. Given this, spline figures are not shown.

We used progressively adjusted logistic regression models to estimate the odds ratios and 95% CIs for having a prevalent elevated hs-cTnT or NT-proBNP level at visit 2 by vitamin D categories and by a continuous distribution of 25(OH)D (per 1-SD higher value). We used multivariable-adjusted Poisson relative risk regression to test the association of 25(OH)D with incident elevation in these 2 myocardial biomarkers at ARIC visit 4 among those without elevated biomarkers at baseline. Finally, we used multivariable-adjusted linear regression to evaluate the association of 25(OH)D levels with change in these myocardial biomarkers levels over time (visit 4 minus visit 2 values).

To account for potential confounders and to explore potential mediators, we used models with increasing degrees of adjustment. We created 2 primary models: Model 1 adjusted for demographic factors (age, sex, and race by center), and model 2 further adjusted for potentially confounding behavioral and socioeconomic variables (education, physical activity, smoking status, and body mass index). We also tested 2 additional models. Model 3 further adjusted for potential mediators in the causal pathway between 25(OH)D and CHD or HF, namely, diabetes mellitus, systolic blood pressure, use of hypertension medication, total and high-density lipoprotein cholesterol, hsCRP, and estimated glomerular filtration rate. Finally, model 4 adjusted for the variables in model 3 plus the vitamin D–related biomarkers of serum calcium, phosphate, and parathyroid hormone.

Wald tests were used to formally test for 2-way multiplicative interactions of 25(OH)D with race, sex, or age in relation to elevated hs-cTnT or NT-proBNP by including cross-product terms in model 2.

In addition, we performed sensitivity analyses using inverse probability weighting to account for attrition due to mortality, loss to follow-up, and incident CHD and HF that occurred between visits 2 and 4. We also performed sensitivity analyses in which participants with incident CHD and HF events between visits 2 and 4 (n=411) were excluded.

The 2-sided *P* values ≤0.05 were considered statistically significant. We performed statistical analyses using SAS version 9.3 (SAS Institute).

Results

The baseline characteristics of the study population (n=11 311) at ARIC visit 2 by vitamin D categories are described in Table 1. In the overall study population, levels of 25(OH)D were deficient (<20 ng/mL) in 32%, intermediate in 45%, and optimal in 23%. The proportion of participants who were black was 46% in the deficient category, 17% in the intermediate category, and 6% in the optimal category. Participants with deficient 25(OH)D <20 ng/mL were more likely to be female and to have more CVD risk factors such as lower physical activity, higher body mass index, higher hsCRP levels, and more prevalent diabetes mellitus.

Cross-Sectional Analyses

The prevalence of elevated hs-cTnT at baseline was 4% (n=433). The multivariable-adjusted cross-sectional associations of vitamin D categories with prevalent elevated hs-cTnT at ARIC visit 2 are shown in Table 2. In models adjusted for demographics only, vitamin D deficiency was associated with increased odds of elevated hs-cTnT (model 1: odds ratio 1.35, 95% CI 1.01–1.81), compared with optimal vitamin D; however, this association was no longer statistically significant after further adjustment for lifestyle factors and CVD risk factors. There were no significant interactions by age, race, or sex.

There were 2375 participants (27% of the women and 13% of the men) who had prevalent elevated NT-proBNP (≥100 pg/mL) at baseline. The multivariable-adjusted cross-sectional associations of vitamin D categories with prevalent elevated NT-proBNP at ARIC visit 2 are shown in Table 3. In the overall cohort, there was no significant association of 25(OH)D with NT-proBNP levels; however, a significant interaction was found by sex (*P*<0.0001, model 2). Men with vitamin D deficiency had greater likelihood of elevated NT-proBNP than men with optimal vitamin D after adjustment for demographic and lifestyle factors (odds ratio 1.42, 95% CI 1.10–1.85; model 2), but this was not true for women (odds ratio 0.97, 95% CI 0.83–1.15). This association remained statistically significant for men after further adjustment for vascular risk factors. There was no interaction by age or race.

Prospective Analyses

As shown in Table 4, participants with higher 25(OH)D levels had relatively less increase in hs-cTnT levels over time after adjustment for demographics, lifestyle, CVD risk factors, and markers of mineral metabolism (model 4, β=−0.24 ng/L [95% CI, −0.41 to −0.07] per 1 SD greater 25(OH)D at visit 2). Compared with optimal vitamin D, deficient vitamin D

Table 1. Baseline Characteristics*[†] by Serum 25(OH)D Categories: The ARIC Study (1990–1992)

Serum 25(OH)D	Deficient, <20 ng/mL	Intermediate, 20–29 ng/mL	Optimal, ≥30 ng/mL
Participants, n	3603	5067	2641
Biomarkers of interest			
25(OH)D (ng/mL), median	15.9	24.8	34.3
hs-cTnT (ng/L), median[‡]	1.5 [4.5]	1.5 [4.5]	1.5 [3.5]
Elevated cTnT (≥14 ng/L)	4.7	3.5	3.3
NT-proBNP (pg/mL), median[‡]	49.2 [66.0]	49.1 [60.9]	52.3 [63.3]
Elevated NT-proBNP (≥100 pg/mL)	21.9	20.1	21.4
Demographics and behaviors			
Age, y	56.1 (5.7)	56.9 (5.7)	57.2 (5.7)
Female, %	69.4	53.3	50.6
Black, %	46.0	17.1	6.3
Education level[§], %			
Less than high school	23.1	19.0	16.7
High school or vocational school	40.3	41.7	45.5
College, graduate or professional school	36.6	39.3	37.8
Smoking status, %			
Current	26.1	19.8	18.6
Former	30.9	37.8	43.4
Never	43.0	42.4	38.0
Physical activity (Baecke index)[§]	2.2 (0.7)	2.5 (0.8)	2.7 (0.9)
Physiological characteristics			
Body mass index, kg/m^2	29.2 (6.2)	27.6 (4.9)	26.1 (4.1)
Diabetes mellitus, %	18.4	12.0	8.1
Systolic blood pressure, mm Hg	123.5 (19.4)	120.4 (18.1)	118.7 (17.5)
Antihypertensive medications, %	33.3	27.2	22.7
Total cholesterol, mg/dL	210.1 (41.1)	209.7 (38.2)	209.3 (37.2)
HDL cholesterol, mg/dL	50.8 (16.9)	49.3 (16.1)	52.2 (17.9)
Cholesterol medications, %	4.6	5.4	5.9
hsCRP (mg/L), median (IQR)	2.7 (1.2, 5.9)	2.0 (1.0, 4.1)	1.8 (0.9, 3.8)
eGFR category, %			
≥90	70.9	66.5	62.5
60–90	26.9	31.6	35.2
<60	2.2	1.9	2.3
Calcium, mg/dL	9.4 (0.4)	9.3 (0.4)	9.3 (0.4)
PTH, pg/mL	47.2 (20.4)	40.7 (14.6)	36.7 (12.2)
Phosphorus, mg/dL	3.6 (0.5)	3.5 (0.5)	3.5 (0.5)

25(OH)D indicates 25-hydroxyvitamin D; ARIC, Atherosclerosis Risk in Communities; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; IQR, interquartile range; NT-proBNP, N-terminal pro–brain natriuretic peptide; PTH, parathyroid hormone.
*All variables had a *P*-value less than 0.05 when comparing differences between groups except for the elevated NT–proBNP, serum calcium, total cholesterol, and use of cholesterol medications.
[†]Data presented as mean (SD) or percentage unless noted.
[‡]IQR.
[§]Assessed at ARIC visit 1 (1987–1989).

Table 2. The Cross-Sectional Associations of 25-Hydroxyvitamin D With Prevalent Elevated hs-cTnT (≥14 ng/L) at ARIC Visit 2 (1990–1992)

Vitamin D Categories	Deficient, <20 ng/mL	Intermediate, 20–29 ng/mL	Optimal, ≥30 ng/mL	Per 1-SD Higher Vitamin D (8.48 ng/mL)
Total, n	3603	5067	2641	11 311
Elevated hs-cTnT, n	169	178	86	433
Model 1*	1.35 (1.01–1.81)[†]	1.03 (0.79–1.36)	1 (Reference)	0.90 (0.80–1.00)
Model 2[‡]	1.17 (0.87–1.59)	0.95 (0.72–1.25)	1 (Reference)	0.95 (0.84–1.07)
Model 3[§]	1.16 (0.85–1.61)	0.97 (0.73–1.29)	1 (Reference)	0.94 (0.83–1.06)
Model 4[ǁ]	1.11 (0.81–1.54)	0.95 (0.72–1.27)	1 (Reference)	0.96 (0.85–1.09)

ARIC indicates Atherosclerosis Risk in Communities.

Results are presented as number with elevated troponin in each vitamin D group and as progressively adjusted odds ratios (95% CIs) according to clinical categories of vitamin D and per continuous increase in vitamin D. hs-cTnT indicates high-sensitivity cardiac troponin T.

*Model 1: Logistic regression adjusted for age, sex, race/center.

[†]Statistically significant ($P<0.05$).

[‡]Model 2: Model 1 plus adjustment for behavioral and socioeconomic variables (education, physical activity, smoking status, and body mass index).

[§]Model 3: Model 2 plus adjustment for potential mediators (diabetes mellitus, systolic blood pressure, use of hypertension medications, total and high-density lipoprotein cholesterol, use of cholesterol-lowering medications, high-sensitivity C-reactive protein, and estimated glomerular filtration rate categories).

[ǁ]Model 4: Model 3 plus adjustment for mineral metabolism–related biomarkers (calcium, phosphorous, parathyroid hormone).

(<20 ng/mL) was statistically significantly associated with greater increase in hs-cTnT levels in fully adjusted model 4 (β=0.54 ng/L, 95% CI 0.08–1.01). These associations of low vitamin D with increased hs-cTnT over time remained similar in a sensitivity analysis in which participants with incident CHD and HF events between visits 2 and 4 (n=411) were excluded. The modest associations also remained similar in a sensitivity analysis that included only participants with measurable hs-cTnT levels at both ARIC visits 2 and 4 (n=3587; model 4, β=−0.44 ng/L [95% CI −0.83 to −0.05] per 1 SD greater 25(OH)D at visit 2).

There was no interaction by age or race. Initially there was a significant interaction by sex (P=0.02 for interaction, model 2), but further exploration found this to be sensitive to 1 outlier, a male participant with low vitamin D and very elevated change in hs-cTnT of 1351 ng/L. When excluding this outlier (as presented in Table 4), there was no longer a statistically significant sex interaction (P=0.33). Among women, deficient vitamin D levels were statistically significantly associated with greater increase in hs-cTnT than was optimal vitamin D across all 4 models tested. Although there were not statistically significant associations in men, the direction and magnitude of associations were the same as those observed in women. The sample size was smaller for men versus women (3770 versus 5219), thus precision was lower among men.

At follow-up, among those without elevated troponin at baseline, there were 517 cases of incident elevated hs-cTnT. Participants (without elevated values at baseline) who developed incident elevated hs-cTnT were more likely to have higher median hs-cTnT values at baseline compared with those who not developed an incident elevated level (8 versus 1.5 ng/L). In the overall cohort, higher 25(OH)D levels

(assessed per 1-SD higher value) were associated with decreased risk of incident elevated hs-cTnT in demographic adjusted models only (relative risk 0.89 [95% CI 0.81–0.99], model 1); however, there was no statistically significant association in more fully adjusted models or when assessed by categories (Table 5). There was a significant interaction present by age (P=0.01 for interaction, model 2). Vitamin D deficiency was associated with incident elevated hs-cTnT in younger but not older adults (dichotomized at the mean age) after adjustment for demographics, lifestyle and CVD risk factors, and markers of mineral metabolism (relative risk 2.18 [95% CI 1.21–3.94] versus 0.78 [95% CI 0.56–1.08] for <56 versus ≥56 years, respectively; model 4). There were no meaningful interactions by race or sex.

Regarding the prospective analysis for NT-proBNP levels, there were no significant associations of vitamin D categories or vitamin D levels assessed continuously (per 1-SD higher value) with change in NT-proBNP (visit 4 level minus visit 2 level) in any of the multivariable models tested (data not shown). In addition, there were no interactions by age, sex, or race for NT-proBNP change. In sensitivity analyses excluding participants with incident CHD or HF between visits 2 and 4, there still were no significant associations.

Between ARIC visits 2 and 4, there were 1677 cases of incident elevated NT-proBNP among those without elevated NT-proBNP at baseline. Participants who developed incident elevated NT-proBNP were more likely to have higher median NT-proBNP values at baseline compared with those who did not develop an incident elevated level (62.3 versus 33.3 pg/mL). In the overall cohort, there were no significant associations of vitamin D categories with incident elevated NT-proBNP (Table 6). There were no significant interactions by

Table 3. The Cross-Sectional Associations of 25-Hydroxyvitamin D With Prevalent Elevated NT-proBNP (≥100 pg/mL) at ARIC Visit 2 (1990–1992)

Vitamin D Categories	Deficient, 20 ng/mL	Intermediate, 20–29 ng/mL	Optimal, ≥30 ng/mL	Per 1-SD Higher Vitamin D (8.48 ng/mL)
Overall, n	3603	5067	2641	11 311
Elevated NT-proBNP	790	1019	566	2375
Model 1*	1.06 (0.93–1.22)	0.94 (0.83–1.06)	1 (Reference)	0.98 (0.93–1.03)
Model 2†	1.07 (0.93–1.23)	0.95 (0.84–1.08)	1 (Reference)	0.97 (0.92–1.02)
Model 3‡	1.15 (0.99–1.33)	1.03 (0.91–1.17)	1 (Reference)	0.94 (0.89–1.00)
Model 4§	1.06 (0.91–1.23)	1.00 (0.88–1.14)	1 (Reference)	0.98 (0.92–1.03)
Men, n	1103	2366	1305	4774
Elevated NT-proBNP	171	279	147	597
Model 1*	1.56 (1.20–2.01)‖	1.12 (0.90–1.39)	1 (Reference)	0.83 (0.75–0.92)‖
Model 2†	1.42 (1.10–1.85)‖	1.08 (0.86–1.34)	1 (Reference)	0.86 (0.77–0.95)‖
Model 3‡	1.50 (1.13–2.00)‖	1.17 (0.93–1.49)	1 (Reference)	0.82 (0.74–0.92)‖
Model 4§	1.34 (1.00–1.80)‖	1.12 (0.88–1.42)	1 (Reference)	0.87 (0.78–0.97)‖
Women, n	2500	2701	1336	6537
Elevated NT-proBNP	619	740	419	1778
Model 1*	0.94 (0.80–1.10)	0.88 (0.76–1.02)	1 (Reference)	1.02 (0.96–1.08)
Model 2†	0.97 (0.83–1.15)	0.90 (0.78–1.05)	1 (Reference)	1.01 (0.95–1.07)
Model 3‡	1.03 (0.87–1.22)	0.96 (0.82–1.12)	1 (Reference)	0.98 (0.92–1.05)
Model 4§	0.97 (0.81–1.16)	0.94 (0.81–1.10)	1 (Reference)	1.01 (0.95–1.08)

Results are presented as number with elevated BNP in each vitamin D group and as progressively adjusted odds ratios (95% CIs), according to clinical categories of vitamin D and per continuous increase in vitamin D for overall population and stratified by sex. ARIC indicates Atherosclerosis Risk in Communities; NT-proBNP, N-terminal pro–brain natriuretic peptide.
*Model 1: Logistic regression adjusted for age, race by center, and sex (in overall model).
†Model 2: Model 1 plus adjustment for behavioral and socioeconomic variables (education, physical activity, smoking status, and body mass index).
‡Model 3: Model 2 plus adjustment for potential mediators (diabetes mellitus, systolic blood pressure, use of hypertension medications, total and high-density lipoprotein cholesterol, use of cholesterol-lowering medications, high-sensitivity C-reactive protein, and estimated glomerular filtration rate categories).
§Model 4: Model 3 plus adjustment for mineral metabolism–related biomarkers (calcium, phosphorous, parathyroid hormone).
‖Statistically significant ($P < 0.05$).

age or race; however, again, an interaction by sex was noted ($P = 0.01$ for interaction, model 2). Men with intermediate vitamin D levels had increased risk of incident elevated NT-proBNP compared with men with optimal vitamin D. The relative risks of the deficient group were similar to (although not stronger than) those observed for the intermediate group, but the deficient group had fewer events and thus less power. No associations of vitamin D categories and incident elevated NT-proBNP were seen among women.

Finally, for all prospective analyses, we performed sensitivity analyses using inverse probability weighting to examine the effect of attrition between visits 2 and 4. The results were mostly similar to the main results presented (inverse probability weighting data not shown). The only major difference was for the analyses of change in hs-cTnT over time by sex (that was presented in Table 4). When repeating this analysis using inverse probability weighting, results for women were statistically significant for model 1 only, but the direction and magnitude of associations for models 2 to 4 remained similar to those observed in the main results.

Discussion

In this large population-based cohort free of CHD and HF at baseline, low vitamin D was associated with greater 6-year change in hs-cTnT levels, and differences by age and sex were found for the associations with incident hs-cTnT and NT-proBNP. Vitamin D deficiency relative to optimal levels was associated with greater incidence of elevated hs-cTnT among younger members (ie, <56 years) of the cohort. In addition, low vitamin D was associated with incident elevated NT-proBNP among men but with a nongraded relation and not among women.

Vitamin D and Subclinical Myocardial Damage

In our study, we found that low 25(OH)D was not independently associated with prevalent elevated hs-cTnT levels after considering CVD risk factors. Prior cross-sectional studies evaluating vitamin D and cardiac troponin T or I levels have been conflicting. Serum 25(OH)D levels were found to be

Table 4. Levels of 25-Hydroxyvitamin D and Change in High-Sensitivity Cardiac Troponin T (ng/L) From Baseline ARIC Visit 2 (1990–1992) to Visit 4 (1996–1998), Excluding 1 Outlier

Vitamin D Categories	Deficient, <20 ng/mL	Intermediate, 20–29 ng/mL	Optimal, ≥30 ng/mL	Linear per 1-SD Higher Vitamin D (8.40 ng/mL)
Overall, n	2711	4105	2173	8989
Model 1*	0.68 (0.24–1.13)[†]	0.30 (−0.08 to 0.69)	0 (Reference)	−0.26 (−0.43 to −0.10)[†]
Model 2[‡]	0.45 (−0.01 to 0.91)	0.18 (−0.21 to 0.57)	0 (Reference)	−0.18 (−0.35 to −0.01)[†]
Model 3[§]	0.42 (−0.04 to 0.88)	0.18 (−0.21 to 0.57)	0 (Reference)	−0.19 (−0.36 to −0.02)[†]
Model 4[‖]	0.54 (0.08–1.01)[†]	0.24 (−0.15 to 0.63)	0 (Reference)	−0.24 (−0.41 to −0.07)[†]
Men, n	805	1900	1065	3770
Model 1*	0.77 (−0.13 to 1.66)	0.44 (−0.26 to 1.14)	0 (Reference)	−0.33 (−0.66 to 0.003)
Model 2[‡]	0.44 (−0.47 to 1.36)	0.23 (−0.48 to 0.94)	0 (Reference)	−0.21 (−0.54 to 0.13)
Model 3[§]	0.35 (−0.56 to 1.26)	0.27 (−0.44 to 0.97)	0 (Reference)	−0.21 (−0.54 to 0.13)
Model 4[‖]	0.50 (−0.42 to 1.42)	0.34 (−0.36 to 1.05)	0 (Reference)	−0.28 (−0.62 to 0.06)
Women, n	1906	2205	1108	5219
Model 1*	0.62 (0.18–1.06)[†]	0.17 (−0.24 to 0.58)	0 (Reference)	−0.23 (−0.39 to −0.07)[†]
Model 2[‡]	0.49 (0.03–0.95)[†]	0.08 (−0.33 to 0.49)	0 (Reference)	−0.19 (−0.36 to −0.02)[†]
Model 3[§]	0.49 (0.03–0.95)[†]	0.06 (−0.35 to 0.47)	0 (Reference)	−0.21 (−0.38 to −0.04)[†]
Model 4[‖]	0.60 (0.13–1.07)[†]	0.10 (−0.32 to 0.51)	0 (Reference)	−0.25 (−0.42 to −0.08)[†]

Results presented in β coefficients and 95% CIs according to clinical categories of vitamin D and per continuous increase in vitamin D for overall population and stratified by sex. ARIC indicates Atherosclerosis Risk in Communities.
*Model 1: Linear regression adjusted for age, race by center, and sex (in overall model).
[†]Statistically significant (*P*<0.05).
[‡]Model 2: Model 1 plus adjustment for behavioral and socioeconomic variables (education, physical activity, smoking status, and body mass index).
[§]Model 3: Model 2 plus adjustment for potential mediators (diabetes mellitus, systolic blood pressure, use of hypertension medications, total and high-density lipoprotein cholesterol, use of cholesterol-lowering medications, high-sensitivity C-reactive protein, and estimated glomerular filtration rate categories).
[‖]Model 4: Model 3 plus adjustment for mineral metabolism–related biomarkers (calcium, phosphorous, parathyroid hormone).

inversely associated with cardiac troponin levels in patients with stable CHD[13] and in hemodialysis patients[14] using traditional assays but were not associated with hs–cTnT in the population-based Cardiovascular Health Study.[15] This discrepancy may have occurred because in cross-sectional studies, the direction of association is not known, and low vitamin D and elevated cTnT may both be markers of a poorer health state.

Prospective analyses can lend more support to a causal relationship. To our knowledge, this study is the first to evaluate the association of vitamin D levels with change in troponin levels over time and with incident elevations in hs–cTnT above the 14-ng/L threshold. We found that low vitamin D levels were associated only with incident elevated hs–cTnT among younger but not older adults. The reason for this finding is uncertain and possibly due to chance, but it may be that vitamin D plays a more important role in primary prevention of atherosclerosis and structural changes of the heart (ie, intermediate phenotypes that place one at risk for subsequent myocardial injury). It may be that in older patients, who already have a higher prevalence of structural cardiac abnormalities (ie, stage B HF) and subclinical atherosclerosis, the role of vitamin D relative to other traditional risk factors is less impactful. Elevated hs–cTnT is more common in older adults because of a higher prevalence of nonacute coronary syndrome conditions like structural heart disease.[33] A level of 14 ng/L is also a much more extreme elevation by percentile of population in a younger person than in an older person. Of note, for patients with acute coronary syndromes, a prior study found that the relative prognostic significance of an elevated troponin level attenuates with age, such that an elevated troponin value confers a higher relative risk among younger compared with older adults.[19] Furthermore, the diagnostic performance of hs–cTnT for acute coronary syndromes is superior in younger versus older adults.[33]

When considering change in hs–cTnT over 6 years on a continuous level, higher (compared with lower) vitamin D levels were associated with less increase in hs–cTnT levels over time after adjustment of CVD risk factors. This association was statistically significant only in women, although qualitatively, the results were similar in men with no significant interaction by sex. The reason for this possible sex difference is uncertain. Some prior work identified higher vitamin D levels as more strongly associated with a lower risk of CHD among women compared with men,[34] and this would be consistent with our

Table 5. The Longitudinal Associations of 25-Hydroxyvitamin D and Incident Elevated hs-cTnT (≥14 ng/L) at the 6-Year Follow-up Visit (1996–1998) According to Vitamin D Levels Measured at ARIC Visit 2 (1990–1992)

Vitamin D Categories	Deficient, <20 ng/mL	Intermediate, 20–29 ng/mL	Optimal, ≥30 ng/mL	Per 1-SD Higher Vitamin D (8.40 ng/mL)
Overall, n	2616	3994	2123	8733
Elevated hs-cTnT	149	243	125	517
Model 1*	1.26 (0.98–1.63)	1.09 (0.88–1.35)	1 (Reference)	0.89 (0.81–0.99)[†]
Model 2‡	1.07 (0.83–1.40)	1.00 (0.80–1.24)	1 (Reference)	0.95 (0.86–1.05)
Model 3§	1.03 (0.78–1.34)	1.00 (0.80–1.25)	1 (Reference)	0.96 (0.86–1.06)
Model 4‖	1.01 (0.77–1.33)	1.00 (0.80–1.25)	1 (Reference)	0.96 (0.86–1.06)
Age <56 years, n	1356	1831	937	4124
Elevated hs-cTnT	60	70	19	149
Model 1*	2.60 (1.51–4.48)[†]	1.78 (1.07–2.97)[†]	1 (Reference)	0.74 (0.61–0.91)[†]
Model 2‡	2.36 (1.35–4.13)[†]	1.64 (0.98–2.74)	1 (Reference)	0.77 (0.63–0.95)[†]
Model 3§	2.25 (1.25–4.04)[†]	1.76 (1.02–3.01)[†]	1 (Reference)	0.78 (0.63–0.96)[†]
Model 4‖	2.18 (1.21–3.94)[†]	1.74 (1.01–2.99)[†]	1 (Reference)	0.79 (0.64–0.98)[†]
Age ≥56 years, n	1260	2163	1186	4609
Elevated hs-cTnT	89	173	106	368
Model 1*	0.98 (0.73–1.33)	0.96 (0.75–1.22)	1 (Reference)	0.95 (0.85–1.07)
Model 2‡	0.82 (0.60–1.11)	0.87 (0.68–1.11)	1 (Reference)	1.02 (0.91–1.15)
Model 3§	0.79 (0.57–1.08)	0.87 (0.68–1.12)	1 (Reference)	1.03 (0.91–1.15)
Model 4‖	0.78 (0.56–1.08)	0.88 (0.68–1.12)	1 (Reference)	1.02 (0.91–1.15)

Results are presented as number with incident elevated troponin in each vitamin D group and as progressively adjusted relative risks (95% CIs) according to clinical categories of vitamin D and per continuous increase in vitamin D for overall population and stratified by age groups. ARIC indicates Atherosclerosis Risk in Communities; hs-cTnT, high-sensitivity cardiac troponin T.
*Model 1: Poisson regression adjusted for age (in overall model), race by center, and sex.
[†]Statistically significant (P<0.05).
‡Model 2: Model 1 plus adjustment for behavioral and socioeconomic variables (education, physical activity, smoking status, and body mass index).
§Model 3: Model 2 plus adjustment for potential mediators (diabetes mellitus, systolic blood pressure, use of hypertension medications, total and high-density lipoprotein cholesterol, use of cholesterol-lowering medications, high-sensitivity C-reactive protein, and estimated glomerular filtration rate categories).
‖Model 4: Model 3 plus adjustment for mineral metabolism–related biomarkers (calcium, phosphorous, parathyroid hormone).

findings, although our prior work with ARIC did not find any interaction by sex for the association of low vitamin D with incident CHD.[3] Sex differences in levels of high-sensitivity troponin have been noted,[35] with men being more likely than women to be above the 14 ng/L cutoff.[36] Despite women having lower levels of hs-cTnT on average, the prognostic value of high-sensitivity troponin for clinical cardiovascular events has been shown to be greater in women compared with men in some studies[20,21] and not in others.[35]

For the overall cohort, this association of 25(OH)D levels with change in hs-cTnT levels was a very modest relationship (0.54 ng/L greater hs-cTnT levels for participants with vitamin D–deficient compared with optimal levels). Rather than consider change in the overall population, perhaps the more clinically relevant question relates to the risk associated with crossing a clinically significant threshold. Consequently, the analysis of vitamin D status and risk of incident elevated hs-cTnT (defined by 14 ng/L, which is recognized clinically as a significant threshold) is probably the more relevant analysis. We found a 2-fold

increased risk conferred by vitamin D deficiency for incident elevated hs-cTnT among younger (but not older) adults.

In summary, if confirmed in other studies, our findings suggest that vitamin D deficiency—a modifiable factor—could be a contributor to chronic subclinical damage, particularly in younger adults and in women. Nevertheless, interpretations from subgroup analyses should be considered cautiously as hypothesis generating only.

Vitamin D and Subclinical Wall Stress

We did not find any cross-sectional associations of vitamin D with prevalent elevated NT-proBNP in the overall ARIC sample, although sex differences were noted, with vitamin D deficiency being associated with elevated NT-proBNP in men but not in women. This result was contrary to our hypothesis, and the reason for this is uncertain. In our cohort, the prevalence of both vitamin D deficiency and elevated NT-proBNP was higher among women than men.

Table 6. The Longitudinal Associations of 25-Hydroxyvitamin D and Incident Elevated NT-proBNP (≥100 pg/mL) at the 6-Year Follow-up Visit (1996–1998) According to Vitamin D Levels Measured at ARIC Visit 2 (1990–1992)

Vitamin D Categories	Deficient, <20 ng/mL	Intermediate, 20–29 ng/mL	Optimal, ≥30 ng/mL	Per 1-SD Higher Vitamin D
Overall, n	2161	3329	1733	7223
Elevated NT-proBNP, n	468	781	428	1677
Model 1*	1.00 (0.87–1.15)	0.99 (0.88–1.11)	1 (Reference)	1.00 (0.95–1.05)
Model 2†	1.00 (0.86–1.15)	0.99 (0.88–1.12)	1 (Reference)	1.00 (0.95–1.05)
Model 3‡	1.02 (0.88–1.18)	1.03 (0.91–1.16)	1 (Reference)	0.98 (0.93–1.04)
Model 4§	1.02 (0.88–1.18)	1.03 (0.91–1.16)	1 (Reference)	0.98 (0.93–1.04)
Men, n	717	1706	959	3382
Elevated NT-proBNP, n	132	340	170	642
Model 1*	1.22 (0.97–1.55)	1.21 (1.01–1.46)‖	1 (Reference)	0.94 (0.86–1.02)
Model 2†	1.19 (0.93–1.51)	1.21 (1.00–1.46)	1 (Reference)	0.95 (0.87–1.04)
Model 3‡	1.18 (0.92–1.51)	1.23 (1.01–1.48)‖	1 (Reference)	0.94 (0.86–1.03)
Model 4§	1.17 (0.91–1.50)	1.22 (1.01–1.48)‖	1 (Reference)	0.94 (0.86–1.03)
Women, n	1444	1623	774	3841
Elevated NT-proBNP, n	336	441	258	1035
Model 1*	0.89 (0.75–1.06)	0.87 (0.74–1.01)	1 (Reference)	1.02 (0.96–1.09)
Model 2†	0.90 (0.75–1.08)	0.88 (0.75–1.02)	1 (Reference)	1.02 (0.95–1.09)
Model 3‡	0.93 (0.78–1.12)	0.92 (0.78–1.07)	1 (Reference)	1.00 (0.94–1.07)
Model 4§	0.93 (0.77–1.12)	0.92 (0.78–1.08)	1 (Reference)	1.00 (0.93–1.07)

Results are presented as number with incident elevated BNP in each vitamin D group and as progressively adjusted relative risks (95% CIs) according to clinical categories of vitamin D and per continuous increase in vitamin D for overall population and stratified by sex. ARIC indicates Atherosclerosis Risk in Communities; NT-proBNP, N-terminal pro–brain natriuretic peptide.
*Model 1: Poisson regression adjusted for age (in overall model), race by center, and sex.
†Model 2: Model 1 plus adjustment for behavioral and socioeconomic variables (education, physical activity, smoking status, and body mass index).
‡Model 3: Model 2 plus adjustment for potential mediators (diabetes mellitus, systolic blood pressure, use of hypertension medications, total and high-density lipoprotein cholesterol, use of cholesterol-lowering medications, high-sensitivity C-reactive protein, and estimated glomerular filtration rate categories).
§Model 4: Model plus +adjustment for mineral metabolism–related biomarkers (calcium, phosphorous, parathyroid hormone).
‖Statistically significant ($P<0.05$).

Other prior studies generally have not found cross-sectional associations of vitamin D with BNP or NT-proBNP. In cross-sectional analyses, 25(OH)D was not associated with BNP in the Hoorn study (older general population of white men and women),[16] in a population of CHD patients,[13,17] or in dialysis patients.[14] In the Cardiovascular Health Study, 25(OH)D was inversely associated with NT-proBNP in unadjusted analyses but not in analyses adjusted for demographic and CVD risk factors.[15] The Hoorn and Cardiovascular Health Studies did not report on interactions by sex between 25(OH)D and BNP in their respective publications. Consequently, our findings of sex differences in this association of low vitamin D and elevated NT-proBNP should be considered hypothesis generating and need to be confirmed in other studies. A prior study, however, found that low vitamin D levels were correlated with left ventricle dilation by echocardiography in men but not in women in a population of stable HF patients[37]; this result is consistent with our findings of stronger associations among men.

In contrast to the published outcome studies linking low vitamin D to incident HF events, we did not find any overall associations of 25(OH)D with incident elevated levels of NT-proBNP. The absence of an association of vitamin D with this sensitive biomarker of wall stress casts doubt on whether the relationship of low vitamin D and HF is causal. Similar to the cross-sectional analyses, there were some associations of vitamin D with incident elevated NT-proBNP among men but not women. These results were unusual because the associations with incident NT-proBNP were statistically significant for only the intermediate vitamin D group (20–29 ng/mL) and not the deficient group (<20 ng/mL), although the strengths of the association (relative risks) were similar between the 2 low vitamin D groups compared with optimal levels.

The reason for this sex difference noted in the prospective associations of vitamin D with incident elevated NT-proBNP is uncertain, and our finding warrants confirmation in other studies. Past data regarding the relationship of NT-proBNP levels with clinical outcomes by sex are conflicting. Some prior

studies did not find that NT-proBNP levels differed by sex,[38] but others found NT-proBNP levels to be higher among women[39] (as we did). In this ARIC cohort, the median NT-proBNP level was 62.9 pg/mL for women and 34.9 pg/mL for men. Some studies found the prognostic value of NT-proBNP or BNP to be similar by sex,[38] whereas others found that BNP levels were a stronger predictor of mortality in women.[40] Prior work with ARIC found that NT-proBNP was more strongly associated with risk factors associated with HF in men compared with women; therefore, sex-specific prediction models for HF were recommended.[12] Of note, we did not previously find any interaction by sex in the association of low 25(OH)D with incident HF in ARIC.[5]

Limitations and Strengths

Certain limitations should be taken into consideration in the interpretation of our study. First, we had only a single measurement of 25(OH)D that may not reflect long-term vitamin D status, but we adjusted 25(OH)D concentrations for seasonal variation. Second, interaction testing by subgroups was meant to be hypothesis generating, and numbers were low among certain groups. Given the multiple tests performed, some interactions might be expected to occur by chance. Although we did not use Bonferroni-corrected P values, we take the cautious stance that these subgroup analyses are exploratory only and warrant further confirmation in other studies, although many of our findings have some biological plausibility and are supported by other studies. We chose a threshold for NT-proBNP of ≥100 pg/mL as elevated, per prior studies,[32] but levels vary by age and other studies have chosen different thresholds.[41] Higher cut points are used for the diagnosis of clinical HF,[42] but we also examined change in NT-proBNP in a linear fashion.

Our study has many strengths. The ARIC study population is well characterized, and we were able to adjust for many confounding lifestyle variables, CVD risk factors (which may be mediators of these associations), and other markers of mineral metabolism. The cohort is large, and we were able to explore effect modification within a priori defined subgroups of age, race, and sex. Most important, to our knowledge this is the first prospective study that investigated the associations of 25(OH)D levels with changes in markers of both myocardial injury and wall stress. Our work will serve as a platform for future work in this field.

Conclusions

Vitamin D deficiency was associated with increased 6-year change in hs-cTnT levels, and differences by age and sex were found for the associations with incident hs-cTnT and NT-proBNP. Although some of these findings were consistent with

our a priori hypotheses, others were unexpected. All of these subgroup analyses should be considered exploratory and hypothesis generating. If these associations are causal, further research is needed to understand the mechanisms by which low 25(OH)D confers increased risk in these subgroups. Vitamin D deficiency is easy to screen for and can be treated with supplementation and modest sunlight exposure. Randomized clinical trials are needed to determine whether treating deficient 25(OH)D can prevent myocardial damage and wall stress, ultimately leading to the prevention of adverse CVD outcomes.

Disclosures

Dr Michos has served as a consultant for Siemens Diagnostics (modest). Dr Selvin has served on an Advisory Board for Roche Diagnostics. Dr Ballantyne has the following disclosures: Grant/Research Support (all significant; all paid to institution, not individual): Abbott Diagnostic, Roche Diagnostic; Consultant (all modest): Abbott Diagnostics, Roche; Other: Provisional patent (patent no. 61721475) entitled "Biomarkers to Improve Prediction of Heart Failure Risk" filed by Baylor College of Medicine and Roche.

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