25-Hydroxyvitamin D Levels and the Risk of Stroke
A Prospective Study and Meta-analysis
Qi Sun, MD, ScD; An Pan, PhD; Frank B. Hu, MD, PhD; JoAnn E. Manson, MD, DrPH; Kathryn M. Rexrode, MD, MPH

Background and Purpose—Despite evidence suggesting that vitamin D deficiency may lead to elevated cardiovascular disease risk, results regarding the association of 25-hydroxyvitamin D (25(OH)D) levels with stroke risk are inconclusive. We aimed to examine this association in a prospective study in women and to summarize all existing data in a meta-analysis.

Methods—We measured 25(OH)D levels among 464 women who developed ischemic stroke and an equal number of control subjects who were free of stroke through 2006 in the Nurses’ Health Study (NHS). We searched MEDLINE and EMBASE for articles published through March 2011 that prospectively evaluated 25(OH)D levels in relation to stroke.

Results—After multivariable adjustment for lifestyle and dietary covariates, lower 25(OH)D levels were associated with an elevated risk of ischemic stroke in the NHS: the OR (95% CI) comparing women in the lowest versus highest tertiles was 1.49 (1.01–2.18; \( P_{\text{trend}} = 0.04 \)). We found 6 other prospective studies that examined 25(OH)D in relation to stroke outcomes. After pooling our results with these prospective studies that included 1214 stroke cases in total, low 25(OH)D levels were associated with increased risk of developing stroke outcomes in comparison to high levels: the pooled relative risk (95% CI) was 1.52 (1.20–1.85; \( F = 0.0\% \), \( P_{\text{heterogeneity}} = 0.63 \)). In 2 studies that explicitly examined ischemic stroke, this association was 1.59 (1.07–2.12; \( I^2 = 0.0\% \), \( P_{\text{heterogeneity}} = 0.80 \)).

Conclusions—These data provide evidence that low vitamin D levels are modestly associated with risk of stroke. Maintaining adequate vitamin D status may lower the risk of stroke in women. (Stroke. 2012;43:00-00.)

Key Words: meta-analysis ■ stroke ■ vitamin D

Hypertension and diabetes are among the leading risk factors for stroke and targets for stroke prevention.1 Interestingly, human observational studies strongly suggested beneficial effects of vitamin D, a hormone that primarily regulates calcium metabolism, on lowering the risk of both conditions.2–4 In addition, mechanistic studies have provided mounting evidence supporting direct vascular effects of vitamin D that may lead to a lower risk of stroke.5–6 Despite the basic science research evidence, epidemiological studies examining vitamin D status, as measured by 25-hydroxyvitamin D (25(OH)D) levels in blood, in relation to risk of stroke morbidity or mortality have been inconsistent. Some,7–9 but not all,10–12 of these studies documented significant, inverse associations between 25(OH)D levels and stroke risk. The inconsistency of the results may be partially explained by insufficient statistical power, because most previous studies only accumulated a small number of stroke cases. Therefore, we conducted the largest prospective study, thus far, to examine the relationship between plasma 25(OH)D levels and risk of ischemic stroke among women who were initially free of stroke in the Nurses’ Health Study (NHS). We also summarized the existing evidence through a meta-analysis to shed light on this important association.

Methods

Study Populations
The NHS cohort consists of 121,700 female registered nurses aged 30 to 55 years who were residing in 11 US states and enrolled in 1976 through responding to a mailed questionnaire on their medical history and lifestyle practices. In 1989 to 1990, blood samples were collected from 32,826 participants. Among these participants, we prospectively identified and confirmed 483 incident ischemic stroke cases from the date of blood draw through June 2006. We used a risk-set sampling scheme to randomly select 1 control for each case from the rest of the population who remained free of stroke when the case occurred; the probability of being selected as a control subject is proportional to the length of follow-up. We further matched cases

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and control subjects for age at blood draw (± 1 year), date of blood draw (±3 months), menopausal status (yes, no), use of postmeno-
apausal hormone (current or nonuser), race (white or other races), and smoking status (past, current, or nonsmoker). After excluding 19 case–control pairs with missing 25(OH)D data, 464 pairs were available for analysis.

The study protocol was approved by the Institutional Review
Board of the Brigham and Women’s Hospital and the Human
Subjects Committee Review Board of Harvard School of Public
Health.

Ascertainment of Ischemic Stroke
In the NHS cohort, we requested permission of access to medical
records of participants who reported having a stroke diagnosis in
follow-up questionnaires. Study physicians, who were blinded as to
the exposure status of these participants, reviewed medical records
including results of CT or MRI. An ischemic nonfatal stroke
diagnosis was confirmed if the medical records demonstrated a
neurological deficit with sudden or rapid onset that persisted for ≥24
hours or until death in accordance with the criteria of the National
Stroke Index.9-14 With an underlying etiology of thromboembolic
mechanism. Fatal stroke cases were identified from reports
of next of kin or postal authorities or by searching the National Death
Index if stroke was indicated as the cause of death in autopsy reports,
hospital records, or death certificates. Fatal ischemic stroke cases
were confirmed by reviewing medical or autopsy records following
the same diagnostic criteria as described previously.

Assessment of Exposure and Covariates
Plasma samples of each case–control pair were handled identically
and analyzed in the same run by the same technicians in a random
sequence under identical conditions. Plasma 25(OH)D levels were
measured using the LIAISON 25 OH Vitamin D TOTAL Assay
(DiaSorin, Stillwater, MN), which is a direct competitive chemilu-
minesence immunoassay performed on the DiaSorin LIAISON
automated analyzer. Quality control samples were dispensed
throughout each analytic run. Based on the measurements of these
case control samples, the average intra-assay coefficient of variation
was 13.9% for 25(OH)D. Assessments of covariates were described in
the online-only Data Supplement (http://stroke.ahajournals.org).

Statistical Methods
We categorized the cases and control subjects into tertiles according
to the distribution of the 25(OH)D levels among the control subjects.
Conditional logistic regressions, in which matching design is taken
into account, were used to examine the association between 25(OH)D and stroke risk. In the multivariable analysis, we controlled
for covariates that may confound the association of interest, includ-
ing body mass index, physical activity, and dietary factors. Probab-
ility values for linear trend were calculated by entering an ordinal
score based on the median value in each tertile of 25(OH)D into the
models. In a secondary analysis, we calculated the season-adjusted
residuals of 25(OH)D levels by regressing this biomarker on the
month at blood draw in a linear regression model. We used the residuals
that are statistically independent of season as exposures of interest and repeated the analysis.

All probability values were 2-sided, and 95% CIs were calculated
for odds ratios (ORs) or relative risks (RRs). Data were analyzed with
the Statistical Analysis Systems software package, Version 9.2
(SAS Institute, Inc, Cary, NC).

Meta-Analysis
We identified and included 6 studies7-12 that explicitly evaluated
25(OH)D levels in relation to incident stroke or stroke mortality
(Figure 1). Study selection and data extraction were described in
the online-only Data Supplement. Meta-analyses were performed by
using Stata 11.0 (Stata Corp, College Station, TX). We fitted a
fixed-effects model to derive summary estimates that were
based on the logarithms of RRs and corresponding SEs in each
individual study. Heterogeneity among the results of these studies
was evaluated using the Cochran Q test and I² statistic. To detect
any nonlinear dose–response relationship, we first used a re-
stricted cubic spline regression model (Stata RC_SPLINE com-
mand) with 3 knots to create spline variables of 25(OH)D levels.
We then fitted 2 log-linear dose–response regression models
(Stata GLST command); in the first model, we only included a
continuous 25(OH)D variable, and in the second model, we
included both linear and spline terms of 25(OH)D levels. Lastly,
we used the likelihood ratio test to examine the significance of
any nonlinearity by comparing the model with the linear term
only with the model with both the linear and the cubic spline
terms. For this dose–response analysis, we requested data on
levels of 25(OH)D and person-time (if Cox regression was used)
for each category for 6 studies.5-12 Five studies7,9-12 responded to
our request, and provided these data. For 1 study that did not
respond to our request,8 we multiplied number of subjects by
average follow-up time to estimate the person-years. Begg funnel
plots and Egger tests were used to assess potential publication bias.15,16

Results
The Nurses’ Health Study
Table 1 shows the baseline characteristics of cases and
control subjects. The cases and control subjects had similar
baseline levels of 25(OH)D. Among control subjects, 41.6%
would be considered vitamin D-deficient with 25(OH)D levels
≤50 nmol/L17 compared with 45.3% of cases. We also
examined the association of 25(OH)D with age, body mass
index, physical activity, systolic blood pressure, dietary
vitamin D intake, race, and season of blood draw among
control subjects. As expected, 25(OH)D levels were signifi-
cantly (P<0.05) correlated with body mass index (partial
Pearson correlation coefficient [r] = −0.23), physical activity
(r = 0.20), and dietary vitamin D intake (r = 0.19). In addition,
white participants had higher 25(OH)D levels than blacks
(mean 25(OH)D levels were 56.9 versus 44.7 nmol/L, respec-
tively), and 25(OH)D levels were higher for women living in
the southern states (mean, 59.7 nmol/L) than those who lived

![Figure 1](http://stroke.ahajournals.org)
Table 1. Baseline Characteristics of Ischemic Stroke Cases and Control Subjects in the Nurses’ Health Study

<table>
<thead>
<tr>
<th>Characteristics*</th>
<th>Cases (n=464)</th>
<th>Control Subjects (n=464)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demography and lifestyle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y‡</td>
<td>60.8±5.9</td>
<td>60.8±6.0</td>
<td>0.93</td>
</tr>
<tr>
<td>European ancestry, %‡</td>
<td>97.6</td>
<td>97.6</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.0±5.1</td>
<td>25.4±4.8</td>
<td>0.09</td>
</tr>
<tr>
<td>Physical activity, MET-hr/wk</td>
<td>15.1±19.6</td>
<td>16.2±18.4</td>
<td>0.41</td>
</tr>
<tr>
<td>Smoking status, %‡§</td>
<td>41.9</td>
<td>41.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Never smoked</td>
<td>41.9</td>
<td>41.6</td>
<td></td>
</tr>
<tr>
<td>Past smoker</td>
<td>40.4</td>
<td>42.0</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>17.7</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>Menopausal status, %‡§</td>
<td>&gt;0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopause</td>
<td>6.3</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>Postmenopause, current hormone users</td>
<td>42.2</td>
<td>42.0</td>
<td></td>
</tr>
<tr>
<td>Postmenopause, not current hormone users</td>
<td>46.8</td>
<td>47.2</td>
<td></td>
</tr>
<tr>
<td>Postmenopause, hormone use status unknown</td>
<td>4.7</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>Aspirin use, %§</td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>&lt;1 tablet/wk</td>
<td>39.6</td>
<td>30.2</td>
<td></td>
</tr>
<tr>
<td>1–2 tablets/wk</td>
<td>25.3</td>
<td>30.4</td>
<td></td>
</tr>
<tr>
<td>3–6 tablets/wk</td>
<td>11.6</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>7–14 tablets/wk</td>
<td>17.6</td>
<td>16.8</td>
<td></td>
</tr>
<tr>
<td>15+ tablets/wk</td>
<td>6.0</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Use of multivitamin, %</td>
<td>37.9</td>
<td>34.3</td>
<td>0.25</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>47.8</td>
<td>34.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of high cholesterol, %</td>
<td>47.6</td>
<td>46.3</td>
<td>0.69</td>
</tr>
<tr>
<td>History of heart disease, %</td>
<td>15.3</td>
<td>14.0</td>
<td>0.58</td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>12.9</td>
<td>6.3</td>
<td>0.0005</td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D, IU/d</td>
<td>353.8±208.7</td>
<td>347.1±211.0</td>
<td>0.63</td>
</tr>
<tr>
<td>Calcium, mg/d</td>
<td>1021.0±414.1</td>
<td>1042.3±378.0</td>
<td>0.41</td>
</tr>
<tr>
<td>Glycemic load</td>
<td>101.3±16.5</td>
<td>100.9±16.4</td>
<td>0.73</td>
</tr>
<tr>
<td>Polysaturated to saturated fat ratio</td>
<td>0.57±0.15</td>
<td>0.58±0.17</td>
<td>0.31</td>
</tr>
<tr>
<td>Trans fat, g/d</td>
<td>1.7±0.4</td>
<td>1.7±0.5</td>
<td>0.77</td>
</tr>
<tr>
<td>Alcohol, g/d</td>
<td>6.7±11.0</td>
<td>6.0±9.7</td>
<td>0.32</td>
</tr>
<tr>
<td>25(OH)D, nmol/L</td>
<td>55.0±25.5</td>
<td>56.8±22.7</td>
<td>0.24</td>
</tr>
</tbody>
</table>

MET-hr indicates metabolic equivalent task-hr; 25(OH)D, 25-hydroxyvitamin D.
*For continuous variables, values were expressed as mean±SD; for categorical variables, percent was used.
†P values were based on Student t test for continuous variables or Pearson χ² test for categorical variables.
‡Matching factors.
§Based on nonmissing data.

In conditional logistic regression accounting for matching factors, women in the lowest 25(OH)D tertile had a nonsignificantly higher risk of ischemic stroke (Table 2). After adjustment for body mass index and physical activity, the association was attenuated to 1.24 (0.89–1.74; P trend=0.20) comparing extreme tertiles. However, when we adjusted for other lifestyle risk factors and history of chronic conditions, the association was strengthened to some extent: the OR for stroke (95% CI) was 1.42 (0.99–2.04; P trend=0.06) for the women in the lowest tertile compared with those in the highest. Finally, after additional adjustment for dietary risk factors, including use of multivitamins, intake of calcium and trans fat, polyunsaturated fat to saturated fat ratio, and glycemic load, the association was further strengthened with an OR (95% CI) of 1.49 (1.01–2.60; P trend=0.03) comparing extreme tertiles. Further adjustment of estimated glomerular filtration rate or C-reactive protein did not materially change the association; the ORs (95% CI) were 1.49 (1.02–2.19; P trend=0.04) and 1.46 (1.00–2.14; P trend=0.05), respectively.

We further conducted several secondary analyses to explore the association of 25(OH)D in certain subgroups or with certain stroke subtypes. First, we examined interaction between plasma 25(OH)D levels and risk factors of stroke or factors that influence 25(OH)D levels, including age, body mass index, physical activity, use of hormone therapy, season of blood draw, latitude of participants’ residence, estimated glomerular filtration rate, and C-reactive protein (online-only Data Supplement Table I). We found a marginally significant interaction by current use of hormone therapy: comparing low versus high tertiles of 25(OH)D, the OR (95% CI) was 1.06 (0.40–2.80) for nonusers, whereas this OR (95% CI) was 1.21 (1.17–4.00; P trend=0.04) among users, the majority (>75%) of whom used oral hormone therapy. Second, we examined the association of 25(OH)D with several subtypes of ischemic stroke: large artery infarction (n=142), lacunar infarction (n=173), or other types of ischemic infarction (n=149). The ORs (95% CI) comparing extreme tertiles of 25(OH)D levels were 1.78 (0.77–4.10; P trend=0.20) for large artery infarction, 2.47 (1.15–5.32; P trend=0.02) for lacunar infarction, and 1.21 (0.95–5.17; P trend=0.05) for all other types of ischemic stroke. Lastly, we used cut points that were used to define vitamin D deficiency/adequacy18 to categorize the study participants and repeated the analysis. Although a similar trend was observed, the association was somewhat attenuated: in comparison to women whose 25(OH)D levels
Table 2. OR (95% CI) of Ischemic Stroke for 25(OH)D Levels at Baseline in the Nurses’ Health Study

<table>
<thead>
<tr>
<th>Plasma Levels of 25(OH)D, nmol/L, at Baseline</th>
<th>Tertile 3 (Highest)</th>
<th>Tertile 2</th>
<th>Tertile 1 (Lowest)</th>
<th>( P_{\text{trend}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range)</td>
<td>77.6 (65.5–264.3)</td>
<td>55.0 (45.8–65.4)</td>
<td>35.0 (9.2–45.7)</td>
<td>. . .</td>
</tr>
<tr>
<td>Case/control, no.</td>
<td>133/156</td>
<td>160/154</td>
<td>171/154</td>
<td></td>
</tr>
<tr>
<td>Model 1*</td>
<td>1.0</td>
<td>1.20 (0.88–1.64)</td>
<td>1.31 (0.95–1.82)</td>
<td>0.10</td>
</tr>
<tr>
<td>Model 2†</td>
<td>1.0</td>
<td>1.16 (0.84–1.59)</td>
<td>1.24 (0.89–1.74)</td>
<td>0.20</td>
</tr>
<tr>
<td>Model 3‡</td>
<td>1.0</td>
<td>1.25 (0.89–1.76)</td>
<td>1.42 (0.99–2.04)</td>
<td>0.06</td>
</tr>
<tr>
<td>Model 4¶</td>
<td>1.0</td>
<td>1.26 (0.89–1.79)</td>
<td>1.49 (1.01–2.18)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

25(OH)D indicates 25-hydroxyvitamin D.
*Model 1 was conditioned on all matching factors, ie, age at blood draw (y), menopausal status (yes, no), use of postmenopausal hormone (current or nonuser), white (yes, no), and smoking status (past, current, or nonsmoker).
†Based on Model 1, Model 2 was further adjusted for body mass index (kg/m²) and physical activity (metabolic equivalent task-hr/wk, in tertiles).
‡Based on Model 2, Model 3 was further adjusted for use of aspirin (<1 tablet/wk, 1–2 tablets/wk, 3–6 tablets/wk, 7–14 tablets/wk, and 15+ tablets/wk), hypertension (yes, no), high cholesterol (yes, no), history of heart disease or diabetes (yes, no), and alcohol consumption (g/d, in tertiles).
¶Based on Model 3, Model 4 was further adjusted for use of multivitamin (yes, no) and intakes of calcium and trans fat, polyunsaturated fat to saturated fat ratio, and glycemic load (all in tertiles).

≥75 nmol/L, the ORs (95% CI) were 0.96 (0.65–1.43) for levels in 50 to 74 nmol/L, 1.11 (0.72–1.70) for levels in 30 to 49 nmol/L, and 1.39 (0.80–2.42) for levels <30 nmol/L.

Meta-Analysis

The characteristics of the 6 other prospective cohort studies that evaluated 25(OH)D levels in relation to various stroke outcomes are shown in online-only Data Supplement Table II. Most studies included both men and women, except Bolland et al’s study11 that included women only. Serum 25(OH)D levels were measured in all studies, whereas study outcomes varied among these studies: 2 studies examined fatal strokes only and 4 other studies evaluated total strokes. Only Kilkkinen et al’s study8 examined ischemic stroke and hemorrhagic stroke separately. Cox proportional hazard regression was used to model the association of interest in all but Pilz et al’s studies,8–12 in which logistic regression was used to model the association. The number of stroke cases ranged from 42 to 293 in these studies. In total, including our study, there were 1214 stroke cases included in the current meta-analysis.

When all data were pooled together, the combined RR (95% CI) of stroke comparing low versus high vitamin D status was 1.52 (1.20–1.85; Figure 2). We did not observe evidence for heterogeneity of the results; the \( F \) statistics was 0.0% and the \( P_{\text{heterogeneity}} \) was 0.63. When we pooled the results from the NHS cohort with Kilkkinen et al’s results specifically for ischemic stroke, the pooled RR (95% CI) was 1.59 (1.07–2.12). Similarly, no evidence of heterogeneity was found between the results of these 2 studies. Of note, in this analysis for ischemic stroke, we used RR comparing ≥31 nmol/L versus >47 nmol/L in the NHS study to be consistent with the cut points used in the Kilkkinen et al study.8 The Begg funnel plot and Egger test did not suggest evidence of publication bias (online-only Data Supplement Figure I).

In the cubic spline regression model, we detected a potentially nonlinear dose–response relationship; the \( P \) for nonlinearity was 0.02. This dose–response relationship is shown in Figure 3. There was a clear threshold effect in that the risk of stroke did not start decreasing until the 25(OH)D levels exceeded certain levels of approximately 40 nmol/L.

We further conducted several sensitivity analyses to examine the robustness of our observations. First, when we used a random-effects model, we observed essentially the same results. Second, when we excluded Drechsler et al’s and Pilz et al’s studies that included patients on dialysis or patients referred to coronary angiography, respectively, the results did not change substantially; the pooled RR (95% CI) was 1.51 (1.18–1.83). Lastly, when we examined the dose–response relationship among prospective cohort studies that used Cox regression to model the association,8–12 a marginally significant probability value for nonlinearity of 0.06 was documented, probably because of low power for this stratified analysis.

Discussion

In this prospective study among women without a history of stroke, with multivariable adjustment for lifestyle and dietary factors, we documented a modest association between low plasma 25(OH)D levels and an increased risk of incident ischemic stroke. Associations appeared most robust for lacunar infarction. This observation was confirmed by a meta-analysis pooling our data with 6 other prospective studies that overall demonstrated the same inverse associations between blood 25(OH)D levels and various stroke outcomes. A potentially nonlinear dose–response relationship between 25(OH)D levels and stroke risk was further suggested by the current investigation.

Independent of the effects of vitamin D on calcium metabolism, emerging evidence suggests several mechanisms through which vitamin D modulates cardiovascular health.
For example, vitamin D receptor-null mice have significantly increased expression of renin and plasma angiotensin, whereas injection of 1,25-dihydroxyvitamin D to wild-type mice suppressed renin production, showing significant effects of vitamin D on this pathway.\textsuperscript{5} Independent of the tightly controlled circulating 1,25-dihydroxyvitamin D levels, intracellularly produced hormone may directly regulate the function and behaviors of vascular smooth muscle cells, endothelial cells, macrophages, and adipocytes.\textsuperscript{19–22} In addition, local production of 1,25-dihydroxyvitamin D appears to be regulated in part by levels of 25(OH)D,\textsuperscript{20} suggesting that the autocrine or paracrine effects of vitamin D may depend on the availability of 25(OH)D as well. Consistently, animal and human experiments provide data supporting the effects of vitamin D treatment/supplementation on increasing endothelium-dependent vascular relaxation,\textsuperscript{23,24} inhibiting vascular smooth muscle cell growth,\textsuperscript{25} improving insulin resistance and β-cell dysfunction,\textsuperscript{26,27} inhibiting production of inflam-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Pooled fixed-effects relative risk (95% CI) of stroke comparing high 25(OH)D levels with low 25(OH)D levels. (A) Total stroke; (B) ischemic stroke. Bars indicate 95% CIs and probability values are \textit{P} for heterogeneity. 25(OH)D indicates 25-hydroxyvitamin D.}
\end{figure}
matory cytokines, and regulating the reaction of monocytes to environmental stressors. These mechanisms may also likely underlie the stronger association observed among current users of hormone replacement therapy; the effects of low 25(OH)D status may be further exaggerated by the adverse effects of oral hormone therapy on inflammation, thromboembolism, and coagulation.

Despite the basic science evidence, only limited prospective data existed regarding the relationship between vitamin D status and risk of stroke. In addition, most of these studies did not document significant findings probably because most of these earlier studies used small sample sizes resulting in limited statistical power for detecting a significant association. However, when we pooled our findings with all previous prospective data, we found a significant association suggesting that low vitamin D status may lead to an increased risk of stroke. Moreover, we did not observe significant heterogeneity of individual study associations; 6 of 7 studies suggested an inverse association. With respect to the associations for subtypes of stroke, only Kilkkinen et al examined this association for ischemic stroke and hemorrhagic stroke separately. They found a significant inverse association between baseline 25(OH)D levels and risk of fatal ischemic stroke (n=175), although the inverse association for fatal hemorrhagic stroke (n=43) was not significant. When we categorized our participants according to the cut points of 25(OH)D levels that corresponded to those in this Finnish study, we observed similar associations for combined fatal and nonfatal ischemic stroke. Thus far, the current study was the only investigation that further examined 25(OH)D levels in relation to subtypes of ischemic stroke, and we found a stronger association for lacunar infarction. The effects of vitamin D on hypertension and diabetes may explain this stronger association because these diseases were believed to be major risk factors for lacunar stroke, although a recent meta-analysis suggested that these 2 conditions were equally associated with lacunar and nonlacunar strokes. Apparently, more studies are warranted to derive a solid estimate on the effects of vitamin D on subtypes of stroke.

Our investigation is subject to a few limitations. First, our study population consisted of registered nurses with mostly European ancestry. It is unknown whether our current findings can be generalized to other races that, in general, have lower 25(OH)D levels than whites. This restricted generalizability pertains to the meta-analysis results as well because the vast majority of participants in the contributing studies were also white. In addition, 25(OH)D deficiency (50 nmol/L) was relatively common (40%) and dietary calcium was high (>1000 mg/day) in our study population. These characteristics further restrict the generalizability of the results. Second, we only measured baseline 25(OH)D levels, and a single measure may not represent long-term levels. However, in a validation study among 71 NHS participants who provided 3 blood samples within 2 to 3 years, 25(OH)D levels at each time point were highly correlated, an intraclass correlation of 0.72 was found. In addition, in the current study among 102 control subjects who provided a second blood sample approximately 10 years after baseline blood draw, an intraclass correlation of 0.48 was observed. These data suggest that a single measure of blood 25(OH)D is a reasonable proxy for vitamin D exposure with correlations over time that are similar to those for cholesterol and blood pressure. Furthermore, measurement error in the 25(OH)D assay, although relatively modest (coefficient of variation=13.9%), may introduce additional random variation that likely attenuates the true association. Third, although the ability to adjust carefully for a wide array of lifestyle and dietary covariates in this study is a strength, residual confounding, in particular due to adiposity, cannot be fully excluded in this observational study. Uncontrolled and residual confounding in studies included in the meta-analysis cannot be excluded either. The results can be biased by...
confounding toward either direction. Fourth, although we did not find statistical heterogeneity of associations in our meta-analysis, these prospective studies are heterogeneous in several aspects such as various stroke outcomes (stroke mortality and total, ischemic, and hemorrhagic strokes), diverse methods for identifying and confirming stroke outcomes, different assays for 25(OH)D measurements, varying baseline health status of the study populations (general population versus patients with specific diseases or conditions), various covariates adjusted in each individual study, and dramatically different levels of 25(OH)D and comparison categories across studies. Large individual-level data are still needed to minimize this between-study heterogeneity and to model any dose–response relationship precisely. Likewise, detection of interactions between vitamin D and other stroke risk factors also requires such data. Fifth, because vitamin D deficiency is quite common among prevalent cerebrovascular patients and a history of stroke is a strong risk factor of recurrent stroke,1 previous investigations on total stroke or stroke mortality7,10–12 that did not exclude baseline patients with stroke may be subject to confounding bias, which can lead to erroneously strong associations. Lastly, some key factors involved in the biology of 25(OH)D, such as 1,25-dihydroxyvitamin D, parathyroid hormone, phosphate, and fibroblast growth factor 23 were not measured in the current analysis. Therefore, we were unable to examine the role of these molecules in the association between 25(OH)D and risk of ischemic stroke. In terms of strengths, the current study used incident ischemic stroke confirmed by medical records, and such homogeneity of outcome may help detect biologically meaningful associations. Other strengths of the current study include large sample size, long follow-up period, and rich data allowing comprehensive analysis.

Conclusions

We found a modest association between low 25(OH)D levels and risk of incident ischemic stroke among US women without a history of stroke, and this observation was consistent with pooled results based on all prior prospective studies. Further studies are warranted to explore this association among other races and for stroke subtypes. In particular, large-scale clinical trials are needed to shed light on the effects of achieving optimal vitamin D status in the primary prevention of stroke. Given that US populations have a high prevalence of both vitamin D insufficiency and stroke, solid evidence is especially needed to guide public health recommendations for lowering the burden of both conditions.

Acknowledgments

We are indebted to Drs Stefan Pilz, Heidi T. May, Jeffrey L. Anderson, Jukka Marniemi, Mark J. Bolland, and Christiane Drexhansler for their contributions to the meta-analysis. Dr Sun has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Sources of Funding

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Disclosures

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References


25-Hydroxyvitamin D Levels and the Risk of Stroke: A Prospective Study and Meta-analysis

Qi Sun, An Pan, Frank B. Hu, JoAnn E. Manson and Kathryn M. Rexrode

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Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2012/03/22/STROKEAHA.111.636910.DC1

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SUPPLEMENTAL MATERIAL

Assessment of covariates

We administered questionnaires at baseline and every two years thereafter to collect and update information on major lifestyle practices, history of chronic diseases, and other exposures, such as body weight, cigarette smoking, alcohol use, physical activity, use of multivitamin and aspirin, menopausal status, and post-menopausal hormone use in the Nurses’ Health Study (NHS) cohort. Body mass index (BMI) as weight in kilograms divided by the square of height in meters (kg/m^2) was calculated to assess overall adiposity. Since 1980, diet has been assessed using a semi-quantitative food frequency questionnaire (FFQ) every 2-4 years. Nutrient intake was calculated based on responses to the FFQ and the nutrient content of foods was derived from the Harvard Food Composition Database. The validity of the FFQs used in the NHS cohort has been extensively examined and demonstrated. In the current analysis, all lifestyle covariates were derived from the questionnaire administered in 1990 or the nearest available year prior to 1990. In addition, we calculated and used the average of nutrient intake assessed since 1980 through 1990 to minimize measurement error and to better represent long-term diet. We derived estimated glomerular filtration rate (eGFR) using a formula^1 that
\[
eGFR = 1.86 \times \text{Creatinine} \ (\text{mg/dL})^{-1.154} \times \text{Age(yr)}^{-0.203}
\]

Meta-analysis

Study selection

Two investigators (QS and AP) conducted independent literature search of the MEDLINE and EMBASE database for studies published through March 2011 that examined blood 25(OH)D levels in relation to stroke outcomes. The searching terms were ("stroke"[Mesh] OR "stroke"[All Fields] OR "cerebrovascular disorders"[Mesh]) AND ("vitamin D"[Mesh] OR "25-hydroxyvitamin D" OR “vitamin D status”) for MEDLINE search and (“stroke” AND “vitamin D”) for EMBASE search. Studies were eligible if they were prospective observational studies that evaluated 25(OH)D levels in plasma or serum in relation to stroke outcomes. Our initial search identified 546 unique publications that were potentially eligible to the meta-analysis. Two reviewers (QS and AP) independently reviewed all abstracts and retrieved full text articles if the abstracts were not excluded from further examination. Any discrepancies between the two reviewers were solved by discussions.

Data extraction

Two authors (QS and AP) independently extracted data from each study using a standardized extraction form. The information that we extracted included name of the first author, publication year, country of origin, characteristics of study participants (age and gender), study design, distribution of 25(OH)D levels, stroke outcomes, sample size, number of cases, estimates of the effect size (RR and 95% CI) in the most fully adjusted model, and covariates included in the fully adjusted model. In this meta-analysis, we used the RRs and 95% CIs comparing lowest vs. highest 25(OH)D categories. In Pilz et al’s original publication,^2 only RRs for each z score of 25(OH)D levels were provided. Per our request, Pilz et al calculated the RR (95% CI) comparing
lowest tertile vs. highest tertile of serum 25(OH)D levels. In addition, Anderson et al\textsuperscript{3} provided 95\% CIs for the RRs in their publication upon request.
**Supplementary Table 1.** Odds ratio* (95% CI) of ischemic stroke for tertiles of plasma 25(OH)D levels by various risk factors, the Nurses’ Health Study.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Plasma 25(OH)D levels (nmol/L)</th>
<th>P&lt;sub&gt;trend&lt;/sub&gt;</th>
<th>P&lt;sub&gt;interaction&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tertile 3 (highest)</td>
<td>Tertile 2</td>
<td>Tertile 1 (lowest)</td>
</tr>
<tr>
<td>Age at baseline (yrs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 65 (307 cases)</td>
<td>1.0</td>
<td>1.24 (0.79, 1.95)</td>
<td>1.84 (1.10, 3.09)</td>
</tr>
<tr>
<td>≥ 65 (157 cases)</td>
<td>1.0</td>
<td>1.13 (0.55, 2.34)</td>
<td>0.90 (0.45, 1.81)</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25 (227 cases)</td>
<td>1.0</td>
<td>1.02 (0.51, 2.03)</td>
<td>1.44 (0.70, 3.00)</td>
</tr>
<tr>
<td>≥ 25 (237 cases)</td>
<td>1.0</td>
<td>0.99 (0.41, 2.40)</td>
<td>1.22 (0.41, 3.65)</td>
</tr>
<tr>
<td>Physical activity (METs-hr/week)†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (242 cases)</td>
<td>1.0</td>
<td>1.74 (0.74, 4.08)</td>
<td>2.55 (1.01, 6.45)</td>
</tr>
<tr>
<td>High (222 cases)</td>
<td>1.0</td>
<td>1.82 (0.75, 4.43)</td>
<td>2.41 (0.90, 6.42)</td>
</tr>
<tr>
<td>History of hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (242 cases)</td>
<td>1.0</td>
<td>1.10 (0.57, 2.09)</td>
<td>1.17 (0.61, 2.26)</td>
</tr>
<tr>
<td>Yes (222 cases)</td>
<td>1.0</td>
<td>1.44 (0.43, 4.79)</td>
<td>2.54 (0.65, 9.82)</td>
</tr>
<tr>
<td>Current user of hormone therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No (268 cases)</td>
<td>1.0</td>
<td>1.14 (0.70, 1.84)</td>
<td>1.06 (0.62, 1.84)</td>
</tr>
<tr>
<td>Yes (196 cases)</td>
<td>1.0</td>
<td>1.69 (0.92, 3.08)</td>
<td>2.16 (1.17, 4.00)</td>
</tr>
<tr>
<td>Season of blood draw</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer‡ (146 cases)</td>
<td>1.0</td>
<td>0.85 (0.25, 2.95)</td>
<td>2.62 (0.40, 16.99)</td>
</tr>
<tr>
<td>Other seasons (318 cases)</td>
<td>1.0</td>
<td>1.85 (1.10, 3.12)</td>
<td>1.88 (1.11, 3.17)</td>
</tr>
<tr>
<td>Residence at blood collection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southern states# (121 cases)</td>
<td>1.0</td>
<td>0.38 (0.05, 2.73)</td>
<td>0.89 (0.15, 5.35)</td>
</tr>
<tr>
<td>Northern states (343 cases)</td>
<td>1.0</td>
<td>0.94 (0.58, 1.53)</td>
<td>1.53 (0.86, 2.73)</td>
</tr>
</tbody>
</table>

* Estimated glomerular filtration rate†
<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (237 cases)</td>
<td>1.0</td>
<td>2.55 (1.14, 5.74)</td>
<td>2.21 (0.86, 5.67)</td>
<td>0.07</td>
<td>0.96</td>
</tr>
<tr>
<td>High (227 cases)</td>
<td>1.0</td>
<td>0.64 (0.25, 1.64)</td>
<td>0.78 (0.30, 2.04)</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>

C-reactive protein†
|                  |       |          |          |       |       |
| Low (214 cases)  | 1.0   | 0.84 (0.40, 1.77) | 2.16 (0.87, 5.33) | 0.18  | 0.21  |
| High (250 cases) | 1.0   | 1.81 (0.74, 4.39) | 2.50 (0.94, 6.70) | 0.07  |       |

*Odds ratios were adjusted for the same set of covariates as adjusted in Model 4, Table 2, with the exception of the stratifying variable per se in each model. To control for residual confounding by continuous stratifying variables, we controlled for a linear term of these variables in their stratified analyses.
†Cut-points for high vs. low were median values.
‡June, July, and August.
§Southern states include California, Florida, and Texas; Northern states include Connecticut, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, and Pennsylvania.
#Because of the small sample size for participants living in Southern states, we adjusted for continuous covariates whenever possible to avoid non-convergence of statistical models.
<table>
<thead>
<tr>
<th>Author</th>
<th>Study participants</th>
<th>Study design</th>
<th>Follow-up period (yr)</th>
<th>Exposure and assay method</th>
<th>Outcome and ascertainment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marniemi et al, 2005&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Location: Finland Total n: 755 Female: 52.2% Age: 65-99 yr</td>
<td>Prospective cohort study</td>
<td>≤10</td>
<td>Serum 25(OH)D levels (mean±SD): Cases: 29.6±17.9 nmol/L Controls: 31.3±19.4 nmol/L Assay: radioimmunoassay (DiaSorin, Stillwater, Minnesota, US).</td>
<td>Total stroke, n=70; identified from the Finnish National Register of Causes of Death and the Finnish National Hospital Discharge Register and confirmed by original hospital and health center patient records.</td>
</tr>
<tr>
<td>Pilz et al, 2008&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Location: German, LURIC Study Total n: 2589 Female: 31.7% Age (median): 61.7 yr for survivors and 69.3 yr for fatal stroke patients</td>
<td>Prospective cohort study</td>
<td>Median: 7.75</td>
<td>Serum 25(OH)D levels (median[interquartile range]): Stroke patients: 27.5 (20.7–44.9) nmol/L Survivors: 41.7 (28.0–59.7) nmol/L Assay: liquid chromatography–tandem mass spectrometry.</td>
<td>Fatal stroke, n=42; identified through death certificates.</td>
</tr>
<tr>
<td>Kilkkinen et al, 2009&lt;sup&gt;5&lt;/sup&gt;</td>
<td>Location: Finland, Mini-Finland Health Survey Total n: 6219 Female: 54.7% Age: ≥30 yr old, median was 49.4 yr</td>
<td>Prospective cohort study</td>
<td>26-28, median: 27.1</td>
<td>Serum 25(OH)D levels (mean±SD): Men: 45.7±20.3 nmol/L Women: 41.5±18.9 nmol/L Assay: radioimmunoassay (DiaSorin, Stillwater, Minnesota, US).</td>
<td>Fatal stroke, n=293; identified through linkage with Statistics Finland.</td>
</tr>
<tr>
<td>Bolland et al, 2010&lt;sup&gt;6&lt;/sup&gt;</td>
<td>Location: New Zealand Total n: 1471 Female: 100% Age (mean): 74.1 yr</td>
<td>Prospective cohort study</td>
<td>≤5</td>
<td>Serum 25(OH)D levels (mean±SD): 50.9±19.1 nmol/L Assay: radioimmunoassay (DiaSorin, Stillwater, Minnesota, US)</td>
<td>Total stroke, n=59; self-reports of stroke cases were confirmed via medical records reviewed by a neurologist.</td>
</tr>
<tr>
<td>Drechsler</td>
<td>Location: German,</td>
<td>Prospective</td>
<td>Median: 4</td>
<td>Serum 25(OH)D levels</td>
<td>Total stroke, n=89; stroke</td>
</tr>
<tr>
<td>Study</td>
<td>Location</td>
<td>Total n</td>
<td>Female</td>
<td>Age (mean)</td>
<td>Study Design</td>
</tr>
<tr>
<td>----------------</td>
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</tr>
<tr>
<td>et al, 2010</td>
<td>US</td>
<td>1108</td>
<td>45.7%</td>
<td>18-80 yr</td>
<td>cohort study</td>
</tr>
<tr>
<td>Anderson et al, 2010</td>
<td>US</td>
<td>26025 (≥50 yr)</td>
<td>75%</td>
<td>66 yr</td>
<td>Prospective cohort study</td>
</tr>
<tr>
<td>Author</td>
<td>Comparison categories</td>
<td>Relative risk (95% CI)</td>
<td>Comparison groups in the current meta-analysis</td>
<td>Transformed relative risk† (95% CI)</td>
<td>Covariates in the fully-adjusted model</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------------------------------------------------------------------------------</td>
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<td>--------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Marniemi et al, 2005⁴</td>
<td>Highest vs lowest tertiles; Median (interquartile range): tertile 1, 13 (11-19); tertile 2, 26 (20-34); tertile 3, 47 (35-166).*</td>
<td>1.00 (0.51, 1.94)</td>
<td>Highest vs lowest tertiles</td>
<td>1.00 (0.52, 1.96)</td>
<td>Age, sex, smoking and functional capacity</td>
</tr>
<tr>
<td>Pilz et al, 2008²</td>
<td>Highest vs lowest tertiles; Median (interquartile range): tertile 1, 22.5 (17.8-27.5); tertile 2, 41.5 (36.5-47.3); tertile 3, 68.0 (59.5-81.8).*</td>
<td>1.94 (0.78, 4.80)*</td>
<td>Lowest vs highest tertiles</td>
<td>Untransformed</td>
<td>Age, sex, low-density and high-density lipoprotein cholesterol, active smokers, body mass index, C-reactive protein, glomerular filtration rate, arterial hypertension, diabetes mellitus, N-terminal pro-B-type natriuretic peptide, physical activity level, calcium, and parathyroid hormone values</td>
</tr>
<tr>
<td>Kilkkinen et al, 2009⁵</td>
<td>Quintile 5 vs quintile 1</td>
<td>0.48 (0.31, 0.75)</td>
<td>Lowest vs highest quintiles</td>
<td>2.08 (1.33, 3.23)</td>
<td>Age, sex, marital status, education, body mass index, alcohol consumption, smoking, leisure-time physical activity, and season of baseline examination</td>
</tr>
<tr>
<td>Bolland et al, 2010⁶</td>
<td>&lt;50 nmol/L vs ≥50 nmol/L</td>
<td>1.4 (0.8, 2.5)</td>
<td>&lt;50 nmol/L vs ≥50 nmol/L</td>
<td>Untransformed</td>
<td>Season, treatment allocation, age, body weight, smoking status, systolic blood pressure, and history of ischemic heart</td>
</tr>
<tr>
<td>Study</td>
<td>Description</td>
<td>Relative Risk (95% CI)</td>
<td>Reference Level</td>
<td>Control Level</td>
<td>Risk Factor (Untransformed)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>------------------------------------------------------------------------------</td>
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<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Drechsler et al, 2010</td>
<td>≤25 nmol/L vs &gt;75 nmol/L Median (interquartile range): 20 (17, 23) for ≤25 nmol/L and 94 (83, 104) for &gt;75 nmol/L.</td>
<td>2.58 (0.74, 8.98)</td>
<td>≤25 nmol/L</td>
<td>vs &gt;75 nmol/L</td>
<td>Age, sex, atorvastatin treatment, season, coronary artery disease, congestive heart failure, systolic blood pressure, smoking, duration of dialysis, ultrafiltration volume, body mass index, levels of low-density and high-density lipoprotein cholesterol, C-reactive protein, HbA1c, use of beta-blockers, ACE inhibitors, diuretics, and levels of parathyroid hormone, calcium, and phosphate</td>
</tr>
<tr>
<td>Anderson et al, 2010</td>
<td>Very low (≤37.5 nmol/L) vs normal (&gt;75 nmol/L): very low, 30.0 (22.5-35.0); low, 57.5 (50-67.5); normal 95.0 (85.0-112.5)*</td>
<td>1.78 (1.20*, 2.66*)</td>
<td>≤37.5 nmol/L</td>
<td>vs &gt;75 nmol/L</td>
<td>Age, gender, hypertension, hyperlipidemia, diabetes mellitus, and peripheral vascular disease</td>
</tr>
</tbody>
</table>

*These data were provided by investigators upon request.
†To convert relative risks comparing the highest vs. lowest levels to those comparing the lowest vs. highest levels, we took the reciprocal values of the relative risks and their 95% CIs.
Supplementary Figure 1. Funnel plot of studies of 25(OH)D in relation to stroke risk. Dotted lines are pseudo 95% CIs.
Supplemental Reference


