Race, vitamin D–binding protein gene polymorphisms, 25-hydroxyvitamin D, and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study\(^1\)-\(^4\)

Jared P Reis, Erin D Michos, Elizabeth Selvin, James S Pankow, and Pamela L Lutsey

**ABSTRACT**

**Background:** Low 25-hydroxyvitamin D [25(OH)D] is associated with diabetes, but few studies have examined racially diverse populations while also accounting for key vitamin D–binding protein (DBP) gene polymorphisms.

**Objective:** We sought to evaluate whether the association between 25(OH)D and incident diabetes varied by race and important DBP single nucleotide polymorphisms (SNPs).

**Design:** We studied 10,222 adults (8120 whites, 2102 blacks) aged 46–70 y at baseline (1990–1992) from the ARIC (Atherosclerosis Risk in Communities) Study with follow-up for incident diabetes ascertained during study visits conducted in 1993–1995 and 1996–1998. Adjusted HRs and their 95% CIs for diabetes were estimated according to 25(OH)D status.

**Results:** During follow-up there were 750 incident cases of diabetes. The association of 25(OH)D with diabetes varied by race (\(P\)-interaction = 0.004). Among whites, the adjusted HR for diabetes corresponding to each additional SD higher 25(OH)D concentration (21.3 nmol/L) was 0.95 (95% CI: 0.91, 0.99). No significant association was observed among blacks (HR: 1.06; 95% CI: 0.99, 1.14). There was evidence that the A allele at rs4588 and the T allele at rs7041, which are reported to be associated with high and low DBP concentrations, respectively, modified the association between 25(OH)D and diabetes among whites (\(P\)-interaction < 0.05 for both) but not blacks (\(P\)-interaction > 0.50 for both).

**Conclusions:** In this large, community-based study, low 25(OH)D concentrations were associated with diabetes among whites but not blacks. Interactions by key DBP SNPs varied between genotypes associated with either high or low DBP concentrations among whites but not blacks. Nevertheless, the findings from this prospective study suggest that there are important differences in the association of 25(OH)D with incident diabetes between white and black adults. *Am J Clin Nutr* doi: 10.3945/ajcn.115.107334.

**Keywords:** diabetes, race, vitamin D binding protein polymorphisms, cohort study

**INTRODUCTION**

Vitamin D, a fat-soluble vitamin formed in the skin from 7-dehydrocholesterol during exposure to solar UV-B irradiation or through oral intake from food and supplements, plays a critical role in regulating plasma calcium concentration through effects on intestinal absorption and bone metabolism (1). Although the association between vitamin D status and bone health is well established, there is emerging evidence from both in vivo and in vitro studies that has suggested extraskeletal effects of vitamin D on insulin secretion and insulin action (2). Clinical studies have provided further evidence to support the hypothesis that low vitamin D status (as measured by serum 25-hydroxyvitamin D [25(OH)D])\(^5\) is associated with impaired \(\beta\) cell function, insulin resistance, and glucose intolerance (3–5). In a recent meta-analysis of findings from 21 prospective studies of 25(OH)D and incident type 2 diabetes, the pooled RR comparing the highest with the lowest category of 25(OH)D was 0.62 (95% CI: 0.54, 0.70) (6). Most, but not all, studies (7) observed an inverse association; however, the pooled relation was robust across the sexes and did not vary significantly by a number of methodologic factors (6). Nevertheless, whether the association between vitamin D status and diabetes varies by race remains unknown due to a limited number of studies conducted in diverse populations.

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\(^{3}\)Supplemental Tables 1 and 2 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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\(^{5}\)Abbreviations used: ARIC, Atherosclerosis Risk in Communities; CKD EPI, Chronic Kidney Disease Epidemiology Collaboration DBP, vitamin D–binding protein; SNP, single nucleotide polymorphism; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D\(_2\), 25-hydroxyvitamin D\(_2\); 25(OH)D\(_3\), 25-hydroxyvitamin D\(_3\).

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Relative to whites, blacks have low vitamin D concentrations but, paradoxically, higher bone mineral density and lower fracture risk (8). Low concentrations of 25(OH)D among blacks are suspected to be due, at least in part, to a greater cutaneous melanin content, which blocks the initial conversion of 7-dihydrocholesterol to previtamin D$_3$ in the skin (9, 10) and a dietary intake that includes a lower consumption of dairy products and other foods fortified with vitamin D (11). However, recent evidence suggests that, although total 25(OH)D concentrations differ between the races, concentrations of bioavailable vitamin D, the fraction not bound to vitamin D–binding protein (DBP), which makes up ~10–15% of total 25(OH)D, may be similar in blacks and whites (12). DBP is the primary vitamin D carrier protein and may impair the actions of vitamin D on target cells (13). Racial variation in key DBP single nucleotide polymorphisms (SNPs; i.e., rs4588 and rs7041), which together explain nearly 80% of the variability in DBP concentrations, may result in similar concentrations of bioavailable vitamin D between the races (12).

The objective of the current study was to determine whether racial differences exist in the association of 25(OH)D with diabetes while also accounting for important DBP SNPs in a diverse, community-based cohort of adults. We hypothesized that the association between 25(OH)D concentrations and diabetes would vary significantly between the races and would be more pronounced among those who are genetically predisposed to having higher DBP concentrations (and conversely lower bioavailable vitamin D).

**METHODS**

**Study population**

The Atherosclerosis Risk in Communities (ARIC) Study is a prospective cohort of 15,792 middle-aged adults from 4 US communities: Forsyth County, NC; Jackson, MS; Minneapolis, MN, and Washington County, MD. Only blacks were recruited in Jackson, MS, whereas participants in the other centers reflected the source population (mostly white). Participants’ first examination (visit 1) took place from 1987 to 1989, with 3 follow-up visits (visits 2–4), each occurring approximately every 3 y. A fifth visit took place from 2011 to 2013. Given the number of years between visits 4 and 5 and the potential for known issues related to attrition, we chose not to include data from visit 5 in the current analysis. All participants provided written informed consent at each examination, and institutional review boards from each center approved the study annually.

Serum 25(OH)D concentrations were measured in samples collected at visit 2 (1990–1992; baseline for this analysis), which was attended by 14,348 participants. Excluded from the analysis were the following participants: those who self-identified as neither black nor white (n = 42) and blacks from the Minnesota and Maryland centers (n = 49) due to small numbers, which would not allow reasonable adjustment for center; those who had prevalent diabetes at visit 2 (n = 2146) or an unknown diabetes status at baseline or follow-up (n = 8); those who did not attend visits 3 or 4 (n = 1019); and those who were missing specimens for measurement of 25(OH)D at visit 2 (n = 862). For the primary analysis, our final analytic sample included 10,222 participants (8120 whites, 2102 blacks). For genetic analyses, we further excluded those who did not consent to participate in genetic research or who had missing genetic data (n = 389; final sample = 9843).

**Clinical measurements**

Standard protocols for data collection were used across study centers and examinations. Participants were asked to fast for at least 12 h before each examination and to avoid smoking or engaging in heavy physical activity for at least 2 h.

**Serum 25(OH)D concentrations**

Serum 25-hydroxyvitamin D$_2$ [25(OH)D$_2$] and 25-hydroxyvitamin D$_3$ [25(OH)D$_3$] were measured in 2012–2013 by using a high-sensitivity mass spectrometer (AB Sciex 5500) at the Advanced Research and Diagnostic Laboratory, University of Minnesota, Minneapolis, MN, from samples that had been in storage at −70°C since collection at visit 2. CVs were 6.9% for 25(OH)D$_3$ and 19.8% for 25(OH)D$_2$. Values for 25(OH)D were calculated as the sum of 25(OH)D$_2$ and 25(OH)D$_3$. Because 25(OH)D concentrations vary by season (14), we adjusted for seasonal variation 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. We performed this adjustment separately for whites and for blacks, because seasonal variation in 25(OH)D concentrations also varies by race (15). This new variable “25(OH)D adjusted for month of blood draw” is an estimate of average annual 25(OH)D concentrations and was used as the exposure variable in all analyses.

**DBP SNPs**

SNP genotypes for rs4588 and rs7041 were obtained from the ITMAT-Broad-CARE Chip, a custom 50K SNP genotyping array, with genotyping performed at the Broad Institute of Massachusetts Institute of Technology and Harvard. Quality control procedures were previously published (16).

**Incident diabetes**

We classified individuals as having diabetes if they met any of the following criteria: fasting glucose concentration of at least 7.0 mmol/L (126 mg/dL), nonfasting glucose concentration of at least 11.1 mmol/L (200 mg/dL), reported current use of glucose-lowering medication, or a positive response to the question, “Has a doctor ever told you that you had diabetes (sugar in the blood)?” Incident cases of diabetes at visits 3 and 4 were identified among participants who did not have diabetes at baseline (visit 2). Fasting glucose was available for all participants at baseline, for 97% at visit 3, and for 89% at visit 4. Although we were unable to differentiate type 2 from type 1 diabetes, it has been estimated that ~95% of cases of diabetes identified in adulthood are type 2 (17, 18). In addition, because the current study included adults aged 46–70 y and excluded those with diabetes at baseline, we believe almost all cases should be type 2 diabetes.
Other variables

Information on age, race, educational level, usual alcohol intake, and parental history of diabetes was based on self-report. Participants were asked to bring to each visit all medications taken in the 2 wk before the examination; all medication names were transcribed and coded. Physical activity was measured with the Baecke questionnaire at visit 1, but not at visit 2, so values from visit 1 were carried forward (19). Height and weight were measured, and BMI at visit 2 was calculated as weight in kilograms divided by height in meters squared. Sitting blood pressure at visit 2 was measured in triplicate with a random-zero sphygmomanometer; the mean of the last 2 measurements was used.

Serum glucose concentration was measured by a modified hexokinase-glucose-6-phosphate dehydrogenase procedure at each visit. Insulin was measured by radioimmunoassay at visit 2. Lipids were measured at the time of visit 2. High-sensitivity C-reactive protein (hs-CRP) was measured in 2012–2013 on visit 2–stored samples by using Roche reagents on a Modular P Chemistry analyzer. Plasma total cholesterol and triglycerides were determined by enzymatic methods. HDL cholesterol was measured after dextran-magnesium precipitation, and the Friedewald equation was used to calculate LDL cholesterol in those with triglyceride concentrations <4.52 mmol/L (400 mg/dL). Serum creatinine was measured at visit 2 by using a modified Jaffe reaction. Cystatin C was measured in 2012–2013 from stored samples collected at visit 2 by using the Gentian cystatin C assay on the Roche Modular P Chemistry analyzer. Estimated glomerular filtration rate was estimated by using the 2012 Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equation, which incorporates both cystatin C and creatinine (20).

Data analysis

Participant characteristics were described by using means or proportions as appropriate. We calculated the incidence rate of diabetes per 1000 person-years according to quintiles of 25(OH)D. For participants without diabetes, we calculated person-years from baseline to the last clinic date attended (visit 3 or 4). For participants with incident diabetes, we assigned the date of occurrence according to the method described by Duncan et al. (21) as the date when the glucose value crossed the diagnostic threshold, estimated by linear interpolation. We used multivariable Cox proportional hazards regression models to estimate HRs and their corresponding 95% CIs according to baseline 25(OH)D status. All multivariable models were adjusted for age, sex, race, education, physical activity, smoking status, alcohol use, and family history of diabetes. A second model additionally adjusted for BMI. We used restricted cubic splines to explore the dose-response association. Tests for a linear trend across 25(OH)D quintiles were performed by modeling the 25(OH)D quintiles in the multivariable models as a continuous term. Potential effect modification of the association between 25(OH)D and incident diabetes by race and key DBP SNPs was evaluated by testing the statistical significance of a multiplicative interaction term in models that also included lower order terms. SNPs were analyzed by using a codominant genetic model with the use of each of the 3 SNP genotypes. We confirmed the proportionality assumption with the inclusion of cross-product terms between 25(OH)D quintiles and ln(time).

To extend estimation of the long-term association between baseline 25(OH)D concentrations and incident diabetes by an additional 10 y, we performed supplemental analyses, including self-reported data on diagnosed diabetes or use of diabetes medications from follow-up participant telephone interviews conducted annually after visit 4 (1999–2008). Tests of statistical significance were 2-tailed, with an α level of 0.05. SAS version 9.3 (SAS Institute) was used to perform all analyses.

RESULTS

Participant characteristics

Participants who did not attend visits 3 and 4 (n = 1019) were generally more likely to be black (32.0% vs. 20.6%), male (47.7% vs. 42.8%), and current smokers (40.2% vs. 20.9%); were less likely to have more than a high school level of education (27.3% vs. 41.3%); and had slightly lower 25(OH)D concentrations (55.1 vs. 60.0 nmol/L). However, they were generally of a similar age (57.6 vs. 56.7 y) and BMI (in kg/m²; 27.3 vs. 27.4).

Among the 10,222 eligible participants, 25(OH)D3 concentrations were very low compared with concentrations of 25(OH)D3. For whites, raw 25(OH)D3 and 25(OH)D3 concentrations were 1.8 (IQR: 1.2–3.5) and 59.6 (IQR: 45.4–74.0) nmol/L, respectively (to convert to ng/mL divide by 2.496). For blacks, these values were 1.4 (IQR: 0.9–2.6) and 40.9 (IQR: 31.1–53.5) nmol/L, respectively. Median total 25(OH)D adjusted for month of blood draw was 64.7 (IQR: 51.3–76.8) nmol/L in whites and 45.7 nmol/L (IQR: 34.7–57.0) in blacks. In general, among both whites and blacks, those with lower vitamin D concentrations tended to be younger, female, and less physically active; to have higher BMI and systolic blood pressure; and to be more likely to use antihypertensive medications (Table 1).

Race, 25(OH)D, and incident diabetes

During a maximum of 9 y of follow-up through visit 4, there were 750 incident cases of diabetes (527 in whites and 223 in blacks). As shown in Table 2 and Figures 1 and 2, the association of 25(OH)D with diabetes varied significantly by race (P-interaction = 0.004). Among whites (Figure 1), the cubic spline regression model showed that lower 25(OH)D concentrations were monotonically associated with a higher diabetes risk. In multivariable analyses (Table 2, model 1), the HR for diabetes per 1-SD higher 25(OH)D concentration was 0.90 (95% CI: 0.86, 0.93). Further adjustment for BMI (model 2) attenuated the HR to 0.95 (95% CI: 0.91, 0.99), but it remained significant. The HR was unchanged after additional adjustment for LDL and HDL cholesterol; systolic blood pressure, antihyperlipidemic and antihypertensive medication use, estimated glomerular filtration rate, or hs-CRP. The HR for diabetes adjusted for model 2 covariates per 1-SD higher adjusted 25(OH)D3 (20.4 nmol/L) was 0.93 (95% CI: 0.90, 0.97). Among blacks, there was no evidence of an association between 25(OH)D and incident diabetes (Table 2, Figure 2). The HR for diabetes adjusted for model 2 covariates per 1-SD higher adjusted 25(OH)D3 was 1.06 (95% CI: 0.99, 1.14).
# TABLE 1
Baseline characteristics of participants according to race and quintiles of serum 25(OH)D: the ARIC study, 1990–1992

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<tr>
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<tbody>
<tr>
<td></td>
<td>Q1 (n = 1060)</td>
<td>Q2 (n = 1525)</td>
</tr>
<tr>
<td><strong>Median, nmol/L</strong></td>
<td>36.7</td>
<td>50.6</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>1.6–44.1</td>
<td>44.2–55.8</td>
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</table>

**Selected characteristics**

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<tbody>
<tr>
<td>Age, y</td>
<td>56.3 ± 5.5</td>
<td>56.8 ± 5.6</td>
</tr>
<tr>
<td>Women, % (n)</td>
<td>71.9 (762)</td>
<td>60.3 (920)</td>
</tr>
<tr>
<td>More than high school education, % (n)</td>
<td>39.0 (412)</td>
<td>41.3 (630)</td>
</tr>
<tr>
<td>Current smoker, % (n)</td>
<td>29.1 (308)</td>
<td>20.0 (304)</td>
</tr>
<tr>
<td>Alcohol, g/wk</td>
<td>40.0 ± 105.1</td>
<td>35.7 ± 80.0</td>
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<tr>
<td>Sport index, units</td>
<td>2.3 ± 0.7</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Family history of diabetes, % (n)</td>
<td>22.2 (235)</td>
<td>22.7 (346)</td>
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</tbody>
</table>

**Clinical characteristics**

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<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>28.2 ± 5.8</td>
<td>27.5 ± 4.9</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>119.1 ± 17.4</td>
<td>119.1 ± 17.6</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>70.6 ± 9.8</td>
<td>70.3 ± 9.6</td>
</tr>
<tr>
<td>Hypertension medication use, % (n)</td>
<td>26.2 (278)</td>
<td>24.5 (375)</td>
</tr>
<tr>
<td>Lipid-lowering medication use, % (n)</td>
<td>5.9 (62)</td>
<td>6.2 (95)</td>
</tr>
<tr>
<td>eGFR, mL · min⁻¹ · 1.73m²⁻²</td>
<td>94.3 ± 15.5</td>
<td>95.3 ± 14.4</td>
</tr>
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</table>

**Blood**

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<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>5.6 ± 0.6</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>126.4 ± 64.6</td>
<td>143.1 ± 106.3</td>
</tr>
<tr>
<td>hs-CRP, mg/dL</td>
<td>4.3 ± 7.7</td>
<td>3.5 ± 6.0</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.4 ± 1.0</td>
<td>3.5 ± 0.9</td>
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1ARIC, Atherosclerosis Risk in Communities; BP, blood pressure; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; Q, quintile; 25(OH)D, 25-hydroxyvitamin D.

2Mean ± SD (all such values).
TABLE 2
Adjusted HRs (95% CIs) for incident diabetes according to baseline serum 25(OH)D in the overall cohort and by race: the ARIC study, 1990–1998

| Serum 25(OH)D | Q1 | Q2 | Q3 | Q4 | Q5 | P-trend | Per 1 SD
<table>
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<tbody>
<tr>
<td>Median, nmol/L</td>
<td>36.0</td>
<td>30.2</td>
<td>61.1</td>
<td>72.0</td>
<td>89.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Range</td>
<td>1.6–44.1</td>
<td>44.2–55.8</td>
<td>55.9–66.3</td>
<td>66.4–78.9</td>
<td>79.0–272.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Overall cohort</td>
<td>207/2044</td>
<td>164/2045</td>
<td>125/2044</td>
<td>154/2045</td>
<td>100/2044</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cases/total, n</td>
<td>99/1060</td>
<td>113/1525</td>
<td>99/1745</td>
<td>131/1843</td>
<td>85/1947</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Event rate</td>
<td>1.92 (1.48, 2.48)</td>
<td>1.51 (1.17, 2.48)</td>
<td>1.19 (0.91, 1.55)</td>
<td>1.47 (1.14, 1.89)</td>
<td>1 (reference)</td>
<td>&lt;0.0001</td>
<td>0.92 (0.89, 0.95)</td>
</tr>
<tr>
<td>Whites</td>
<td>1.37 (1.05, 1.80)</td>
<td>1.22 (0.94, 1.58)</td>
<td>1.03 (0.79, 1.34)</td>
<td>1.33 (1.03, 1.71)</td>
<td>1 (reference)</td>
<td>0.09</td>
<td>0.97 (0.93, 1.00)</td>
</tr>
<tr>
<td>Cases/total, n</td>
<td>99/1060</td>
<td>113/1525</td>
<td>99/1745</td>
<td>131/1843</td>
<td>85/1947</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Event rate</td>
<td>2.32 (1.72, 3.13)</td>
<td>1.71 (1.28, 2.27)</td>
<td>1.27 (0.95, 1.71)</td>
<td>1.58 (1.20, 2.08)</td>
<td>1 (reference)</td>
<td>&lt;0.0001</td>
<td>0.90 (0.86, 0.93)</td>
</tr>
<tr>
<td>Blacks</td>
<td>1.59 (1.17, 2.16)</td>
<td>1.32 (0.99, 1.77)</td>
<td>1.06 (0.79, 1.42)</td>
<td>1.39 (1.06, 1.83)</td>
<td>1 (reference)</td>
<td>0.01</td>
<td>0.95 (0.91, 0.99)</td>
</tr>
<tr>
<td>Cases/total, n</td>
<td>108/984</td>
<td>51/520</td>
<td>26/299</td>
<td>23/202</td>
<td>15/97</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Event rate</td>
<td>20.8</td>
<td>18.4</td>
<td>16.5</td>
<td>21.5</td>
<td>30.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Model 1</td>
<td>0.75 (0.43, 1.30)</td>
<td>0.62 (0.34, 1.11)</td>
<td>0.59 (0.31, 1.11)</td>
<td>0.72 (0.37, 1.39)</td>
<td>1 (reference)</td>
<td>0.64</td>
<td>1.02 (0.95, 1.09)</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.59 (0.34, 1.04)</td>
<td>0.53 (0.29, 0.96)</td>
<td>0.36 (0.20, 1.07)</td>
<td>0.35 (0.14, 1.32)</td>
<td>1 (reference)</td>
<td>0.12</td>
<td>1.06 (0.99, 1.14)</td>
</tr>
</tbody>
</table>

1Cox proportional hazards regression models were used to compute multivariable-adjusted HRs with corresponding 95% CIs with Q5 of 25(OH)D serving as the reference group. Model 1 adjusts for age, sex, race (except for race-stratified analyses), education (less than high school, high school, more than high school), physical activity (Baecke sport activity index), smoking status (current, former, never), alcohol use (g/wk), and family history of diabetes (yes or no). Model 2 adjusts for Model 1 plus BMI (kg/m2). The P value for interaction by race was 0.004. ARIC, Atherosclerosis Risk in Communities; Q, quintile; 25(OH)D, 25-hydroxyvitamin D.

21 SD = 21.3 nmol/L.

3Incidence rate per 1000 person-years.

DBP polymorphisms, 25(OH)D, and incident diabetes

The frequency of DBP gene polymorphisms varied by race. For rs4588, the A allele, reported to be associated with higher DBP concentrations (and conversely lower bioavailable vitamin D), was present in 29% of whites and 11% of blacks. For rs7041, the G allele, which is also associated with high DBP concentrations, was 56% in whites and 16% in blacks. Median (IQR) values for the AA, AC, and CC groups for rs4588 were 54.9 (43.7–66.1), 59.6 (46.8–73.0), and 61.3 (47.1–76.1) nmol/L, respectively. These values were 66.8 (54.2–80.3), 62.0 (48.9–75.3), and 51.4 (39.0–64.5) nmol/L for GG, TG, and TT groups for rs7041, respectively.

Table 3 shows the adjusted HRs and 95% CIs for diabetes per 1-SD higher 25(OH)D concentration stratified by rs4588 and rs7041 genotype and by race. Because of the rare frequency of homozygotes for the A allele at rs4588 and the G allele at rs7041 among blacks (n = 19 and 52, respectively) and the limited frequency of diabetes in these groups (n = 4, for both), we excluded these individuals from genotype analysis. In race-stratified analyses, the presence of the A allele at rs4588 appeared to modify the association between 25(OH)D and diabetes among whites (P-interaction = 0.01) but not blacks (P-interaction = 0.60). An inverse association was observed among those in the AA group but not those in the AC or CC groups.

The association between 25(OH)D and diabetes appeared to be stronger among those with the T allele at rs7041 among whites (P-interaction = 0.02). The rs7041 genotype did not modify the association between 25(OH)D and diabetes risk among blacks (P-interaction = 0.82).

Supplemental analyses of baseline 25(OH)D with long-term diabetes risk

When the follow-up period was extended from 1999 to 2008 to include self-reported data on diabetes collected during the annual telephone interviews after visit 4, there were 1355 additional cases of incident diabetes identified among whites and 497 additional cases among blacks. Among whites, the association between 25(OH)D and incident diabetes incorporating these additional cases was slightly attenuated compared with the visit-based diabetes ascertainment–only findings [HR per 1-SD higher 25(OH)D: 0.98; 95% CI: 0.96, 1.00; P-trend across quintiles = 0.03] (Supplemental Table 1). There was no association observed among blacks (P-trend = 0.66) (Supplemental Table 1). Findings stratified by rs4588 and rs7041 genotypes were also similar to the results from the visit-based definition of diabetes, even with the inclusion of black participants homozygous for the A allele at rs4588 and the G allele at rs7041 (Supplemental Table 2).

DISCUSSION

In this large, community-based cohort of mostly middle-aged white and black adults, we found that the association between serum 25(OH)D concentrations and incident diabetes varied significantly by race and DBP genotype. Lower 25(OH)D concentrations were monotonically associated with a higher risk of diabetes among whites, independent of adiposity and numerous potential confounding factors. With regard to differences by DBP genotype, we identified conflicting results. There was evidence that the association between 25(OH)D and diabetes among

whites was stronger among those with the A allele at rs4588, which has been reported to be associated with higher DBP concentrations, as well as among those with the T allele at rs7041, which conversely has been shown to be associated with lower DBP concentrations. Among blacks, there was no evidence of an association regardless of DBP genotype. These findings suggest that there are important differences in the association of vitamin D status with diabetes between white and black adults.

Although several studies have investigated the association between 25(OH)D and incident diabetes, few included black individuals, a population at high risk of both vitamin D deficiency and diabetes (22, 23). In an analysis from the Diabetes Prevention Program, which included 1159 white and 419 black adults, low 25(OH)D concentrations were associated with a greater risk of diabetes, which did not vary significantly between white and nonwhite (including black, Hispanic, Asian, and other racial-ethnic groups) participants (24). In another pooled analysis of 3 nested case-control studies in 4661 white and 228 black post-menopausal women from the Women’s Health Initiative, no association was observed between 25(OH)D and incident diabetes regardless of race (25).

Our results, which suggest differences in the association of 25(OH)D with diabetes risk between white and black adults, replicate the findings of a cross-sectional study from the NHANES III (26). In that analysis, strong inverse dose-response associations were observed between 25(OH)D and prevalent diabetes among whites and Mexican Americans but not in blacks (26). Other studies also showed that the association of low vitamin D with related outcomes, including peripheral arterial disease (27), stroke (28), and heart disease (29), may be stronger in whites than in blacks. The reasons for the lack of an inverse association with diabetes in blacks are unclear but may be due, at least in part, to decreased sensitivity to the effects of vitamin D and/or related hormones (30). For example, black individuals have higher circulating concentrations of 1,25-dihydroxyvitamin D and parathyroid hormone at a given concentration of 25(OH)D (31, 32). In addition, black adults have a greater bone mineral density and a lower frequency of skeletal fractures despite having elevated parathyroid hormone concentrations, which should promote increased bone resorption (8, 33, 34). Alternate potential mechanisms include racial differences in the frequency of vitamin D receptor gene polymorphisms and consequent metabolic effects (35).

Approximately 85–90% of 25(OH)D circulates tightly bound to DBP, which may impair the ability of vitamin D to act on target cells (36). The remainder, referred to as bioavailable
vitamin D, circulates mostly loosely bound to albumin, with a small proportion in the free form. Racial differences in rs4588 and rs7041 result in similar concentrations of bioavailable vitamin D between the races, despite lower total concentrations among blacks (12). Those who are genetically predisposed to high DBP concentrations should also have the lowest concentrations of bioavailable vitamin D. Thus, we hypothesized that low 25(OH)D and a genetic predisposition to high DBP concentrations (and consequently low bioavailable vitamin D concentrations) would act synergistically to increase the risk of diabetes. We found evidence that these 2 DBP SNPs modified the association between 25(OH)D and diabetes among whites but not blacks. Associations of low 25(OH)D with diabetes were stronger among white adults with the A allele at rs4588 as well as the T allele at rs7041. However, the A allele has been associated with higher DBP concentrations (~106.7 μmol/L per copy), whereas the T allele has been associated with lower DBP concentrations (~366 μmol/L per copy) (12). To our knowledge, no other study has tested whether the association between 25(OH)D and diabetes may vary by these DBP SNPs. DBP polymorphisms were associated with prevalent diabetes in some (37) but not all (38) studies. Future studies are needed to determine whether those with the lowest concentrations of bioavailable vitamin D are at a particularly high risk of diabetes.

The inverse dose-response association observed among white adults in the current study is consistent with the summary findings of a recent meta-analysis of 21 prospective studies of 25(OH)D and diabetes (6). Insulin resistance and impaired β-cell function, 2 fundamental features of diabetes, have been reported with vitamin D insufficiency (4, 39). Vitamin D receptors for 1,25-dihydroxyvitamin D are present on pancreatic β cells, and severe vitamin D deficiency can impair insulin secretion in animal models (2, 40). However, the results from small clinical trials and post hoc analyses of larger trials on the effects of vitamin D supplementation on glycemia or diabetes have been inconsistent (41, 42). A recent meta-analysis of these studies suggested that vitamin D supplementation had no effect on glucose homeostasis or diabetes prevention (43). Notable limitations of these trials included their small sample size, short duration of follow-up, variable study quality, and focus on intermediate outcomes.

In the current study, we found that the association between 25(OH)D and diabetes among whites was substantially attenuated, but remained significant after adjustment for level of adiposity. Adipose tissue cells sequester vitamin D, making stores less available to become biologically activated, leading to lower 25(OH)D among overweight or obese individuals (44). On the other hand, vitamin D may directly affect adiposity and other metabolic variables that mediate the pathway from vitamin D status to diabetes (4). However, accumulating evidence suggests that 25(OH)D may be low as a result of obesity and not vice versa (45, 46).

Strengths of the current study include the following: a community-based sampling method; a biracial cohort; extensive data on potential confounders; a large sample size, which increased precision and permitted simultaneous adjustment and stratification by multiple variables; and the standardized data collection protocols and rigorous quality control of the ARIC study. Nevertheless, at least 3 limitations

### Table 3

<table>
<thead>
<tr>
<th>rs4588</th>
<th>rs7041</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>GG</td>
</tr>
<tr>
<td>Cases/total, n</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Whites</td>
<td>51/644 0.75 (0.64, 0.90) 207/3177 0.94 (0.88, 1.01)</td>
</tr>
<tr>
<td>Blacks</td>
<td>295/363 0.70 (0.61, 0.79)</td>
</tr>
</tbody>
</table>

Cox proportional hazards regression models were used to compute multivariable-adjusted HRs with corresponding 95% CIs per 1-SD higher baseline serum 25(OH)D. Adjusts for age, sex, education (less than high school, high school, or more than high school), physical activity (Baecke sport activity index), smoking status (current, former, or never), alcohol use (g/wk), family history of diabetes (yes or no), and BMI (kg/m²). 1 SD = 21.3 nmol/L. ARIC, Atherosclerosis Risk in Communities; Q, quintile; 25(OH)D, 25-hydroxyvitamin D.
deserve mention. First, our study included middle-aged and older adults at baseline and therefore our results may not be generalizable to younger populations. Second, we measured serum 25(OH)D concentrations at only a single time at baseline and therefore we were unable to determine whether changes in 25(OH)D may have occurred during the follow-up period. As a result, our findings may underestimate the true association between 25(OH)D and diabetes. Third, we did not directly measure DBP concentrations and thus we were unable to calculate bioavailable vitamin D concentrations. However, the DBP SNPs included in the current study have been shown to explain nearly 80% of the variability in DBP concentrations (12), providing a strong proxy measure of DBP concentration, and, when combined with information on 25(OH)D, an estimate of bioavailable vitamin D.

In conclusion, in this large, community-based study, we found that low 25(OH)D concentrations were associated with a higher risk of diabetes among whites independent of adiposity and numerous potential confounding factors. No significant association was observed among blacks. Although this association also varied by key DBP SNPs among whites, results were inconsistent between SNPs. Nevertheless, the findings from this prospective study suggest that there are important differences in the association of 25(OH)D with incident diabetes between white and black adults.

The authors’ responsibilities were as follows—JPR: conceptualized the study, designed the analysis, analyzed and interpreted the data, drafted the manuscript, and critically revised the manuscript for important intellectual content; EDM, ES, and JSP: interpreted the data and critically revised the manuscript, and critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. JPR is the guarantor of this work and, as such, had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript. None of the authorsdeclared a conflict of interest.

REFERENCES


40. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 2008;88:491S-9S.


