



Published in final edited form as:

Atherosclerosis. 2017 February ; 257: 123–129. doi:10.1016/j.atherosclerosis.2017.01.016.

Serum 25-hydroxyvitamin D is associated with incident peripheral artery disease among white and black adults in the ARIC study cohort

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Abstract

Background and aims—Low 25-hydroxyvitamin D [25(OH)D] concentrations have been associated with peripheral artery disease (PAD). Prevalence of low 25(OH)D and PAD differ between whites and blacks. However, these associations have not been studied prospectively or in a population based cohort. We tested the hypothesis that low 25(OH)D is associated with greater risk of incident PAD in white and black adults.

Methods—25(OH)D was measured in serum collected at ARIC visit 2 (1990 – 1992). We followed 11,789 ARIC participants free of PAD at visit 2 through 2011 for incident PAD events. 25(OH)D (ng/mL) was categorized as deficient (<20), insufficient (20 to <30) or sufficient (≥ 30). PAD was defined by an ankle brachial index (ABI) of <0.9 at ARIC visits 3 or 4 or a hospital

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Author contributions

IRR and PLL planned and designed the analysis. IRR analyzed the data under the supervision of PLL. IRR wrote the first draft of the paper. All authors reviewed manuscript and provided critical scientific input. IRR and PLL had responsibility for final content. All authors approved of final manuscript draft.

Conflict of interest

I. R. Rapson reports grants from National Institutes of Health, during the conduct of the study; E.D. Michos reports honorarium from Seimens Diagnostics outside the submitted work; A. Alonso reports grants from National Institutes of Health, during the conduct of the study; A. T. Hirsch reports grants from AstraZeneca, grants from Pluristem, grants and other support from Merck, and other support from Bayer, outside the submitted work; J. P. Reis has nothing to disclose; P.L. Lutsey reports grants from National Institutes of Health, during the conduct of the study.

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diagnosis with an ICD-9 code indicating PAD during follow-up. Analysis used multivariable-adjusted Cox proportional hazards regressions.

Results—Over a mean follow-up of 17.1 years 1,250 incident PAD events were identified. 22% of whites and 61% of blacks were 25(OH)D deficient. After adjustment for demographic characteristics the hazard ratio (95% CI) of PAD in participants with deficient versus sufficient 25(OH)D was 1.49 (1.26, 1.76). Inclusion of BMI, physical activity, and smoking status attenuated the association [1.25 (1.06, 1.48)]. The association between 25(OH)D and PAD was qualitatively stronger in blacks (*p* for interaction = 0.20).

Conclusions—Deficient 25(OH)D was associated with increased risk of PAD in black and white participants. Whether treatment of low vitamin D through supplementation or modest sunlight exposure prevents PAD is unknown.

Introduction

Atherosclerotic lower extremity peripheral arterial disease (PAD) is usually characterized by at least one high grade (>75%) stenosis in the arteries that supply the legs, which present in the infrarenal aorta or more distal arteries. Prevalence of PAD among US adults aged 40 or older is approximately 7%, which equates to 8.5 million individuals.[1, 2] Black race is a strong PAD risk factor; the prevalence of PAD is approximately 1.7 times higher in blacks than in whites.[3] Individuals with PAD may suffer from leg pain while exercising, and in severe cases PAD patients experience constant leg pain, gangrene, or amputation. In addition to negatively impacting quality of life, PAD has been associated with increased risk of major coronary events, cardiovascular mortality, and mortality.[4] Although up to two-thirds of PAD cases are asymptomatic, these individuals are still at elevated risk of death and atherosclerotic cardiovascular disease (CVD) events.[5]

Vitamins D₂ and D₃ are acquired through exposure to ultraviolet B light or ingestion and are converted to serum 25-hydroxyvitamin D [25(OH)D]. Although 1,25(OH)₂D is the active hormone, 25(OH)D has traditionally been viewed as the best biomarker for assessing vitamin D status. The Endocrine Society defines sufficient concentrations of 25(OH)D as 30–100 ng/mL, insufficiency as 21–29 ng/ml, and deficiency as below 20 ng/ml.[6] However, these cut-points are not based on recommendations for cardiovascular health. Deficient 25(OH)D levels are common, and prevalence varies by race/ethnicity; an analysis that used 2003–2006 National Health and Nutrition Examination Survey (NHANES) data found that 28% of whites and 81% of blacks had concentrations of < 20 ng/mL.[7]

Research has associated low 25(OH)D with increased risk of CVD events[8, 9], and a 35% increased risk of CVD death.[10] There is, however, a growing body of evidence suggesting that the association between 25(OH)D and CVD outcomes is stronger in whites than blacks. [11–14] Adverse CVD outcomes observed in individuals with low levels of 25(OH)D are thought to be mediated through traditional CVD risk factors such as hypertension [15–20], diabetes [9, 21–24], and inflammation.[25–31]

To date, little research has evaluated whether low 25(OH)D is associated with the development of PAD. The studies that have looked at this relation are limited in that they

were cross-sectional, in clinical populations, or limited to a white population.[32–34] Using data from the community-based and prospective Atherosclerosis Risk in Communities (ARIC) study we tested the hypothesis that deficient 25(OH)D compared to sufficient levels is associated with greater risk of incident PAD, and that the association is stronger among whites than blacks.

Materials and methods

Study design

The ARIC study is a prospective cohort designed to determine the causes of atherosclerosis and cardiovascular outcomes.[35] Using probability sampling, a total of 15,792 participants aged 45–64 years were initially enrolled in 1987–89 (visit 1), of whom 11,478 were white and 4,266 were black. Recruitment took place at 4 sites (suburban Minneapolis, MN; Forsyth Co., NC; Washington Co., MD; Jackson, MS). Participants have been followed over 27 years, with annual phone interviews (semi-annual since 2012) and 4 follow-up clinical study visits. Visits occurred at approximately year 3 (visit 2, 1990–1992), 6 (visit 3, 1993–95), 9 (visit 4, 1996–98), and 24 (visit 5, 2011–2013). 25(OH)D was measured in participant serum collected at visit 2; therefore, for the present analysis, visit 2 was used as baseline. Participants were followed for outcomes until the last date of active surveillance collection, death, or December 31, 2011.

ABI, the ratio of SBP in the ankle to the SBP in the upper arm, was not measured at visit 2. Since ABI was not measured concurrently with 25(OH)D, as a surrogate we defined prevalent PAD according to visit 1 ABI or hospital detection between visits 1 and 2. Participants were excluded from the analysis if they had prevalent PAD at visit 1 or hospital detected PAD between visits 1 and 2 (n=503), missing ABI information at ARIC visit 1 (n=457), an ABI of greater than 1.4 at visit 1 (indicative of arterial stiffness that interferes with ABI as a diagnostic tool for PAD) (n = 315), were neither African American nor white or if they were African Americans from the MN and MD centers (n = 78), did not attend visit 2 (n = 1,444), had missing data for 25(OH)D (n = 1,198), or missing information on other key covariates (n=8). The final analytic sample included 11,789 participants. The institutional review committees at each study center approved the study protocol, and all participants provided informed consent.

25(OH)D measurement

Serum 25(OH)D was measured in 2012–2013 using previously unfrozen serum samples from visit 2 by liquid chromatography high-sensitivity mass spectrometry (Waters Alliance e2795; Waters, Milford, MA, USA) at the University of Minnesota Molecular Epidemiology and Biomarker Research Laboratory. Annual average serum 25(OH)D levels were estimated by accounting for seasonality using a residuals approach, as in previous ARIC papers.[36] Serum 25(OH)D levels were analyzed categorically, as deficient (<20 ng/mL), insufficient (20 to <30 ng/mL), and sufficient (≥ 30 ng/mL). Blind duplicate serum samples from ARIC visit 2 were used to calculate the coefficient of variation (CV) and correlation (r): 25(OH)D₃ CV=6.9, r=0.97; 25(OH)D₂ CV=20.8, r = 0.98.[36] Measurements of 25(OH)D₃ and 25(OH)D₂ were summed to calculate total serum 25(OH)D.

Incident PAD ascertainment

Incident PAD was defined as an ABI of less than 0.9 at ARIC visit 3 or 4, or a hospital discharge diagnosis of PAD, leg amputation, or leg revascularization procedure (leg endarterectomy, aorto-iliac-femoral bypass surgery, or leg bypass surgery) during follow-up.

ABI was measured in all participants at visit 1 and a random sample of participants at visit 3 (n=3,787) and 4 (n=5,143).[37] Trained staff used the Dinamap 1846 automated oscillometric device (Criticon, Tampa, FL) to measure ankle SBP at the posterior tibial artery with the participant prone, and brachial SBP in the right arm with the participant supine.[38] At visit 1 average ankle and brachial SBPs were calculated from two measurements in a randomly selected leg and (usually) the right arm. At visits 3 and 4 one ankle SBP and one brachial SBP were measured. ABI was defined as the ratio of the ankle SBP to the brachial SBP.

Participants were contacted by phone (annually through 2012) to identify intermittent claudication symptoms and all hospitalizations. Hospital discharge diagnosis International Classification of Disease, Ninth Revision codes of 443.9 (peripheral vascular disease, unspecified), 84.11 (toe amputation), 84.12 (foot amputation), 84.15 (below-knee amputation), 84.17 (above-knee amputation), 38.18 (leg endarterectomy), 39.25 (aorto-iliac-femoral bypass), and 39.29 (leg bypass surgery) were considered to be hospitalized PAD. Participants were assumed not to have had incident PAD if ABI was not low at visits 3 or 4 and no event was recorded through active surveillance. Date of incidence was defined as the first date of PAD based on either low ABI at visit 3 or 4, or hospital discharge with PAD.

Covariates

Covariate information was from visit 2 (1990–1992), unless otherwise noted. Race, sex, age, education level (<high school degree, high school degree or some college, college graduate at visit 1), and smoking status (current, former, never) were self-reported. Physical activity was measured on a scale from 1 (low) to 5 (high) using the Baecke sports questionnaire at visit 1.[39] Medications for treatment of hypertension, diabetes, or high cholesterol were self-reported and transcribed from bottles participants brought to the visit. Height, weight, and SBP were measured. Fasting cholesterol and triglyceride levels were measured according to ARIC standard procedures.[40] Glucose was measured at visit 2 using a hexokinase assay. Diabetes mellitus was defined as fasting blood glucose level of ≥ 126 mg/dL, a non-fasting blood glucose level of ≥ 200 mg/dL, a self-reported physician diagnosis of diabetes, or the use of anti-diabetes medication in the previous 2 weeks. Serum creatinine was measured using the Jaffe method at visit 2. Cystatin C, serum calcium, and serum phosphorus were measured using a Roche Modular P Chemistry analyzer. Estimated glomerular filtration (eGFR) was calculated from serum creatinine and cystatin C levels using the CKD-EPI equation.[41] Serum parathyroid hormone (PTH) was measured using a Roche Elecsys 2010 Analyzer with a sandwich immunoassay method. High sensitivity C-reactive protein (hs-CRP) was measured using a Roche latex particle enhanced immunoturbidimetric assay kit and Modular P Chemistry analyzer. Serum fibroblast growth factor 23 was measured on a 2-site enzyme-linked immunosorbent assay

(KainosLaboratories, Inc., Tokyo, Japan). Vitamin D binding protein SNPs rs7041 and rs4588 SNP genotypes were obtained using the ITMATBroad-CARe Chip.[12]

Statistical methods

Descriptive statistics of all potential covariates and effect modifiers, stratified by categorical 25(OH)D exposure groups, were calculated. The primary analysis used Cox proportional hazards regression to model the relationship between 25(OH)D categories and time to incident PAD. Person-time accrued from visit 2 until a PAD event occurred, end of follow-up (December 31st 2011), the participant was lost to follow-up, or died, whichever occurred earlier. The relation between 25(OH)D and incident PAD was adjusted for several sets of covariates in nested models. Model 1 adjusted for age, educational category, race, sex. Model 2 further adjusted for BMI, physical activity, and smoking status. We used Cox regression with restricted cubic splines to visually depict the association between 25(OH)D and incident PAD separately in black and white participants. 5 knots were placed at the 5th, 25th, 50th, 75th, and 95th percentiles of race specific 25(OH)D. The value at the 10th percentile was used as the referent, similar to previous publications.[33]

In addition to adjustment for baseline demographic and behavioral variables, secondary analysis looked at the association between 25(OH)D and incident PAD after adjustment for known PAD risk factors, some of which may be mediators. Model 3 added to model 2 prevalent diabetes, hypertension medication use, SBP, LDL cholesterol, HDL cholesterol, cholesterol medication use, and hs-CRP. Additionally, we separately added to model 2 eGFR, serum calcium, serum fibroblast growth factor 23, serum parathyroid hormone, and serum phosphorus. As a sensitivity analysis, we restricted the analysis to participants who were free of PAD and had ABI of ≥ 0.9 at visit 3 ($n = 3,579$). The outcome in this analysis was incident PAD after visit 3.

Effect modification by age, race, sex and key SNPs associated with vitamin D binding protein levels (i.e. rs7041, rs4588) was tested by including cross product terms in the models. Regardless of the presence of race-interactions, *a priori* we planned to present race-stratified models, given inherent interest.

Analyses were performed with SAS 9.3 (SAS Institute, Inc. Cary, North Carolina). Spline regression was performed with STATA 13.1 (StataCorp, College Station, Texas). Analyses were two sided with a type 1 error of 0.05 or less considered statistically significant.

Results

A total of 11,789 participants were included in the final analysis; 2839 (24.0%) were black, 6668 (56.6%) were female, and the mean \pm SD age was 56.8 ± 5.7 years. Mean 25(OH)D level was 24.3 ± 8.5 ng/ml overall, and was higher in white (26.1 ± 8.3) than black (18.9 ± 6.7) participants. Among white participants, 29% had a 25(OH)D level ≥ 30 ng/mL (sufficient), 49% had a level 20 to <30 ng/mL (insufficient), and 22% had a level <20 ng/mL (deficient). Only 6% of black participants had a 25(OH)D level ≥ 30 ng/mL, 32% had a level 20 to <30 ng/mL, and 61% had a level <20 ng/mL.

Baseline characteristics by 25(OH)D categories are summarized in Table 1. Briefly, participants with deficient 25(OH)D tended to be black, female, less educated, current smokers, and have an overall worse CVD risk factor profile.

The mean follow-up was 17.1 (SD 5.7) years, with a maximum of 22.8 years. A total of 1,250 (10.6%) participants developed incident PAD, with 15% (n=186) of events detected at visit 3, 24% (n=305) detected at visit 4, and 61% (n=759) detected through hospital surveillance. The unadjusted incidence rate was 6.2 per 1000 person-years. Among white participants, there were 907 events for an incidence rate of 5.9 per 1000 person-years. Among black participants there were 343 events for an incidence rate of 7.3 per 1000 person-years.

The association between 25(OH)D and incident PAD is visually depicted using race-stratified restricted cubic splines (Fig. 1) and presented according to 25(OH)D categories (Table 2). As shown in the race-stratified spline results, the hazard of PAD was inversely associated with level of 25(OH)D in both black and white participants, although the association appeared somewhat stronger in blacks. As shown in Table 2, after adjustment for baseline demographic characteristics (model 1) 25(OH)D level was inversely associated with incident PAD (2 df, Chi-square $p<0.0001$) and there was evidence of linear dose-response (1df, $p<0.0001$). The hazard of incident PAD in participants with deficient and insufficient 25(OH)D compared to participants with sufficient 25(OH)D was 1.49 (95% CI: 1.26, 1.76) and 1.14 (95% CI: 0.98, 1.32) respectively.

Inclusion of BMI, physical activity, and cigarette smoking status (model 2) attenuated the association between 25(OH)D and PAD, though it remained statistically significant ($p=0.02$); HR for deficient vs. sufficient 25(OH)D was 1.25 (95% CI 1.06, 1.48). Adjustment for cardiovascular risk factors (model 3) further attenuated the association (HR: 1.15 95% CI: 0.97, 1.37, $p=0.22$). There were no multiplicative interactions of the association between 25(OH)D and incident PAD by age, race, sex or SNPs rs7041 or rs4588. Despite the lack of significant race interaction (model 2 interaction $p=0.20$), as specified *a priori*, race-stratified results are presented. The magnitude of the association between deficient vs. sufficient 25(OH)D and incident PAD was qualitatively stronger in blacks (HR: 1.84 95% CI: 1.07, 3.19) than whites (HR: 1.23 95% CI: 1.02, 1.48), after accounting for demographics and behavioral factors.

We also explored the impact of further adjustment for variables that are known to be biochemically related to 25(OH)D level (i.e. serum calcium, FGF-23, PTH phosphorus, eGFR). Adding these biomarkers to model 2 did not substantially change the association between 25(OH)D and incident PAD. Results of these analyses are shown in Table 3.

In sensitivity analyses results were similar when the analyses were restricted to non-smokers (data not shown). Results were qualitatively stronger compared to the main analyses when we restricted to the 3,579 participants who at baseline were free of PAD and at visit 3 had a measured ABI of ≥ 0.9 (to exclude the potential for incident ABI <0.9 between visit 1 and 2). With this restriction, the HR of incident PAD after visit 3 in participants with deficient 25(OH)D compared to participants with sufficient 25(OH)D was 1.66 (95% CI: 1.14, 2.40)

after adjustment for demographic characteristics (model 1), and 1.46 (95% CI: 1.00, 2.15) after further adjustment for BMI, physical activity, and smoking status (model 2).

Discussion

In this analysis of nearly 12,000 participants from the community-based ARIC study cohort, those with deficient levels of 25(OH)D were at approximately 30% greater risk of developing incident PAD, relative to those with sufficient 25(OH)D levels, after accounting for demographics and lifestyle factors. Although there was not a significant interaction by race, the association between low 25(OH)D level and incident PAD was qualitatively stronger in blacks than it was in whites. As predicted, adjustment for known PAD risk factors attenuated the association of 25(OH)D and PAD. These variables may be mediators of the relation or share common cause with low 25(OH)D.

Our finding that low 25(OH)D was associated with an approximately 30% increased risk of PAD is consistent with previous research. The idea that, among individuals with PAD, the presence and development of atherosclerosis may be influenced by disorders of calcium metabolism is not new. It has been previously reported that, relative to healthy controls, patients with PAD have lower bone density and abnormal bone turnover.[42] Fetuin-A, which is a member of the cystatin superfamily of cysteine protease inhibitors involved in vascular pathology and bone metabolism, has also been linked to PAD.[43]

Looking specifically at studies of 25(OH)D and PAD, a study that used the electronic medical records of 41,504 patients in the Intermountain Healthcare system who had 25(OH)D measured for clinical indications (e.g. osteoporosis risk), found approximately 40% increased risk of PAD in patients with low 25(OH)D (< 15 ng/ml) compared to those with sufficient concentrations (>30 ng/mL).[34] Cross-sectional analyses that used NHANES data found an 80% higher prevalence of PAD in participants in the lowest quartile of 25(OH)D compared to the highest quartile after adjustments.[32] However cross-sectional analysis may be complicated by reverse causation if patients with PAD are unable to participate in outdoor physical activity due to limitations from their PAD, and thus have less sunlight exposure and lower 25(OH)D levels. Therefore, our prospective study of incident PAD enhances existing knowledge about the association between 25(OH)D and PAD, as results from studies of patients may be biased and lack generalizability, and results from cross-sectional research cannot establish temporality.

Although interaction by race was not statistically significant, our finding that the magnitude of association was qualitatively higher in black participants was opposite of the hypothesized effect. It is unclear why, in the ARIC population, the association was qualitatively stronger in blacks than whites. Importantly, there were few events among blacks with sufficient 25(OH)D levels, and confidence intervals for hazard estimates were wide.

Prior research on 25(OH)D and PAD is limited in that it has been cross-sectional, or prospective using a clinical sample of white patients.[32–34] This analysis used the ARIC study, a community based prospective cohort of blacks and whites with more than 20 years

of follow-up and standardized procedures for collection of numerous variables related to cardiovascular health. This analysis also has limitations. First, 25(OH)D was measured only once, and there was a long time-span between that measurement and when some of the PAD events accrued, therefore exposure misclassification may have occurred. However, exposure misclassification would likely weaken the observed association. Second, ABI (measured at visits 1, 3 and 4) was not measured concurrently with 25(OH)D (measured at visit 2). For the primary analyses we used low ABI at visit 1 or diagnosis of PAD between visits 1 and 2 as a surrogate for PAD at visit 2. To address the limitation of lack of concurrent measurements, we conducted a sensitivity analysis that was restricted participants who did not have PAD at visit 3. Results in this sensitivity analysis were qualitatively stronger than those in the full cohort, with a HR of 1.46 (vs. 1.25) for deficient compared to sufficient participants after adjustments (model 2). Third, it is possible that 25(OH)D is not the optimal biomarker for assessing vitamin D status.[44, 45] Fourth, the ARIC study did not collect information on sunlight exposure or about how much time people spent outdoors. However, concentrations of 25(OH)D are downstream from sun exposure and nutritional intake, and are influenced by individual-level factors (e.g. amount of melanin in the skin which blocks the conversion of 7-dehydrocholesterol to vitamin D in the skin). As such, concentrations of 25(OH)D are important when thinking about the potential role of vitamin D in the development of PAD. Fifth, ABI was measured in one leg at visits 1, 3, and 4. Measurement of ABI in one leg could cause misclassification of participants with uni-lateral PAD as non-PAD cases. At visits 3 and 4 ABI was only performed on a randomly sample of participants. These would both cause the measured ABI incidence to be lower than the true rate, however this may be alleviated by the fact that >60% of cases were detected through hospital surveillance. Finally, although we controlled for a number of baseline demographic, behavioral, and health characteristics, this is an observational study and residual confounding may remain.

In this large prospective analysis, deficient levels of 25(OH)D were associated with an increased hazard of PAD in whites and blacks, and the association remained after adjustment for demographic and lifestyle variables. Estimates of the 25(OH)D and PAD association were stronger in black than white participants, however confidence intervals for the estimates in blacks were wide, and tests of multiplicative interaction by race were non-significant. It is possible that increasing levels of vitamin D in blacks and whites through supplementation or modest sunlight exposure could result in lower incidence of PAD, however additional research is needed to support these findings.

Acknowledgments

The authors thank the staff and participants of the ARIC study for their important contributions. The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). This research was further supported by grants from the NIH National Heart, Lung, and Blood Institute (R01 HL103706 to Dr. Lutsey) and the NIH Office of Dietary Supplements (R01 HL103706-S1 to Dr. Lutsey). Reagents for the C-reactive protein assays were donated by the manufacturers. Genotyping was supported through the NHLBI CArE (Candidate Gene Resource) grant (N01-HC-65226).

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Highlights

- The first population based bi-racial prospective cohort study on 25(OH)D and PAD.
- Deficient 25(OH)D was associated with increased PAD in whites and blacks.
- The relation of 25(OH)D and PAD was qualitatively stronger in blacks.
- It is unknown if treatment of low vitamin D prevents PAD.

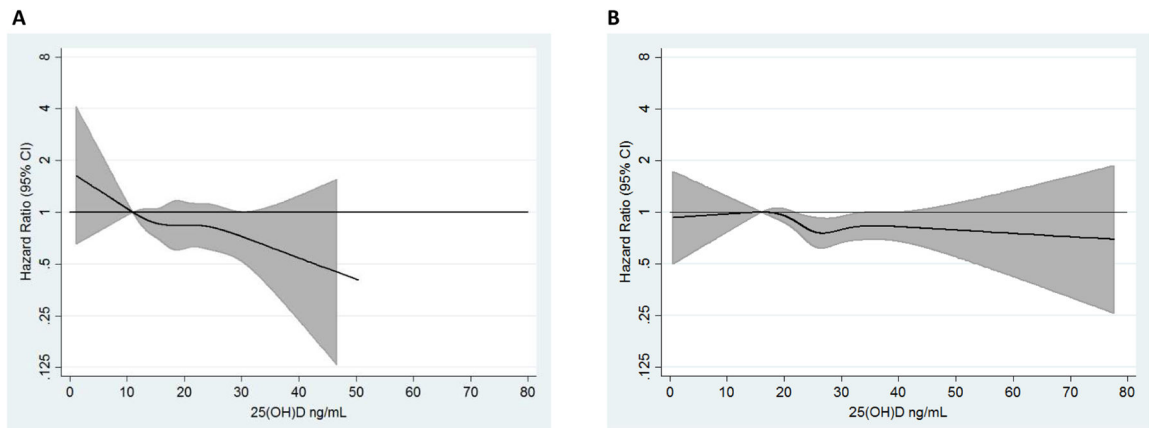


Fig. 1. Multivariable-adjusted hazard ratio of PAD at different levels of 25(OH)D based on restricted cubic spline

(A) Black and (B) white participants. Restricted cubic splines adjusted for age, education level, sex, BMI, physical activity, and smoking status. Knots at the 5th, 25th, 50th, 75th, and 95th percentiles of the race specific 25(OH)D. The 10th percentile was the referent.

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Table 1

Baseline (1990–1992) participant characteristics by 25(OH)D levels in the Atherosclerosis Risk in Communities Study.

	25(OH)D level, ng/mL		
	Deficient <20	Insufficient 20 to <30	Sufficient 30
N (%)	3790 (32)	5260 (45)	2739 (23)
Demographic characteristics			
Age (years), mean ± SD	56.3 ± 5.7	57.0 ± 5.7	57.3 ± 5.7
Female, N (%)	2595 (68)	2728 (52)	1345 (49)
Race, N (%)			
Black	1742 (46)	921 (18)	176 (6)
White	2048 (54)	4339 (82)	2563 (94)
Education, N (%)			
11 years	911 (24)	1046(20)	500 (18)
12–16 years	1519 (40)	2191 (42)	1229 (45)
17+ years	1354 (36)	2015(38)	1005 (37)
Behavioral characteristics and BMI			
BMI (kg/m ²), mean ± SD	29.4 ± 6.2	27.7 ± 4.9	26.3 ± 4.2
Smoking, N (%)			
Current	976 (26)	1033 (20)	526 (19)
Never	1200 (32)	2034 (39)	1204 (44)
Former	1600 (42)	2187 (42)	1009 (37)
Physical activity (1–5), mean ± SD	2.2 ± 0.7	2.5 ± 0.8	2.7 ± 0.9
Physiologic characteristics			
Calcium (mg/dl), mean ± SD	9.4 ± 0.4	9.3 ± 0.4	9.3 ± 0.4
eGFR (ml/min per 1.73 m ²), mean ± SD	97.6 ± 18.1	95.3 ± 15.9	93.0 ± 15.8
FGF-23 (pg/ml), mean ± SD	43.5 ± 20.3	44.5 ± 16.0	45.3 ± 16.7
Parathyroid hormone (pg/ml), mean ± SD	48.2 ± 24.5	41.2 ± 17.6	36.8 ± 12.6
Phosphorus (mg/dl), mean ± SD	3.6 ± 0.5	3.5 ± 0.5	3.5 ± 0.5
Physiologic characteristics			
hs-CRP (mg/l), mean ± SD	5.2 ± 8.2	3.9 ± 6.5	3.7 ± 6.6
Diabetes mellitus, N (%)	732 (19)	694 (13)	240 (9)
HDL (mg/dl), mean ± SD	50.4 ± 17.0	48.7 ± 16.2	51.3 ± 17.8
LDL (mg/dl), mean ± SD	133.7 ± 37.9	134.0 ± 36.3	131.7 ± 35.7
SBP (mm Hg), mean ± SD	123.7 ± 18.0	120.6 ± 18.0	118.9 ± 17.6
Medication			
Hypertension medication, N (%)	1413 (37)	1642 (31)	748 (27)
Cholesterol medication, N (%)	349 (9)	185 (4)	185 (7)

Table 2

Hazard ratios of peripheral artery disease by categorical 25(OH)D levels.

Hazard ratios (95% confidence intervals) for 25(OH)D level (ng/mL)				
	Sufficient 30	Insufficient 20 to <30	Deficient <20	<i>p</i> ^a
All participants				
Events, N (%)	256 (9)	533 (10)	461 (12)	
Model 1	1	1.14 (0.98, 1.32)	1.49 (1.26, 1.76)	<.0001
Model 2	1	1.08 (0.92, 1.25)	1.25 (1.06, 1.48)	0.02
Model 3	1	1.05 (0.90, 1.22)	1.15 (0.97, 1.37)	0.22
Whites				
Events, N (%)	242 (9)	423 (10)	242 (12)	
Model 1	1	1.10 (0.94, 1.29)	1.48 (1.23, 1.77)	<.0001
Model 2	1	1.03 (0.88, 1.21)	1.23 (1.02, 1.48)	0.06
Model 3	1	1.02 (0.87, 1.20)	1.13 (0.93, 1.37)	0.41
Blacks				
Events, N (%)	14 (8)	110 (12)	219 (13)	
Model 1	1	1.58 (0.91, 2.77)	1.94 (1.12, 3.34)	0.03
Model 2	1	1.70 (0.97, 2.97)	1.84 (1.07, 3.19)	0.09
Model 3	1	1.50 (0.85, 2.63)	1.64 (0.95, 2.85)	0.19

Model 1 is adjusted for age, education level, sex, and race (overall results only).

Model 2 is adjusted for age, education level, sex, race (overall results only), BMI, physical activity, and smoking status.

Model 3 is adjusted for age, education level, sex, race (overall results only), BMI, physical activity, smoking status, SBP, HDL, LDL, CRP, diabetes mellitus, cholesterol medication use, and hypertension medication use.

^a2 degree of freedom type 3 Wald Chi square test of significance.

Table 3

Hazard of peripheral artery disease by categorical 25(OH)D exposure levels after adjustment for baseline variables and biomarkers biochemically associated with 25(OH)D.^a

Hazard ratios (95% confidence intervals) for 25(OH)D level (ng/mL)				
Additional adjustment variable	Sufficient 30	Insufficient 20 to <30	Deficient <20	<i>p</i> ^b
eGFR	1	1.09 (0.93, 1.29)	1.25 (1.04, 1.51)	0.05
Calcium	1	1.08 (0.92, 1.25)	1.26 (1.06, 1.49)	0.02
FGF-23	1	1.08 (0.93, 1.26)	1.27 (1.07, 1.50)	0.01
PTH	1	1.06 (0.91, 1.23)	1.22 (1.03, 1.44)	0.05
Phosphorus	1	1.07 (0.92, 1.25)	1.26 (1.06, 1.49)	0.02

^aAdjusted for age, education level, sex, race, BMI, physical activity, smoking status, and the listed variable.

^b2 degree of freedom type 3 Wald Chi square test of significance.