25-Hydroxyvitamin D and Symptomatic Ischemic Stroke: An Original Study and Meta-Analysis

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Objective: We tested the hypothesis that low plasma concentrations of 25-hydroxyvitamin D are associated with increased risk of symptomatic ischemic stroke in the general population.
Methods: We measured plasma 25-hydroxyvitamin D in 10,170 individuals from the general population, the Copenhagen City Heart Study. During 21 years of follow-up, 1,256 and 164 persons developed ischemic and hemorrhagic stroke, respectively. In a meta-analysis of ischemic stroke, we included 10 studies, 58,384 participants, and 2,644 events.
Results: Stepwise decreasing plasma 25-hydroxyvitamin D concentrations were associated with stepwise increasing risk of ischemic stroke both as a function of seasonally adjusted percentile categories and as a function of clinical categories of 25-hydroxyvitamin D (p for trend $\leq 2 \times 10^{-3}$). In a Cox regression model comparing individuals with plasma 25-hydroxyvitamin D concentrations between the 1st and 4th percentiles to individuals with 25-hydroxyvitamin D concentrations between the 50th and 100th percentiles, multivariate adjusted hazard ratio of ischemic stroke was 1.82 (95% confidence interval, 1.41–2.34). Comparing individuals with clinical categories of severe vitamin D deficiency (<25.0nmol/l [<10.0ng/ml]) to individuals with optimal vitamin D status (≥75.0nmol/l [≥30.0ng/ml]), the multivariate adjusted hazard ratio of ischemic stroke was 1.36 (1.09–1.70). 25-Hydroxyvitamin D concentrations were not associated with risk of hemorrhagic stroke. In a meta-analysis comparing lowest versus highest quartile of 25-hydroxyvitamin D concentrations, the multivariate adjusted odds ratio of ischemic stroke was 1.54 (1.43–1.65) with a corresponding hazard ratio of 1.46 (1.35–1.58) in prospective general population studies.
Interpretation: In this large population-based prospective study, we observed stepwise increasing risk of symptomatic ischemic stroke with decreasing plasma 25-hydroxyvitamin D concentrations. This finding was substantiated in a meta-analysis.
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Reduced plasma 25-hydroxyvitamin D (25-OH-vitD) concentrations as a diagnostic marker of vitamin D deficiency have been associated with several well-established risk factors for ischemic stroke, such as arterial hypertension, thrombosis, atherosclerosis, and inflammation,[1] and a limited number of observational studies have directly associated reduced 25-OH-vitD concentrations with increased risk of ischemic stroke, although results have been inconsistent.[2–10]

In the present study, we tested the hypothesis that reduced concentrations of 25-OH-vitD are associated with increased risk of symptomatic ischemic stroke in the general population. We included 10,170 individuals from the Copenhagen City Heart Study with baseline 25-OH-vitD measurements, and followed them for up to 30 years, during which time 1,256 and 164 developed ischemic and hemorrhagic stroke, respectively. Also, the association of reduced 25-OH-vitD concentrations with increased risk of ischemic stroke/stroke was summarized in a meta-analysis including previous and present studies.

Subjects and Methods

The study was approved by a Danish ethical committee (No. KF-V.100.2039/91) and conducted according to the

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Additional supporting information can be found in the online version of this article.

Declaration of Helsinki. Written informed consent was obtained from participants.

Study Cohort

The Copenhagen City Heart Study is a prospective study of the general population of Copenhagen, Denmark, initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994, 2001–2003, and 2011 with ongoing re-examinations.[11,12] At each examination, participants completed a questionnaire, underwent a physical examination, and provided blood samples. We included individuals aged 20 to 100 years from the 1981–1983 examination randomly selected to represent an age- and gender-stratified distribution of the general population of the City of Copenhagen (n = 87,172), irrespective of disease status; 18,089 were invited, 12,698 participated, and 10,170 had 25-OH-vitD measurements and of these, 1,256 and 164 individuals developed ischemic and hemorrhagic stroke during follow-up. Median follow-up time up to May 2011 was 21 years (range, 0–29 years). All individuals were followed from baseline in 1981–1983 until the occurrence of stroke, death, or May 9, 2011, whichever came first. Follow-up was 100% complete.

Information on diagnosis of cerebrovascular disease, including ischemic and hemorrhagic stroke (World Health Organization [WHO]; International Classification of Diseases [ICD], 8th edition: codes 431–438; and International Classification of Diseases, 10th edition: codes I60–I68 and G45) was collected from 1976 until May 2011 from questionnaires at the initial examination and at the 3 follow-up examinations, and by reviewing hospital admissions with diagnoses entered in the national Danish Patient Registry, and causes of death entered in the national Danish Causes of Death Registry.[13] For participants with registered cerebrovascular disease, records from general practitioners and hospital records were requested, and the diagnosis of ischemic and hemorrhagic stroke was validated by 2 independent doctors with special interest in stroke[14] and blinded to the test results. Ischemic stroke was defined according to WHO criteria as rapidly developed signs of focal (or global) disturbance of cerebral function lasting >24 hours (unless interrupted by death), with no apparent nonvascular cause.[15] Hemorrhagic stroke and subarachnoidal hemorrhage were excluded from the ischemic stroke group. To distinguish among infarction, intracerebral hemorrhage, and subarachnoidal hemorrhage, either computed tomography or magnetic resonance imaging scan, spinal fluid examination, autopsy, or surgical description was necessary. If the scan did not show an infarction or hemorrhage, but the individual had symptoms that met the criteria of the stroke definition, then the event was diagnosed as an ischemic stroke. The diagnosis of ischemic and hemorrhagic stroke was not applied in cases in which a scan revealed signs of prior cerebrovascular disease, but without history of any symptoms, if symptoms were nonfocal, or if symptoms lasted <24 hours.

Biochemical Analyses

Plasma 25-OH-vitD (D2+D3) was measured using a competitive chemiluminescent immunoassay, CLIA (DiaSorin, Stillwater, MN), with intra- and interassay coefficients of variation of 10% and 11%, respectively, and a lower detection limit of 5nmol/l. Colorimetric assays (Boehringer Mannheim, Mannheim, Germany or Konelab, Espoo, Finland) were used to measure creatinine, total cholesterol, and high-density lipoprotein (HDL) cholesterol in plasma. Total cholesterol and HDL cholesterol were measured the same day as the blood sample was collected in 1981–1983. On these samples stored at −20°C without previous thawing, creatinine and 25-OH-vitD were measured in 2010–2011.

Other Covariates

For stratified analysis, covariates were dicotomized as follows: body mass index was expressed as measured weight in kilograms divided by measured height in meters squared and coded as low (<25kg/m^2) or high (≥25kg/m^2). Physical activity was coded as low or high using information on physical activity during work and leisure time; low physical activity was predominantly sedentary work and <2 hours of leisure time physical activity per week. Smoking status was classified as current, former, or never smokers. Alcohol consumption was based on average weekly consumption of beer, wine, and liquor during the preceding month categorized into low to abstinent (<14/21U) or high (≥14/21U per week for women/men); 1U alcohol = ∼12g. Hypertension was diastolic blood pressure ≥90mmHg (≥85mmHg for diabetics) and/or systolic blood pressure ≥140mmHg (≥135mmHg for diabetics)[16] and/or use of antihypertensive medication perscribed specifically for hypertension. Diabetes mellitus was nonfasting blood glucose concentrations ≥11.0mmol/l[17] and/or insulin or other antidiabetic treatment. Atrial fibrillation was diagnosed from electrocardiographic recordings obtained at the 4 study examinations and/or from the national Danish Patient Registry (ICD8: 427.93, 427.94; ICD10: I48.9). Hormone replacement therapy and lipid-lowering therapy were self-reported. Estimated glomerular filtration rate[18] was low (<60ml/min per 1.73m^2) or high (≥60ml/min per 1.73m^2).

Statistical Analysis

Data were analyzed using Stata/SE. To adjust for seasonal variation in 25-OH-vitD concentrations, we assigned individuals to percentiles of 25-OH-vitD concentration by month of sample collection. Participants were grouped according to 25-OH-vitD concentrations into the upper 2 quartiles (the 50th–100th percentiles; interquartile range within the group: 47–71nmol/l) as a reference group to achieve a large group representative of the population, the third quartile as an intermediate group (the 25th–49th percentiles; 28–46nmol/l), and the lowest fourth quartile (1st–24th percentiles; 14–23 nmol/l); to explore the association with very low concentrations, we further divided the lowest quartile into the 10th to 24th percentiles (18–26nmol/l), the 5th to 9th percentiles (13–17nmol/l), and the 1st to 4th percentiles (7.5–12nmol/l). We also assigned categories of 25-OH-vitD concentrations according to widely used clinical cutpoints[19]: severe vitamin D deficiency as 25-OH-vitD <25.0nmol/l (<10.0ng/ml), moderate vitamin D deficiency as

TABLE : Characteristics of Participants in the Danish General Population, the Prospective Copenhagen City Heart Study

		25-Hydroxyvitamin D Percentile Categories			
Characteristics	**All**	**50th–100th**	**25th–49th**	**1st–24th**	p_{trend}
No. of participants	10,170	5,085	2,532	2,553	
25-hydroxyvitamin D, nmol/l	44 (26–58)	62 (47–71)	34 (28–40)	19 (14–23)	<0.001
Age, yr	56 (48–65)	56 (48–64)	56 (49–65)	57 (50–65)	<0.001
Women	56%	57%	55%	55%	0.02
Body mass index, kg/m^2	25 (22–28)	25 (24–27)	26 (25–28)	26 (25–29)	<0.001
High physical activity	66%	70%	66%	60%	<0.001
Current smoking	58%	52%	59%	68%	<0.001
High alcohol consumption	13%	12%	12%	15%	0.36
Total cholesterol, mmol/l	5.9 (5.1–6.6)	5.9 (5.1–6.6)	6.0 (5.1–6.7)	6.0 (5.1–6.7)	0.003
High-density lipoprotein cholesterol, mmol/l	1.2 (0.9–1.3)	1.2 (1.0–1.4)	1.2 (1.1–1.3)	1.1 (1.1–1.3)	<0.001
Hypertension	63%	60%	64%	68%	<0.001
Diabetes mellitus	3%	2%	4%	5%	<0.001
Atrial fibrillation	5%	5%	5%	6%	0.04
Hormone replacement therapy	9%	10%	8%	7%	<0.001
Lipid-lowering therapy	0%	0%	0%	0%	N.A.
Antihypertensive medication	11%	11%	12%	10%	0.54
Low estimated glomerular filtration rate	35%	34%	35%	35%	0.15

Continuous variables are reported as median and interquartile range, and categorical variables are reported in percentage. Tests for trend across percentile categories are nonparametric tests by Cuzick for continuous variable and Cuzick extension of Wilson rank sum test for categorical variables (for all tests, 2-sided, Bonferroni-corrected p values$<0.05/16 = 0.0031$). Information on covariates reported in this table was >99% complete. Data are from the 1981–1983 examination of the Copenhagen City Heart Study. To convert 25-hydroxyvitamin D from nanomoles per liter to nanograms per liter, divide by 2.496; to convert cholesterol from millimoles per liter to milligrams per deciliter, divide by 0.0259. N.A. = not applicable.

25.0 to 49.9nmol/l (10.0–19.9ng/ml), vitamin D insufficiency as 50.0 to 74.9nmol/l (20.0–29.9ng/ml), and optimal vitamin D range as ≥75.0nmol/l (≥30.0ng/ml). Tests for trend across percentile categories were nonparametric tests by Cuzick for continuous variables and Cuzick extension of Wilson rank sum test for categorical variables (for all tests, 2-sided, Bonferroni-corrected p values $< 0.05/15 = 0.0033$).

Cumulative incidence of ischemic stroke was plotted against age using the Kaplan–Meier method, and log-rank trend tests were used to examine differences across groups of 25-OH-vitD percentiles or concentrations.

Cox proportional hazard regression models with age as a time scale and use of left truncation (delayed entry) were used to examine the association between 25-OH-vitD concentrations and risk of ischemic or hemorrhagic stroke; individuals with ischemic/hemorrhagic stroke before study entry were excluded. Multivariate Cox regression analyses included adjustment for physical activity, hypertension, diabetes mellitus, atrial fibrillation, hormone replacement therapy, lipid-lowering therapy, and use of antihypertensive medication as dichotomous variables, smoking as current, former, or never, and body mass index, alcohol consumption, plasma total cholesterol, HDL cholesterol, and estimated glomerular filtration rate as continuous variables; month of blood sampling was also adjusted for in analyses of clinical categories. Data from examinations in 1981–1983, 1991–1994, and 2001–2003 were used as time-dependent covariates for multivariate adjustment. We tested the assumption of proportional hazards by plotting −ln (survival probability) against ln (analysis time), and by the use of Schoenfeld global test; we did not detect violations of this assumption.

The association between 25-OH-vitD percentiles and risk of ischemic stroke was examined overall, and in strata of the

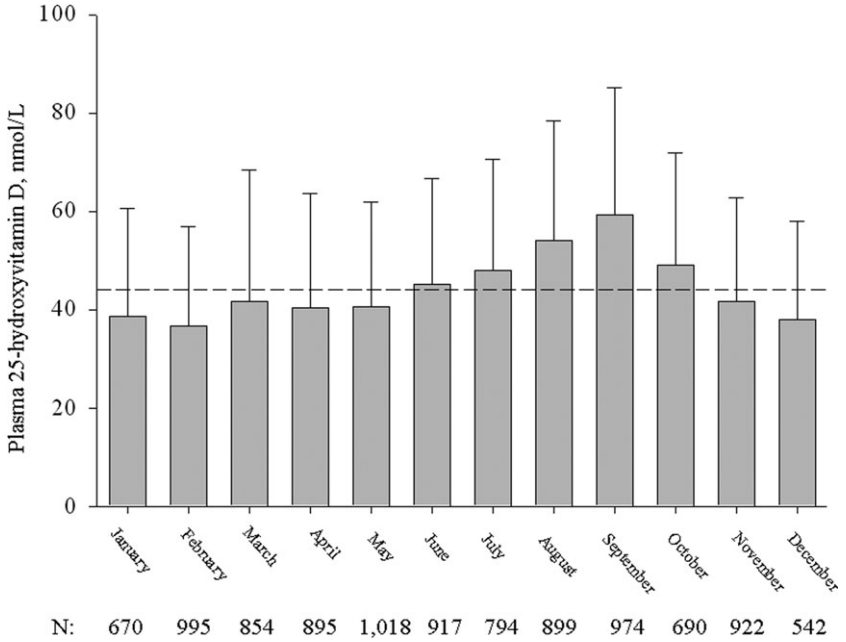

FIGURE 1: Mean 25-hydroxyvitamin D concentration in nanomoles per liter as a function of sampling month in 10,170 individuals from the Danish general population, the Copenhagen City Heart Study. Whiskers represent +1 standard deviation.

same covariates as in the multivariate adjustment above, but with the covariate stratified for excluded from the adjustment. Interaction between 25-OH-vitD concentrations and other covariates on ischemic stroke was evaluated by including 2-factor interaction terms, 1 at a time, in the multivariate Cox regression model.

Meta-Analysis

The meta-analysis was performed according to the MOOSE guidelines,[20] and to summarize results from present and previous studies, we identified relevant peer-reviewed population-based prospective, cross-sectional, and patient-based studies on the association between 25-OH-vitD concentrations and risk of ischemic stroke or stroke by an electronic search of published work in PubMED up until June 2012, using combinations of key words (Supplementary Data), and by scanning relevant reference lists. Both studies using ischemic stroke and the composite endpoint of stroke/fatal stroke were included. This search strategy identified 9 previous studies on ischemic stroke/stroke.[2–10]

To compare risk estimates from the different studies, we estimated the log hazard ratio, β, of the risk between the lowest versus the highest quartile group of 25-OH-vitD concentrations in the studies, assuming a log-linear association with the endpoint over the midrange of baseline values of 25-OH-vitD.[21] Standard errors were calculated from the approximation: $s^2 = 2.54 \times (1/n_1 + 1/n_2)$, where n_1 is the number of events and n_2 is the number of disease-free participants.

Meta-analyses were performed using random and fixed effect models overall and by study design. Publication bias was evaluated by funnel plots, Begg rank correlation test, and Egger regression test. Heterogeneity was evaluated by I^2 statistics.

Results

The Table shows baseline characteristics of all participants and by percentile categories of 25-OH-vitD concentrations. During follow-up, 1,256 and 164 ischemic and hemorrhagic stroke events occurred. Individuals with 25-OH-vitD concentrations between the 1st and 24th percentiles were more often physically inactive, current smokers, and high alcohol consumers and more often had hypertension or diabetes mellitus than individuals with 25-OH-vitD concentrations above the 24th percentile. Seasonal variation in 25-OH-vitD concentration is shown in Figure 1.

Ischemic Stroke

Cumulative incidence of ischemic stroke as a function of age increased stepwise with decreasing 25-OH-vitD concentrations (log-rank trend, $p = 3 \times 10^{-9}$; Fig 2).

Stepwise decreasing 25-OH-vitD concentrations were associated with stepwise increased risk of ischemic stroke both as a function of seasonally adjusted percentile categories and as a function of clinical categories (Fig 3; p for trend: 2×10^{-3} to 9×10^{-5}). Comparing individuals with 25-OH-vitD concentrations between the 1st and 4th percentiles (interquartile range, 7.5–12nmol/l) to individuals with 25-OH-vitD concentrations between the 50th and 100th percentiles (47–

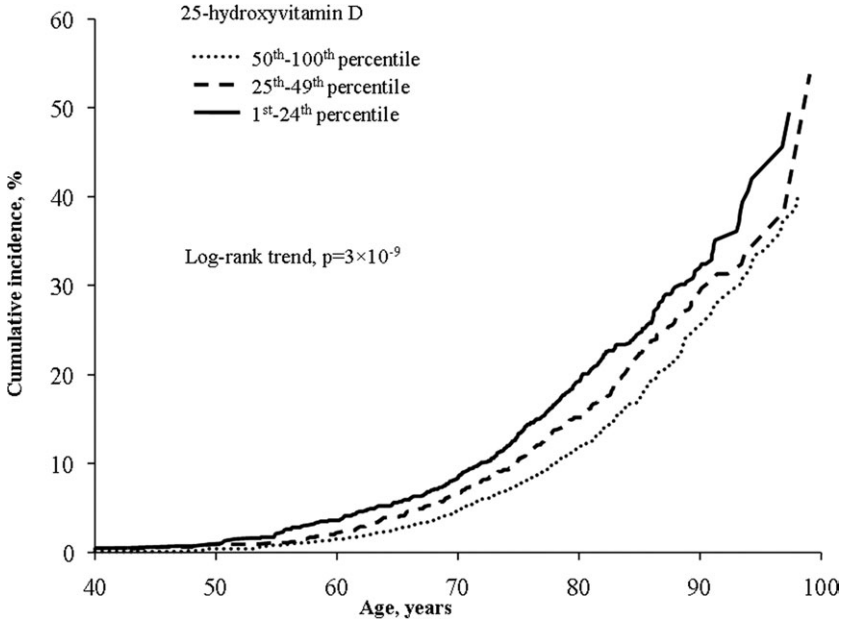

FIGURE 2: Cumulative incidence by the Kaplan–Meier method of ischemic stroke as a function of age in years and by 25-hydroxyvitamin D percentile categories of the 1st to 24th percentiles, 25th to 49th percentiles, and 50th to 100th percentiles. Log-rank trend test is for trend across percentile categories.

71nmol/l), age- and gender-adjusted hazard ratio for ischemic stroke was 1.88 (95% confidence interval, 1.46–2.41). The corresponding hazard ratio after multivariate adjustment for potential confounders, including body mass index, physical activity, smoking, alcohol consumption, plasma total cholesterol, HDL cholesterol, hypertension, diabetes mellitus, atrial fibrillation, hormone replacement therapy, lipid-lowering therapy, antihypertensive medication, and estimated glomerular filtration rate, was 1.82 (1.41–2.34). For clinical categories, comparing

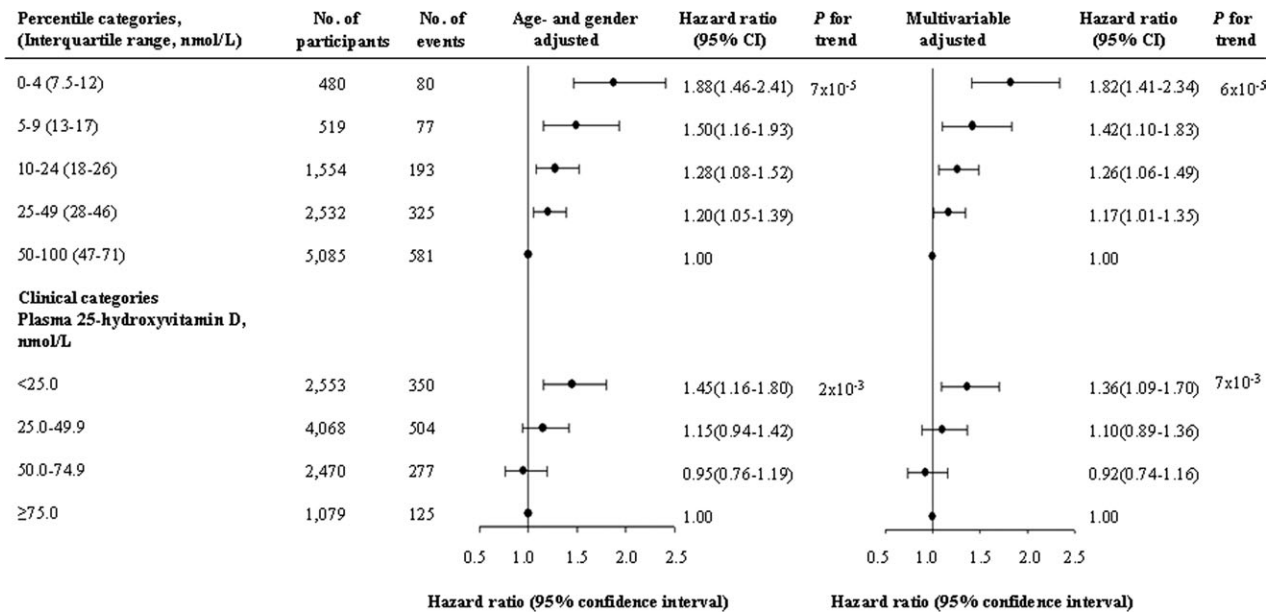

FIGURE 3: Risk of ischemic stroke as a function of 25-hydroxyvitamin D (25-OH-vitD) by seasonally adjusted percentile categories (top) and by clinical categories of absolute 25-OH-vitD concentration in nanomoles per liter (bottom). Multivariate adjustment was for age, gender, body mass index, physical activity level, smoking, alcohol consumption, total cholesterol level, high-density lipoprotein cholesterol level, hypertension, diabetes mellitus, atrial fibrillation, hormone replacement therapy, lipid-lowering therapy, use of antihypertensive medication, and estimated glomerular filtration rate; in analysis of clinical categories, month of blood sampling was also adjusted for. Black dots represent hazard ratio, and error bars the 95% confidence interval (CI). Probability values for trend are by Cuzick extension of a Wilcoxon rank sum test.

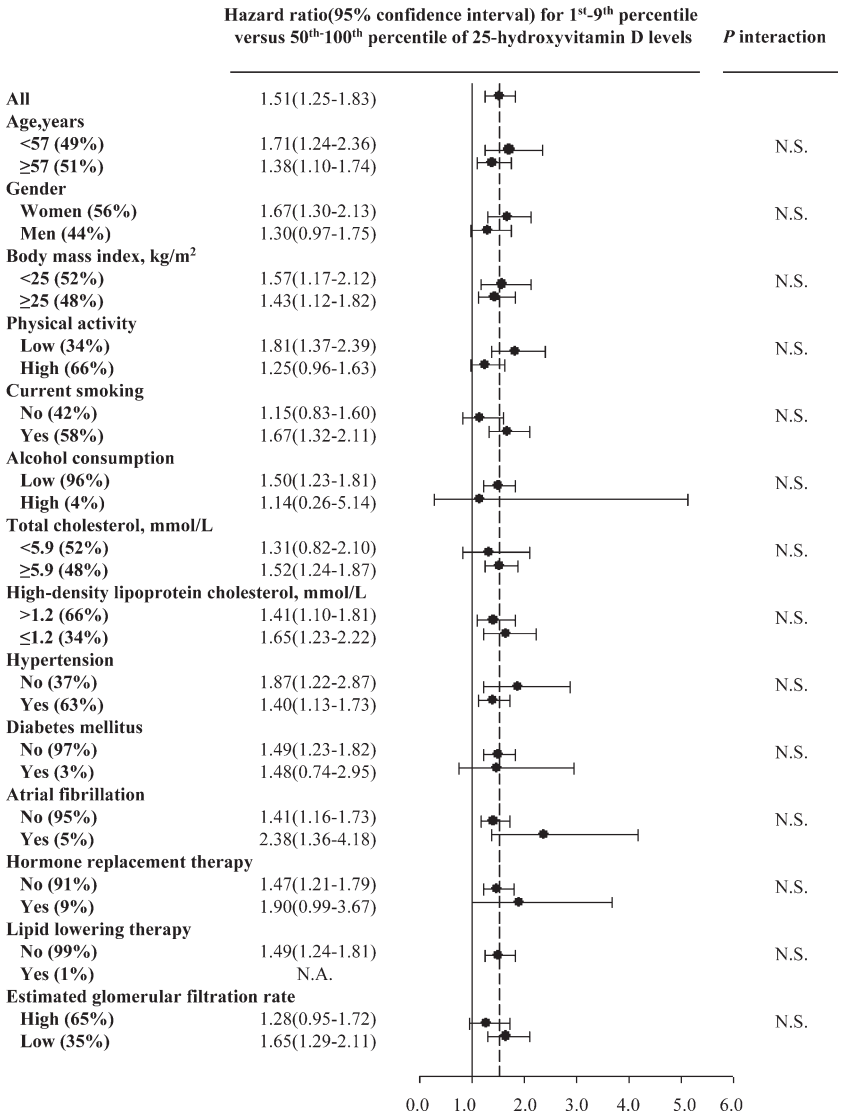

FIGURE 4: Risk of ischemic stroke for the 1st to 9th percentiles versus 50th to 100th percentiles of 25-hydroxyvitamin D concentrations. Black dots represent hazard ratio with 95% confidence interval, adjusted multivariably for age, gender, body mass index, physical activity level, current smoking, alcohol consumption, plasma total cholesterol, high-density lipoprotein cholesterol, hypertension, diabetes mellitus, atrial fibrillation, hormone replacement therapy, lipid-lowering therapy, and estimated glomerular filtration rate. Probability values are for test of interaction between 25-hydroxyvitamin D in percentiles and covariates on risk of ischemic stroke with Bonferroni correction for multiple comparisons; *p* values were multiplied by 14. N.A. = not applicable; N.S. = not significant as original *p* value multiplied by 14 was >1.0.

individuals with severe vitamin D deficiency to individuals with optimal vitamin D status, the age- and gender-adjusted hazard ratio was 1.45 (1.16–1.80), and after multivariate adjustment the corresponding hazard ratio was 1.36 (1.09–1.70; see Fig 3).

In stratified analyses, individuals in the 1st to 9th versus 50th to 100th percentiles of 25-OH-vitD concentrations had increased risk of ischemic stroke in all strata of other covariates (Fig 4). In accordance with this, tests of interaction between 25-OH-vitD percentiles and other covariates on risk of ischemic stroke showed no evidence for interaction.

Hemorrhagic Stroke

25-OH-vitD concentrations were not associated with risk of hemorrhagic stroke (Supplementary Fig 1).

Meta-Analysis

In a meta-analysis including 10 studies with a total of 58,384 participants and 2,644 ischemic stroke/stroke events, the odds ratios for ischemic stroke in the lowest versus highest quartile of 25-OH-vitD concentrations were 1.54 (95% confidence interval, 1.43–1.65; fixed effect) and 1.67 (1.43–1.96; random effect; Fig 5). The corresponding hazard ratios in prospective studies were

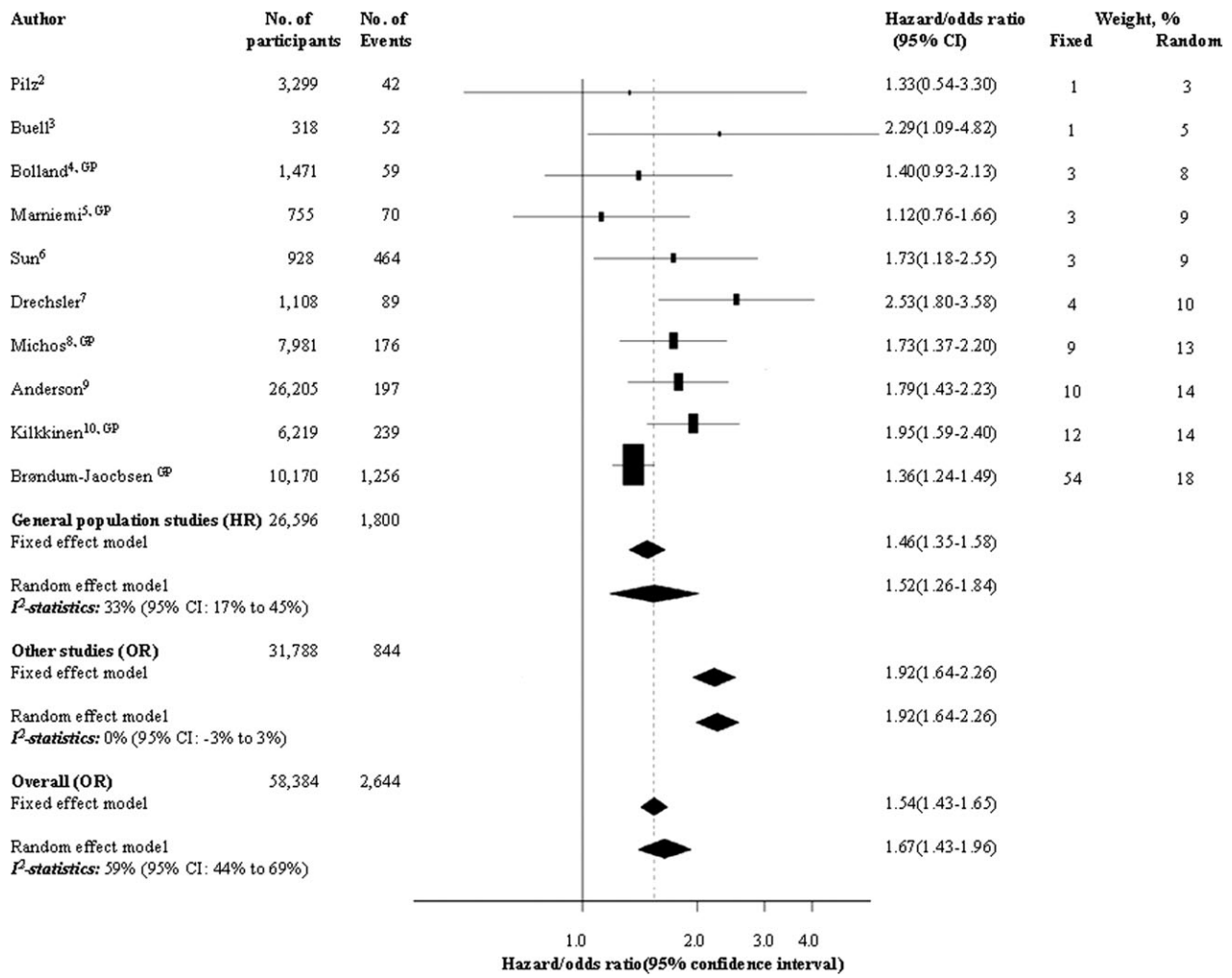

FIGURE 5: Meta-analysis of studies of 25-hydroxyvitamin D concentrations and risk of ischemic stroke. In the forest plot, the black box area is proportional to the fixed effect weight of each study, and horizontal lines correspond to the 95% confidence interval (CI). Black diamonds and the dashed vertical line represent the summary estimate, and the confidence interval for summary estimate corresponds to the width of the black diamond. The solid vertical line corresponds to a hazard ratio (HR) of 1.0, equivalent to no association. GP = general population study; HR = hazard ratio; OR = odds ratio.

1.46 (1.35–1.58) and 1.52 (1.26–1.84). There was a moderate degree of heterogeneity ($I^2 = 59\%$; 95% confidence interval, 44–69%; see Fig 5) and no evidence of publication bias (Supplementary Fig 2).

Discussion

In this large population-based prospective study, we observed a stepwise increase in risk of symptomatic ischemic stroke with stepwise decreasing 25-OH-vitD concentrations, as a function of both percentile and clinical categories. This finding was substantiated in a meta-analysis in which the present study contributed 48% of all ischemic stroke events.

The biological mechanism explaining the association between vitamin D status and ischemic stroke is most likely linked to the association between reduced 25-OH-vitD concentrations and traditional risk factors for ischemic stroke. Other than age, hypertension is the most important risk factor for ischemic stroke,[22] and experimental studies have shown that low 25-OH-vitD concentrations lead to increased renin gene transcription,[23] causing hypertension predisposing to ischemic stroke, but also atherosclerosis and atrial fibrillation, thus facilitating cerebral embolism. However, several other consequences of low 25-OH-vitD concentrations have been associated with increased risk of ischemic stroke such as:

1. Thrombosis. In vitamin D receptor knockout mice, enhanced thrombogenicity was observed by upregulation of thrombomodulin expression and downregulation of tissue factor expression.[24–26]
2. Atherosclerosis in the carotid arteries. Reduced 25-OH-vitD concentrations have been associated with

carotid intima–media thickness, although results are conflicting.[27,28]
3. Inflammation. This is considered a risk factor for ischemic stroke, and reduced 25-OH-vitD is associated with overall increased inflammatory activity.[29]

In addition to ischemic stroke, low 25-OH-vitD concentrations have also been associated with increased risk of ischemic heart disease,[30] peripheral vascular disease,[31] and overall cardiovascular mortality.[32] Moreover, there is an ongoing debate on a potential dual role of vitamin D on vascular calcification,[33] and epidemiological studies have reported increased risk of cardiovascular disease in individuals with both low and high 25-OH-vitD concentrations.[34]

Although slightly attenuated, our results are in agreement with the overall result from the present and a parallel meta-analysis[6] not including the present study. In some of the included studies, diagnoses of ischemic stroke/stroke relied solely on information from national death registries and thus did not meet the same validation criteria as previously described in the present study.[12,13,35]

The robustness of the association between reduced 25-OH-vitD concentrations and increased risk of ischemic stroke is demonstrated by our observation of stepwise increases in risk both across 5 percentile categories and across clinical categories, and by the finding that adjustment for several known confounders only attenuated the risk slightly. However, from the present data we cannot exclude the possibility of confounding by unknown sources or reverse causation, and to establish causality either randomized controlled trials like the ongoing VITAL study[36] or Mendelian randomization studies are needed.

Strengths of our study include the large prospective sample from a homogenous general population recruited from a limited geographic area with detailed information on several potential confounders, a high participation rate, a long follow-up time with no loss to follow-up, a high number of events, and the use of extensively validated diagnoses of ischemic and hemorrhagic stroke. Because participants were enrolled and blood samples drawn during 1981–1983 when focus on vitamin D supplementation or dietary intake (including fortified products) was very limited, our data are for practical purposes free of confounding by these factors.

Potential limitations include that some degradation of 25-OH-vitD may have occurred during storage at −20°C; however, such degradation is unlikely to have affected the result of the present study to a large extent for 2 reasons. First, 25-OH-vitD has been observed to be minimally degradable during storage at −20°C and through multiple freeze–thaw-cycles.[37,38] Second, the well-preserved seasonal variation observed in our study suggests that degradation is limited. Another potential limitation is selection bias, which is also unlikely, as we randomly selected participants from the general population. Additionally, although our validation of ischemic and hemorrhagic stroke was thorough, we cannot completely exclude some misclassification of diagnoses in the present study, and we cannot exclude that a fraction of participants may have had silent or unregistered ischemic strokes.[39] Importantly, however, such misclassification would bias the result toward the null hypothesis and therefore cannot explain the present findings. Unavailable data on potential confounding factors, including skin color, use of vitamin supplements, serum parathyroid hormone, and calcium concentrations, represent yet another limitation. Regarding reverse causation/confounding, it is well known from epidemiological studies that low 25-OH-vitD concentrations are seen in individuals with low physical activity, in smokers, and in obese individuals, all of which are risk factors for ischemic stroke, and therefore low 25-OH-vitD concentrations may merely be a proxy for these risk factor. However, we have adjusted for these factors in the multivariate adjusted Cox regression model. For hemorrhagic stroke, we had less statistical power than for ischemic stroke (164 vs 1,256 events), and therefore we cannot completely exclude that the negative finding for hemorrhagic stroke could be caused by insufficient statistical power. Also, in white but not in black Americans, optimal vitamin D status has been shown to attenuate the age-associated increase in systolic blood pressure.[40] Finally, as 25-OH-vitD concentrations differ between individuals with different skin color and depend on the amount of sun exposure, our results may not necessarily apply to all populations, but would certainly be applicable to populations with similar skin color and living in countries with similar sun exposure as in Northern Europe.

In conclusion, in this large population-based prospective study, we observed a stepwise increase in risk of symptomatic ischemic stroke with stepwise decreasing 25-OH-vitD concentrations. This finding was substantiated in a meta-analysis. To establish causality, either Mendelian randomization studies or conventional randomized clinical intervention trials are needed. Before such evidence is available, it should be recognized that vitamin D is important for musculoskeletal health, and that poststroke patients are particularly prone to develop

musculoskeletal diseases for which vitamin D is an effective treatment.

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Authorship

B.G.N. initiated the study, which was designed in detail by P.B.-J., B.G.N., and M.B. All authors had access to all data. P.S. collected raw data. Database handling and statistical analyses were by P.B.-J., B.G.N., and M.B. P.B.-J., B.G.N., and M.B. contributed to analyses and interpretation of data. P.B.-J. wrote the first draft of the paper, which was revised and finally accepted by the other 3 authors.

Potential Conflicts of Interest

Nothing to report.

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