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Vitamin D deficiency and incident stroke risk in community-living black and white adults

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Abstract

Background—Black individuals are at greater risk of stroke and vitamin D deficiency than white individuals. Epidemiologic studies have shown that low 25-hydroxyvitamin D (25(OH)D) concentrations are associated with increased risk of stroke, but these studies had limited representation of black individuals.

Methods—We examined the association of 25(OH)D with incident stroke in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study, a cohort of black and white adults ≥45 years of age. Using a case-cohort study design, plasma 25(OH)D was measured in 610 participants who developed incident stroke (cases) and in 937 stroke-free individuals from a stratified cohort random sample of REGARDS participants (comparison cohort).

Results—In multivariable models adjusted for socio-demographic factors, co-morbidities and laboratory values including parathyroid hormone, lower 25(OH)D concentrations were associated with higher risk of stroke (25(OH)D >30 ng/mL reference; 25(OH)D concentrations 20–30 ng/mL, hazard ratio [HR] 1.33, 95% confidence interval [95%CI] 0.89,1.96; 25(OH)D <20 ng/mL, HR 1.85, 95%CI 1.17,2.93). There were no statistically significant differences in the association of lower 25(OH)D with higher risk of stroke in black vs. white participants in fully adjusted models (HR comparing lowest vs. higher 25(OH)D category 2.62, 95%CI 1.18, 5.83 in blacks vs. 1.64,

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95%CI 0.83, 3.24 in whites, $P_{interaction}$=0.82). The associations were qualitatively unchanged when restricted to ischemic or hemorrhagic stroke subtypes or when using race-specific cut-offs for 25(OH)D categories.

Discussion—Vitamin D deficiency is a risk factor for incident stroke and the strength of this association does not appear to differ by race.

Keywords

stroke; vitamin D; epidemiology

INTRODUCTION

Higher circulating vitamin D concentrations are linked to better markers of cardiovascular health. Epidemiologic studies have shown that higher 25-hydroxyvitamin D (25(OH)D) concentrations are associated with lower prevalence of hypertension, diabetes, and inflammation, and lower incidence of cardiovascular disease events and mortality.[1-5] Experimental data show that vitamin D lowers blood pressure via direct inhibition of the renin angiotensin aldosterone system,[6] enhances insulin sensitivity,[7, 8] and reduces secretion of pro-inflammatory cytokines,[9, 10] providing biological plausibility for an association of 25(OH)D deficiency with cardiovascular disease.

Most of the studies that examined the association of 25(OH)D with cardiovascular disease risk used a composite of events as the primary outcome. Few studies examined the association of 25(OH)D with stroke as a primary outcome, and those that did were limited by a lack of racial diversity.[11–13] This is important in that low 25(OH)D concentrations are more common in black individuals than in white individuals and are strongly associated with hypertension.[3] Since hypertension plays a critical role in explaining much of the excess risk of stroke in black vs. white Americans,[14, 15] this suggests that lower 25(OH)D concentrations may partly contribute to the higher risk of stroke in blacks as compared to whites. Further, prior studies have shown substantial racial differences in the association of 25(OH)D with cardiovascular disease outcomes including hypertension, coronary heart disease and fatal stroke events.[3, 16, 17] However, no studies have specifically examined if the association of 25(OH)D with incident stroke differs by race. Accordingly, the primary focus of the current study was to examine the association of 25(OH)D with incident stroke in a large, bi-racial cohort of community-dwelling adults.

METHODS

The Reasons for Geographic and Racial Differences in Stroke (REGARDS) study is a population-based investigation of stroke incidence in United States (US) adults. Details of the study design have been reported elsewhere.[18] Briefly, the REGARDS study recruited participants between 2003 and 2007 and has been continuously following participants since baseline. The recruitment goal for REGARDS was to enroll a large national study of participants aged 45 years and older that would be evenly balanced in terms of race (black and white), geography (Southeastern US and the rest of the nation), and sex. Potential participants were initially mailed a letter inviting them to participate in a baseline telephone

interview lasting approximately 45 minutes. The telephone response rate was 33% and cooperation rate was 49%, similar to other cohort studies.[19, 20] Following initial verbal consent during the telephone interview, a trained health professional went to the participant's home to collect blood and urine samples, and obtain blood pressure measurements, an electrocardiogram (ECG), other key study variables, and written consent. A pill bottle review was conducted to record information on all medications that participants reported taking during the 2 weeks preceding the in-home study visit. Blood was stored and analyzed at the central lab at the University of Vermont and ECGs were centrally read at Wake Forest University. The study was approved by Institutional Review Boards at all participating institutions. The final study sample included 30,239 participants (42% black and 55% female).

Primary Exposure

The exposure of interest was 25(OH)D concentrations measured in baseline plasma samples using a commercially-available ELISA (Immunodetection Systems, Fountain Hills, AZ). The assay range was 5–150 ng/ml. Intra-assay CVs were 8.82–12.49%.

Outcome of Interest

The outcome of interest was incident stroke. Every six months participants or their proxies are interviewed by telephone to determine stroke related hospitalizations. Medical records were pursued for all hospitalizations suspected to be related to stroke, transient ischemic attack, or stroke symptoms. Medical records were first reviewed by a trained neurological clinician to verify the completeness and to remove clear non-stroke cases; suspected strokes were then forwarded on for review by a team of stroke experts. Stroke events were defined according to the World Health Organization definition of stroke.[21] Events not meeting this definition but characterized by symptoms lasting < 24 hours, with neuroimaging consistent with acute ischemia or hemorrhage, were classified as "clinical strokes" and included as stroke events. Strokes were classified as ischemic or hemorrhagic. Ischemic strokes were further sub-classified into etiologic subtypes of small vessel occlusion, large vessel atherosclerosis, cardioembolic or unclassified; hemorrhagic strokes were further sub-classified as intracerebral hemorrhage or subarachnoid hemorrhage.[22] All stroke subtype classifications were based upon the potential stroke etiology discovered during post-stroke evaluation as per other stroke epidemiology studies.[23–25]

Derivation of Case Cohort

We used a case-cohort study design. This approach provides an unbiased estimate of the relative hazard of an outcome(s) without requiring measurement of biomarkers in all participants and is considered a gold standard for minimizing the cost of expensive assays without compromising the power advantage of large cohort studies.[26] Cases included all participants who developed an incident stroke during follow-up through September, 2011. The cohort random sample (comparison group) was selected using stratified sampling to ensure sufficient representation of high-risk groups. All participants with at least one follow-up contact (n=29,653) were categorized into 20 strata based on age (45–54, 55–64, 65–74, 75–84, 85 years), race (black or white), and sex (male or female).[27] In each stratum, participants were randomly selected to fulfill the desired distribution: 50% black, 50%

white, 50% female, 50% male, 20% age 45–54, 20% age 55–64, 25% age 65–74, 25% age 75–84, and 10% age ≥85.

Covariates of Interest

Age, race, sex, smoking history, annual family income, and educational attainment were determined by self-report. Systolic and diastolic blood pressure were defined as the average of two seated measures taken after a 5 minute rest. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference (in centimeters) was measured using a tape measure positioned midway between the lowest rib and the iliac crest with the participant standing. History of coronary heart disease (CHD) was defined as having any of the following: evidence of myocardial infarction on the baseline ECG, self-report of a prior history of a cardiac procedure (coronary artery bypass surgery or percutaneous coronary intervention), or self-reported history of myocardial infarction. Diabetes was defined as self-reported use of insulin or oral hypoglycemic agents, fasting blood glucose concentration of 126 mg/dL or higher, or a non-fasting blood glucose concentration of 200 mg/dL or higher. History of atrial fibrillation was ascertained from self-report or by detection in ECG recordings obtained during the baseline study visit. Left ventricular hypertrophy (LVH) was classified using ECG criteria.[28] Phosphorus and calcium concentrations were measured in baseline plasma samples using standard assays. Serum intact parathyroid hormone concentrations (PTH) were measured using a commercially available ELISA (Roche Elecsys 2010, Roche Diagnostics, Indianapolis, IN). Serum total cholesterol, high-density lipoprotein cholesterol (HDL) and triglycerides were measured by colorimetric reflectance spectrophotometry, and low-density lipoprotein cholesterol was calculated using the Friedewald equation. Estimated glomerular filtration rate (eGFR) was determined from serum creatinine measurements using the Chronic Kidney Disease (CKD) Epidemiology Collaboration equation.[29] Urine albumin measured by the BNII ProSpec nephelometer (Siemens AG) and urine creatinine measured by the rate Jaffé method (Roche/Hitachi, Basel, Switzerland) were used to calculate urine albumin to creatinine ratio (ACR). Chronic kidney disease (CKD) was defined as an eGFR < 60 ml/min/1.73m^2 or an ACR ≥30 mg/g.

Statistical Analysis

Descriptive statistics were used to compare participant characteristics across categories of 25(OH)D within the cohort random sample using appropriate weights to account for the stratified sampling design. Vitamin D categories were defined using clinically relevant cut-off values (< 20 ng/ml, 20 – 30 ng/ml, > 30 ng/ml).

After confirming the proportionality of hazards, weighted Cox regression models for case-cohort studies[30] were used to estimate the hazard ratio of incident stroke as a function of baseline 25(OH)D in sequential models. Model 1 adjusted for age, sex, race, and an age *x* race interaction term because associations of race with stroke are greater at younger ages, as previously reported.[14] Model 2 adjusted for variables in Model 1 plus systolic blood pressure, season of blood draw, BMI, clinical factors (history of diabetes, history of CHD, atrial fibrillation, current smoking, LVH, and markers of CKD [eGFR, log-transformed ACR]) and use of anti-hypertensive medications, aspirin and statins. Model 3 adjusted for

variables in Model 2 plus markers of mineral metabolism (phosphorus, calcium and PTH concentrations) and lipid status (HDL, LDL, total cholesterol and triglycerides). In a sensitivity analysis, we further adjusted for uncontrolled blood pressure defined as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg.[31] In all models, 25(OH)D was analyzed in categories, with the highest category (> 30 ng/ml) serving as the referent group. Given wide variability in the distribution of 25(OH)D concentrations by race, we examined for effect modification by race by testing the statistical significance ($p<0.10$ was considered significant) of a multiplicative interaction term in the model and examined the same associations using race-specific tertiles of 25(OH)D in black and white participants, separately. In pre-specified analyses, we examined the association of 25(OH)D with stroke risk in models stratified by ischemic vs. hemorrhagic strokes and in models further stratified by ischemic and hemorrhagic stroke subtypes. For the latter analyses, when more than one etiologic subtype was considered present, that case was counted in each subtype group. A two-tailed P value < 0.05 was considered statistically significant except for the analyses of interaction. All analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics of Study Participants

A total of 654 participants who developed a stroke over a median of 3.1 (interquartile range 1.6, 3.9) years of follow-up and 1,069 stroke-free participants in the stratified cohort random sample were included in the study. After excluding 97 participants who had a history of stroke at the baseline visit and 79 participants who had missing 25(OH)D concentrations, a total of 1,547 participants constituted the final analyzed sample (610 cases and 937 participants in the cohort random sample).

Table 1 reports the baseline characteristics of participants in the cohort random sample by categories of 25(OH)D. Lower concentrations of 25(OH)D were associated with female sex, black race, higher BMI, greater waist circumference, higher blood pressure, lower socioeconomic status, current smoking, higher prevalence of diabetes, LVH and CKD, lower prevalence of CHD, greater use of anti-hypertensive medications, lower use of aspirin, and higher PTH concentrations.

Associations of 25(OH)D with incident stroke

Table 2 depicts the hazard ratios (HR) of incident stroke by categories of 25(OH)D. Lower concentrations of 25(OH)D were associated with higher risk of incident stroke in models adjusted for age, race, age x race interaction, and sex (25(OH)D >30 ng/ml reference; 25(OH)D 20–30 ng/ml, hazard ratio [HR] 1.07, 95% confidence interval [CI] 0.81, 1.42; 25(OH)D >30 ng/ml, HR 1.61, 95%CI 1.18, 2.22). The magnitude and strength of the associations were unchanged after adjustment for season of blood draw, systolic blood pressure, BMI, diabetes, current cigarette smoking, CHD, atrial fibrillation, LVH, eGFR, log-transformed ACR and use of anti-hypertensive medications, aspirin and statins (HR comparing 25(OH)D concentrations <20 ng/ml to 25(OH)D >30 ng/ml 1.60, 95%CI 1.11,2.33). Further adjustment for other markers of mineral metabolism, HDL, LDL, total

cholesterol, and triglycerides increased the magnitude and statistical strength of these associations (HR comparing 25(OH)D concentrations <20 vs. >30 ng/ml 1.85, 95%CI 1.17, 2.93). The results did not meaningfully change when further adjusted for the presence or absence of uncontrolled blood pressure in study participants (HR 1.79, 95%CI 1.13, 2.84).

No statistically significant differences in the association of lower 25(OH)D with higher risk of incident stroke were observed in blacks as compared to whites in models adjusted for age, sex, season of blood draw, blood pressure, BMI, diabetes, LVH, current smoking, CHD, eGFR, log-transformed ACR, use of anti-hypertensive medications, aspirin and statins, and serum calcium, phosphorus, PTH, HDL, LDL, total cholesterol, and triglyceride concentrations ($P_{interaction}$=0.82), though the magnitude of the HR comparing the lowest to highest category of 25(OH)D was numerically higher in blacks than whites (HR 2.62, 95%CI 1.18, 5.83 in blacks vs. 1.64, 95%CI 0.83, 3.24 in whites). Results were qualitatively unchanged when using race-specific cut-points for 25(OH)D categories (Table 3).

Associations of 25(OH)D with stroke subtypes

A total of 536 strokes were classified as ischemic in etiology and 74 as hemorrhagic. When the analysis was restricted to ischemic strokes, the results were similar to analyses using all strokes types—lower 25(OH)D concentrations were associated with higher risk of incident stroke in fully adjusted models (HR comparing 25(OH)D <20 vs. >30 ng/ml 1.84, 95%CI 1.14, 2.97, Table 4). When restricted to hemorrhagic strokes, the magnitude of the association was similar (HR comparing 25(OH)D <20 vs. >30 ng/ml 1.82, 95%CI 0.91, 3.65), though it was not statistically significant in models adjusted for age, race, age *x* race interaction, and sex.

A total of 134 ischemic strokes were further sub-classified as being cardioembolic in etiology, 84 as being due to large vessel atherosclerosis, 104 as being due to small vessel occlusion, and 244 were unclassified (the total was greater than 536 because more than one etiologic subtype was identified for some events). In models restricted to different ischemic stroke subtypes, lower categories of 25(OH)D levels were associated with higher risk of incident stroke after adjustment for age, race, age *x* race interaction, and sex in all subtypes, but this association was only statistically significant for small vessel disease and unclassified ischemic stroke subtypes.

A total of 61 hemorrhagic strokes were further sub-classified as intracerebral hemorrhages and 13 as subarachnoid hemorrhages. In models adjusted for age, race, age *x* race interaction, and sex, lower 25(OH)D concentrations were associated with higher risk of intracerebral hemorrhage, though the association just missed statistical significance (HR comparing 25(OH)D <20 vs. >30 ng/ml 2.09, 95%CI 0.96, 4.56; *P*trend=0.05). There was no discernible association of lower 25(OH)D concentrations with higher risk of subarachnoid hemorrhage.

DISCUSSION

In this study of community-dwelling adults, lower 25(OH)D concentrations were associated with higher risk of incident stroke. This association was independent of traditional stroke

risk factors and was similar in magnitude and strength in black and white participants. These results indicate that lower 25(OH)D is an important risk factor for incident stroke in community-dwelling adults irrespective of black or white race.

Two meta-analyses pooling data from all available prospective studies examining the association of 25(OH)D with stroke (combined total stroke events=3858), found independent associations of lower 25(OH)D concentrations with higher risk of stroke.[11, 13] Importantly, however, the studies contributing the most number of stroke events in these analyses had a relatively limited number of black participants, and the few studies that included racially diverse populations had relatively few stroke events. As a result, these studies were limited in their ability to examine potential racial differences in the association of 25(OH)D with stroke risk. This is important in that low 25(OH)D concentrations are much more common in black individuals than white individuals; black individuals have a disproportionately high risk of stroke fatality as compared to white individuals; and several prior studies have shown marked heterogeneity in the association of 25(OH)D with cardiovascular disease events by race. In an analysis of black and white participants of the Third National Health and Nutrition Examination Survey (NHANES), lower 25(OH)D concentrations were associated with higher risk of fatal stroke in white but not in black participants.[17] Similarly, in a report from the Multi-Ethnic Study of Atherosclerosis (MESA) and Cardiovascular Health Study (CHS) studies, lower 25(OH)D concentrations were associated with higher risk of CHD in white but not black participants.[16] The results of the current study stand in contrast to these prior finding by showing that low 25(OH)D is an important risk factor for stroke in both black and white adults.

There are a number of biological mechanisms that might underlie an association of low 25(OH)D with stroke risk. The active form of vitamin D (calcitriol) directly inhibits renin gene expression.[6] The physiological importance of this pathway was demonstrated in vitamin D receptor knock-out animals that develop severe hypertension and associated down-stream consequences such as left ventricular hypertrophy.[32] Human studies similarly showed that lower 25(OH)D concentrations were associated with renin angiotensin aldosterone system activation[33] and some, but not all studies, showed that treatment with vitamin D lowered blood pressure.[34–37] Since hypertension is a powerful risk factor for stroke, it is possible that the association of low 25(OH)D with stroke risk may be explained in part by higher blood pressure. Notably, however, the magnitude of the association of 25(OH)D with stroke risk was unchanged in multivariable models adjusted for systolic blood pressure, suggesting that higher blood pressure does not explain the association of lower 25(OH)D with greater stroke risk. Importantly, animal studies have shown direct, neuroprotective effects of vitamin D in ischemia-reperfusion models of cerebral infarction,[38] suggesting that potential beneficial effects of vitamin D may be occurring at the cellular level.

Few clinical trial data exist on the efficacy of vitamin D supplementation on reducing stroke. The data that are available have not supported the efficacy of vitamin D supplementation for reducing stroke risk.[39] However, these data were derived from studies that did not have stroke outcomes as a primary end-point and thus, were not designed to determine whether vitamin D supplementation may reduce stroke risk in the general population. The ongoing

Vitamin D and Omega-3 Trial (VITAL) is the largest trial to date with a primary outcome of reducing major cardiovascular events, including stroke events.[40] While not a primary focus of the study, VITAL anticipates recruiting a sufficient number of black individuals to determine whether the effect of vitamin D on cardiovascular outcomes differs by race. Given the results of the current study, if vitamin D is shown to be efficacious in reducing stroke risk among black individuals, this may be an important focus of future interventions meant to narrow disparities in stroke outcomes since mean 25(OH)D concentrations are much lower in black than white individuals.

Our study had important limitations. We had only one baseline measure of 25(OH)D. Inclusion of only black or white adults limits our ability to extrapolate these findings to other races/ethnicities. Recent data suggest that traditional measures of vitamin D may be a poor proxy for true vitamin D status, especially among black individuals, because standard 25(OH)D assays do not discriminate between relatively inert vitamin D bound to its primary carrier protein (vitamin D binding protein) and the more biologically active free or bioavailable vitamin D.[41] Because we did not have direct or indirect measurements of bioavailable vitamin D, we could not examine associations of bioavailable vitamin D with stroke risk and whether these differ from the more traditional 25(OH)D measures used in this study. Our study also had a number of strengths including standardized collection of baseline data as well as prospective data including physician-adjudicated stroke events and ischemic stroke subtypes.

In summary, lower 25(OH)D concentrations were an independent risk factor for incident stroke irrespective of black or white race. Given the much higher prevalence of 25(OH)D deficiency in blacks as compared to whites, if ongoing or future clinical trials support the efficacy of vitamin D supplementation in reducing stroke events, these results suggest that treating vitamin D deficiency may be an effective method for reducing racial disparities in stroke risk in the US.

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References

1. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007 May; 49(5):1063–9. [PubMed: 17372031]
2. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990–2000. Diabetes. 2008 Oct; 57(10):2619–25. [PubMed: 18591391]

3. Judd SE, Nanes MS, Ziegler TR, Wilson PW, Tangpricha V. Optimal vitamin D status attenuates the age-associated increase in systolic blood pressure in white Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr. 2008 Jan; 87(1):136–41. [PubMed: 18175747]
4. Martins D, Wolf M, Pan D, et al. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2007 Jun 11; 167(11):1159–65. [PubMed: 17563024]
5. Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med. 2008 Aug 11; 168(15):1629–37. [PubMed: 18695076]
6. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002 Jul; 110(2):229–38. [PubMed: 12122115]
7. Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. The FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2003 Mar; 17(3):509–11. [PubMed: 12551842]
8. Zella JB, McCary LC, DeLuca HF. Oral administration of 1,25-dihydroxyvitamin D3 completely protects NOD mice from insulin-dependent diabetes mellitus. Archives of biochemistry and biophysics. 2003 Sep 1; 417(1):77–80. [Comparative Study Research Support, Non-U.S. Gov't]. [PubMed: 12921782]
9. Lemire JM, Adams JS, Sakai R, Jordan SC. 1 alpha,25-dihydroxyvitamin D3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. J Clin Invest. 1984 Aug; 74(2):657–61. [PubMed: 6611355]
10. Lemire JM, Archer DC, Beck L, Spiegelberg HL. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr. 1995 Jun; 125(6 Suppl): 1704S–8S. [PubMed: 7782931]
11. Brondum-Jacobsen P, Nordestgaard BG, Schnohr P, Benn M. 25-hydroxyvitamin D and symptomatic ischemic stroke: an original study and meta-analysis. Ann Neurol. 2013 Jan; 73(1): 38–47. [PubMed: 23225498]
12. Pilz S, Dobnig H, Fischer JE, et al. Low vitamin d levels predict stroke in patients referred to coronary angiography. Stroke. 2008 Sep; 39(9):2611–3. [PubMed: 18635847]
13. Sun Q, Pan A, Hu FB, Manson JE, Rexrode KM. 25-Hydroxyvitamin D levels and the risk of stroke: a prospective study and meta-analysis. Stroke. 2012 Jun; 43(6):1470–7. [PubMed: 22442173]
14. Howard G, Cushman M, Kissela BM, et al. Traditional risk factors as the underlying cause of racial disparities in stroke: lessons from the half-full (empty?) glass. Stroke. 2011 Dec; 42(12):3369–75. [PubMed: 21960581]
15. Howard G, Lackland DT, Kleindorfer DO, et al. Racial differences in the impact of elevated systolic blood pressure on stroke risk. JAMA Intern Med. 2013 Jan 14; 173(1):46–51. [PubMed: 23229778]
16. Robinson-Cohen C, Hoofnagle AN, Ix JH, et al. Racial differences in the association of serum 25-hydroxyvitamin D concentration with coronary heart disease events. JAMA. 2013 Jul 10; 310(2): 179–88. [PubMed: 23839752]
17. Michos ED, Reis JP, Post WS, et al. 25-Hydroxyvitamin D deficiency is associated with fatal stroke among whites but not blacks: The NHANES-III linked mortality files. Nutrition. 2012 Apr; 28(4):367–71. [PubMed: 22261577]
18. Howard VJ, Cushman M, Pulley L, et al. The reasons for geographic and racial differences in stroke study: objectives and design. Neuroepidemiology. 2005; 25(3):135–43. [PubMed: 15990444]
19. Jackson R, Chambless LE, Yang K, et al. Differences between respondents and nonrespondents in a multicenter community-based study vary by gender ethnicity. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. J Clin Epidemiol. 1996 Dec; 49(12):1441–46. [PubMed: 8970495]

20. Morton LM, Cahill J, Hartge P. Reporting participation in epidemiologic studies: a survey of practice. Am J Epidemiol. 2006 Feb 1; 163(3):197–203. [PubMed: 16339049]
21. Stroke--1989. Recommendations on stroke prevention, diagnosis, and therapy. Report of the WHO Task Force on Stroke and other Cerebrovascular Disorders. Stroke. 1989 Oct; 20(10):1407–31. [PubMed: 2799873]
22. Adams HP Jr, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993 Jan; 24(1):35–41. [PubMed: 7678184]
23. Schneider AT, Kissela B, Woo D, et al. Ischemic stroke subtypes: a population-based study of incidence rates among blacks and whites. Stroke. 2004 Jul; 35(7):1552–6. [PubMed: 15155974]
24. Wiklund PG, Brown WM, Brott TG, et al. Lack of aggregation of ischemic stroke subtypes within affected sibling pairs. Neurology. 2007 Feb 6; 68(6):427–31. [PubMed: 17283317]
25. Stead LG, Gilmore RM, Bellolio MF, et al. Cardioembolic but not other stroke subtypes predict mortality independent of stroke severity at presentation. Stroke Res Treat. 2011; 2011:281496. [PubMed: 22007347]
26. Barlow WE, Ichikawa L, Rosner D, Izumi S. Analysis of case–cohort designs. J Clin Epidemiol. 1999 Dec; 52(12):1165–72. [PubMed: 10580779]
27. Cushman M, Judd SE, Howard VJ, et al. N-terminal pro-B-type natriuretic peptide and stroke risk: the reasons for geographic and racial differences in stroke cohort. Stroke. 2014 Jun; 45(6):1646–50. [PubMed: 24757103]
28. Soliman EZ, Howard G, Prineas RJ, McClure LA, Howard VJ. Calculating Cornell voltage from nonstandard chest electrode recording site in the Reasons for Geographic And Racial Differences in Stroke study. Journal of electrocardiology. 2010 May-Jun;43(3):209–14. [PubMed: 20004413]
29. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Annals of internal medicine. 2009 May 5; 150(9):604–12. [PubMed: 19414839]
30. Onland-Moret NC, van der AD, van der Schouw YT, et al. Analysis of case-cohort data: a comparison of different methods. J Clin Epidemiol. 2007 Apr; 60(4):350–5. [PubMed: 17346608]
31. Tanner RM, Calhoun DA, Bell EK, et al. Prevalence of apparent treatment-resistant hypertension among individuals with CKD. Clin J Am Soc Nephrol. 2013 Sep; 8(9):1583–90. [PubMed: 23868902]
32. Xiang W, Kong J, Chen S, et al. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. Am J Physiol Endocrinol Metab. 2005 Jan; 288(1):E125–32. [PubMed: 15367398]
33. Vaidya A, Sun B, Larson C, Forman JP, Williams JS. Vitamin D3 therapy corrects the tissue sensitivity to angiotensin ii akin to the action of a converting enzyme inhibitor in obese hypertensives: an interventional study. J Clin Endocrinol Metab. 2012 Jul; 97(7):2456–65. [PubMed: 22539586]
34. Forman JP, Scott JB, Ng K, et al. Effect of vitamin D supplementation on blood pressure in blacks. Hypertension. 2013 Apr; 61(4):779–85. [PubMed: 23487599]
35. Judd SE, Raiser SN, Kumari M, Tangpricha V. 1,25-dihydroxyvitamin D3 reduces systolic blood pressure in hypertensive adults: a pilot feasibility study. The Journal of steroid biochemistry and molecular biology. 2010 Jul; 121(1–2):445–7. [Randomized Controlled Trial]. [PubMed: 20420907]
36. Nasri H, Behradmanesh S, Ahmadi A, Rafieian-Kopaei M. Impact of oral vitamin D (cholecalciferol) replacement therapy on blood pressure in type 2 diabetes patients; a randomized, double-blind, placebo controlled clinical trial. J Nephropathol. 2014 Jan; 3(1):29–33. [PubMed: 24644541]
37. Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab [Clinical Trial Randomized Controlled Trial]. 2001 Apr; 86(4):1633–7.
38. Wang Y, Chiang YH, Su TP, et al. Vitamin D(3) attenuates cortical infarction induced by middle cerebral arterial ligation in rats. Neuropharmacology. 2000 Mar 3; 39(5):873–80. [Research Support, U.S. Gov't, P.H.S.]. [PubMed: 10699453]

39. Mao PJ, Zhang C, Tang L, et al. Effect of calcium or vitamin D supplementation on vascular outcomes: a meta–analysis of randomized controlled trials. Int J Cardiol. 2013 Oct 30; 169(2): 106–11. [PubMed: 24035175]

40. Manson JE, Bassuk SS, Lee IM, et al. The VITamin D and OmegA-3 TriaL (VITAL): rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. Contemp Clin Trials. 2012 Jan; 33(1):159–71. [PubMed: 21986389]

41. Powe CE, Evans MK, Wenger J, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013 Nov 21; 369(21):1991–2000. [PubMed: 24256378]

Table 1

Baseline characteristics according to 25-hydxovyitamin D (25(OH)D) concentrations in the cohort random sample (analyses weighted to the full cohort). Results are depicted as mean (95% confidence interval), median [interquartile range] or frequencies.

	25(OH)D > 30 ng/ml	25(OH)D 20 – 30 ng/ml	25(OH)D < 20 ng/ml	*P*-trend
Weighted N	8442	9944	7939	
Age	64.8 (63.8,65.9)	65.0 (64.0,66.0)	64.1 (63.0,65.1)	0.32
Male (%)	51	46	36	<0.001
Black (%)	17	32	74	<0.001
Body Mass Index (kg/m^2)	27.5 (26.9,28.1)	29.0 (28.5,29.6)	31.4 (30.6,32.2)	<0.001
Waist circumference (cm)	91.9 (90.3, 93.4)	95.0 (93.5, 96.4)	100.5 (98.8, 102.3)	<0.001
Systolic blood pressure (mm Hg)	125.0 (123.1,126.8)	126.7 (125.1,128.3)	130.7 (128.7,132.7)	0.002
Diastolic blood pressure (mm Hg)	74.8 (73.6,75.9)	76.2 (75.3,77.2)	78.6 (77.5,79.7)	<0.001
Less than high school education (%)	6	11	18	<0.001
Annual income < $20,000/year (%)	13	13	28	<0.001
Co-morbidities				
Current smoking (%)	12	11	19	<0.001
Diabetes (%)	10	20	33	<0.001
Coronary heart disease (%)	20	16	13	<0.001
Atrial fibrillation (%)	9	9	8	0.16
Chronic kidney disease (%)	16	17	23	<0.001
Left ventricular hypertrophy (%)	7	6	11	<0.001
Medication Use				
Anti-hypertensives (%)	51	55	68	<0.001
Aspirin (%)	50	39	38	<0.001
Statins (%)	33	31	33	0.72
Laboratory values				
Calcium (mg/dL)	9.3 (9.2,9.3)	9.1 (9.0, 9.2)	9.2 (9.1, 9.3)	0.25
Phosphorus (mg/dL)	3.5 (3.4, 3.6)	3.5 (3.4, 3.5)	3.5 (3.4, 3.5)	0.94
Intact parathyroid hormone (pg/ml)	35.5 [27.3, 44.7]	40.1 [31.64, 50.0]	50.1 [36.8, 71.5]	<0.001
Total cholesterol (mg/dL)	187.7 (183.8, 191.6)	192.9 (188.2, 197.5)	193.0 (188.3,197.7)	0.24
High-density lipoprotein (mg/dL)	53.2 (51.1, 55.3)	494. (48.3, 51.5)	52.1 (50.2, 54.1)	0.07
Low-density lipoprotein (mg/dL)	113.1 (109.1, 117.1)	110.0 (10.6,7, 113.3)	115.9 (111.8, 119.9)	0.16
Triglycerides (mg/dL)	132.3 (123.1, 141.5)	135.4 (127.4, 143.4)	121.5 (115.0, 128.0)	0.19

Abbreviations: 25(OH)D, 25-hydxovyitamin D; NT-proBNP, N-terminal pro B-type natriuretic peptide.

Table 2

Hazard ratio (95% confidence interval) of incident stroke according to baseline 25-hydoxyvitamin D (25(OH)D) categories.

	25(OH)D > 30 ng/ml	**25(OH)D 20 – 30 ng/ml**	**25(OH)D < 20 ng/ml**	***P* trend**
Weighted N	8442	9944	7939	26325
Events	167	213	230	610
Model 1 *	ref	1.07 (0.81, 1.42)	1.61 (1.18, 2.21)	0.003
Model 2 †	ref	1.21 (0.87, 1.69)	1.60 (1.11, 2.33)	0.01
Model 3 ‡	ref	1.33 (0.89, 1.96)	1.85 (1.17, 2.93)	0.009

*Adjusted for age, race, age*race interaction and sex

†Adjusted for age, race, age*race interaction, sex, season of blood draw, systolic blood pressure, body mass index, diabetes, left ventricular hypertrophy, current smoking, atrial fibrillation, coronary heart disease, estimated glomerular filtration rate, log-transformed albumin to creatinine ratio and use of anti-hypertensive medications, statins, and aspirin.

‡Adjusted for terms in Model 2 + serum calcium, phosphorus, intact parathyroid hormone, high-density lipoprotein, low-density lipoprotein, total cholesterol and triglyceride concentrations.

Table 3

Hazard ratio (95% confidence interval) of incident stroke according to baseline, race-specific tertiles of 25-hydoxyvitamin D (25(OH)D) in black and white participants.

	25(OH)D >23 ng/ml blacks >32 ng/ml whites	25(OH)D 15 – 23 ng/ml blacks 24 – 32 ng/ml whites	25(OH)D < 15 ng/ml blacks < 24 ng/ml whites	*P* trend
Blacks (Weighted N=10,595)				
Weighted N	3602	3505	3488	
Events	66	103	85	
Model 1 *	ref	1.74 (1.17, 2.57)	1.62 (1.06, 2.47)	0.02
Model 2 †	ref	1.76 (1.07, 2.89)	1.49 (0.88, 2.54)	0.14
Model 3 ‡	ref	2.21 (1.24, 3.95)	2.06 (1.04, 4.07)	0.03
Whites (Weighted N=15,730)				
Weighted N	5414	5153	5163	
Events	115	103	138	
Model 1 *	ref	0.89 (0.61, 1.29)	1.15 (0.80, 1.65)	0.42
Model 2 †	ref	0.92 (0.57, 1.48)	1.23 (0.78, 1.91)	0.36
Model 3 ‡	ref	1.07 (0.61, 1.88)	1.48 (0.83, 2.62)	0.19

*Adjusted for age and sex

†Adjusted for age, sex, season of blood draw, systolic blood pressure, body mass index, diabetes, left ventricular hypertrophy, current smoking, atrial fibrillation, coronary heart disease, estimated glomerular filtration rate, log-transformed albumin to creatinine ratio and use of anti-hypertensive medications, statins, and aspirin.

‡Adjusted for terms in Model 2 + serum calcium, phosphorus, intact parathyroid hormone, high-density lipoprotein, low-density lipoprotein, total cholesterol and triglyceride concentrations.

Table 4

Hazard ratio (95% confidence interval) of incident stroke according to baseline 25-hydoxyvitamin D (25(OH)D) categories stratified by stroke subtype.

	25(OH)D > 30 ng/ml	25(OH)D 20 – 30 ng/ml	25(OH)D < 20 ng/ml	*P* trend
Restricted to all ischemic strokes (n=536)				
Events	150	183	203	536
Model 1 *	ref	1.03 (0.77, 1.37)	1.59 (1.15, 2.19)	0.004
Model 2 †	ref	1.18 (0.83, 1.66)	1.59 (1.09, 2.34)	0.02
Model 3 ‡	ref	1.27 (0.85, 1.91)	1.84 (1.14, 2.97)	0.01
Restricted to cardioembolic ischemic strokes (n=134)				
Events	45	44	45	134
Model 1 *	ref	0.84 (0.53, 1.33)	1.34 (0.79, 2.27)	0.43
Restricted to large vessel disease ischemic strokes (n=84)				
Events	28	31	25	84
Model 1 *	ref	1.09 (0.62, 1.94)	1.64 (0.89,3.01)	0.13
Restricted to small vessel disease ischemic strokes (n=104)				
Events	43	37	24	104
Model 1 *	ref	1.42 (0.82, 2.47)	2.03 (1.11, 3.70)	0.02
Restricted to unclassified ischemic strokes (n=244)				
Events	99	78	67	244
Model 1 *	ref	0.96 (0.66, 1.39)	1.67 (1.11, 2.52)	0.02
Restricted to all hemorrhagic strokes (n=74)				
Events	17	30	27	74
Model 1 *	ref	1.48 (0.78, 2.81)	1.82 (0.91, 3.65)	0.08
Restricted to intra-cerebral hemorrhagic strokes (n=61)				
Events	13	27	21	61
Model 1 *	Ref	1.79 (0.89, 3.63)	2.09 (0.96, 4.56)	0.05
Restricted to sub-arachnoid hemorrhagic strokes (n=13)				
Events	4	3	6	13
Model 1 *	ref	0.53 (0.11, 2.53)	0.96 (0.24, 3.79)	0.99

* Adjusted for age, race, age*race interaction and sex

† Adjusted for age, race, age*race interaction, sex, season of blood draw, systolic blood pressure, body mass index, diabetes, left ventricular hypertrophy, current smoking, atrial fibrillation, coronary heart disease, estimated glomerular filtration rate, log-transformed albumin to creatinine ratio and use of anti-hypertensive medications, statins, and aspirin.

‡ Adjusted for terms in Model 2 + serum calcium, phosphorus, intact parathyroid hormone, high-density lipoprotein, low-density lipoprotein, total cholesterol and triglyceride concentrations.