Serum 25-Hydroxyvitamin D Levels and the Prevalence of Peripheral Arterial Disease
Results from NHANES 2001 to 2004

Michal L. Melamed, Paul Muntner, Erin D. Michos, Jaime Uribarri, Collin Weber, Jyotirmay Sharma, Paolo Raggi

Objective—The purpose of this study was to determine the association between 25-hydroxyvitamin D (25(OH)D) levels and the prevalence of peripheral arterial disease (PAD) in the general United States population.

Methods and Results—We analyzed data from 4839 participants of the National Health and Nutrition Examination Survey 2001 to 2004 to evaluate the relationship between 25(OH)D and PAD (defined as an ankle-brachial index <0.9). Across quartiles of 25(OH)D, from lowest to highest, the prevalence of PAD was 8.1%, 5.4%, 4.9%, and 3.7% (P trend <0.001). After multivariable adjustment for demographics, comorbidities, physical activity level, and laboratory measures, the prevalence ratio of PAD for the lowest, compared to the highest, 25(OH)D quartile (<17.8 and ≥29.2 ng/mL, respectively) was 1.80 (95% confidence interval: 1.19, 2.74). For each 10 ng/mL lower 25(OH)D level, the multivariable-adjusted prevalence ratio of PAD was 1.35 (95% confidence interval: 1.15, 1.59).

Conclusions—Low serum 25(OH)D levels are associated with a higher prevalence of PAD. Several mechanisms have been invoked in the literature to support a potential antiatherosclerotic activity of vitamin D. Prospective cohort and mechanistic studies should be designed to confirm this association. (Arterioscler Thromb Vasc Biol. 2008;28:1179-1185)

Key Words: 25-hydroxyvitamin D • peripheral arterial disease • phosphate • PTH • cardiovascular disease

Peripheral arterial disease (PAD) affects approximately 5 million US adults, and it is associated with a high risk of morbidity and mortality from cardiovascular disease. Established risk factors for PAD include typical atherosclerosis risk factors such as dyslipidemia, diabetes mellitus, hypertension, smoking, and reduced kidney function. In addition, nontraditional cardiovascular risk factors such as elevated C-reactive protein (CRP) and fibrinogen have been associated with PAD.

Basic science research indicates that low vitamin D levels may be linked with cardiovascular risk. Vascular smooth muscle cells possess the 1-α hydroxylase enzyme that locally activates circulating vitamin D. In animal models, active vitamin D is an inhibitor of the renin-angiotensin system and myocadial cell hypertrophy. Cellular experiments show that active vitamin D and its analogs exhibit anticoagulant activity. Conflicting data are available on the association between low 25(OH)D levels and cardiovascular disease in the general population.

Among adults, 25-hydroxyvitamin D (25(OH)D) levels ≥30 ng/mL are considered optimal. These levels are associated with reduced fracture rates and have been postulated to be associated with better health outcomes. The goal of the current study was to evaluate the association between serum 25(OH)D levels and the prevalence of PAD in the general population. To accomplish this goal, we analyzed data from a nationally representative sample of the United States adult population aged ≥40 years in NHANES 2001 to 2004. In addition, elevated serum calcium, phosphate, and intact parathyroid hormone (iPTH) levels have been associated with cardiovascular disease in the general population. Because these serum markers are associated with vitamin D levels, we assessed the association between these serum markers and PAD in a secondary analysis.

Study Population
NHANES 2001 to 2004 was a nationally representative cross-sectional survey of the civilian noninstitutionalized United States population performed by the National Center for Health Statistics. All participants underwent standardized interviews, physical examinations, and laboratory testing. Ankle-brachial index (ABI) was measured in NHANES 2001 to 2004 participants ≥40 years of age. Serum 25(OH)D levels were measured for 4839 participants, ≥40 years of age, who had complete data on all other study variables.

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Study Variables
Study procedures in NHANES 2001 to 2004 consisted of an in-home interview followed by a medical examination and blood sample collection at a mobile examination center. Of relevance to the current analysis, variables collected during the in-home interview included age, gender, race-ethnicity, cigarette smoking, education, leisure-time physical activity, and a history of diabetes mellitus and myocardial infarction. For the current analysis, self-reported race-ethnicity was categorized as non-Hispanic white, non-Hispanic black, Mexican-American, and other. Participants who reported having smoked ≥100 cigarettes during their lifetime were classified as current or former smokers if they answered affirmatively or negatively, respectively, to the question “Do you smoke cigarettes now?” Diabetes mellitus was defined as a self-report of a physician diagnosis, not during pregnancy, with concurrent use of oral hypoglycemic or insulin medication. A history of myocardial infarction was based on participants’ self-report of a previous physician diagnosis on the medical history questionnaire. Being physically active was defined as participating in 30 or more minutes of moderate or vigorous activity or strength training in the 30 days preceding the NHANES visit.

The NHANES 2001 to 2004 examination procedures included measurement of height, weight, and blood pressure. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Normal weight, overweight, and obesity were defined as a BMI of <25 kg/m², 25 to <30 kg/m², and ≥30 kg/m², respectively. Three blood pressure measurements were taken during a single examination visit by a physician using a protocol adapted from the American Heart Association. Based on the average of all 3 blood pressure measurements, hypertension was defined as systolic or diastolic blood pressure ≥140 mm Hg or 90 mm Hg, respectively, or self-reported current use of blood pressure lowering medication. Medication and supplement use data were obtained for use of statins, antihypertensives, antidiabetes medications, and vitamin D supplement—mentation through questionnaires and pill bottle reviews. Detailed descriptions of blood collection and processing procedures are provided in the NHANES Laboratory/Medical Technologists Procedures Manual.19 Serum creatinine concentration was measured using the modified kinetic method of Jaffé, calibrated to the assays used for the development of the Modification of Diet in Renal Disease (MDRD) Study equation, and glomerular filtration rate was estimated with the simplified MDRD equation.

Participant characteristics were calculated by PAD status. The statistical significance of differences for these characteristics was determined using least squares and maximum likelihood for continuous and dichotomous variables, respectively. Next, the prevalence of PAD was calculated by quartile of 25(OH)D, with the statistical significance of trends across quartile assessed using maximum likelihood. Prevalence ratios of PAD associated with quartile of serum 25(OH)D were calculated using log binomial regression models. An initial model included adjustment for age, gender, and race-ethnicity with a subsequent model including additional adjustment for education, current and former cigarette smoking, leisure-time physical activity, diabetes mellitus, total to HDL cholesterol ratio, body mass index, systolic blood pressure, log homocysteine, glycohemoglobin, statin use, antihypertensive medication use, vitamin D supplement use, history of myocardial infarction, elevated CRP, and chronic kidney disease. To further explore the dose–response relationship of 25(OH)D with PAD, we used restricted quadratic splines with knots at the 10th, 50th, and 90th percentiles of the 25(OH)D distribution (12.4, 23.4, 34.3 ng/mL, respectively). The multivariable-adjusted association between 25(OH)D, as continuous variable, and PAD was determined overall and for subgroups defined by age, gender, race-ethnicity, BMI, physical activity, diabetes mellitus, a history of myocardial infarction, chronic kidney disease, and vitamin D supplement use. For analyses as a continuous variable, the prevalence ratio of PAD was calculated for 1 standard deviation lower serum 25(OH)D (10 ng/mL). Differences in the association of 25(OH)D and PAD across subgroup were tested by using a multiplicative term in the regression models. Additionally, the associations between serum calcium, phosphate, and iPTH, each modeled by quartile and as continuous variables, and PAD were determined. Data were analyzed using SUDAAN (version 9.0; Research Triangle Institute) to account for the complex NHANES sampling design including unequal probabilities of selection, oversampling, and nonresponse.

Results
Participant Characteristics
Participants with PAD were older, more likely to be non-Hispanic black, have less than a high school education, be a former smoker, and have diabetes mellitus, higher glycohemoglobin levels, hypertension, a history of myocardial infarction, chronic kidney disease, use statins, and have elevated homocysteine and CRP (Table 1). Additionally, those with PAD were less likely to be physically active. There was no difference in serum calcium, phosphate, or iPTH levels between participants with and without PAD. In contrast, mean 25(OH)D levels were significantly lower among participants with PAD (P < 0.001).

Associations Between Serum 25 (OH) Vitamin D Levels and PAD
A graded association was present between lower 25(OH)D levels and a higher prevalence of PAD (Table 2). This association was present after age, gender, race-ethnicity, and multivariable adjustment. Compared to their counterparts in the highest 25(OH)D quartile (≥29.2 ng/mL), participants in the lowest 25(OH)D quartile (<17.8 ng/mL) were 2.18 (95%
The confidence interval [CI]: 1.50, 3.16) and 1.80 (1.19, 2.74) times more likely to have PAD, after age, gender, race-ethnicity, and multivariable adjustment, respectively. In a multivariable adjusted spline regression model, a progressive increase in PAD was evident at lower levels of 25(OH)D levels (Figure 1).

Subgroup Analysis

After multivariable adjustment, the prevalence ratio of PAD associated with each 10 ng/mL lower 25(OH)D was 1.35 (95% CI: 1.15, 1.59; Figure 2). The association between 25(OH)D and PAD was similar (probability value for interaction >0.10) in all subgroups except physical activity. Specifically, the prevalence ratio of PAD per each 10 ng/mL lower 25(OH)D was 1.61 (95% CI: 1.19, 2.18) and 1.13 (95% CI: 0.95, 1.35) for those who were and were not physically active, respectively (P interaction=0.06).

Associations of Serum Calcium, Phosphate, and iPTH Levels and PAD

Higher quartiles of serum calcium, phosphate, and iPTH levels were not associated with PAD (Table 3). Additionally, modeled as a continuous variable, there was no association between serum calcium, phosphate, or iPTH and PAD, overall, or in subgroups (data not shown).

Discussion

In this nationally representative sample of US adults, there was a strong, graded association between lower levels of 25(OH)D levels and PAD. This association was present after adjustment for several cardiovascular risk factors and potential confounders. In contrast, no association was present between elevated serum calcium, phosphate or iPTH levels, and PAD in this population.
Epidemiological studies have shown an inverse association between blood pressure and vitamin D levels and a direct association between increasing latitude, as a surrogate of lower vitamin D levels, and blood pressure. One small clinical trial suggested that oral vitamin D supplementation reduces systolic blood pressure. Additionally, in another trial, patients randomized to ultraviolet light exposure 3 times a week experienced a 180% increase in 25(OH)D levels and improved blood pressure control. However, another small study did not show this effect on blood pressure.

The association of 25(OH)D deficiency with glucose intolerance and the metabolic syndrome is another potential mechanism for increased cardiovascular disease risk. In a study of older adults with impaired fasting glucose, supplementation with vitamin D and calcium attenuated the progression of insulin resistance and hyperglycemia. However, in the current study, a graded association between 25(OH)D and PAD remained present after adjustment for diabetes mellitus and glycohemoglobin.

The association of both 25(OH)D and 1,25(OH)2 vitamin D levels with cardiovascular events remains controversial. In some studies, low 25(OH)D levels have been associated with increased prevalence of coronary heart disease (CHD), stroke, and congestive heart failure. However, low 25(OH)D has been associated with a protective association in other studies. For example, in a case–control study of 143 patients with CHD by Rajasree, 25(OH)D levels were associated with a multivariable-adjusted odds ratio for CHD of 3.18 (95% CI: 1.31, 7.73). In contrast, in a case–control study of acute myocardial infarction by Scragg, 25(OH)D levels above the median were protective against CHD (multivariable adjusted odds ratio: 0.43, 95% CI: 0.27, 0.69). In the Scragg study, 25(OH)D levels were notably lower than in the study by Rajasree. Among 1739 Framingham Offspring Study participants free of cardiovascular disease at baseline, a 25(OH)D level of 15 ng/mL was associated with a multivariable-adjusted 62% higher hazard of developing a first cardiovascular event. Interestingly, PAD, defined as the onset of new claudication, was included in the outcome definition. However, PAD only comprised 8 of the 120 events in the study.

There are several lines of evidence suggesting that vitamin D may play a role in the pathogenesis of cardiovascular disease.

### Table 2. Age, Gender, Race-Ethnicity, and Multivariable Adjusted† Prevalence Ratios of Peripheral Arterial Disease (Ankle/Brachial Index <0.9) by Quartiles of Serum 25(OH) Vitamin D

<table>
<thead>
<tr>
<th>Quartile of Serum 25(OH) Vitamin D (range)</th>
<th>n</th>
<th>Median 25(OH)D</th>
<th>Prevalence, % (95% CI)</th>
<th>Prevalence ratio Age, gender, race-ethnicity adjusted</th>
<th>Prevalence ratio Multivariable† adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Lowest, &lt;17.8)</td>
<td>1445</td>
<td>12.9</td>
<td>8.1 (6.4 to 9.9)</td>
<td>2.18 (1.50 to 3.16)***</td>
<td>1.80 (1.19 to 2.74)***</td>
</tr>
<tr>
<td>2 (17.8 to 23.4)</td>
<td>1292</td>
<td>20.4</td>
<td>5.4 (4.2 to 6.6)</td>
<td>1.42 (0.92 to 2.20)</td>
<td>1.49 (0.95 – 2.34)</td>
</tr>
<tr>
<td>3 (23.5 to 29.1)</td>
<td>1153</td>
<td>26.0</td>
<td>4.9 (3.8 to 5.9)</td>
<td>1.28 (0.92 – 1.76)</td>
<td>1.28 (0.91 to 1.81)</td>
</tr>
<tr>
<td>4 (Highest, ≥29.2)</td>
<td>949</td>
<td>33.8</td>
<td>3.7 (2.5 to 4.8)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
</tbody>
</table>

Cut-points for the 25(OH)D quartiles were based on the weighted distribution. Therefore, the unweighted sample size (n) is not equal within each quartile. CI indicates confidence interval. ***P value <0.001 compared to the reference group of 25(OH) vitamin D ≥29.2 ng/mL.

†Multivariable model adjusted for: age, gender, race-ethnicity, education, current and former cigarette smoking, leisure-time physical activity, diabetes mellitus, total to HDL cholesterol ratio, body mass index, systolic blood pressure, glycohemoglobin, statin use, antihypertensive medication use, vitamin D supplement use, history of myocardial infarction, log homocysteine, elevated CRP, and chronic kidney disease.
Cardiac myocytes possess vitamin D receptors. Several in vitro studies have shown that active vitamin D inhibits cardiac myocyte hypertrophy. Paricalcitol, an active vitamin D compound, attenuated the development of left ventricular hypertrophy in Dahl salt-sensitive rats. Vitamin D has been shown to be an inhibitor of the renin-angiotensin system. In addition, several small studies have shown that supplementation with various forms of vitamin D may improve the cytokine profile (CRP, TNF-α levels) of patients with vitamin D deficiency and congestive heart failure. Cellular experiments showed that active vitamin D and its analogs exhibit anticoagulant activity. Finally, the vitamin D-24-hydroxylation transgenic rat, a model of vitamin D deficiency attributable to continuous degradation of active vitamin D, develops aortic atherosclerosis. Interestingly, an early experimental model of atherosclerosis was the cholesterol and vitamin D-fed rat. These rats were given an extremely high dose of vitamin D2 (1.8 million units/kg), and developed aortic atherosclerosis. It is interesting that animal models of both excessive and insufficient vitamin D develop atherosclerosis and that there is conflicting human data on very high levels and very low levels of 25(OH)D being associated with cardiovascular disease suggesting that there may be an optimal level that is neither too high nor too low.

The results of the current study show that the association between low 25(OH)D levels and the prevalence of PAD was consistent across multiple subgroups. In the subgroup analyses, the association was stronger for non-Hispanic whites and for participants without diabetes mellitus. However, the interaction terms were not significant for non-Hispanic blacks versus whites (P interaction = 0.31) or diabetes status (P interaction = 0.19). Although one should be cautious interpreting the results from subgroup analyses because of the reduced sample size and statistical power, these results are similar to those reported by Scragg et al. This line of investigation deserves further research.

The association we report is cross-sectional, similar to 2 previous smaller studies of 25(OH)D and PAD. In a study of 161 patients with angiographically proven PAD, those with Stage II PAD, defined as the presence of claudication symptoms, had higher mean 25(OH)D levels (23.4 ng/mL) compared to patients with Stage IV PAD, defined as the presence of ulcers (9.4 ng/mL). Similarly, in a separate study of 95 patients with angiographically proven PAD, patients with Stage II PAD had higher 25(OH)D levels compared to Stage IV PAD. The results of the current study extend these findings to a large nationally representative population with clinical and subclinical PAD.

High iPTH levels have been associated with cardiovascular disease in the general population. Elevated serum phosphate levels have also been associated with cardiovascular events, a composite outcome which included peripheral vascular disease, in a community-based study. Despite these suggestive findings, we did not find an association between serum calcium, phosphate, and iPTH levels and the prevalence of PAD.
There are potential limitations to the current study. Most notably, the study was cross-sectional. As in any cross-sectional study, one must be cautious in interpreting the direction of the association. It has been hypothesized that patients with PAD may be less mobile and therefore receive less sun exposure and have lower 25(OH)D levels. Although this may be the case for severe PAD, all participants in the current study were noninstitutionalized and mobile enough to attend the NHANES visit. An additional limitation is the lack of data in NHANES on sun exposure, geographic location, and the season during which participants attended their study visits. Another potential limitation is that angiography was not used to detect PAD, which was impractical in a large, population-based survey. Despite these limitations, the current study maintains several strengths. Using the ABI allowed us to identify the presence of subclinical PAD. NHANES included a broad range of data collection. This allowed us to study 25(OH)D, calcium, phosphate, and iPTH and perform statistical analyses after adjustment for several potential confounding factors. Additional strengths include standardized protocols with rigorous quality control for data collection and inclusion of a large nationally representative study sample. The large sample size allowed the investigation of the association between 25(OH)D and PAD in several important subgroups. The consistency of the results in these subgroups is noteworthy.

In summary, in this nationally representative study, US adults with low 25(OH)D levels had a higher prevalence of PAD. This association was strong, graded, and present after adjustment for multiple cardiovascular risk factors. Prospective and mechanistic studies are needed to confirm these associations.

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Disclosures

E.D.M. has served as a consultant to Abbott pharmaceuticals.

References


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