Vitamin D Status and Risk of Stroke
The Rotterdam Study

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Background and Purpose—Recent findings suggest that vitamin D, a neuroprotective prohormone, is involved in the pathogenesis of cardiovascular disease. However, previous studies investigating the association between vitamin D and stroke have shown inconsistent findings. In view of these discrepancies, we determined the association of vitamin D status with stroke using data from a population-based study.

Methods—Within the RS (Rotterdam Study), an ongoing prospective population-based study, we measured serum 25-hydroxyvitamin D concentrations between 1997 and 2008 in 9680 participants (56.8% women) aged ≥45 years. We assessed a history of stroke at baseline and subsequently followed for incident stroke until January 1, 2016. Regression models were used to investigate the association of serum 25-hydroxyvitamin D with prevalent and incident stroke separately, adjusted for age, sex, study cohort, season of blood sampling, and other cardiovascular risk factors.

Results—Of 9680 participants, 339 had a history of stroke at baseline. Serum 25-hydroxyvitamin D concentration was associated with prevalent stroke, adjusted odds ratio per SD decrease, 1.31; 95% CI, 1.14–1.51. After excluding participants with prevalent stroke, we followed 9338 participants for a total of 98 529 person-years. During follow-up, 735 participants developed a stroke. Lower serum 25-hydroxyvitamin D concentration was not associated with a higher stroke risk, adjusted hazard ratio per SD decrease, 1.06; 95% CI, 0.97–1.16. However, severe vitamin D deficiency did show a significant association: hazard ratio, 1.25; 95% CI, 1.05–1.50.

Conclusions—In this population-based cohort, we found an association between vitamin D and prevalent stroke. Only severe vitamin D deficiency was associated with incident stroke. This suggests that lower vitamin D levels do not lead to a higher stroke risk but are instead a consequence of stroke. **(*Stroke*. 2019;50:2293-2298. DOI: 10.1161/STROKEAHA.119.025449.)**

Key Words: 25-hydroxyvitamin D ■ cardiovascular disease ■ risk factors ■ seasons ■ vitamin D deficiency

Vitamin D (25-hydroxyvitamin D [25(OH)D] is most commonly known for its effects on bone metabolism and calcium homeostasis.[1] In recent years, it has received attention for its nonskeletal effects. Accumulating evidence suggests that vitamin D has additional health benefits such as reducing risk of cardiovascular disease and even cognitive disorders.[1] Vitamin D is also neuroprotective because of its involvement in regulating the release of neurotrophic factors and in maintaining the blood-brain barrier integrity.[2–5] Since vitamin D insufficiencies can be treated, vitamin D may have an important public health implication in the prevention of neurological disorders, including stroke.

In recent decades, several studies have been performed on the relationship between vitamin D and stroke risk, showing inconsistent results. A recent meta-analysis showed a 62% increased risk of stroke in individuals on the lowest end of the spectrum of 25(OH)D serum levels compared with the highest end, with the included studies individually using different cutoff values in their analyses.[6] In contrast, studies evaluating more common dichotomous serum level cutoffs found no effect on stroke risk.[7,8] In addition to using different cutoffs, another explanation for the inconsistent findings may be differing follow-up periods. Studies with a short follow-up may be less suited to analyze long-term effects of serum vitamin D levels on stroke risk.[9–12] Although the most recent meta-analysis of observational studies found an association between vitamin D status and stroke risk, this was not confirmed in the recently published data from the VITAL trial (Vitamin D and Omega-3 Trial).[13] In view of these discrepancies, we determined the association of vitamin D status with stroke using data from the population-based RS (Rotterdam Study). We present results on both prevalent and incident stroke. Furthermore, we assessed both long-term and short-term effects of vitamin D and finally explored the effect of using different vitamin D cutoffs on stroke risk.

Methods

Study Population

We conducted this study using data from the RS, a large prospective population-based cohort in the Netherlands with participants aged ≥45.

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The study began in 1990 with 7983 participants and was further extended in 2000 and 2006 with 3011 and 3932 participants, respectively. Participants are invited for follow-up examinations every 3 to 4 years and are continuously monitored through electronic linkage of the study database with corresponding medical records. Further details of the study are described elsewhere.[14] Blood samples for our analyses were taken in 3828 participants from the first cohort during the third visit (RS-I-3: 1997–1999, 79.8% of surviving participants) and during the first visits of cohorts 2 and 3 with 2473 and 3445 participants, respectively (RS-II-1: 2000–2001, 82.1%; RS-III-1: 2006–2008, 87.6%). For further details regarding the inclusion process, see Figure 1.

The RS has been approved by the medical ethics committee at the Erasmus University of Rotterdam and the Ministry of Health, Welfare and Sport of the Netherlands. The study is implemented in the Population Studies Act: Rotterdam Study (Wet Bevolkingsonderzoek ERGO). All participants provided written informed consent for participation in the study and for researchers to access medical information from their personal physicians. Requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to Department of Epidemiology, Erasmus MC University Medical Center at f.vanrooij@erasmusmc.nl.

Serum 25-Hydroxyvitamin D Concentration

Vitamin D status is commonly determined by measuring 25(OH) D levels,[15] and these were measured in the study participants using an electrochemiluminescence binding assay (COBAS Roche Diagnostics GmbH, Germany). Detailed test characteristics have been described elsewhere.[16,17]

Stroke Assessment

We defined stroke based on the definition set by the World Health Organization. These include a syndrome of rapidly developing clinical symptoms, with an apparent vascular cause, of focal or global disturbance of cerebral function lasting for ≥24 hours or leading to death.[18,19] We assessed prevalent stroke at baseline during an interview with a trained physician and verified this data with medical records. After enrollment, we continuously monitored participants for incident stroke through linkage of the study database with files from general practitioners. Files from nursing home physicians and from general practitioners of participants who moved out of the study district were also checked. Additional information, such as clinical notes and neuroimaging reports, were obtained from hospital records. These records were used to categorize stroke as ischemic or hemorrhagic. If this information was insufficient to differentiate between these types, then the stroke was classified as unspecified. Subarachnoid hemorrhages due to ruptured aneurysms were not considered stroke events.[19] Potential strokes were reviewed by research physicians and verified by an experienced stroke neurologist. Follow-up for incident stroke was conducted until January 1, 2016. Participants were followed from study entry until stroke, death, last health status update when they were known to be stroke-free, or January 1, 2016, whichever came first. Follow-up was complete for 96.8% of potential person-years.

Covariate Assessment

Covariates were originally selected on whether they have a biologically plausible role in either the risk of developing a stroke or as a determinant of vitamin D concentration or both.[20] Detailed information on covariates was assessed by personal interview and physical examination with a trained physician and blood sampling during visits to the research center. This information was gathered at the same examination round as the blood collection for serum 25(OH) D concentration measurements. We defined season of blood collection by dividing collection dates into the following categories: March 21 to June 20; June 21 to September 20, September 21 to December 20, and December 21 to March 20. Level of education was categorized as primary education and advanced education at first through third level. Information regarding ethnicity (white or not), smoking habits, and education level were obtained by trained interviewers during home interviews. We calculated body mass index using the measured weight of participant at baseline in kg, divided by the measured height of the participant at baseline in m squared. Smoking habit was categorized as never smoking, former smoking, or currently smoking. Blood pressure was measured in a seating position after a resting period of 5 minutes on the right upper arm using a random-zero sphygmomanometer. Hypertension was defined as the use of antihypertensive medication or otherwise as the average of 2 measurements performed with a 2-minute interval, ≥140/90 mm Hg. Hypercholesterolemia was defined as a total cholesterol ≥6.2 mmol/L or the use of serum lipid-reducing agents. Estimated kidney function was calculated with the CKD-EPI equation (Chronic Kidney Disease Epidemiology Collaboration).[21] Physical activity was assessed using a validated adapted version of Zutphen Physical Activity Questionnaire and expressed in MET (Metabolic Equivalent of Task) hours/week within cohorts RS-I-3 and RS-II-1.[22] For RS-III-1, we used data from the LASA-questionnaire (Longitudinal Aging Study Amsterdam).[23] To combine this data for use in our analyses, we used the standardized *Z* scores of these questionnaires and included them in one scaled variable. History of myocardial infarction and heart failure status was

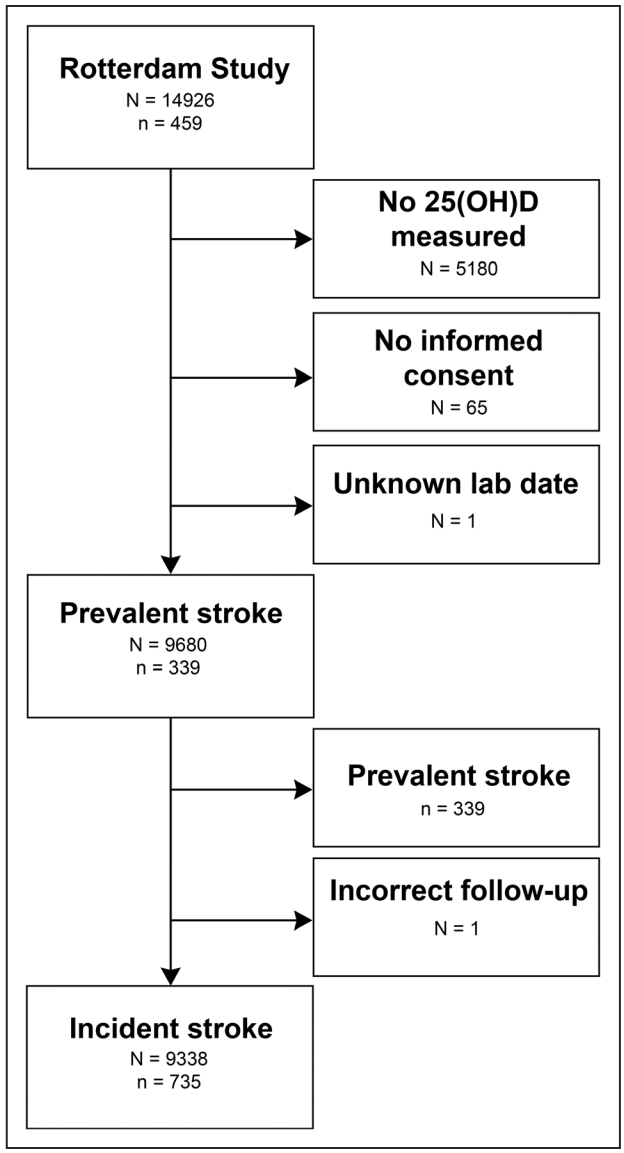

Figure 1. Flowchart of the study population. n denotes number of events; and N, number of people at risk. 25(OH)D indicates 25-hydroxyvitamin D; and RS, Rotterdam Study.

assessed by self-report during home interviews and were verified by ongoing monitoring of medical records. We used an automated enzymatic procedure (Boehringer Mannheim System) to assess levels of serum calcium and total cholesterol.

Statistical Analysis

We devised 2 different models for adjustment to study the association between vitamin D and stroke. The first model was adjusted for age, sex, and cohort. In the second model, we additionally adjusted for ethnicity, highest attained education level, body mass index, smoking habit, hypertension, hypercholesterolemia, estimated kidney function, history of myocardial infarction, history of heart failure, physical activity level, season of blood collection, and serum calcium concentration. The association between vitamin D and prevalent stroke was analyzed with binary logistic regression models, and we used Cox-regression models for incident stroke. We excluded prevalent strokes in the longitudinal analyses. We also assessed the assumptions for proportional hazards, linearity, and additivity and found no major violations.

Serum 25(OH)D concentrations were analyzed per SD decrease and categorized into tertiles with the highest tertile as reference. We additionally used a priori specified, clinically relevant concentration cutoffs where we grouped our participants into categories of vitamin D inadequacy, using guidelines set by the National Academy of Medicine in the United States and by the Endocrine Society.[24,25] Insufficiency was defined as 25(OH)D <75 nmol/L, deficiency as levels of <50 nmol/L, and severe deficiency as <30 nmol/L.

Finally, we examined the association of vitamin D with stroke subtypes: ischemic and hemorrhagic stroke.

To illustrate the effect of different cutoffs for vitamin D and stroke risk, we performed a cutoff analysis where we shifted the cutoffs per 5 nmol/L increments to compare those below versus those above the cutoff. And to address short- and long-term effects of lower serum 25(OH)D levels on stroke risk, we performed an additional analysis where we divided our participants by follow-up time in 3-year increments.

All analyses were performed in IBM SPSS version 24.[26] Missing values among covariates were imputed using 5-fold multiple imputation. We used determinant, outcome, and included covariates for imputation and predictors of missing data. We had <1.5% missings for all covariates except for hypercholesterolemia (28.8%), physical activity (12.7%), and ethnicity (3.6%).

Results

Baseline Characteristics

Table 1 shows baseline characteristics used in the cross-sectional analysis. At baseline, 339 people had a history of stroke. During 98 529 observed person-years (mean follow-up time 10.6 years, interquartile range, 7.8), 735 people had a stroke. Of these, 526 were ischemic events, 83 were hemorrhagic, and 126 were unspecified.

Vitamin D and Prevalent Stroke

At baseline, participants with lower serum 25(OH)D concentration were more likely to have had a stroke compared to participants with higher 25(OH)D concentration: odds ratio per SD decrease 1.36 (95% CI, 1.19–1.55). These results attenuated but were still statistically significant after further adjustment: odds ratio, 1.31 (95% CI, 1.14–1.51). According to previously established categories, participants with low vitamin D were more likely to have had a stroke at baseline in all categories, with severely vitamin D deficiency (<30 nmol/L versus ≥30 nmol/L) having the highest odds ratio, 1.48 (95% CI, 1.12–1.96; Table 2).

Table 1. Baseline Characteristics of the Study Population (N=9680)

Characteristic	Mean/n	SD/%
Age, y	65.1	9.9
Women	5502	56.8%
White ethnicity	9266	95.7%
BMI, kg/m^2	27.3	4.2
Education		
Primary	1226	12.7%
Low	3920	40.5%
Intermediate	2804	28.4%
High	1730	17.8%
Physical activity (SD)	0.1	1.0
Hypertension	4767	48.3%
Hypercholesterolemia	4941	51.0%
Kidney function*	79.7	14.9
Smoking		
Never	2986	30.3%
Former	4521	45.9%
Current	2173	22.0%
History of heart failure	254	2.6%
History of myocardial infarction	485	5.0%
Season of sampling		
Spring	3133	32.4%
Summer	1566	16.2%
Autumn	2898	29.9%
Winter	2083	21.5%
Serum calcium, mmol/L	2.4	0.1
Vitamin D category†		
Sufficient	2304	23.8%
Insufficient	2782	28.7%
Deficient	2748	28.4%
Severely deficient	1846	19.1%

N denotes count where applicable, else mean values; and SD, SD where applicable, else percentage of group. BMI indicates body mass index; and CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

*Estimated glomerular filtration rate (CKD-EPI).[21]

†Vitamin D sufficiency ≥75 nmol/L; insufficiency <75 ≥50 nmol/L; deficiency <50 ≥30 nmol/L; severe deficiency <30 nmol/L. Missing values are imputed.

Vitamin D and Incident Stroke

Per SD lower serum 25(OH)D level was related to an increased risk of stroke after adjusting for age, sex, and cohort: hazard ratio (HR), 1.09 (95% CI, 1.01–1.19) per SD decrease (Table 2). This effect became statistically nonsignificant after further adjustment: HR, 1.06 (95% CI, 0.97–1.16). These results were similar for the subtypes ischemic stroke (adjusted HR, 1.06; 95% CI, 0.96–1.18 per SD decrease) and hemorrhagic stroke (adjusted HR, 0.96; 95% CI, 0.75–1.23 per SD decrease). Severe vitamin D deficiency (<30 versus

≥30 nmol/L) was the only clinical category that showed a significant increase in stroke risk after further adjustment: HR, 1.25 (95% CI, 1.05–1.50).

Figure 2 shows a forest plot of results for the cutoff analyses. Adjusted HRs become statistically significant with serum 25(OH)D level cutoff values starting from 35 nmol/L and lower: HR, 1.21 (95% CI, 1.03–1.44) until 25 nmol/L: HR, 1.30 (95% CI, 1.06–1.59).

Finally, we also found no increase in stroke risk with longer follow-up time increments (Table I in the online-only Data Supplement).

Discussion

In this population-based study, we found that serum 25(OH)D levels are associated with prevalent stroke, but not with incident stroke. Only severe vitamin D deficiency was associated with incident stroke.

Previous studies on the association between vitamin D and stroke may be inconsistent[6–8] for several reasons: first, previous studies used different cutoff values of serum 25(OH)D in their analyses, and this may have influenced their findings. A tertile comparison, for example, with the lowest tertile below 45 nmol/L, might not yield a statistically significant association,[10] whereas a quintile comparison, with the lowest quintile of 25(OH)D below the 35 nmol/L cutoff, may indeed lead to significant results.[27] In view of these observations, our data did suggest an association when we lowered the cutoff value for vitamin D deficiency to <35 nmol/l (Figure 2). However, for cutoff values below <20 nmol/L the association again became nonsignificant, probably because of small number of cases.

A second possible explanation for the inconsistent findings is that vitamin D deficiency is only harmful in the short-term. This harmful effect on stroke risk would only be visible within a short follow-up time. Indeed, previous studies with shorter follow-up periods have shown statistically significant associations for higher stroke risk in vitamin D deficient individuals.[9,10,12,28] In line with these findings, our sensitivity analysis with follow-up increments showed an increased stroke risk, although statistically nonsignificant, within 3 years of follow-up (Table I in the online-only Data Supplement), with decreasing effect estimates in longer follow-up increments. This would explain why we do not find an effect of lower vitamin D in our total follow-up period. Similarly, a Danish study by Skaaby et al[7] had a comparable mean follow-up time to our study and also found no increased risk of stroke in vitamin D deficient individuals.

Another Danish study with comparable follow-up times found an increased stroke risk (HR, 1.23; 95% CI, 1.06–1.42) in individuals aged 20 to 100 years with serum 25(OH)D values <25 nmol/L compared with ≥50 nmol/L.[29] The severe deficiency studied here could explain the observed association, as we also found a significantly increased risk of stroke when assessing severe vitamin D deficiency. Another cohort study within Chinese individuals from Hong Kong compared lowest versus highest quintiles of serum 25(OH)D) and found an increased risk for developing stroke with lower vitamin D levels (HR, 1.78; 95% CI, 1.16–2.74).[30] This study also had a comparable follow-up, but the retrospective nature of the study makes it more prone to bias than a prospective design.

The results from our analyses with prevalent stroke offer a third explanation, as these suggest that low vitamin D serum levels are a consequence of stroke. People who develop a stroke may have limited vitamin D production because of reduced exposure to sunlight and diet quality, among other factors.[24,31] Another explanation for these findings is that lower vitamin D predominantly may only be associated with nonfatal stroke instead of fatal stroke, as prevalent strokes are nonfatal, whereas incident stroke includes both types. Finally, the recent published VITAL trial,[13] a randomized, placebo-controlled trial, comparing the use of vitamin D supplements and marine omega-3 fatty acids with placebo in the elderly, found no decrease in incidence of cardiovascular events, including stroke, in the intervention group during the mean follow-up of 5.3 years. The findings of the VITAL trial suggest that any benefit of vitamin D supplementation is too mild to have an impact on stroke risk, unless the individuals are already severely vitamin D deficient, as rightfully suggested by the VITAL trial investigators. Direct and indirect effects of vitamin D have been proposed for pathophysiological pathways leading to stroke.[2,3,32] Directly, vitamin D is involved in maintaining endovascular function[33–35] and regulating

Table 2. Results Analyses of Serum 25(OH)D and Stroke

	Prevalent Stroke	Incident Stroke
	N/n=9680/339	N/n=9338/735
Total Sample	OR (95% CI)	HR (95% CI)
Model 1*		
Per SD decrease	1.36 [1.19–1.55]†	1.09 [1.01–1.19]†
Insufficient‡	1.55 [1.13–2.11]†	1.12 [0.92–1.36]
Deficient	1.56 [1.23–1.97]†	1.11 [0.95–1.30]
Severely deficient	1.60 [1.24–2.07]†	1.30 [1.09–1.54]†
Lowest tertile	1.81 [1.35–2.44]†	1.18 [0.97–1.43]
Middle tertile	1.36 [1.00–1.84]	1.02 [0.84–1.24]
Highest tertile	1.00 (ref)	1.00 (ref)
Model 2		
Per SD decrease	1.31 [1.14–1.51]†	1.06 [0.97–1.16]
Insufficient	1.41 [1.02–1.94]†	1.07 [0.87–1.31]
Deficient	1.43 [1.11–1.84]†	1.05 [0.90–1.24]
Severely deficient	1.48 [1.12–1.96]†	1.25 [1.05–1.50]†
Lowest tertile	1.63 [1.18–2.24]†	1.10 [0.90–1.34]
Middle tertile	1.32 [0.96–1.80]	0.98 [0.80–1.19]
Highest tertile	1.00 (ref)	1.00 (ref)

N denotes number of people at risk; and n, number of events. 25(OH)D indicates 25-hydroxyvitamin D; HR, hazard ratio; and OR, odds ratio.

*Model 1: adjusted for age, sex, study cohort; model 2: adjusted for model 1 and for ethnicity, BMI, highest level of education, physical activity (in SD scores), prevalent hypertension, prevalent hypercholesterolemia, estimated kidney function, smoking habit, prevalent heart failure, prevalent myocardial infarction, season of sampling, and serum calcium concentration.

†$P<0.05$.

‡Vitamin D insufficiency: <75 nmol/L vs ≥75 nmol/L; deficiency: <50 nmol/L vs ≥50 nmol/L; severe deficiency: <30 nmol/L vs ≥30 nmol/L.

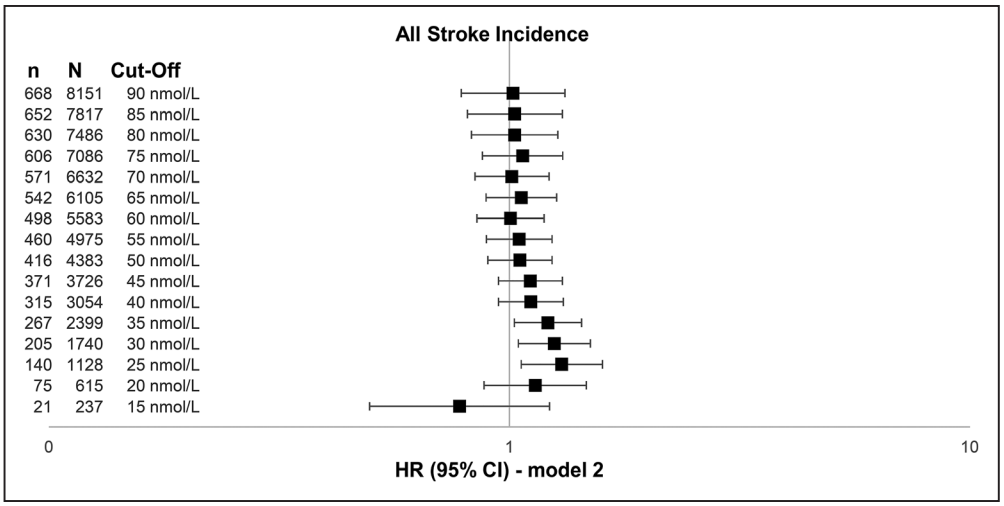

Figure 2. Forest plot of incident all stroke cutoff analysis using adjusted models. n denotes number of events under cutoff point; and N, number of people at risk under cutoff point. Results adjusted for age, sex, study cohort, ethnicity, body mass index, highest level of education, physical activity (in SD scores), prevalent hypertension, prevalent hypercholesterolemia, estimated kidney function, smoking habit, prevalent heart failure, prevalent myocardial infarction, season of sampling, and serum calcium concentration. HR indicates hazard ratio.

inflammatory activity within vascular walls.[36,37] Indirectly, vitamin D is involved in regulating renin production through the Renin-Angiotensin-Aldosterone system-pathway, thereby attenuating hypertension.[38] Although hypertension and atherosclerosis are important factors in the pathophysiology of stroke,[2,3,39] other established cardiovascular risk factors have also been linked to vitamin D status, such as serum lipid status and insulin sensitivity.[32,40] Indeed, any association within our data became statistically nonsignificant after adjustment for any of these risk factors.

Our study has several limitations. We were unable to perform repeated vitamin D measurements over time to assess if the vitamin D status remained stable. However, previous epidemiological studies on serum 25(OH)D have suggested that a single measurement is an adequate biomarker that represents long-term levels of vitamin D.[41,42] Furthermore, this study was performed within a predominantly white population, limiting its generalizability to other populations with a different ethnic makeup, as shown by Judd et al.[10] Strengths of this study are its prospective design with a long and almost complete follow-up. We additionally assessed a large selection of potential confounders and adjusted our results accordingly. Furthermore, we used an established method to measure 25(OH)D serum levels. Finally, stroke cases were assessed using an elaborate adjudication method.

Conclusions

Within the population-based Rotterdam Study, we found an association between vitamin D and prevalent stroke, whereas no association was found with incident stroke. Only severe vitamin D deficiency suggested an association. This suggests that lower vitamin D levels do not lead to a higher stroke risk but are instead a consequence of stroke.

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Disclosures

None.

References

1. Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ*. 2014;348:g2035. doi: 10.1136/bmj.g2035
2. Muscogiuri G, Annweiler C, Duval G, Karras S, Tirabassi G, Salvio G, et al. Vitamin D and cardiovascular disease: from atherosclerosis to myocardial infarction and stroke. *Int J Cardiol*. 2017;230:577–584. doi: 10.1016/j.ijcard.2016.12.053
3. Norman PE, Powell JT. Vitamin D and cardiovascular disease. *Circ Res*. 2014;114:379–393. doi: 10.1161/CIRCRESAHA.113.301241
4. Borgi L, McMullan C, Wohlhueter A, Curhan GC, Fisher ND, Forman JP. Effect of Vitamin D on endothelial function: a randomized, double-blind, placebo-controlled trial. *Am J Hypertens*. 2017;30:124–129. doi: 10.1093/ajh/hpw135

5. McMullan CJ, Borgi L, Curhan GC, Fisher N, Forman JP. The effect of vitamin D on renin-angiotensin system activation and blood pressure: a randomized control trial. *J Hypertens*. 2017;35:822–829. doi: 10.1097/HJH.0000000000001220
6. Zhou R, Wang M, Huang H, Li W, Hu Y, Wu T. Lower vitamin d status is associated with an increased risk of ischemic stroke: a systematic review and meta-analysis. *Nutrients*. 2018;10:277.
7. Skaaby T, Husemoen LL, Pisinger C, Jørgensen T, Thuesen BH, Fenger M, et al. Vitamin D status and incident cardiovascular disease and all-cause mortality: a general population study. *Endocrine*. 2013;43:618–625. doi: 10.1007/s12020-012-9805-x
8. Perna L, Schöttker B, Holleczek B, Brenner H. Serum 25-hydroxyvitamin D and incidence of fatal and nonfatal cardiovascular events: a prospective study with repeated measurements. *J Clin Endocrinol Metab*. 2013;98:4908–4915. doi: 10.1210/jc.2013-2424
9. Anderson JL, May HT, Horne BD, Bair TL, Hall NL, Carlquist JF, et al; Intermountain Heart Collaborative (IHC) Study Group. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. *Am J Cardiol*. 2010;106:963–968. doi: 10.1016/j.amjcard.2010.05.027
10. Judd SE, Morgan CJ, Panwar B, Howard VJ, Wadley VG, Jenny NS, et al. Vitamin D deficiency and incident stroke risk in community-living black and white adults. *Int J Stroke*. 2016;11:93–102. doi: 10.1177/1747493015607515
11. Drechsler C, Pilz S, Obermayer-Pietsch B, Verduijn M, Tomaschitz A, Krane V, et al. Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events, and mortality in haemodialysis patients. *Eur Heart J*. 2010;31:2253–2261. doi: 10.1093/eurheartj/ehq246
12. Bolland MJ, Bacon CJ, Horne AM, Mason BH, Ames RW, Wang TK, et al. Vitamin D insufficiency and health outcomes over 5 y in older women. *Am J Clin Nutr*. 2010;91:82–89. doi: 10.3945/ajcn.2009.28424
13. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al; VITAL Research Group. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med*. 2019;380:33–44. doi: 10.1056/NEJMoa1809944
14. Ikram MA, Brusselle GGO, Murad SD, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2018 update on objectives, design and main results. *Eur J Epidemiol*. 2017;32:807–850. doi: 10.1007/s10654-017-0321-4
15. Cashman KD, van den Heuvel EG, Schoemaker RJ, Prévéraud DP, Macdonald HM, Arcot J. 25-Hydroxyvitamin D as a biomarker of Vitamin D status and its modeling to inform strategies for prevention of Vitamin D deficiency within the population. *Adv Nutr*. 2017;8:947–957. doi: 10.3945/an.117.015578
16. Leino A, Turpeinen U, Koskinen P. Automated measurement of 25-OH vitamin D3 on the roche modular E170 analyzer. *Clin Chem*. 2008;54:2059–2062. doi: 10.1373/clinchem.2008.111732
17. Vitezova A, Voortman T, Zillikens MC, Jansen PW, Hofman A, Uitterlinden AG, et al. Bidirectional associations between circulating vitamin D and cholesterol levels: the Rotterdam Study. *Maturitas*. 2015;82:411–417. doi: 10.1016/j.maturitas.2015.08.005
18. Hatano S. Experience from a multicentre stroke register: a preliminary report. *Bull World Health Organ*. 1976;54:541–553.
19. Wieberdink RG, Ikram MA, Hofman A, Koudstaal PJ, Breteler MM. Trends in stroke incidence rates and stroke risk factors in Rotterdam, the Netherlands from 1990 to 2008. *Eur J Epidemiol*. 2012;27:287–295. doi: 10.1007/s10654-012-9673-y
20. VanderWeele TJ. Principles of confounder selection. *Eur J Epidemiol*. 2019;34:211–219. doi: 10.1007/s10654-019-00494-6
21. Levey AS, Stevens LA. Estimating GFR using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation: more accurate GFR estimates, lower CKD prevalence estimates, and better risk predictions. *Am J Kidney Dis*. 2010;55:622–627. doi: 10.1053/j.ajkd.2010.02.337
22. Caspersen CJ, Bloemberg BP, Saris WH, Merritt RK, Kromhout D. The prevalence of selected physical activities and their relation with coronary heart disease risk factors in elderly men: the Zutphen Study, 1985. *Am J Epidemiol*. 1991;133:1078–1092. doi: 10.1093/oxfordjournals.aje.a115821
23. Stel VS, Smit JH, Pluijm SM, Visser M, Deeg DJ, Lips P. Comparison of the LASA physical activity questionnaire with a 7-day diary and pedometer. *J Clin Epidemiol*. 2004;57:252–258. doi: 10.1016/j.jclinepi.2003.07.008
24. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al; Endocrine Society. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96:1911–1930. doi: 10.1210/jc.2011-0385
25. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine: what clinicians need to know. *J Clin Endocrinol Metab*. 2011;96:53–58. doi: 10.1210/jc.2010-2704
26. IBM Corp. *Spss Statistics for Windows: Version 24.0*. Armonk, NY: IBM; 2016.
27. Schneider AL, Lutsey PL, Selvin E, Mosley TH, Sharrett AR, Carson KA, et al. Vitamin D, vitamin D binding protein gene polymorphisms, race and risk of incident stroke: the Atherosclerosis Risk in Communities (ARIC) study. *Eur J Neurol*. 2015;22:1220–1227. doi: 10.1111/ene.12731
28. Zittermann A, Ernst JB, Prokop S, Fuchs U, Dreier J, Kuhn J, et al. Effect of vitamin D on all-cause mortality in heart failure (EVITA): a 3-year randomized clinical trial with 4000 IU vitamin D daily. *Eur Heart J*. 2017;38:2279–2286. doi: 10.1093/eurheartj/ehx235
29. Afzal S, Nordestgaard BG. Vitamin d, hypertension, and ischemic stroke in 116 655 individuals from the general population: a genetic study. *Hypertension*. 2017;70:499–507.
30. Leung RY, Han Y, Sing CW, Cheung BM, Wong IC, Tan KC, et al. Serum 25-hydroxyvitamin D and the risk of stroke in Hong Kong Chinese. *Thromb Haemost*. 2017;117:158–163. doi: 10.1160/TH16-07-0551
31. Lieber AC, Hong E, Putrino D, Nistal DA, Pan JS, Kellner CP. Nutrition, energy expenditure, dysphagia, and self-efficacy in stroke rehabilitation: a review of the literature. *Brain Sci*. 2018;8:E218.
32. Carvalho LS, Sposito AC. Vitamin D for the prevention of cardiovascular disease: are we ready for that? *Atherosclerosis*. 2015;241:729–740. doi: 10.1016/j.atherosclerosis.2015.06.034
33. Breslavsky A, Frand J, Matas Z, Boaz M, Barnea Z, Shargorodsky M. Effect of high doses of vitamin D on arterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients. *Clin Nutr*. 2013;32:970–975. doi: 10.1016/j.clnu.2013.01.020
34. Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. *J Clin Endocrinol Metab*. 2009;94:4023–4030. doi: 10.1210/jc.2008-1212
35. Mayer O Jr, Filipovský J, Seidlerová J, Vaněk J, Dolejšová M, Vrzalová J, et al. The association between low 25-hydroxyvitamin D and increased aortic stiffness. *J Hum Hypertens*. 2012;26:650–655. doi: 10.1038/jhh.2011.94
36. Chen Y, Liu W, Sun T, Huang Y, Wang Y, Deb DK, et al. 1,25-Dihydroxyvitamin D promotes negative feedback regulation of TLR signaling via targeting microRNA-155-SOCS1 in macrophages. *J Immunol*. 2013;190:3687–3695. doi: 10.4049/jimmunol.1203273
37. Takeda M, Yamashita T, Sasaki N, Nakajima K, Kita T, Shinohara M, et al. Oral administration of an active form of vitamin D3 (calcitriol) decreases atherosclerosis in mice by inducing regulatory T cells and immature dendritic cells with tolerogenic functions. *Arterioscler Thromb Vasc Biol*. 2010;30:2495–2503. doi: 10.1161/ATVBAHA.110.215459
38. 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;110:229–238. doi: 10.1172/JCI15219
39. Furie KL, Kasner SE, Adams RJ, Albers GW, Bush RL, Fagan SC, et al; American Heart Association Stroke Council, Council on Cardiovascular Nursing, Council on Clinical Cardiology, and Interdisciplinary Council on Quality of Care and Outcomes Research. Guidelines for the prevention of stroke in patients with stroke or transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:227–276. doi: 10.1161/STR.0b013e3181f7d043
40. Prabhakaran S, Chong JY. Risk factor management for stroke prevention. *Continuum (Minneap Minn)*. 2014;20(2 Cerebrovascular Disease):296–308. doi: 10.1212/01.CON.0000446102.82420.64
41. Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. *Am J Epidemiol*. 2010;171:903–908. doi: 10.1093/aje/kwq005
42. Sonderman JS, Munro HM, Blot WJ, Signorello LB. Reproducibility of serum 25-hydroxyvitamin d and vitamin D-binding protein levels over time in a prospective cohort study of black and white adults. *Am J Epidemiol*. 2012;176:615–621. doi: 10.1093/aje/kws141