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## Vitamin D, vitamin D binding protein gene polymorphisms, race and risk of incident stroke: the Atherosclerosis Risk in Communities (ARIC) study

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### Abstract

**Background and purpose**—Low vitamin D levels, measured by serum 25-hydroxyvitamin D [25(OH)D], are associated with increased stroke risk. Less is known about whether this association differs by race or D binding protein (DBP) single nucleotide polymorphism (SNP) status. Our objective was to characterize the associations of and interactions between 25(OH)D levels and *DBP* SNPs with incident stroke. It was hypothesized that associations of low 25(OH)D with stroke risk would be stronger amongst persons with genotypes associated with higher DBP levels.

**Methods**—25(OH)D was measured by mass spectroscopy in 12 158 participants in the Atherosclerosis Risk in Communities (ARIC) study (baseline 1990–1992, mean age 57 years, 57% female, 23% black) and they were followed through 2011 for adjudicated stroke events. Two *DBP* SNPs (rs7041, rs4588) were genotyped. Cox models were adjusted for demographic/behavioral/socioeconomic factors.

**Results**—During a median of 20 years follow-up, 804 incident strokes occurred. The lowest quintile of 25(OH)D (<17.2 ng/ml) was associated with higher stroke risk [hazard ratio (HR) 1.34 (1.06–1.71) versus highest quintile]; this association was similar by race (*P* interaction 0.60).

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There was weak evidence of increased risk of stroke amongst those with 25(OH)D < 17.2 ng/ml and either rs7041 TG/GG [HR = 1.29 (1.00–1.67)] versus TT genotype [HR = 1.19 (0.94–1.52)] (*P* interaction 0.28) or rs4588 CA/AA [HR = 1.37 (1.07–1.74)] versus CC genotype [HR = 1.14 (0.91–1.41)] (*P* interaction 0.11).

**Conclusions**—Low 25(OH)D is a risk factor for stroke. Persons with low 25 (OH)D who are genetically predisposed to high DBP (rs7041 G, rs4588 A alleles), who therefore have lower predicted bioavailable 25(OH)D, may be at greater risk for stroke, although our results were not conclusive and should be interpreted as hypothesis generating.

## Keywords

race; stroke; vitamin D

## Introduction

Low levels of vitamin D, as measured by serum 25-hydroxy-vitamin D [25(OH)D], have been estimated to affect approximately 1 billion people worldwide [1] and are associated with increased risk of stroke [2–4]. However, most previous studies have been performed in older white populations [2–4]. Blacks tend to have lower levels of 25(OH)D compared to whites [5]. Little is known about potential racial differences in the association between 25(OH)D and stroke, although there is some evidence that low 25(OH)D is a stronger cardiovascular disease risk factor in whites than blacks [6–9].

Approximately 85%–90% of circulating 25(OH)D is tightly bound to vitamin D binding protein (DBP) and is generally believed to be inactive. Of the remaining circulating 25(OH)D, 10%–15% is loosely bound to albumin and <1% is free. Recent work has shown that bioavailable 25(OH)D – which includes free and albumin-bound 25(OH)D – is more tightly associated with clinical outcomes, including bone and mineral metabolism, than total 25(OH)D [10–12]. Blacks and whites are believed to have similar concentrations of estimated bioavailable 25(OH)D, because blacks have lower levels of both total 25(OH)D and DBP compared to whites [5]. There are two common single nucleotide polymorphisms (SNPs) on the *DBP* gene, rs7041 and rs4588, which have been shown to explain ~80% of the variability in serum DBP levels [5]. Blacks have been shown to be more likely than whites to have a T allele at rs7041 and to have a C allele at rs4588, which both result in lower levels of serum DBP [5]. Although the *DBP* SNPs have not been independently associated with ischaemic stroke [13], it is possible that these SNPs modify the relationship between 25(OH)D levels and stroke risk, potentially reflecting differences in underlying bioavailable 25(OH)D.

Our objective was to characterize the associations of and interactions between 25(OH)D levels, race and *DBP* SNPs with incident stroke occurring over approximately 20 years of follow-up in the community-based Atherosclerosis Risk in Communities (ARIC) study. It was hypothesized that lower concentrations of 25(OH) D would be associated with greater stroke risk, and that these associations would be modified by race (higher risk in whites versus blacks) [6] and by rs7041 and rs4588 SNPs (higher risk with rs7041 G versus T allele

and rs4588 A versus C allele, i.e. those genetically predisposed to higher DBP levels and thus those with lower levels of bioavailable vitamin D) [5].

## Methods

### Study population

The ARIC study is an ongoing, community-based prospective cohort of 15 792 adults aged 45–65 years at baseline (1987–1989) from four US communities: suburbs of Minneapolis, Minnesota, Washington County, Maryland, Forsyth County, North Carolina, and Jackson, Mississippi [14]. Four additional in-person study visits have taken place in 1990–1992 (visit 2), 1993–1995 (visit 3), 1996–1998 (visit 4) and 2011–2013 (visit 5). The ARIC study has been approved by the Institutional Review Boards of all participating institutions. Participants gave written informed consent at each study visit and for the use of genetic data.

25(OH)D levels were measured from serum samples obtained at visit 2 (1990–1992), which serves as the baseline for the present study. Of the 14 348 participants who attended visit 2, 275 with prevalent stroke, 1178 missing 25(OH)D data, 645 missing genetic data or who did not consent for the use of genetic data, and 92 missing other variables of interest were excluded, leaving a total of 12 158 included participants.

### Measurement of 25(OH)D and associated biomarkers

25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> levels were measured from serum samples stored at –70°C until analyzed in 2012–2013 using liquid chromatography tandem high-sensitivity mass spectrometry (Waters Alliance e2795; Waters, Milford, MA, USA). Using samples collected in duplicate tubes and stored, the coefficient of variation (processing plus assay variation) for 25(OH)D<sub>2</sub> was 20.8% and for 25(OH)D<sub>3</sub> was 6.9%. The Pearson correlations from these blind duplicate samples were 0.98 for 25(OH)D<sub>2</sub> and 0.97 for 25(OH)D<sub>3</sub>. 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> were added together for total 25(OH) D concentration.

Using the same stored serum samples, calcium, phosphorus and parathyroid hormone (PTH) were also measured (calcium and phosphate, Roche Modular P-Chemistry Analyzer; PTH, Elecsys 2010, Roche Diagnostics, Indianapolis, IN, USA). At the time of visit 2, serum magnesium was measured based on the procedure of Gindler and Heth and used the metallochromic dye calmagite [1-(1-hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid] [15].

### DBP SNPs

Two common SNPs located in the coding region of the *DBP* gene (rs7041 and rs4588) [5] were genotyped using the ITMAT-Broad-CARE Chip, a custom 50 K SNP genotyping array, at the Broad Institute of Massachusetts Institute of Technology and Harvard. Quality control procedures were published previously [16]. Few blacks had rs7041 GG and rs4588 AA genotypes (2.8% and 1.1%, respectively). Thus, rs7041 was categorized as TT (reference) versus TG/GG and rs4588 as CC (reference) versus AC/AA. In sensitivity analyses, an additive genetic model (TT versus TG versus GG for rs7041; CC versus AC versus AA for rs4588) was considered. Additionally, haplo-types were considered: Gc1s/Gc1s (rs7041 GG

and rsr588 CC), Gc1F/Gc1S (rs7041 TG and rsr588 CC), Gc2/Gc2 (rs7041 TT and rsr588 AA), Gc2/Gc1F (rs7041 TT and rsr588 AC) and Gc1F/Gc1F (rs7041 TT and rsr588 CC) [5].

### Ascertainment of incident stroke

All stroke-related hospitalizations and deaths occurring through 31 December 2011 in ARIC participants were identified by annual telephone follow-up call and community surveillance of all ARIC hospitalizations. Hospital records for all possible stroke-related hospitalizations were obtained (ICD-9 codes 430–438 until 1997 and ICD-9 codes 430–436 afterwards). Definite/probable hospitalized strokes were classified by a combination of computer algorithm and physician review, using standardized criteria [17]. Strokes were subclassified as ischaemic versus hemorrhagic.

### Covariates

All variables used in the analyses were assessed at visit 2 (1990–1992), unless otherwise stated. All continuous variables were centered at the median. Covariates in our main regression model included age (continuous), sex (male; female), race/center (Minnesota whites; Maryland whites; North Carolina whites; North Carolina blacks; Mississippi blacks), education (<high school; high school or equivalent; college, graduate or professional school; assessed at visit 1), physical activity (score range 1–5, based on replies to the modified Baecke Physical Activity questionnaire [18]; assessed at visit 1), cigarette smoking (current; former; never) and body mass index [continuous; weight (kg)/height (m<sup>2</sup>)].

Covariates in supplemental models included diabetes (self-reported physician diagnosis, medication use, fasting serum glucose 126 mg/dl or nonfasting glucose 200 mg/dl), hypertension (self-reported physician diagnosis, medication use, systolic blood pressure 140 mmHg or diastolic blood pressure 90 mmHg), total and high density lipoprotein (HDL) cholesterol (mg/dl), estimated glomerular filtration rate (continuous; calculated using the Chronic Kidney Disease Epidemiology Collaboration formula [19]), as well as serum calcium (continuous), phosphorus (continuous), PTH (continuous) and magnesium (continuous).

### Statistical analysis

25(OH)D concentrations vary by season and by race [1]. Therefore 25(OH)D was adjusted for seasonal change by computing the residuals from a linear regression model (25(OH)D, dependent variable; month of visit, independent variable). The residuals were added back to the mean to create an estimated annual 25(OH) D value. This adjustment was performed separately for whites and for blacks. The estimated annual 25(OH)D value was used in all analyses and was divided into quintiles based on the distribution in the overall population (<17.2 ng/ml; 17.2 to <21.7 ng/ml; 21.7 to <26 ng/ml; 26.0 to <31 ng/ml; 31.0 ng/ml).

Baseline characteristics (1990–1992) of the study population were described using means and proportions across quintiles of 25(OH)D and distributions were compared using *t* tests for continuous variables and chi-squared statistics for categorical variables. Cox proportional hazards models were used to estimate the hazard ratios for the association of

25(OH)D categories with incident stroke. The proportional hazards assumption was checked using Schoenfeld residuals and  $\ln(-\ln)$  survival plots. The association of 25(OH)D with incident stroke was also modeled continuously using a restricted cubic spline model. Two-way multiplicative interactions of 25(OH)D by race, serum magnesium or *DBP* gene polymorphisms rs7041 and rs4588 were formally tested using Wald tests and stratified analyses were presented if there was any evidence for interaction. However, *a priori* it was decided to present results overall and stratified by race based on prior studies [6]. When testing for interaction, the lowest quintile of 25 (OH)D was compared to the other four higher quintiles to increase statistical power (this cutpoint corresponds to a threshold effect seen in our primary analyses of 25 (OH)D and incident stroke). In sensitivity analyses, analyses were performed separately for incident ischaemic and hemorrhagic strokes.

Two primary models were created: model 1 was adjusted for demographic factors [age, sex, race/field center (overall models) or center (race-stratified models)]; model 2 was adjusted for variables included in model 1 + behavioral/socioeconomic variables (education, physical activity, smoking, body mass index). Two additional analyses were performed: (i) model 2 + potential mediators (diabetes, systolic and diastolic blood pressure, use of hypertension medication, total and HDL cholesterol, and estimated glomerular filtration rate), and (ii) model 2 + potential mediators + biomarkers related to vitamin D metabolism (calcium, phosphorus, PTH and magnesium).

All reported *P* values were two-sided and  $P < 0.05$  was considered statistically significant. Analyses were performed using Stata Version 13 (StataCorp, College Station, TX, USA).

## Results

In the overall study population, mean age was 57 years, 57% were female and 23% were black. Median 25(OH) D concentration was 23.9 ng/ml overall and 18.2 ng/ml amongst blacks versus 25.6 ng/ml amongst whites ( $P < 0.001$ ). Compared to those with 25(OH)D concentration in the highest quintile, those with 25(OH)D concentration in the lowest quintile were significantly younger, were more likely to be female and had lower levels of physical activity (all  $P < 0.001$ ). Those in the lowest 25(OH)D quintile were also significantly more likely to have diabetes and hypertension compared to those in the highest quintile (all  $P < 0.001$ ) (Table 1). Characteristics of the study population by 25(OH)D quintile stratified by race were similar to the overall patterns (Table S1).

During a median of 20 years of follow-up, there were 804 incident strokes. After adjustment for demographic factors, being in the lowest quintile of 25(OH) D was associated with a significantly greater risk of stroke compared to the highest quintile [hazard ratio (HR) = 1.49 (1.18–1.89)] (Table 2). This association was attenuated but remained statistically significant after additional adjustment for behavioral/socioeconomic factors [HR = 1.34 (1.06–1.71)]. Results were not appreciably altered when further adjusted for possible mediators and biomarkers related to vitamin D metabolism. Similar patterns were seen stratified by race ( $P$  interaction 0.60), although power was lower in race-specific analyses, particularly amongst blacks. No evidence of interaction by serum magnesium concentration ( $P$  interaction 0.86) was seen. Figure 1 shows the continuous association of 25(OH)D with incident stroke risk

overall and by race. Similar to the quintile analysis, there was evidence for an association between 25(OH)D and increased risk of stroke primarily amongst those with low values of 25(OH)D, suggesting a possible threshold effect at approximately 15–20 ng/ml. Results for incident ischaemic stroke ( $n = 711$  events) were similar to the associations for total stroke events. Analyses for hemorrhagic stroke were not significant but were limited by small numbers of events ( $n = 93$ ) (Table S2).

*DBP* polymorphism frequencies varied by 25(OH)D level and by race (Table S1). TG/GG genotype frequencies for rs7041 were lower amongst those with lower concentrations of 25(OH)D, whereas AC/AA genotype frequencies for rs4588 were higher amongst those with lower concentrations of 25(OH)D. Overall, TG/GG genotypes for rs7041 were seen in 30% of blacks and 81% of whites and AC/AA genotypes for rs4588 were seen in 20% of blacks and 48% of whites. rs7041 and rs4588 were not significantly associated with incident stroke when analyzed as main effects (Table S3). Compared to those with 25(OH)D  $\geq 17.2$  ng/ml and either rs7041 TT genotype or rs4588 CC genotype, those with 25(OH)D  $< 17.2$  ng/ml were at higher risk for stroke regardless of genotype. However, there was some weak evidence of increased risk of stroke amongst those with 25(OH)D  $< 17.2$  ng/ml and rs7041 TG/GG genotype versus TT genotype [TG/GG HR = 1.29 (1.00–1.67) vs. TT HR = 1.19 (0.94–1.52),  $P$  interaction 0.28] (Fig. 2a) and amongst those with 25(OH)D  $< 17.2$  ng/ml and rs4588 CA/AA genotype versus CC genotype [CA/AA HR = 1.37 (1.07–1.74) vs. CC HR = 1.14 (0.91–1.41),  $P$  interaction 0.11] (Fig. 2b) in models adjusted for demographic/behavioral/socioeconomic factors. Similar results were seen in sensitivity analyses using an additive genetic model for rs7041 and rs4588. Similar results were also seen in analyses using haplotypes ( $P$  interaction 0.16).

## Discussion

In this community-based middle-aged sample of white and black adults, lower compared with higher serum concentrations of 25(OH)D were associated with greater incident stroke over approximately 20 years of follow-up. This association was similar by race but there was suggestive evidence, albeit not statistically significant, that associations between low 25(OH)D and incident stroke were stronger amongst participants genetically predisposed to higher serum DBP (i.e. those with presence of the rs7041 G allele or the rs4588 A allele).

Our study is consistent with previous studies showing an association between low concentrations of 25(OH)D and stroke [2–4]. In contrast to a prior study [6], no difference in this relationship by race was seen. However, some weak evidence for effect modification by *DBP* genetic polymorphisms was observed. Lower 25(OH)D levels were associated with a slightly higher risk of stroke amongst those with rs7041 TG/GG versus TT genotype and with rs4588 AC/AA versus CC genotype. Individuals with an rs7041G allele (versus a T allele) and individuals with an rs4588 A allele (versus a C allele) have been shown to have higher DBP levels and therefore lower levels of bioavailable 25(OH)D for a given 25(OH)D level [5]. Our results for both SNP rs7041 and rs4588 suggest that those with genotypes that may reflect higher DBP in the setting of low 25(OH)D concentration, probably indicative of lower bioavailable 25(OH)D, had increased risk of stroke compared to those with genotypes reflecting lower DBP.



Certain limitations should be taken into consideration in the interpretation of this study. First, there was only a single measurement of 25(OH)D, which has been shown to vary over time within individuals [20–22], but 25(OH)D concentrations were adjusted for seasonal change. Secondly, serum DBP concentrations were not measured and it was therefore not possible to estimate bioavailable vitamin D. However, associations and effect modification by *DBP* polymorphisms rs7041 and rs4588 were examined, but these analyses have limited power. Our study also has a number of strengths, including a large bi-racial population, comprehensive assessment of confounders, physician adjudicated stroke events using standardized criteria, and approximately 20 years of follow-up.

In conclusion, in this community-based sample of middle-aged whites and blacks, it was found that lower 25(OH)D was associated with increased incidence of stroke. Individuals with low 25(OH)D who are genetically predisposed to higher DBP (rs7041 G allele, rs4588 A allele), and who therefore have lower predicted bioavailable 25(OH)D, may be at greater risk for stroke, although our results were not conclusive and should be interpreted as hypothesis generating. Further studies on the association of bioavailable vitamin D and DBP with incident stroke are warranted.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

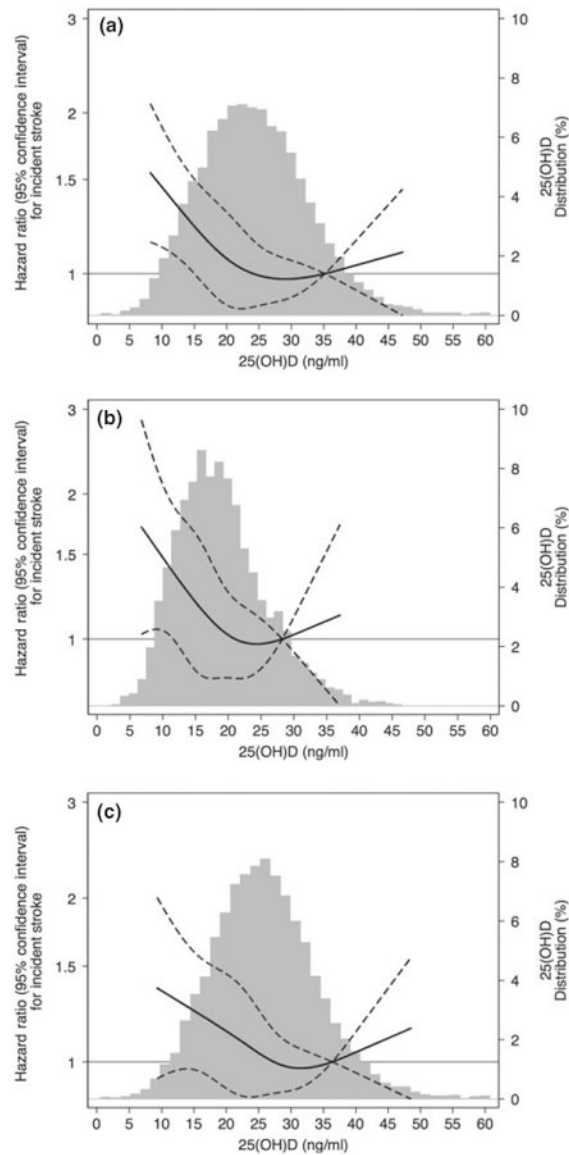
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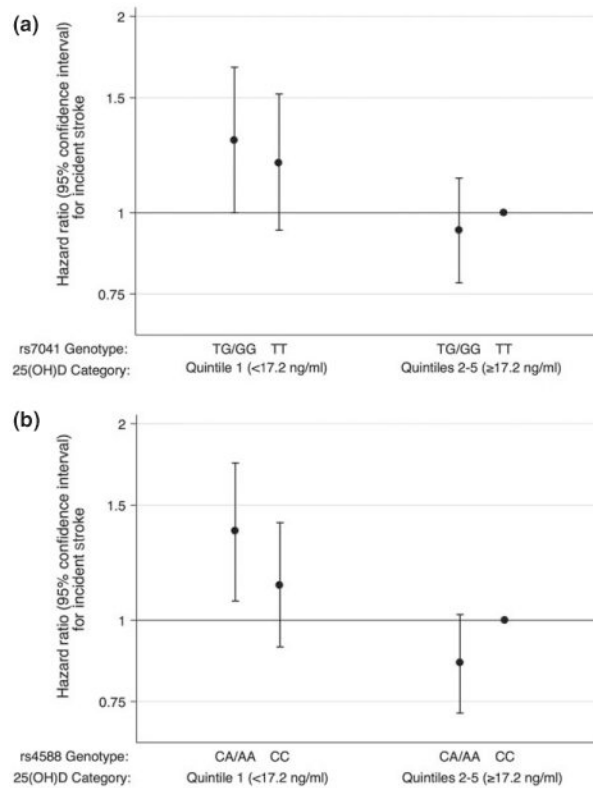
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**Figure 1.**

Adjusted [model is adjusted for age, sex, race/center (overall models) or center (race-stratified models), education, physical activity, smoking status, body mass index] restricted cubic spline model showing the hazard ratios (95% confidence intervals) for the association of 25(OH)D with incident stroke overall (a) and in blacks (b) and whites (c). Follow-up 1990–1992 through 2011. The solid line represents the hazard ratios and the dashed lines represent the 95% confidence intervals. Knots at 5th, 35th, 65th and 95th percentiles. Restricted cubic spline centered at the 90th percentile and truncated at 1st and 99th percentile of 25(OH)D. Histogram shows the distribution of concentrations of 25(OH)D.



**Figure 2.**

Adjusted (model is adjusted for age, sex, race/center, education, physical activity, smoking status, body mass index) hazard ratios (95% confidence intervals) for the association of 25(OH)D with incident stroke overall stratified by *DBP* rs7041 (a) and by rs4588 (b). Follow-up 1990–1992 through 2011. Participants with *DBP* rs7041 TT genotype and higher quintiles (Q2–Q5) of 25(OH)D are the reference group in (a). Participants with *DBP* rs4588 CC genotype and higher quintiles (Q2–Q5) of 25(OH)D are the reference group in (b).

**Table 1**  
**Participant characteristics by 25(OH)D quintile, ARIC visit 2 (1990–1992)**

Quintiles of 25(OH)D					
	Quintile 1 (<17.2 ng/ml) (n = 2431)	Quintile 2 (17.2 to <21.7 ng/ml) (n = 2432)	Quintile 3 (21.7 to <26.0 ng/ml) (n = 2431)	Quintile 4 (26.0 to <31.0 ng/ml) (n = 2432)	Quintile 5 (≥ 31.0 ng/ml) (n = 2432)
25(OH)D (ng/ml), median	14.0	19.6	23.9	28.4	35.2
Vitamin D binding protein gene polymorphisms, %					
rs7041					
TT	51.1	38.0	28.4	22.9	14.4
TG/GG	48.9	62.0	71.6	77.1	85.6
rs4588					
CC	59.2	54.3	55.5	57.0	65.9
AC/AA	40.8	45.7	44.5	43.0	34.1
Age (years), mean (SD)	56.1 (5.7)	56.8 (5.7)	57.0 (5.7)	57.1 (5.7)	57.4 (5.7)
Female, %	73.3	61.9	53.2	48.4	49.6
Race/center, %					
Minneapolis, MN, whites	17.7	24.9	31.7	31.7	32.0
Washington County, MD, whites	18.5	24.9	28.6	28.8	30.1
Forsyth County, NC, whites	13.4	19.2	22.8	28.0	31.9
Forsyth County, NC, blacks	6.2	3.3	1.6	0.9	0.6
Jackson, MS, blacks	44.2	27.8	15.3	10.7	5.4
Education, % <sup>a</sup>					
<High school	24.2	22.6	19.4	18.8	18.1
High school or vocational school	39.4	41.2	40.5	43.8	45.2
College, graduate or professional school	36.5	36.1	40.1	37.4	36.8
Smoking status, %					
Never	42.4	42.9	42.6	38.8	36.9
Former	29.8	34.6	38.1	42.2	43.5
Current	27.9	22.5	19.3	19.0	19.6
Physical activity index, mean (SD) <sup>d</sup>	2.2 (0.7)	2.3 (0.7)	2.5 (0.8)	2.6 (0.8)	2.7 (0.9)
Body mass index (kg/m <sup>2</sup> ), mean (SD)	29.9 (6.7)	28.5 (5.4)	28.0 (5.0)	27.3 (4.6)	26.1 (4.2)

Quintiles of 25(OH)D					
	Quintile 1 (<17.2 ng/ml) (n = 2431)	Quintile 2 (17.2 to <21.7 ng/ml) (n = 2432)	Quintile 3 (21.7 to <26.0 ng/ml) (n = 2431)	Quintile 4 (26.0 to <31.0 ng/ml) (n = 2432)	Quintile 5 (≥31.0 ng/ml) (n = 2432)
Diabetes, %	20.7	17.3	13.5	10.9	8.1
Hypertension, %	44.9	37.6	32.7	30.9	27.1
Total cholesterol (mg/dl), mean (SD)	210 (41.4)	212 (40.1)	210 (38.7)	209 (38.2)	210 (37.7)
HDL cholesterol (mg/dl), mean (SD)	50.2 (17.0)	49.7 (16.5)	48.8 (15.9)	48.8 (16.3)	51.8 (17.9)
Estimated GFR <60 ml/min/1.73 m <sup>2</sup> , %	2.1	2.0	1.4	1.3	2.7
PTH (pg/ml), mean (SD)	50.4 (25.0)	43.9 (29.4)	41.8 (19.7)	39.6 (17.9)	36.5 (12.4)
Calcium (mg/dl), mean (SD)	9.4 (0.5)	9.4 (0.4)	9.3 (0.4)	9.4 (0.4)	9.3 (0.4)
Phosphorus (mg/dl), mean (SD)	3.6 (0.5)	3.6 (0.5)	3.5 (0.5)	3.5 (0.5)	3.5 (0.5)
Magnesium (mEq/l), mean (SD)	1.6 (0.2)	1.6 (0.2)	1.6 (0.2)	1.6 (0.2)	1.6 (0.2)

25(OH)D, 25-hydroxyvitamin D; ARIC, Atherosclerosis Risk in Communities; GFR, glomerular filtration rate; HDL, high density lipoprotein; PTH, parathyroid hormone.

The following variables contained missing data: diabetes (n = 18), hypertension (n = 35), total cholesterol (n = 17), HDL cholesterol (n = 38), PTH (n = 156), calcium (n = 155), phosphorus (n = 155) and magnesium (n = 2).

<sup>a</sup> Assessed at ARIC visit 1 (1987–1989).

**Table 2**  
**Adjusted<sup>a</sup> hazard ratios (95% confidence intervals) for incident stroke by 25(OH)D quintile; follow-up 1990–1992 through 2011**

Quintiles of 25(OH)D					
	Quintile 1 (<17.2 ng/ml)	Quintile 2 (17.2 to <21.7 ng/ml)	Quintile 3 (21.7 to <26.0 ng/ml)	Quintile 4 (26.0 to <31.0 ng/ml)	Quintile 5 (≥31.0 ng/ml)
<b>Overall</b>					
Median 25(OH)D (ng/ml)	14.0	19.6	23.9	28.4	35.2
<i>n</i> events/ <i>n</i> total	203/2431	167/2432	148/2431	150/2432	136/2432
Model 1	<b>1.49 (1.18–1.89)</b>	1.16 (0.91–1.46)	1.06 (0.84–1.34)	1.10 (0.87–1.38)	Reference
Model 2	<b>1.34 (1.06–1.71)</b>	1.07 (0.85–1.36)	1.02 (0.80–1.29)	1.06 (0.84–1.34)	Reference
<b>Blacks</b>					
Median 25(OH)D (ng/ml)	13.6	19.3	23.5	28.2	34.3
<i>n</i> events/ <i>n</i> total	129/1225	70/755	37/411	34/282	15/145
Model 1	1.36 (0.79–2.35)	1.02 (0.58–1.79)	0.95 (0.52–1.73)	1.25 (0.68–2.30)	Reference
Model 2	1.39 (0.80–2.41)	1.03 (0.59–1.81)	0.97 (0.53–1.77)	1.25 (0.68–2.30)	Reference
<b>Whites</b>					
Median 25(OH)D (ng/ml)	14.4	19.8	23.9	28.4	35.3
<i>n</i> events/ <i>n</i> total	74/1206	97/1677	111/2020	116/2150	121/2287
Model 1	<b>1.52 (1.13–2.04)</b>	1.22 (0.93–1.60)	1.09 (0.84–1.41)	1.05 (0.82–1.36)	Reference
Model 2	1.27 (0.94–1.73)	1.12 (0.85–1.48)	1.03 (0.80–1.34)	1.02 (0.79–1.32)	Reference

<sup>a</sup>Model 1: age, sex, race/center (overall model) or center (race-stratified models). Model 2: model 1 + education, physical activity, smoking status, body mass index.

Bold values indicate  $P < 0.05$ .