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Vitamin D Deficiency may be an Independent Risk Factor for Arterial Disease

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WHAT THIS PAPER ADDS

- Vitamin D receptors have a wide tissue distribution including the vascular wall. This suggests that vitamin D status might play a role in the pathogenesis of arterial disease. The current study shows a high prevalence of vitamin D deficiency in patients with occlusive as well as in those with aneurysmatic arterial disease. The study further demonstrates a strong association between low vitamin D status and the severity of atherosclerosis. Since this relationship was independent of traditional cardiovascular risk factors and irrespective of the type of arterial disease, these data suggest a direct effect of vitamin D deficiency on the arterial wall.

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ABSTRACT

Objectives: The aim of this study was to assess the vitamin D status in patients with occlusive or aneurysmatic arterial disease in relation to clinical cardiovascular risk profiles and markers of atherosclerotic disease.
Methods: We included 490 patients with symptomatic peripheral arterial disease (PAD, $n = 254$) or aortic aneurysm ($n = 236$). Cardiovascular risk factors and comorbidities carotid intima–media thickness (CIMT), ankle–brachial index (ABI), serum high-sensitive C-reactive protein (hs-CRP) and vitamin D were assessed. Patients were categorised into severely ($\leq$25 nmol l^{-1}) or moderately (26–50 nmol l^{-1}) vitamin D deficient, vitamin D insufficient (51–75 nmol l^{-1}) or vitamin D sufficient (>75 nmol l^{-1}).
Results: Overall, 45% of patients suffered from moderate or severe vitamin D deficiency. The prevalence of vitamin D deficiency was similar in patients with PAD and those with an aortic aneurysm. Low levels of vitamin D were associated with congestive heart failure and cerebrovascular disease. Adjusting for clinical cardiovascular risk factors, multivariable regression analyses showed that low vitamin D status was associated with higher CIMT ($P = 0.001$), lower ABI ($P < 0.001$) and higher hs-CRP ($P = 0.022$).
Conclusions: The current study shows a strong association between low vitamin D status and arterial disease, independent of traditional cardiovascular risk factors and irrespective of the type of vascular disease, that is, occlusive or aneurysmatic disease.

Several large epidemiological studies have concluded that vitamin D deficiency is associated with excess mortality.[1,2] It is becoming increasingly clear that vitamin D has a much broader range of actions in the human body in addition to its well-known effects on calcium homeostasis and bone metabolism. There is accumulating evidence that vitamin D deficiency has important extraskeletal effects, including the cardiovascular system.[3,4] Several clinical studies have reported a high prevalence of vitamin D deficiency in patients with peripheral arterial disease (PAD),[5] coronary artery disease[6] and stroke,[7] as well as the association of vitamin D deficiency with cardiovascular mortality.[2,8,9] Furthermore, low vitamin D status is related to major cardiovascular risk factors, such as hypertension, obesity and diabetes mellitus.[4,10,11]

The aforementioned studies suggest that vitamin D deficiency promotes atherosclerosis.[4,12] However, it is not known whether this

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is a direct effect of vitamin D on the arterial wall, and/or the result of a vitamin D deficiency-associated increase in established cardiovascular risk factors. It is also unclear whether the severity of arterial disease is related to the severity of vitamin D deficiency. Furthermore, it is not known whether patients with aneurysmatic arterial disease also display vitamin D deficiency.

To answer these questions, we assessed the vitamin D status in a large population of patients with occlusive or aneurysmatic arterial disease, and related this to clinical cardiovascular risk profiles as well as to markers for the severity of arterial disease.

Materials and Methods

Study population

The study population consisted of patients with PAD or aortic aneurysmatic disease treated between 2004 and 2011 in the Erasmus University Medical Center in Rotterdam, the Netherlands. Patients with PAD were defined as having symptomatic atherosclerotic lower extremity arterial disease with an ankle–brachial index (ABI) of $\leq$0.9. Patients with aortic aneurysms were defined as having an aortic diameter >30 mm. Common carotid artery intima–media thickness (CIMT), ABI and high-sensitive C-reactive protein (hs-CRP) were routinely measured in all vascular-surgery patients. Patients with routinely measured serum vitamin D levels at the vascular outpatient clinic were included, whereas patients using vitamin D supplementation were excluded from this study. The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board.

Baseline characteristics

A detailed history was obtained from every patient, including traditional risk factors such as age, sex, hypertension (defined as a blood pressure $\geq$140/90 mmHg in non-diabetics, $\geq$130/80 mmHg in diabetics or use of anti-hypertensive medication), hypercholesterolaemia (defined as a low-density lipoprotein (LDL) cholesterol $\geq$3.5 mmol l^{-1} or use of lipid-lowering medication), chronic obstructive pulmonary disease (COPD; defined as a history of COPD or stage $\geq$1 according to the Global Initiative for Obstructive Lung Disease (GOLD) classification), diabetes mellitus (defined as a fasting plasma glucose $\geq$7.0 mmol l^{-1}, non-fasting glucose $\geq$11.1 mmol l^{-1} or use of anti-diabetic medication) and smoking status. Furthermore, the atherosclerotic and cardiac risk factors as embedded in the Revised Cardiac Risk (RCR) index were obtained.[13] The RCR index includes congestive heart failure (defined as a history of congestive heart failure), ischaemic heart disease (defined as a history of myocardial infarction, coronary revascularisation or the presence of pathologic Q-waves on the electrocardiogram), cerebrovascular disease (defined as a history of ischaemic/haemorrhagic stroke or transient ischaemic attack), kidney disease (defined as a serum creatinine $\geq$2.0 mg dl^{-1}) and insulin-dependent diabetes mellitus. The use of prescription medications was recorded and included statins, beta-blockers, rennin–angiotensin–aldosterone system (RAAS) inhibitors and diuretics.

Atherosclerotic markers

The severity of atherosclerotic disease was assessed by measurements of the CIMT, ABI and hs-CRP. The CIMT was measured using the guidelines from the 'Mannheim Carotid Intima–Media Thickness Consensus'.[14,15] Several measurements from the left and the right common carotid artery were made. The highest CIMT value was used for analysis, while measurements of plaques (defined as a focal structure encroaching into the arterial lumen of at least 0.5 mm)[14] were excluded from analysis. The ABI was measured at rest using a portable counter-top Doppler 8-MHz vascular probe (Imexdop CT + Vascular Doppler; Nicolet Vascular, Madison, WI, USA). The ABI was calculated by dividing the higher of the right and left systolic ankle pressures (posterior tibial or dorsal pedal artery) by the highest systolic brachial blood pressure, according to the TransAtlantic Inter-Society Consensus for Management of PAD (TASC) guidelines.[16] Serum hs-CRP was measured using immunochemistry (Beckman Coulter, Woerden, the Netherlands).

Vitamin D measurements

Serum vitamin D was measured in fresh blood samples using a 25-hydroxyvitamin D radioimmunoassay (Diasorin Inc., Stillwater, MN, USA). Within-run coefficient of variation (CV) was 8.6–12.5% and total imprecision CV was 8.2–11.0%. Patients were categorised into four groups based on commonly used cut-off values:[17–19] severely ($\leq$25 nmol l^{-1}) or moderately (26–50 nmol l^{-1}) vitamin D deficient, vitamin D insufficient (51–75 nmol l^{-1}) or vitamin D sufficient (>75 nmol l^{-1}). To convert nanomolar to nanogram per millilitre, one should divide by 2.496.

Statistical analysis

Dichotomous data are described as counts and percentages. Continuous variables are described as mean $\pm$ standard deviation (SD), or median and interquartile ranges (IQRs) in the case of non-Gaussian distribution. Categorical data were compared using chi-square tests. Continuous variables were compared using analysis of variance (ANOVA), or Kruskal–Wallis tests as appropriate. Linear univariable and multivariable regression analyses were performed in separate models using CIMT, ABI or the natural logarithm of hs-CRP as dependent variable. Vitamin D concentrations per 10 nmol l^{-1} was used as independent variable and adjustments were made for age, gender, congestive heart failure, ischaemic heart disease, cerebrovascular disease, renal function by estimated glomerular filtration rate (eGFR), diabetes mellitus, COPD, hypertension, smoking and type of arterial disease. To address the seasonal fluctuation of vitamin D levels, further adjustments were made for calendar season of vitamin D measurement. For all tests, a P-value <0.05 (two-sided) was considered significant. All analyses were performed using PASW (Predictive Analytics Software) version 17.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

A total of 490 patients were included in the study. As many as 254 patients (51.8%) were diagnosed with PAD of the lower extremities and 236 patients (48.2%) were diagnosed with a thoracic and/or abdominal aortic aneurysm (AAA). The mean age of the population was 67 $\pm$ 11 years and the average value of vitamin D concentration was 57 $\pm$ 93 nmol l^{-1}, as presented in Table 1. A total of 62 patients (12.7%) were severely vitamin D deficient, 160 patients (32.7%) were moderately deficient, 138 patients (28.2%) were vitamin D insufficient and 130 patients (26.5%) had sufficient vitamin D levels. There were no differences between patients with PAD and those with an aortic aneurysm with regard to the frequencies of vitamin D deficiency ($P = 0.258$, Fig. 1) or the mean vitamin D concentration (57 $\pm$ 31 and 59.2 $\pm$ 27 nmol l^{-1}, $P = 0.390$). Mean ABI in the patients with aneurysmatic disease was 0.88 and 47% of these patients had an ABI $\leq$0.9. No significant differences in vitamin D concentration were found between AAA patients with normal ABI or low ABI (mean 63 vs. 55 nmol l^{-1}, $P = 0.066$). Further,

Table 1
Baseline characteristics according to vitamin D status.

	Total population	Vitamin D status				*P*-value for trend
		Severely deficient	Moderately deficient	Insufficient	Sufficient	
		≤25 nmol/L	26–50 nmol/L	51–75 nmol/L	>75 nmol/L	
	n = 490	*n* = 62	*n* = 160	*n* = 138	*n* = 130	
Vitamin D level (nmol/L), mean(±SD)	57 ± 93	17 ± 6	39 ± 7	62 ± 7	96 ± 19	–
Baseline characteristics						
Male gender (%)	355 (72.4)	42 (67.7)	114 (71.3)	111 (80.4)	88 (67.7)	0.083
Age (years±SD)	66.8 ± 10.7	64.3±11.6	66.9 ± 11.2	67.8 ± 9.6	66.7 ± 10.6	0.212
Body mass index (kg/m^2), mean(±SD)	26.4 ± 4.4	26.1 ± 5.3	26.4 ± 4.6	26.8 ± 4.2	26.0 ± 4.0	0.495
eGFR (ml/min/1.73 m^2), mean(±SD)	78.32 ± 26.29	86.07 ± 30.75	75.18 ± 28.74	78.46 ± 23.48	78.35 ± 22.97	0.053
Type of arterial disease						
Peripheral arterial disease (%)	254 (51.8)	39 (62.9)	81 (50.6)	72 (52.1)	62 (47.6)	0.258
Thoracic and/or abdominal aortic aneurysm (%)	236 (48.2)	23 (37.1)	79 (49.4)	66 (47.8)	68 (52.3)	
Cardiovascular diseases						
Congestive heart failure (%)	40 (8.1)	12 (19.3)	16 (10.0)	6 (4.3)	6 (4.6)	0.001
Ischaemic heart disease (%)	185 (37.7)	27 (43.5)	69 (43.1)	50 (36.2)	39 (30.0)	0.112
Cerebrovascular disease (%)	85 (17.3)	13 (20.9)	35 (21.8)	27 (19.5)	10 (7.6)	0.009
Cardiovascular risk factors						
Kidney disease (≥2.0 mg/dl)	46 (9.1)	4 (6.4)	22 (13.7)	11 (7.9)	9 (6.9)	0.103
Diabetes mellitus (%)	100 (20.4)	20 (32.2)	32 (20.0)	25 (18.1)	23 (17.6)	0.103
Hypertension (%)	329 (67.1)	41 (66.1)	105 (65.6)	103 (74.6)	80 (61.5)	0.152
Hypercholesterolaemia (%)	455 (92.8)	58 (93.5)	152 (95.0)	126 (91.3)	119 (91.5)	0.573
Smoking – current (%)	209 (42.6)	36 (58.0)	71 (44.3)	62 (44.9)	40 (30.7)	0.014
Smoking – ever (%)	379 (77.3)	54 (87.0)	120 (75.0)	110 (79.7)	95 (73.0)	0.129
COPD (%)	171 (34.8)	22 (35.4)	59 (36.8)	43 (31.1)	47 (36.1)	0.691
RCR index						
RCR score, mean(±SD)	1.16 ± 1.01	1.45 ± 1.14	1.27 ± 1.10	1.11 ± 0.84	0.93 ± 0.97	0.004
0–1 risk factors (%)	333 (67.9)	36 (58.0)	100 (62.5)	98 (71.1)	99 (76.1)	0.001
2 risk factors (%)	105 (21.4)	14 (22.5)	38 (23.7)	33 (23.9)	20 (15.3)	
≥3 risk factors (%)	52 (10.6)	12 (19.3)	22 (13.7)	7 (5.0)	11 (8.4)	
Medication						
Statins (%)	411 (83.8)	54 (87.0)	139 (81.2)	112 (81.1)	106 (81.5)	0.548
Beta-blockers (%)	383 (78.1)	50 (80.6)	124 (77.5)	110 (79.7)	99 (76.1)	0.903
RAAS inhibitors (%)	235 (47.9)	32 (51.6)	78 (48.7)	71 (51.4)	54 (41.5)	0.384
Diuretics (%)	122 (24.8)	14 (22.5)	44 (27.5)	37 (26.8)	27 (20.7)	0.536
Antiplatelets (%)	327 (66.7)	49 (79.0)	99 (61.8)	92 (66.6)	87 (66.9)	0.124

Abbreviations: eGFR; estimated glomerular filtration rate, COPD; chronic obstructive pulmonary disease, RCR index; Revised Cardiac Risk index, RAAS inhibitors; renin-angiotensin system inhibitors.

although seasonal variation in vitamin D deficiency was observed in the overall population, no differences between patients with PAD and aneurysms were observed, as presented in Fig. 2.

Cardiovascular comorbidities

Patient groups with decreasing vitamin D levels had an increasing prevalence of congestive heart failure ($P = 0.001$), cerebrovascular disease ($P = 0.009$) and were more frequent current smokers ($P = 0.014$), as presented in Table 1. Overall high-risk cardiovascular profiles were significantly associated with lower vitamin D levels, as illustrated by a stepwise increase in RCR scores for groups with increasing vitamin D deficiency ($P = 0.004$).

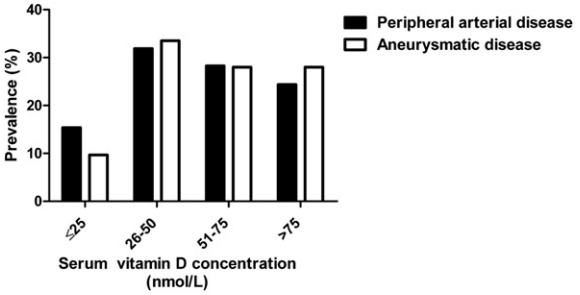

Figure 1. Prevalence of vitamin D deficiency according to type of arterial disease.

Atherosclerotic markers

The mean (±SD) CIMT in all patients was 0.97 ± 0.31 mm and a stepwise decrease was observed from 1.06 ± 0.37 mm in patients with severe vitamin D deficiency to 0.90 ± 0.27 in patients with sufficient vitamin D levels ($P = 0.007$), as presented in Table 2. The mean ABI was 0.70 ± 0.26 and increased stepwise in each group from 0.56 ± 0.28 in patients with severe vitamin D deficiency to 0.77 ± 0.24 in patients with sufficient vitamin D levels ($P < 0.001$). Furthermore, median hs-CRP in all groups was 4.3 mg l^{-1} (IQR: 2.2–7.8 mg l^{-1}). High concentrations of hs-CRP were especially

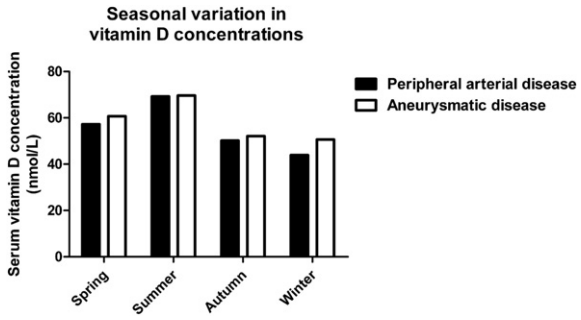

Figure 2. Seasonal variation in vitamin D deficiency according to type of arterial disease.

Table 2
Atherosclerotic markers according to vitamin D status.

Atherosclerotic markers	Vitamin D status					*P*-value for trend
	Total population	Severely deficient	Moderately deficient	Insufficient	Sufficient	
		≤25 nmol/L	26–50 nmol/L	51–75 nmol/L	>75 nmol/L	
CIMT (mm) (mean ± SD)	0.97 ± 0.31	1.06 ± 0.37	1.01 ± 0.34	0.94 ± 0.27	0.90 ± 0.27	0.007
ABI (mean ± SD)	0.70 ± 0.26	0.56 ± 0.28	0.68 ± 0.25	0.72 ± 0.26	0.77 ± 0.24	<0.001
hs-CRP (mg/L) [IQR]	4.3 [2.2–7.8]	7.5 [2.5–12.7]	4.0 [2.3–7.9]	3.8 [1.9–6.8]	4.8 [2.2–7.8]	0.040

Abbreviations: CIMT; common carotid intima-media thickness, ABI; ankle-brachial index, hs-CRP; high-sensitive C-reactive protein.

observed in patients with severe vitamin D deficiency with a median of 7.5 mg l^{-1} (2.5–12.7 mg l^{-1}) ($P = 0.040$).

Multivariable linear regression analyses were performed to determine the association between vitamin D concentration and CIMT, ABI and hs-CRP independently of clinical risk factors. Significant associations for vitamin D concentration per 10 nmol l^{-1} were observed for CIMT (beta −0.017 mm, 95% confidence interval (95%CI): −0.027:−0.007, $P = 0.001$), ABI (beta 0.015, 95%CI: 0.007:0.022, $P < 0.001$) and hs-CRP (beta −0.047 mg l^{-1}, 95%CI: −0.086:−0.007, $P = 0.022$) (Table 3).

Discussion

The current study shows a strong association between low vitamin D status and the severity of arterial disease, independent of traditional cardiovascular risk factors and irrespective of the type of vascular disease, i.e. occlusive or aneurysmatic disease.

Vitamin D_3 is synthesised in the skin from cholesterol under the action of ultraviolet B light.[3] Furthermore, vitamin D can be ingested as cholecalciferol (vitamin D_3) or ergocalciferol (vitamin D_2). Vitamin D is subsequently converted to 25-hydroxyvitamin D (calcidiol) in the liver or stored in adipose tissue. In the kidneys, 25-hydroxyvitamin D is converted to 1,25-dihydroxyvitamin D (calcitriol), which is the biologically active form of vitamin D.[3] The blood concentration of 25-hydroxyvitamin D reflects the dietary intake of vitamin D_2 or D_3 and the amount of vitamin D_3 produced in the skin, and is considered as the best indicator of vitamin D storage.[17] As there is still some debate on the best classification of vitamin D status,[17–20] we used a currently proposed vitamin D classification including clinical relevant cut-off values to describe vitamin D status in our patient cohort.

The observed prevalence of vitamin D deficiency (i.e., ≤50 nmol l^{-1}) of 45% in patients with arterial disease is comparable to previous reports on vitamin D levels in patients with PAD.[21–24] As vitamin D deficiency has been identified as an independent risk factor for mortality,[1,2] the question arises if and how vitamin D deficiency is related to the occurrence of cardiovascular events. In line with previous reports,[7,25] we found that vitamin D deficiency is associated with the occurrence of congestive heart failure and cerebrovascular disease in univariable analyses. In addition, as compared to patients with sufficient vitamin D levels, patients with severe vitamin D deficiency had a significantly higher RCR index, a well-known predictor of postoperative cardiovascular events in patients undergoing non-cardiac surgery.[13]

Next, we attempted to identify how vitamin D status is related to the severity of arterial disease. We observed a strong association between vitamin D and the atherosclerotic markers, CIMT and ABI. The CIMT and ABI provide information about the progression of atherosclerosis. In previous reports, Flu et al. showed the prognostic value of CIMT and ABI, independent of the RCR index.[26,27] Targher et al. observed a similar association between vitamin D deficiency and CIMT in patients with diabetes mellitus,[28] and Reis et al. reported a significant association between vitamin D deficiency and the internal, rather than the common, CIMT.[29] To our knowledge, only two other studies reported ABI measurements in patients with vitamin D deficiency.[5,30] Although both studies reported mild associations, our study clearly shows the stepwise decrease in ABI per vitamin D deficiency category, and a significant correlation in multivariable linear regression models. In addition, whereas other studies reported varying results regarding CRP and vitamin D deficiency,[30–32] the current study shows that serum hs-CRP levels are elevated in patients with severe vitamin D deficiency.

In contrast to previous studies, we found that vitamin D deficiency was not related to hypertension, obesity, diabetes and dyslipidaemia. Furthermore, the correlation between low vitamin D status and markers of atherosclerotic severity was independent of cardiovascular risk factors.

Interestingly, the association between low vitamin D status and the severity of arterial disease was independent of type of vascular disease. To our knowledge, this relationship between vitamin D status and aneurysm formation has thus far not been reported in humans. Although aortic aneurysms have traditionally been attributed to atherosclerosis, there is increasing epidemiological, biochemical and genetic evidence that aneurysmal arterial disease is different from occlusive atherosclerotic disease, a common denominator being ageing of the arterial wall.

Taken together, the data in the current study suggest that the relationship between low vitamin D status and arterial disease is mediated by an independent effect of vitamin D deficiency on the arterial wall. Vitamin D receptors are not exclusively detected in the bone and mineral pathway, but have a wide tissue distribution, including vascular smooth-muscle cells and vascular endothelial cells.[17] The diverse physiologic actions of vitamin D on the vascular wall include reduction of smooth muscle-cell proliferation,[33] reduction of macrophage secretion of pro-inflammatory cytokines interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) and increased secretion of the anti-inflammatory cytokine IL-10, and therefore reducing the state of vascular inflammation.[34–36] In an

Table 3
Multivariable linear regression models for associations between vitamin D and atherosclerotic markers.

Atherosclerotic markers	*n*		Beta for vitamin D[b]	95%CI for Beta	*P*-value
CIMT	439	Unadjusted	−0.019	−0.029 : −0.009	<0.001
		Adjusted[a]	−0.017	−0.027 : −0.007	0.001
ABI	365	Unadjusted	0.017	0.008 : 0.026	<0.001
		Adjusted[a]	0.015	0.007 : 0.022	<0.001
hs-CRP	391	Unadjusted	−0.044	−0.082 : −0.005	0.027
		Adjusted[a]	−0.047	−0.086 : −0.007	0.022

Abbreviations: CIMT; common carotid intima-media thickness, ABI; ankle-brachial index, hs-CRP; high-sensitive C-reactive protein.

[a] Adjusted for: age, gender, congestive heart failure, ischaemic heart disease, cerebrovascular disease, renal function using eGFR, diabetes mellitus, chronic obstructive pulmonary disease, hypertension, smoking, type of arterial disease and calendar season of 25-hydroxyvitamin D measurement.

[b] Vitamin D per 10 nmol/L.

atherosclerotic mouse model, it has been demonstrated that oral vitamin D_3 reduces the formation of atherosclerotic plaques by the suppression of proatherogenic T lymphocytes.[37] In addition, low circulating levels of vitamin D have been associated with endothelial dysfunction in humans.[38,39] Furthermore, it has previously been reported that people with vitamin D deficiency have increased vascular calcification, a sign of advanced atherosclerosis,[40,41] as well as increased aortic stiffness.[42] These vitamin D-related effects all promote arterial disease.[4,12] Experimental studies provide increasing evidence that factors regulating mineral ion homeostasis, such as vitamin D, affect the ageing process, including vascular ageing.[43]

There are several limitations that need to be considered. Due to the nature of this study, it remains uncertain whether the association between low vitamin D status and arterial disease is causal, or whether it is just a bystander. Furthermore, several potentially confounding factors could have influenced our analyses, the most important ones being race, diet and sunlight exposure. As our study population consisted mostly of Caucasians, race was not a factor in our analyses. Moreover, as lower vitamin D levels are observed in non-Caucasian populations, the true prevalence of vitamin D deficiency in PAD patients may actually have been underestimated. The influence of low dietary intake, thereby not only reducing vitamin D but also other nutrients, was not taken into account in this study. However, low vitamin D in the European population is mainly caused by low sunlight exposure rather than diet.[17,44] Therefore, in the multivariable models we corrected for the season of vitamin D measurement to minimise confounding by seasonal variations in sunlight exposure.

In conclusion, this study demonstrates that low vitamin D status is a risk factor for the severity of arterial disease, independent of traditional cardiovascular risk factors and irrespective of the type of vascular disease, i.e. occlusive or aneurysmatic disease. It might be hypothesised that primary and secondary preventive strategies to reduce vascular disease should focus on vitamin D status, in addition to blood-pressure reduction, lipid and glucose control, weight loss and lifestyle changes. A beneficial effect of vitamin D supplementation on blood-pressure reduction has been demonstrated in several clinical trials.[45,46] Although improving vitamin D status might be a promising public-health strategy to reduce cardiovascular disease and improve survival,[47,48] there is still much debate about the requirement levels of vitamin D in relation to extra-skeletal outcomes.[20] Further large-scale, randomised clinical trials are needed to test the effects of vitamin D on cardiovascular disease and to further elucidate the biology of vitamin D on the arterial wall.

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Conflict of Interest

None.

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