Race and Vitamin D Binding Protein Gene Polymorphisms Modify the Association of 25-Hydroxyvitamin D and Incident Heart Failure

The ARIC (Atherosclerosis Risk in Communities) Study

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ABSTRACT

OBJECTIVES This study sought to determine if low serum 25-hydroxyvitamin D (25(OH)D) is associated with incident heart failure (HF) and if the association is: 1) partly mediated by traditional cardiovascular risk factors; 2) stronger among whites than blacks; and 3) stronger among those genetically predisposed to having high levels of vitamin D binding protein (DBP).

BACKGROUND Suboptimal 25(OH)D is a potential cardiovascular risk factor.

METHODS A total of 12,215 ARIC (Atherosclerosis Risk in Communities) study participants free of HF at baseline (1990 to 1992; median age, 56; 24% black) were followed through 2010. Total serum 25(OH)D was measured at baseline using liquid chromatography-mass spectrometry. Incident HF events were identified by a hospital discharge code of ICD9-428 and parallel International Classification of Diseases codes for HF deaths.

RESULTS 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. In both races, those with low 25(OH)D and the rs7041 G allele, which predisposes to high DBP, were at greater risk (p-interaction = 0.01).

CONCLUSIONS Low serum 25(OH)D was independently associated with incident HF among whites, but not among blacks. However, in both races, low 25(OH)D was associated with HF risk among those genetically predisposed to high DBP. These findings provide novel insight into metabolic differences that may underlie racial variation in the association between 25(OH)D and cardiovascular risk. (J Am Coll Cardiol HF 2015;3:347-56) © 2015 by the American College of Cardiology Foundation.
vitamin D is a fat-soluble vitamin obtained through cutaneous synthesis stimulated by sun exposure and through oral intake from food and supplements. Insufficient vitamin D, as assessed by low circulating 25-hydroxyvitamin D (25(OH)D), has recently drawn attention as a potential cardiovascular disease (CVD) risk factor (1,2), although this remains controversial (3,4). If suboptimal 25(OH)D influences CVD risk, it likely does so predominantly by elevating established CVD risk factors, namely hypertension (5), diabetes (6), and inflammation (7). Although several studies have explored associations of 25(OH)D with risk of coronary heart disease (CHD) and stroke (1), much less is known about associations between 25(OH)D and incidence of heart failure (HF) (8,9).

If an association between 25(OH)D and HF exists, it is unclear whether it varies by race or ethnicity. Relative to whites, blacks have low 25(OH)D levels but paradoxically higher bone density and lower fracture risk (10). Additionally, there is some suggestion that associations of low 25(OH)D with risk of diabetes (11), peripheral artery disease (12), stroke (13), and CHD (14) are stronger in whites than blacks. However, prior studies of other CVD phenotypes were often limited in that they were cross-sectional and/or had limited power for race- and ethnicity-stratified analyses. Whether associations between 25(OH)D and HF differ by race is unknown.

Racial differences in vitamin D metabolism are believed to underlie racial and ethnic interactions in associations between 25(OH)D and outcomes. Foremost, recent work suggests that although concentrations of 25(OH)D differ between blacks and whites, levels of bioavailable vitamin D are similar (15). Racial variation in key vitamin D binding protein (DBP) single-nucleotide polymorphisms (SNPs) (i.e., rs7041 and rs4588), which are missense mutations and together explain 80% of the variation in DBP levels, result in blacks and whites having similar levels of bioavailable vitamin D despite disparate levels of 25(OH)D (15). As alternate mechanisms, black individuals have higher circulating concentrations of 1,25(OH)2D at a given level of 25(OH)D (16), and vitamin D receptor gene affinity and polymorphism frequencies vary by race (17).

Using observational data from the prospective ARIC (Atherosclerosis Risk in Communities) cohort we tested the hypotheses that low serum 25(OH)D is associated with incident HF and that this association: 1) is stronger among whites than blacks; 2) is partly mediated by traditional cardiovascular risk factors; and 3) is stronger among those genetically predisposed to having high levels of DBP. In exploratory analyses we also examined whether levels of the 3-epi-25(OH)D2 epimer are associated with HF risk.

METHODS

SELECTION AND DESCRIPTION OF PARTICIPANTS. The ARIC study is a community-based prospective cohort that in 1987 to 1989 recruited a total of 15,792 men and women, age 45 to 64 years, from 4 U.S. communities: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland (18). Only blacks were recruited in Jackson, whereas participants in the other field centers reflected the underlying population (mostly white in Minnesota and Maryland, white and black in North Carolina). Four cohort re-examinations have taken place: 1990 to 1992 (visit 2), 1993 to 1995 (visit 3), 1996 to 1998 (visit 4), and 2011 to 2013 (visit 5). Local institutional review boards approved the ARIC protocol, and all participants gave informed consent.

Serum 25(OH)D was measured in samples collected at ARIC visit 2 (1990 to 1992), which was attended by 14,348 participants. Thus, visit 2 is baseline for the present analysis. Excluded from the analysis are participants who self-identified as neither black nor white (n = 42), blacks from the Minnesota and Maryland centers (n = 49), those who had prevalent HF at visit 2 or were missing variables needed to define prevalent HF (n = 945), or with missing 25(OH)D data because specimens were not available for the C-reactive protein and serum albumin assays were donated by the manufacturers. Genotyping was supported through the National Heart, Lung, and Blood Institute CARE (Candidate Gene Resource) grant (N01-HE-65226). Dr. Lutsey has received grant support from the National Institutes of Health National Heart, Lung, and Blood Institute (R01 HL073706) and the National Institutes of Health Office of Dietary Supplements (R01 HL073706-S1). Dr. Selvin has received grant support from the National Institute of Diabetes and Digestive and Kidney Diseases (R01 DK089574). All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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for measurement (n = 1,097). For the primary analysis our final analytic sample included 12,215 participants. For genetic analyses we further excluded those who did not consent to participate in genetic research or had missing genetic data (n = 487; final sample = 11,728).

25(OH)D AND NONGENETIC VARIABLES. At visit 2, ARIC participants underwent interviews, fasting venipuncture, and measurement of blood pressure and anthropometrics. Participants brought to the visit all medications taken in the 2 weeks before the examination; medication names were transcribed and coded. Physical activity was not assessed at visit 2, so values from visit 1 were carried forward. Height and weight were measured, and body mass index (BMI) calculated as weight/height². Sittting blood pressure was measured in triplicate with a random-zero sphygmomanometer; the mean of the last 2 measurements was analyzed. Diabetes was defined by fasting blood glucose ≥126 mg/dl, nonfasting glucose ≥200 mg/dl, a self-report of physician diagnosis, or current medication use for diabetes.

Participants were asked to fast for 12 h before the blood draw. Plasma and serum were collected at visit 2 and frozen at -70°C until analyzed. Serum 25(OH)D₂, 25(OH)D₃, and the 3-epi-25(OH)D₃ epimer were measured using a high-sensitivity mass spectrometer (AB Sciex 5500, Framingham, Massachusetts) at the Advanced Research and Diagnostic Laboratory, University of Minnesota, Minneapolis, Minnesota, in 2012 to 2013. Epimers have identical chemical structures except for a single site of molecular asymmetry (in this case C-3α- vs. C-3β-hydroxy); it is not presently known to what extent 3-epi-25(OH)D₃ is physiologically active. Using split samples sent 1 week apart, the blind duplicate coefficient of variation and Pearson correlation coefficients were as follows: for 25(OH)D₃, coefficient of variation = 6.9, r = 0.97; for 25(OH)D₂, coefficient of variation = 20.8, r = 0.98; and for 3-epi 25(OH)D₃, coefficient of variation = 16.5, r = 0.76. Lipids were measured at the time of ARIC visit 2 (1990 to 1992). Plasma total cholesterol, triglycerides, and high-density lipoprotein for cholesterol were measured using typical approaches; low-density lipoprotein for cholesterol was calculated. Serum magnesium was measured by the Ginder and Heth procedure. In 2012 to 2013 high sensitivity C-reactive protein and serum albumin were measured on a Modular P Chemistry analyzer (Roche Diagnostics, Indianapolis, Indiana), serum parathyroid hormone (PTH) on a Roche Elecsys 2010 analyzer (Roche Diagnostics) using a sandwich immunomassay method, and serum fibroblast growth factor (FGF)-23 on a 2-site enzyme-linked immunosorbent assay (Kainos Laboratories, Inc., Tokyo, Japan). Cystatin C was measured in 2012 to 2013 using the Gentian cystatin C assay on the Roche Modular P Chemistry analyzer, and serum creatinine in 1990 to 1992 using a modified kinetic Jaffé reaction. Estimated glomerular filtration rate (eGFR) was calculated using the 2012 CKD EPI equation, which incorporates both cystatin C and creatinine (19). eGFR was categorized according to established clinical cutoffs: ≥90, 60 to 89, and ≤59 ml/min/1.73 m².

Prevalent HF was defined for exclusion by any of the following: an affirmative response to “Were any of the medications you took during the last 2 weeks for heart failure?,” stage 3 or “manifest heart failure” by Gothenburg criteria (20,21), or incident HF hospitalization between visits 1 and 2. Pre-existing CHD was defined by self-reported prior physician diagnosis of myocardial infarction (MI) or coronary revascularization, prevalent MI by 12-lead electrocardiogram at visit 1, or an incident adjudicated CHD event between visits 1 and 2. Incident CHD was used as a time-varying covariate, and defined as the first occurrence of a validated definite or probable hospitalized MI or a definite CHD death.

VITAMIN D BINDING PROTEIN SNPS. SNP genotypes for rs7041 and rs4588 were obtained from the ITMAT-Broad-CARe Chip, a custom 50K SNP genotyping array, with genotyping performed at the Broad Institute of the Massachusetts Institute of Technology and Harvard. Quality control procedures have been previously published (22).

OUTCOME ASCERTAINMENT. Hospitalizations and deaths through December 31, 2010 were identified through: 1) annual telephone calls to ARIC cohort participants (or proxy); 2) active surveillance of local hospital discharge indexes; 3) searching state death records; and 4) linkage to the National Death Index. HF incidence was defined as the first occurrence of either a hospitalization that included an International Classification of Diseases-9th Revision (ICD-9) discharge code of 428 (428.0 to 428.9) among the primary or secondary diagnoses or else a death certificate with an ICD-9 code of 428 or an ICD-10 code of 150 among any of the listed diagnoses or underlying causes of death (21).

STATISTICAL ANALYSIS. Visit 2 serum 25(OH)D was calculated as the sum of 25(OH)D₂ and 25(OH)D₃. We accounted for seasonal variation in 25(OH)D levels by computing the residuals from a linear regression model with 25(OH)D as the dependent variable and month of blood draw (modeled categorically) as the independent variable. By definition, these residuals are uncorrelated with month of blood draw. The grand mean was then added to the 25(OH)D residuals...
obtained from this model. This adjustment was performed separately for whites and for blacks, because seasonal variation in 25(OH)D also differs by race. This new variable “25(OH)D adjusted for month of blood draw” is an estimate of average annual 25(OH)D levels and was used as the exposure variable in all analyses. Secondly we also explored associations of the vitamin D epimer (3-epi-25[OH]D₃) with risk of incident HF.

Characteristics of participants at visit 2 are reported separately for blacks and whites, stratified by quintile of 25(OH)D. For the primary analysis, Cox proportional hazards regression was used to determine associations between 25(OH)D and incident HF. Person-time accrued from the date of the participants’ visit 2 examination until incident HF, loss-to-follow-up, death, or December 31, 2010, whichever came first. Restricted cubic splines were used to explore the dose–response association. We tested the linear trend across the 25(OH)D quintiles by modeling the median of each quintile as a linear term, and separately tested the hazard ratio (HR) per 1 SD (8.53 ng/ml) increment of 25(OH)D. Our first model adjusted for demographics. Model 2 additionally adjusted for educational attainment, physical activity, smoking status, and BMI. Model 3 further adjusted for traditional cardiovascular risk factors. In additional models, we also adjusted separately for eGFR categories, serum magnesium, PTH, FGF-23, serum albumin, and incident CHD, the latter as a time-varying covariate. Cross-product terms were included in the models to evaluate interactions; stratified results are reported, as appropriate. For SNPs, an additive genetic model was used. In sensitivity analyses, we restricted our analysis to participants whose self-reported health was good or excellent at visit 2. The proportional hazards assumption was evaluated quantitatively by testing the interaction between 25(OH)D quintiles and ln(time), and qualitatively by inspection of ln(-ln) survival curves. SAS version 9.2 (SAS Institute Inc., Cary, North Carolina) was used.

RESULTS

The analytic sample of 12,215 participants was on average 57 years old, 24% were black, 56% were female, 34% had prevalent hypertension, and 5% had prevalent CHD. Race-stratified associations between serum 25(OH)D levels and potential covariates are presented in Table 1 (quintiles 1, 3, and 5 only) and in Online Table 1 (all quintiles). Median 25(OH)D was 25.6 ng/ml in whites, and 18.2 ng/ml in blacks. In both racial groups participants with low 25(OH)D tended to be younger, female, less active, more overweight, more likely to have diabetes, and have higher high-sensitivity C-reactive protein and PTH. Among whites, low 25(OH)D was also associated with more adverse blood pressure and lipid profiles.

RACE, 25(OH)D, AND INCIDENT HF. Over a median follow-up of 18 years (maximum 21), a total of 1,799 incident HF events accrued (1,252 among whites, 547 among blacks). The association between 25(OH)D and incident HF varied by race (model 1, p-interaction = 0.02) as visually depicted by the restricted cubic spline models (Figures 1A and 1B) and shown in Table 2. As shown in Figure 1A, among whites there seemed to be a threshold effect whereby HF risk was increased at 25(OH)D levels below 20 ng/ml, but at levels above 20 ng/ml risk was constant. In multivariable analyses (Table 2) whites in the lowest quintile of 25(OH)D were at 1.98 (95% confidence interval [CI]: 1.65 to 2.38) times greater risk of incident HF, after accounting for age and sex. Further adjustment for behaviors and BMI attenuated the HR to 1.42 (95% CI: 1.17 to 1.72), whereas with additional adjustment for numerous cardiovascular risk factors that may be on the causal pathway between 25(OH)D and HF the HR was 1.27 (95% CI: 1.04 to 1.55). Estimates were similar with further adjustment for eGFR category, PTH, FGF23, serum albumin, serum magnesium, prevalent CHD, and incident MI as a time-varying covariate (data not shown). Results were also similar in sensitivity analyses, which restricted the sample to participants who self-reported being in good or excellent health at visit 2 (n total/HF events = 10,316/1,276: model 1 HR(Q1 vs. Q5) = 1.85 [95% CI: 1.49 to 2.29]; p-trend < 0.0001).

Among blacks, regardless of the degree of adjustment, there was no evidence of an association between 25(OH)D and incident HF (Table 2, Figure 1B). There was no evidence of interaction in the association of 25(OH)D with incident HF by age, sex, serum magnesium, prevalent hypertension, or prevalent CHD in either blacks or whites (p-interaction >0.05 for all). In sensitivity analyses when race-specific quintiles were used, a similar pattern was observed (data not shown). There was also no evidence that the 3-epi-25(OH)D₃ epimer was associated with HF risk, after accounting for 25(OH)D in either the full population (Online Table 2), or race-stratified analyses. The 3-epi-25(OH)D₃ and 25(OH)D are correlated at r = 0.46.

DBP GENE POLYMORPHISMS, 25(OH)D, AND INCIDENT HF. Frequencies of key DBP gene polymorphisms varied by race. Among whites for rs7041 the G allele frequency was 56%, whereas in blacks it was 16%. For rs4588 the A allele frequency was 28% in whites and 10% in blacks.
DBP gene polymorphism rs7041 modified the association between 25(OH)D and incident HF in the full ARIC sample (p-interaction = 0.001 across all models), even after adjusting for race. Stratified results are presented in Table 3, Figure 2, and Online Table 3; note the different referent categories for Table 3 and Figure 2. Among those with the rs7041 GG alleles, who would be genetically predisposed to higher DBP levels, the HR for those in the lowest versus highest quintile of 25(OH)D was 2.53 (95% CI: 1.80 to 3.55) after demographic adjustments. For those who were GT for rs7041 the HR for extreme 25(OH)D quintiles was 1.63 (95% CI: 1.27 to 2.08), whereas among those who were TT the HR was 1.18 (95% CI: 0.85 to 1.64). With additional adjustment for behaviors and cardiovascular risk factors, the association among those who were GG remained statistically significant (1.67 [95% CI: 1.15 to 2.42]) but was null for those with the TG and TT alleles.

In race stratified analyses (Table 3, Online Table 3), there was suggestive evidence that rs7041 modified the association between 25(OH)D and HF in both whites and blacks: whites p-interaction model 1 = 0.01, model 2 = 0.01, model 3 = 0.01; blacks p-interaction model 1 = 0.03, model 2 = 0.02, model 3 = 0.01. In whites, similar to the full ARIC population, associations were strongest among participants who were GG. Because of the low G allele frequency among

<table>
<thead>
<tr>
<th>TABLE 1 Participant Characteristics by Baseline Serum 25(OH)D Quintiles* in Whites and Blacks: The ARIC Study 1990-1992</th>
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<tbody>
<tr>
<td>25(OH)D quintiles</td>
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<tr>
<td>Median, ng/ml</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>N</td>
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</tbody>
</table>

Demographics
- Age, yrs: 56.7 ± 5.6, 57.0 ± 5.7, 57.3 ± 5.7, 55.3 ± 5.5, 56.6 ± 6.0, 57.9 ± 5.8
- Female, %: 69.4, 50.5, 49.8, 74.2, 59.0, 37.9
- Education level, %<br>  - <High school: 14.6, 14.3, 16.4, 32.7, 43.0, 49.0
  - High school: 49.0, 43.7, 46.4, 30.4, 26.1, 21.6
  - >High school: 36.4, 42.0, 37.2, 36.9, 30.9, 29.4

Behavioral characteristics
- Sport index: 2.3 ± 0.7, 2.5 ± 0.8, 2.8 ± 0.9, 2.1 ± 0.7, 2.2 ± 0.7, 2.3 ± 0.7
- Smoking status, %<br>  - Current: 30.4, 19.5, 18.6, 25.5, 20.9, 30.1
  - Former: 33.1, 39.3, 44.4, 26.3, 30.5, 37.9
  - Never: 36.5, 41.2, 37.0, 48.2, 48.6, 32.0

Physiologic characteristics
- BMI, kg/m²: 28.6 ± 6.1, 27.6 ± 4.7, 25.9 ± 4.0, 30.7 ± 6.9, 29.2 ± 5.5, 28.9 ± 4.9
- Prevalent diabetes, %: 16.0, 10.9, 7.4, 23.2, 23.5, 16.3
- Systolic BP, mm Hg: 121 ± 18, 119 ± 18, 118 ± 17, 127 ± 21, 125 ± 21, 125 ± 23
- Hypertension meds, %: 27.8, 25.6, 23.8, 44.7, 46.9, 41.2
- Lipid-lowering meds, %: 6.5, 6.7, 7.3, 2.9, 3.9, 4.0
- HDL-C, mg/dl: 47.7 ± 16.1, 47.8 ± 15.9, 51.9 ± 18.0, 53.4 ± 17.7, 53.9 ± 15.4, 51.8 ± 16
- LDL-C, mg/dl: 133 ± 38, 133 ± 35, 132 ± 35, 134 ± 39, 137 ± 41, 135 ± 37
- hs-CRP, mg/l: 4.7 ± 8.1, 3.6 ± 6.2, 3.5 ± 6.9, 6.0 ± 7.7, 5.1 ± 8.1, 5.3 ± 7.2
- Magnesium, mEq/l: 1.6 ± 0.2, 1.6 ± 0.2, 1.6 ± 0.2, 1.6 ± 0.2, 1.6 ± 0.2, 1.6 ± 0.2
- PTH, pg/ml: 46.3 ± 18.8, 40.8 ± 14.6, 36.1 ± 11.9, 53.8 ± 33.0, 43.6 ± 21.9, 40.4 ± 13.8
- eGFR, ml/min/1.73 m²: 93.9 ± 16.1, 95.0 ± 14.9, 93.2 ± 15.3, 102.3 ± 19.5, 101.0 ± 20.0, 101.6 ± 19.7
- eGFR category, %<br>  - >90: 62.4, 64.9, 61.0, 77.4, 76.1, 62.4
  - 60-89: 35.5, 33.6, 36.3, 19.9, 20.5, 32.2
  - <60: 2.1, 1.5, 2.7, 2.7, 3.4, 5.4
- FGF-23, pg/ml: 52.7 ± 366.4, 43.5 ± 15.1, 44.9 ± 15.6, 72.0 ± 985.3, 64.5 ± 379.9, 71.5 ± 348.2
- Prevalent CHD, %: 4.8, 4.6, 5.5, 3.3, 3.4, 4.0

Values are mean ± SD or %, unless otherwise indicated. *Quintiles 1, 3, and 5 are presented here to conserve space, all 5 quintiles are presented in Online Table 1.

25(OH)D = 25-hydroxyvitamin D; ARIC = Atherosclerosis Risk in Communities study; BMI = body mass index; BP = blood pressure; CHD = coronary heart disease; eGFR = estimated glomerular filtration rate; FGF = fibroblast growth factor; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; LDL-C = low-density lipoprotein cholesterol; PTH = parathyroid hormone.
blacks we combined the GG and TG categories. The association between 25(OH)D and incident HF was stronger among blacks with either GG or TG alleles, relative to those with the TT alleles.

There was no evidence that rs4588 modified the association between 25(OH)D and incident HF (data not shown; p-interaction >0.5 in all models).

**DISCUSSION**

In this large community-based sample of 9,311 white and 2,904 black participants of the ARIC study we found important variation in the association between 25(OH)D and incident HF by both race and key DBP gene polymorphisms. Low 25(OH)D was

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**TABLE 2** Adjusted Hazard Ratios (95% Confidence Intervals) of Baseline Serum 25(OH)D With Incident Heart Failure in the Overall Sample and by Race: The ARIC Study 1990-2010

<table>
<thead>
<tr>
<th>Serum 25(OH)D</th>
<th>Quintile 1</th>
<th>Quintile 2</th>
<th>Quintile 3</th>
<th>Quintile 4</th>
<th>Quintile 5</th>
<th>p-Trend (per 1-SD* Change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (ng/ml)</td>
<td>14.0</td>
<td>19.6</td>
<td>23.9</td>
<td>28.4</td>
<td>35.1</td>
<td>1,799/12,215</td>
</tr>
</tbody>
</table>

**Full cohort**

| n events/n total | 424/2,443 | 384/2,443 | 395/2,443 | 296/2,443 | 300/2,443 | 1,799/12,215 |
| Incidence rate†  | 10.7      | 9.4       | 9.5       | 7.1        | 7.1        | 1,799/12,215  |
| Model 1          | 1.67 (1.42-1.95) | 1.35 (1.15-1.57) | 1.36 (1.17-1.58) | 1.01 (0.86-1.19) | 1.00 (reference) | <0.0001 |
| Model 2          | 1.28 (1.09-1.51) | 1.16 (0.99-1.36) | 1.23 (1.06-1.44) | 0.95 (0.81-1.12) | 1.00 (reference) | 0.0003 |
| Model 3          | 1.15 (0.97-1.36) | 1.05 (0.89-1.23) | 1.18 (1.01-1.38) | 0.90 (0.76-1.06) | 1.00 (reference) | 0.03 |

**Whites**

| n events/n total | 201/1,173 | 237/1,673 | 303/2,023 | 240/2,154 | 271/2,290 | 1,252/9,311 |
| Incidence rate†  | 10.6      | 8.3       | 8.8       | 6.5        | 6.8        | 1,252/9,311 |
| Model 1          | 1.98 (1.65-2.38) | 1.38 (1.16-1.64) | 1.36 (1.15-1.60) | 0.98 (0.83-1.17) | 1.00 (reference) | <0.0001 |
| Model 2          | 1.42 (1.17-1.72) | 1.15 (0.96-1.37) | 1.21 (1.02-1.43) | 0.92 (0.78-1.10) | 1.00 (reference) | 0.0001 |
| Model 3          | 1.27 (1.04-1.55) | 1.07 (0.89-1.28) | 1.18 (1.00-1.40) | 0.88 (0.74-1.06) | 1.00 (reference) | 0.005 |

**Blacks**

| n events/n total | 223/1,270 | 147/770 | 92/422 | 56/289 | 29/153 | 547/2,904 |
| Incidence rate†  | 10.8      | 11.8    | 13.2    | 12.2    | 12.1    | 547/2,904 |
| Model 1          | 1.15 (0.78-1.71) | 1.10 (0.74-1.64) | 1.20 (0.79-1.82) | 1.07 (0.68-1.67) | 1.00 (reference) | 0.57 |
| Model 2          | 1.08 (0.73-1.61) | 1.10 (0.74-1.64) | 1.24 (0.82-1.89) | 1.08 (0.69-1.69) | 1.00 (reference) | 0.93 |
| Model 3          | 0.91 (0.61-1.35) | 0.91 (0.60-1.36) | 1.04 (0.68-1.58) | 0.93 (0.59-1.47) | 1.00 (reference) | 0.46 |

Model 1 adjusted for age (years) and sex. Analyses of the full cohort are also adjusted for race. Model 2 adjusted for model 1 + educational attainment, physical activity (Baecke sport activity index), smoking status (current, former, never), and body mass index (kg/m²). Model 3 adjusted for model 2 + prevalent diabetes, hypertension medication use, systolic blood pressure, LDL cholesterol, HDL cholesterol, cholesterol medication use, and hs-CRP. *1 SD = 8.53 ng/ml. †Unadjusted incidence rate per 1,000 person-years. Abbreviations as in Table 1.
associated with greater HF risk among whites, even after accounting for numerous potential mediators, suggesting that low 25(OH)D may influence HF risk independent of these established cardiovascular risk factors. 25(OH)D was unrelated to HF risk in blacks. Furthermore, we provide evidence suggesting that, in both racial groups, presence of the rs7041 G allele may be synergistic with low 25(OH)D in increasing HF risk. This allele is less common in blacks, thus predisposing blacks to lower DBP levels and more bioavailable vitamin D relative to whites. Hence, variation in bioavailable D may underlie the race interaction observed between 25(OH)D concentrations and HF risk.

These findings extend the results of prior studies in that: 1) very little research has evaluated the prospective association between 25(OH)D and incident HF; 2) studies of 25(OH)D and cardiovascular risk have generally included relatively few events among blacks, a pertinent group given that they have both low levels of 25(OH)D and are at greatest risk of HF; and 3) by exposing an interaction between DBP SNPs and 25(OH)D on HF risk, which may partly explain racial variation in the association between 25(OH)D and HF.

**TABLE 3** Adjusted Hazard Ratios (95% Confidence Intervals) of Baseline Serum 25(OH)D With Incident Heart Failure Stratified by rs7041, in the Overall Sample and by Race: The ARIC Study 1990-2010

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<tbody>
<tr>
<td>Full cohort</td>
<td></td>
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<tr>
<td>TT n events/n total</td>
<td>199/1,183</td>
<td>139/902</td>
<td>107/668</td>
<td>79/539</td>
<td>48/344</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.01 (0.72-1.41)</td>
<td>0.88 (0.63-1.23)</td>
<td>1.00 (0.71-1.40)</td>
<td>0.94 (0.66-1.34)</td>
<td>1.00 (reference)</td>
<td>0.95</td>
</tr>
<tr>
<td>TG n events/n total</td>
<td>140/829</td>
<td>152/1,009</td>
<td>177/1,091</td>
<td>139/1,098</td>
<td>146/1,132</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.18 (0.92-1.52)</td>
<td>1.08 (0.85-1.36)</td>
<td>1.17 (0.94-1.47)</td>
<td>1.01 (0.80-1.27)</td>
<td>1.00 (reference)</td>
<td>0.17</td>
</tr>
<tr>
<td>GG n events/n total</td>
<td>56/299</td>
<td>74/441</td>
<td>87/597</td>
<td>61/700</td>
<td>97/896</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.83 (1.28-2.63)</td>
<td>1.67 (1.21-2.29)</td>
<td>1.32 (0.98-1.77)</td>
<td>0.77 (0.56-1.06)</td>
<td>1.00 (reference)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Whites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT n events/n total</td>
<td>46/314</td>
<td>44/387</td>
<td>56/393</td>
<td>47/378</td>
<td>27/257</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.32 (0.80-2.19)</td>
<td>0.97 (0.59-1.58)</td>
<td>1.29 (0.81-2.05)</td>
<td>1.03 (0.64-1.65)</td>
<td>1.00 (reference)</td>
<td>0.40</td>
</tr>
<tr>
<td>TG n events/n total</td>
<td>93/552</td>
<td>116/814</td>
<td>140/978</td>
<td>118/999</td>
<td>140/1,077</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.25 (0.94-1.64)</td>
<td>1.03 (0.80-1.32)</td>
<td>1.03 (0.81-1.31)</td>
<td>0.95 (0.74-1.22)</td>
<td>1.00 (reference)</td>
<td>0.16</td>
</tr>
<tr>
<td>GG n events/n total</td>
<td>52/269</td>
<td>68/420</td>
<td>86/584</td>
<td>60/692</td>
<td>96/890</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.87 (1.30-2.70)</td>
<td>1.60 (1.16-2.22)</td>
<td>1.33 (0.98-1.79)</td>
<td>0.78 (0.56-1.08)</td>
<td>1.00 (reference)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blacks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT n events/n total</td>
<td>153/869</td>
<td>95/515</td>
<td>51/275</td>
<td>32/161</td>
<td>21/87</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.77 (0.48-1.23)</td>
<td>0.72 (0.45-1.16)</td>
<td>0.73 (0.44-1.22)</td>
<td>0.82 (0.48-1.43)</td>
<td>1.00 (reference)</td>
<td>0.45</td>
</tr>
<tr>
<td>TG/GG n events/n total</td>
<td>51/307</td>
<td>42/216</td>
<td>38/126</td>
<td>22/107</td>
<td>7/61</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>1.93 (0.86-4.35)</td>
<td>2.36 (1.05-5.28)</td>
<td>3.22 (1.43-7.26)</td>
<td>2.15 (0.91-5.08)</td>
<td>1.00 (reference)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Model 2 adjusted for age (years), sex, educational attainment, physical activity (Baecke sport activity index), smoking status (current, former, never), and body mass index (kg/m²). Analyses of the full cohort are also adjusted for race. Available in Online Table 3: model 1 (age-, sex-, and where appropriate race-adjusted) and model 3 (cardiovascular disease risk factor adjusted).

Abbreviations as in Table 1.
Our findings in whites, that low 25(OH)D is associated with greater risk of incident HF independent of traditional cardiovascular risk factors, are consistent with results from the prior prospective studies. However, the ARIC population differs in that it was community-based, whereas the Intermountain population consisted of people in whom 25(OH)D levels were drawn for clinical indications (e.g., osteoporosis risk) (8), and in the German population more than 65% had prevalent CHD (9). People with comorbidities may have lower sunlight exposure because they may be less likely to participate in outdoor recreational activities, and thus have lower 25(OH)D levels. In ARIC our findings were robust even when the analysis was restricted to those self-reporting good or excellent health.

There are no prior studies of 25(OH)D and HF incidence in blacks with which to compare our findings. However, similar race interactions between 25(OH)D and cardiovascular outcomes have been reported (13,14).

**DBP GENE POLYMORPHISMS, 25(OH)D, AND INCIDENT HF.**

Racial variation in the DBP SNP rs7041 may be an important contributor to the race interaction we observed between 25(OH)D and HF risk. Frequency of the G allele varies dramatically by race (56% in whites, and 16% in blacks in ARIC, which is similar to other studies [15]). Each copy of this allele is associated with approximately 189 μg/ml higher levels of DBP (15), which should result in lower levels of bioavailable vitamin D. As recently shown by Powe et al. (15), blacks have lower DBP levels and lower 25(OH)D concentrations compared with whites, resulting in similar levels of bioavailable vitamin D. Approximately 85% to 90% of circulating 25(OH)D is tightly bound to DBP and therefore is generally believed to be unavailable for use. The remaining circulating 25(OH)D (of which 10% to 15% is bound to albumin and <1% free) is considered bioavailable.

Our finding that associations between low 25(OH)D and incident HF were stronger among participants who were GG for rs7041 (HR: 2.53) relative to those who were GT (HR: 1.63) or TT (HR: 1.18) suggests that HF risk at a given concentration of 25(OH)D may be greater in those genetically predisposed to have higher DBP, and likely subsequently lower bioavailable vitamin D. Importantly, there was evidence of this interaction among both blacks and whites.

We did not observe an interaction between rs4588 and 25(OH)D on risk of incident HF. However, power was lower because the rs4588 A allele frequency is only 28% in whites and 10% in blacks, and each copy of the A allele is associated with a more modest increase in levels of DBP (~55 μg/ml), relative to each copy of the rs7041 G allele (~189 μg/ml) (15).

To our knowledge, no other study has assessed whether DBP SNPs modify the association between 25(OH)D and cardiovascular risk. One prior study evaluated the main effect between DBP, SNPs and stroke risk, but found no association (27).

**STRENGTHS AND LIMITATIONS.** The most prominent strength of this investigation is the large community-based sample of black and white adults. Nearly 1,800 incident HF events accrued (more than 500 among blacks), therefore allowing us reasonable power to evaluate interactions and perform key subgroup analyses. Also, the ARIC population is well characterized, allowing adjustment for numerous potential confounders and mediators. The most important limitations of this work are its observational nature, and the fact that DBP concentrations were not available; therefore, we were unable to calculate bioavailable vitamin D. Also, 25(OH)D was only measured once, and thus regression dilution bias may have attenuated relative hazard estimates (28).

Although not possible for this manuscript given ARIC’s design, it has been recommended that prospective cohort studies measure serum 25(OH)D concentrations every 2 to 4 years to obtain more accurate estimates of associations between 25(OH)D and outcomes (29).
Lastly, HF events were identified through ICD codes from hospital discharge and death certificates, and thus cases of HF that were managed exclusively in outpatient settings would have been missed. However, ARIC has shown HF ICD codes to have high validity (30).

CONCLUSIONS

In this prospective community-based cohort low 25(OH)D concentrations (particularly <20 ng/ml) were associated with greater risk of incident HF among whites, independent of traditional cardiovascular risk factors thought to mediate the association. Among blacks, there was no evidence of a relationship. Our results also suggest that, regardless of race, low 25(OH)D was a more potent risk factor among individuals genetically predisposed to high DBP levels, and by extension lower bioavailable vitamin D. These findings provide novel insight into metabolic differences that may underlie racial variation in the association between 25(OH)D and cardiovascular risk. Vitamin D supplementation is not presently recommended for CVD prevention (4); our results suggest that were it recommended, a “one dose fits all” approach may be inappropriate. Supplementation, if recommended, would likely only benefit those who are deficient, and levels of bioavailable D may need to be taken into account.

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PERSPECTIVES

CLINICAL COMPETENCY: Low 25(OH)D is associated with elevated risk of developing heart failure in white, but not black, individuals.

TRANSLATIONAL OUTLOOK: It is possible that bioavailable D is the aspect of vitamin D metabolism most closely linked to cardiovascular health; additional research is clearly warranted. Furthermore, evaluation of bioavailable D may explain why associations between 25(OH)D and outcomes are stronger in whites than blacks.

REFERENCES


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and survival (from the Atherosclerosis Risk in Communities Study). Am J Cardiol 2008;101:1016–22.


**KEY WORDS** ARIC, heart failure, race, vitamin D, vitamin D binding protein

**APPENDIX** For supplemental tables, please see the online version of this article.