Plasma 25-Hydroxyvitamin D Levels and Risk of Incident Hypertension Among Young Women

John P. Forman, Gary C. Curhan, Eric N. Taylor

Abstract—Numerous cross-sectional studies demonstrate an inverse association between plasma 25-hydroxyvitamin D [25(OH)D] and blood pressure or hypertension. Prospective data, however, are limited. Among 1484 women aged 32 to 52 years who did not have hypertension at baseline, we prospectively analyzed the association between plasma levels of 25(OH)D and the odds of incident hypertension using a nested case-control study design. We matched cases and controls on age, race, and month of blood collection and further adjusted for body mass index, physical activity, family history of hypertension, oral contraceptive use, and plasma levels of parathyroid hormone, calcium, phosphorous, creatinine, and uric acid. Median plasma 25(OH)D levels were lower in the cases (25.6 ng/mL) than in the controls (27.3 ng/mL; \( P < 0.001 \)). Women in the lowest compared with highest quartile of plasma 25(OH)D had an adjusted odds ratio for incident hypertension of 1.66 (95% CI: 1.11 to 2.48; \( P \) for trend = 0.01). Compared with women with sufficient levels, those with vitamin D deficiency (<30 ng/mL; 65.7% of the study population) had a multivariable odds ratio of 1.47 (95% CI: 1.10 to 1.97). Plasma 25(OH)D levels are inversely and independently associated with the risk of developing hypertension. (Hypertension. 2008;52:828-832.)

Key Words: risk factors ■ hypertension ■ epidemiology ■ vitamin D

The effect of vitamin D on calcium and phosphate homeostasis is well known.\(^1\) However, the intracellular vitamin D receptor is broadly distributed among a variety of tissues, including leukocytes, vascular smooth muscle cells (VSMCs), and juxtaglomerular cells, implying other potential effects of vitamin D.\(^2-4\) In addition, the 1α-hydroxylase enzyme (which converts 25-hydroxyvitamin D [25(OH)D] to 1,25-dihydroxyvitamin D2 [1,25(OH)\(_2\)D]) is also widely distributed and expressed in endothelial cells, VSMCs, macrophages,\(^5\) and various locations in the kidney.\(^6-9\) Experimental studies demonstrate that 1,25(OH)\(_2\)D inhibits renin expression,\(^4,10\) enhances insulin secretion and sensitivity,\(^11,12\) and blocks proliferation of VSMCs.\(^13\)

Although cross-sectional studies demonstrate that 25(OH)D levels, and skin exposure to UVB radiation (the major source of vitamin D),\(^14,15\) are associated with lower blood pressure,\(^16-22\) prospective data are limited. An interventional study conducted in 148 vitamin D–deficient elderly women demonstrated a 9% decrease in systolic blood pressure with supplemental vitamin D and calcium compared with calcium alone.\(^23\) In a previous prospective study of older women (first Nurses’ Health Study) and men (Health Professionals Follow-up Study), we demonstrated an association between deficient levels of 25(OH)D and risk of incident hypertension after adjusting for age, race, body mass index (BMI), and physical activity. However, that study had limited statistical power (190 incident cases),\(^24\) and we did not measure plasma parathyroid hormone (PTH), calcium, or creatinine, factors that are associated with 25(OH)D and that may influence the risk of hypertension.\(^25-28\)

To determine the independent association between plasma 25(OH)D levels and risk of incident hypertension, we conducted a prospective nested case-control study of 1484 young, healthy women from the second Nurses’ Health Study.

Methods

Study Population

The Nurses’ Health Study 2 is an ongoing prospective cohort study of 116 671 female registered nurses that began in 1989. Participants are followed via biennial questionnaires that gather information on health-related behaviors and medical events. Follow-up of participants was >90% through 2005. During the years 1997 to 1999, 29 616 participants contributed blood samples that were stored in liquid nitrogen (−130°C). We conducted a nested case-control study of incident hypertension among those women who contributed blood samples and who did not have prevalent hypertension at the time of blood collection. The institutional review board at Brigham and Women’s Hospital reviewed and approved this study.

We selected cases and controls from among those who met the following criteria at the time of blood collection: (1) fasting for ≥8 hours; (2) no diagnosis of hypertension; (3) no use of antihypertensive medications; (4) no diagnosis of cancer (except nonmelanoma skin cancer); (5) no diagnosis of either coronary heart disease or diabetes; and (6) BMI < 30 kg/m\(^2\). This last eligibility criterion is important, because high BMI is a powerful predictor of hypertension\(^29,30\) and of 25(OH)D levels.\(^22,31\)

Using risk-set sampling, we selected 750 cases who subsequently developed hypertension and 750 controls who did not develop hypertension. Controls were matched to cases on the following...
factors: age (within 1 year), race, date of blood sample collection (within 1 month), day of menstrual cycle if premenopausal (within 2 days), and time of day of the blood collection (within 2 hours). In addition, controls were required to have had ≥1 clinician examination during the 2 years before being selected as a control. After excluding 8 case-control pairs with missing biomarker data, the final study population included 742 case-control pairs (N=1484).

### Biomarker Measurement

The plasma concentration of 25(OH)D was determined by an enzyme immunoassay from Immundiagnostic Systems Inc. This assay is sensitive down to a 25(OH)D concentration of 2.0 ng/mL. The coefficient of variation (CV) using quality control samples was 8.8%. Other plasma factors measured in participants of this study included the following: calcium (colorimetric assay, CV 3.2%), phosphorous (photometric assay, CV 3.6%), uric acid (oxidization with uricase to form allantoin and H2O2, CV 3.4%), and creatinine (modified Jaffe method, CV 6.5%).

### Ascertainment of Other Covariates

Age and BMI (weight in kilograms divided by the height in meters squared) were obtained from the supplemental questionnaire that accompanied the submitted blood samples. Smoking status (never, past, or current), physical activity (in metabolic equivalent task scores), current oral contraceptive use (yes or no), and alcohol intake (grams per day) were ascertained from the biennial questionnaire that accompanied the submitted blood samples. Smoking status (never, past, or current) was self-classified. Family history of hypertension was examined the odds of hypertension among women with 25(OH)D were initially examined in quartiles, with the highest quartile defined as the reference group. In an additional analysis, we defined this diagnosis in 2005 and in every previous year. The sensitivity of self-reported hypertension was 94%. The specificity of a nurse reporting no diagnosis of hypertension was 85%.

Women were considered to have prevalent hypertension at the time of blood collection if they reported hypertension on the biennial questionnaire immediately after their blood collection or on any previous questionnaire. This study was prospective; women with prevalent hypertension or women who reported taking antihypertensive medications on the questionnaire immediately after blood collection were not considered for selection as a case or controls.

### Statistical Analyses

Because the continuous baseline variables, including the levels of 25(OH)D and PTH, were not normally distributed, differences between these variables among cases and controls were analyzed using the Wilcoxon rank-sum test. Differences in categorical variables between cases and controls were compared using the \( \chi^2 \) test. METS indicates metabolic equivalent task scores.

### Table 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=1484)</th>
<th>Cases (n=742)</th>
<th>Controls (n=742)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D, ng/mL</td>
<td>26.6 (21.0 to 32.3)</td>
<td>25.6 (20.3 to 31.0)</td>
<td>27.3 (21.7 to 33.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Demographic variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>43 (40 to 46)</td>
<td>43 (40 to 46)</td>
<td>43 (40 to 46)</td>
<td>Matching factor</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1 (22.0 to 26.6)</td>
<td>25.1 (22.8 to 27.5)</td>
<td>23.2 (21.1 to 25.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity, METS</td>
<td>12.3 (5.0 to 25.9)</td>
<td>11.2 (4.6 to 23.7)</td>
<td>13.3 (5.4 to 27.2)</td>
<td>0.006</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>5.1</td>
<td>5.8</td>
<td>4.5</td>
<td>0.24</td>
</tr>
<tr>
<td>Past smoker, %</td>
<td>22.6</td>
<td>23.3</td>
<td>22.0</td>
<td>0.53</td>
</tr>
<tr>
<td>Alcohol intake, g/d</td>
<td>1.6 (0 to 5.5)</td>
<td>1.5 (0 to 5.8)</td>
<td>1.8 (0 to 5.1)</td>
<td>0.94</td>
</tr>
<tr>
<td>Family history of hypertension, %</td>
<td>55.2</td>
<td>62.8</td>
<td>47.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oral contraceptive use, ever, %</td>
<td>85.1</td>
<td>86.8</td>
<td>83.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Current oral contraceptive use, %</td>
<td>5.1</td>
<td>6.4</td>
<td>3.8</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Physiologic variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH, pg/mL</td>
<td>30.9 (23.6 to 39.3)</td>
<td>31.7 (24.1 to 40.4)</td>
<td>30.1 (23.1 to 38.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.3 (9.0 to 9.6)</td>
<td>9.3 (9.0 to 9.6)</td>
<td>9.3 (9.0 to 9.6)</td>
<td>0.88</td>
</tr>
<tr>
<td>Phosphorous, mg/dL</td>
<td>4.4 (4.1 to 4.8)</td>
<td>4.4 (4.1 to 4.8)</td>
<td>4.5 (4.1 to 4.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>3.9 (3.4 to 4.6)</td>
<td>4.1 (3.5 to 4.7)</td>
<td>3.7 (3.2 to 4.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.79 (0.72 to 0.86)</td>
<td>0.78 (0.71 to 0.86)</td>
<td>0.80 (0.73 to 0.87)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as median (interquartile range). Categorical variables are expressed as percentage. Continuous variables were analyzed using the Wilcoxon rank-sum test and categorical variables with the \( \chi^2 \) test. METS indicates metabolic equivalent task scores.
The population-attributable risk was calculated for vitamin D deficiency using the adjusted OR from the multivariable model and with the sufficient group defined as the “unexposed” group. A baseline incidence rate of 14.6 cases per 1000 women annually (1.46% of the population per year) for the unexposed group was determined using the incidence rate for the parent cohort (Nurses’ Health Study 2).

Results

Baseline Characteristics

The baseline characteristics of the entire study population by case status are shown in Table 1. The median age of the population was 43 years, and because this was a matching factor, did not differ. The median BMI was higher among the cases (25.1 kg/m²) compared with the controls (23.2 kg/m²). Cases were also less physically active and were more likely to have a family history of hypertension and to have used oral contraceptives.

The median plasma level of 25(OH)D was lower among cases (25.6 ng/mL) compared with the controls (27.3 ng/mL; P<0.001). The median plasma levels of PTH (31.7 pg/mL among cases and 30.1 pg/mL among controls; P=0.02) and uric acid (4.1 mg/dL among cases and 3.7 mg/dL among controls; P<0.001) were higher among cases. Calcium, phosphorous, and creatinine levels were not significantly different between case and controls.

Plasma 25(OH)D and PTH levels were correlated. However, this correlation was present only among the subgroup of women with vitamin D deficiency. The Spearman correlation coefficient was −0.22 (P<0.0001) among women whose plasma 25(OH)D level was <30 ng/mL and was −0.08 (P=0.06) among women whose level was ≥30 ng/mL.

25(OH)D Level and Risk of Incident Hypertension

In logistic regression models conditioned on matching factors (age, race, month of blood sample collection, day of menstrual cycle if premenopausal, and hour of the blood collection), Model 2 is conditioned on matching factors and adjusted for BMI, physical activity, family history of hypertension, oral contraceptive use (past and current), creatinine, PTH, calcium, phosphorous, and uric acid. ref indicates reference.

Table 3. Plasma 25(OH)D Deficiency and Odds of Incident Hypertension

The prevalence of vitamin D deficiency (<30 ng/mL) in our study population was 65.7%. We analyzed the odds of developing hypertension among women who were vitamin D deficient compared with those with sufficient levels (Table 3). Vitamin D–deficient women had a 47% increased odds (OR: 1.47; 95% CI: 1.10 to 1.97) after multivariable adjustment.

We also analyzed plasma 25(OH)D levels as a continuous variable. Every 5-ng/mL lower 25(OH)D level was associated with an adjusted OR for incident hypertension of 1.08 (95% CI: 1.01 to 1.15). However, in the subgroup of women with vitamin D deficiency, every 5-ng/mL lower 25(OH)D level was associated with an adjusted OR of 1.11; the OR among women with sufficient vitamin D status was 0.97 for every 5-ng/mL lower 25(OH)D level (P for interaction=0.12).

Population-Attributable Risk

Assuming an incidence rate for hypertension among young women of 14.6 cases per 1000 women annually (derived from...
the parent cohort), the estimated incidence rate among young women with vitamin D deficiency is 21.5 cases per 1000 women annually. Given that 65.7% of women were vitamin D deficient, the population risk attributable to vitamin D deficiency is 4.53 new cases of hypertension per 1000 young women annually. If this association is causal, then vitamin D deficiency may account for 23.7% of all new cases of hypertension developing among young women every year.

**Discussion**

Among 1484 nonobese young women without hypertension, diabetes, or coronary disease at baseline, lower plasma 25(OH)D levels independently predicted clinically important differences in the odds of subsequently developing hypertension. Furthermore, almost two thirds of the population had vitamin D deficiency; if this association was causal, then a substantial proportion of hypertension incidence among young women could be attributed to suboptimal levels of 25(OH)D.

Cross-sectional observations support a relation between vitamin D and blood pressure. Older studies examined UVB radiation as a surrogate marker of vitamin D synthesis in the skin, which declines with increasing distance from the equator and is lower in the winter compared with the summer. In the Intersalt Study, which examined >10,000 participants from around the world, systolic and diastolic blood pressures were significantly and positively associated with distance from the equator. Additional evidence comes from geographical differences in blood pressure among individuals of African origin, with those residing in northern regions having higher blood pressure than those residing closer to the equator. Several studies have shown seasonal variations within the same population, with blood pressure peaking in winter and falling in summer. More recently, in an analysis of the third National Health and Nutrition Examination Survey, the prevalence of hypertension was 30% higher among individuals in the lowest compared with the highest quartile of 25(OH)D.

Prospective studies of this association are few and small in size. For example, Krause et al randomly assigned 18 patients with mild hypertension to receive UVB exposure or UVA (UVA does not produce vitamin D) 3 times weekly for 6 weeks. Along with a 162% rise in plasma 25(OH)D in the UVB group, both systolic and diastolic blood pressures fell by 6 mm Hg. No change was observed with UVA exposure. Another small interventional trial conducted in 148 vitamin D–deficient elderly women demonstrated that 800 IU/d of oral vitamin D for 6 weeks lowered systolic blood pressure by 9.3%. In a prospective cohort study of older men and women, we demonstrated previously that vitamin D–deficient individuals had a 3.2-fold higher risk of hypertension incidence compared with those with optimal levels after adjusting for age, race, BMI, and physical activity; however, that study had limited statistical power (only 190 cases), and lacked measurement of other potential confounders, such as PTH, renal function, and other circulating biomarkers.

Several potential mechanisms for an association between 25(OH)D and hypertension have been suggested. First, Li et al showed that 1,25(OH)2D, the result of 1-hydroxylation of 25(OH)D, inhibits renin expression in mice. Second, lower levels of 25(OH)D are associated with insulin resistance, and vitamin D therapy may enhance insulin secretion and insulin sensitivity. Insulin resistance has been proposed to be involved in the pathogenesis of hypertension. Third, 1,25(OH)D inhibits growth of cultured VSMCs in vitro. Thus the vitamin D-hypertension association may be mediated the renin-angiotensin system, insulin resistance, and vascular function.

Furthermore, the 1α-hydroxylase enzyme that converts 25(OH)D to 1,25(OH)2D is expressed in a variety of tissues, including human endothelial cells, human VSMCs, macrophages, and throughout the kidney. Therefore, 25(OH)D may have biological effects that are independent of measurable circulating 1,25(OH)2D levels; this challenges the traditional notion that biological activity of vitamin D is primarily dependent on conversion in the renal proximal tubule.

Our study has limitations that deserve mention. First, we relied on self-reported hypertension and did not directly measure the blood pressure of our participants; however, all of the participants are registered nurses, and we demonstrated that hypertension reporting by participants of this cohort is highly sensitive. Second, the specificity of hypertension reporting may have resulted in the misclassification of a few truly hypertensive individuals as being nonhypertensive controls; however, such misclassification would tend to diminish the magnitude of the OR. Therefore, our findings may indeed be an underestimate of the true association. Third, we purposely restricted our sample to women with BMI values <30 kg/m². Although this limits the generalizability of our findings to nonobese women, obesity is such a powerful risk factor for both hypertension and reduced 25(OH)D levels that inclusion of a large number of obese women may have obscured a true association. Fourth, we had a single measurement of 25(OH)D; because levels may fluctuate over time, longer periods of follow-up may result in more random misclassification. However, as with misclassification of hypertension status, this would tend to diminish the magnitude of the OR, thereby underestimating the true association. Fifth, our study population was almost entirely white and, therefore, not necessarily generalizable to nonwhites. Finally, we had insufficient power to demonstrate that the association between plasma 25(OH)D levels and incident hypertension differed according to vitamin D status (deficient versus sufficient). Nevertheless, the shape of the association depicted in the Figure, as well as the lack of any association among women with sufficient vitamin D levels, suggests a nonlinear association.

**Perspectives**

Our prospective analysis suggests that lower plasma 25(OH)D levels are independently associated with a higher risk of incident hypertension. Given the high prevalence of vitamin D deficiency plus the availability of relatively cheap and effective interventions to raise 25(OH)D levels, these results could have substantial public health implications. Our findings should be tested in randomized trials to determine whether vitamin D supplementation could be effective in reducing blood pressure.
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Disclosures
None.

References


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