# Association of Vitamin D Deficiency With Peripheral Arterial Disease: A Meta-Analysis of Literature Studies

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**Context:** Patients with vitamin D deficiency have increased cardiovascular (CV) morbidity and mortality. Contrasting data are available about the association between peripheral arterial disease (PAD) and vitamin D status.

**Objective:** To perform a meta-analysis of studies evaluating the association between vitamin D status and PAD.

**Data sources:** Studies were systematically searched in the PubMed, Web of Science, Scopus, and EMBASE databases.

**Results:** Ten studies with data on vitamin D levels in 2079 patients with PAD and 18,233 non-PAD control subjects and six studies on the prevalence of PAD in 23,171 subjects with vitamin D deficiency (<20 ng/mL), 48,311 subjects with vitamin D insufficiency (20 to 30 ng/mL), and 27,910 with normal vitamin D levels (>30 ng/mL) were included. Compared with control subjects, patients with PAD showed significantly lower vitamin D levels [mean difference: −2.24 ng/mL, 95% confidence interval (CI): −3.38 to −1.10, $P < 0.001$; $I^2 = 86.5\%$, $P < 0.001$]. Moreover, a higher prevalence of PAD was found in subjects with vitamin D insufficiency [odds ratio (OR): 1.098, 95% CI: 1.010 to 1.195, $P = 0.029$; $I^2$: 0%, $P = 0.600$] and in subjects with vitamin D deficiency (OR: 1.484, 95% CI: 1.348 to 1.635, $P < 0.001$; $I^2$: 7.65%, $P = 0.367$) compared with control subjects with normal vitamin D levels. Sensitivity analyses and the analysis of data on the cumulative risk of PAD according to vitamin D levels derived from multivariate analysis consistently confirmed the results.

**Conclusions:** Patients with PAD have lower vitamin D levels than control subjects, and both vitamin D deficiency and vitamin D insufficiency are significantly associated with PAD. Reduced vitamin D levels might represent an independent risk factor for PAD and, in turn, for CV events. (***J Clin Endocrinol Metab*** **103: 2107–2115, 2018)**

Deficiency of 25-OH-vitamin D3 (vitamin D) is an often unrecognized and frequently untreated clinical condition that is associated with osteopenia, osteoporosis, and an increased risk of fracture (1). In addition to its effects on bone health, recent evidence shows an association between vitamin D deficiency and nonskeletal major chronic diseases, especially cardiovascular (CV) diseases (2–4), with poor vitamin D status being associated with CV mortality (5), major CV risk factors (6–10), and increased subclinical carotid atherosclerosis (11).

Peripheral arterial disease (PAD) affects about 200 million patients worldwide, with a prevalence of ~30% in subjects aged over 70 years (12). Patients with PAD experience functional disability (intermittent claudication) and ischemia-related complications (rest pain, lower limb ulceration, gangrene, and amputation) and exhibit

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Abbreviations: ABI, ankle brachial index; CI, confidence interval; CV, cardiovascular; MD, mean difference; NOS, Newcastle-Ottawa scale; OR, odds ratio; PAD, peripheral arterial disease.

an increased risk of CV events (13–15). In addition to traditional CV risk factors (12–16), vitamin D deficiency was shown to be independently associated with PAD (17, 18). However, this association has been challenged in other studies (14, 19, 20), and no conclusive evidence is available on the association between vitamin D status and PAD. The only available meta-analysis on this topic (21) showed lower levels of vitamin D in patients with PAD than in control subjects, but it included only ~50% of available studies, was affected by publication bias, and did not provide data about the prevalence of PAD in different vitamin D levels categories. Thus, in the current study, we performed a systematic review with meta-analysis of literature studies to assess levels of vitamin D in patients with PAD and the prevalence of PAD for different categories of vitamin D levels.

## Materials and Methods

### Search strategy

To identify all available studies, a detailed search pertaining to the association of vitamin D status with PAD was conducted according to PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (22). A systematic search was performed in the electronic databases (PubMed, Web of Science, Scopus, EMBASE) using the following search terms in all possible combinations: vitamin D, 25(OH)vitamin D, 25-hydroxy-vitamin D3, hypovitaminosis D, peripheral artery disease, PAD, peripheric artery disease, peripheric arterial disease, lower limb ischemia, and lower limb atherosclerosis. The last search was performed on 17 January 2018. The search strategy was developed without language or publication year restriction.

The reference lists of all retrieved articles were manually reviewed. In the case of missing data, study authors were contacted by e-mail to try to retrieve original data. Two independent authors (G.I. and F.F.) analyzed each article and performed the data extraction independently. In the case of disagreement, a third investigator was consulted (M.N.D.D.M.). Discrepancies were resolved by consensus. Selection results showed a high inter-reader agreement ($\kappa = 0.97$) and have been reported according to the PRISMA flowchart (Supplemental Fig. 1).

### Data extraction and quality assessment

According to the prespecified protocol, all studies evaluating the association of vitamin D status with PAD were included. Case reports, case series without a control group, reviews, and animal studies were excluded. We included in the analysis all studies providing values (means with standard deviation or standard error) of vitamin D in patients with PAD and in control subjects and/or the prevalence of PAD in subjects stratified according to vitamin D status. Vitamin D deficiency was defined as 25(OH)D levels <20 ng/mL, vitamin D insufficiency was defined as 25(OH)D levels ranging from 20 to 30 ng/mL, and normal vitamin D status was defined as 25(OH)D levels >30 ng/mL (1). In each study, data regarding sample size, major clinical and demographic variables, values of vitamin D, and prevalence of PAD were extracted.

As primary analysis, we evaluated mean levels of vitamin D in patients with PAD and in control subjects without PAD. As secondary analysis, we evaluated the prevalence of PAD in patients with vitamin D deficiency [*i.e.*, 25(OH)D <20 ng/mL] and vitamin D insufficiency [*i.e.*, 25(OH)D from 21 to 29 ng/mL] as compared with subjects with 25(OH)D ≥30 ng/mL.

Given the characteristics of the included studies, the evaluation of methodological quality of each study was performed with the Newcastle-Ottawa scale (NOS), which was developed to assess quality of nonrandomized observational studies (23). The scoring system encompasses three major domains (selection, comparability, exposure) and a resulting score range between 0 and 8; a higher score represents a better methodological quality. Results of the NOS quality assessment are reported in Supplemental Tables 1 and 2.

### Statistical analysis and risk of bias assessment

Statistical analysis was carried out using Comprehensive Meta-Analysis, Version 2 (Biostat, Englewood, NJ). Differences among cases and control subjects were expressed as mean difference (MD) with pertinent 95% confidence intervals (CIs) for continuous variables and as odds ratio (OR) with pertinent 95% CI for dichotomous variables. Vitamin D is expressed in ng/mL.

The overall effect was tested using Z scores, and significance was set at $P < 0.05$. Statistical heterogeneity between studies was assessed with $\chi^2$ Cochran Q test and with the $I^2$ statistic, which measures the inconsistency across study results and describes the proportion of total variation in study estimates that is due to heterogeneity rather than sampling error. An $I^2$ value of 0% indicates no heterogeneity; 25% indicates low, 25% to 50% indicates moderate, and 50% indicates high heterogeneity (24).

Absolute risk of PAD presence in subjects with vitamin D deficiency/insufficiency and control subjects was calculated in each group as (number of subjects with PAD)/(total number of subjects). The attributable risk was defined as (risk of PAD in subjects with vitamin D deficiency/insufficiency – risk of PAD in control subjects)/(risk of PAD in subjects with vitamin D deficiency or insufficiency).

Publication bias was assessed by the Egger test and represented graphically by funnel plots of the standard difference in means vs the standard error. Visual inspection of funnel plot asymmetry was performed to address a possible small-study effect, and Egger test was performed to address publication bias over and above any subjective evaluation. A $P$ value <0.10 was considered statistically significant (25). In the case of a large publication bias, the Duval and Tweedie trim and fill method was used to allow for the estimation of an adjusted effect size (26). To be as conservative as possible, the random-effect method was used to take into account the variability among included studies.

### Meta regression analyses

We hypothesized that differences among the included studies may be affected by demographic variables (mean age, male sex) and clinical data (body mass index, diabetes, hypertension, hypercholesterolemia, smoking habit). To assess the possible effect of such variables in explaining different results observed across studies, we planned to perform meta-regression analyses after implementing regression models with difference in vitamin D values or prevalence of PAD as dependent variables ($y$) and the above-mentioned covariates as independent variables ($x$). This analysis was performed with Comprehensive Meta-Analysis, Version 2 (Biostat).

## Results

After excluding duplicate results, the search retrieved 36 articles. Of these studies, 13 were excluded because they were off the topic after scanning the title and/or the abstract, because they were reviews/comments/case reports, or because they lacked data of interest. Eight studies were excluded after full-length paper evaluation (Supplemental Fig. 1).

Fifteen studies were included in the final analysis, with 10 reporting vitamin D levels in 2079 patients with PAD and 18,233 non-PAD control subjects (19, 27–35) and six studies on the prevalence of PAD in 23,171 subjects with vitamin D <20 ng/mL, 48,311 subjects with vitamin D ranging from 20 to 30 ng/mL, and 27,910 with vitamin D >30 ng/mL (17, 20, 31, 36–38). One study (34) provided separate data for black and white patients. For consistency with the other studies, data have been merged in a unique population including both races.

Four studies (five datasets) (17, 31, 36, 38) provided age- and sex-adjusted ORs for the presence of PAD in different vitamin D level categories. The study by Steinvil *et al.* (36) provided an adjusted OR for the presence of PAD split for male and female subjects. The two populations have been analyzed as separate datasets.

Overall, six studies (17, 31, 34–37) had a retrospective design, and nine studies (19, 20, 27–30, 32, 33, 38) were prospective cohort studies. Major characteristics of study populations are shown in Tables 1 and 2.

### Vitamin D levels in patients with PAD and control subjects

A total of 10 studies (19, 27–35) showed significantly lower vitamin D levels in 2079 patients with PAD than in 18,233 non-PAD control subjects (MD: −2.24 ng/mL, 95% CI: −3.38 to −1.10, $P < 0.001$) (Fig. 1). Heterogeneity among these studies was statistically significant ($I^2 = 86.5\%$, $P < 0.001$), and no reduction in the overall heterogeneity was found after excluding one study at a time. A similar result was confirmed when the analysis was repeated after excluding retrospective studies (31, 34, 35) (MD: −1.69 ng/mL, 95% CI: −3.08 to −0.30, $P = 0.017$; $I^2 = 68.9\%$, $P = 0.004$). When specifically including studies using ankle brachial index (ABI) <0.9 as the criteria to define PAD (19, 29–35), reduced vitamin D levels were confirmed in patients with PAD compared with non-PAD control subjects (MD: −1.59 ng/mL, 95% CI: −2.98 to −0.19, $P = 0.026$; $I^2 = 90.6\%$, $P < 0.001$).

The median NOS for study quality was 6, and, after excluding the three studies with a score <6 and judged as "low quality" (19, 27, 28), results were confirmed (MD: −1.76 ng/mL, 95% CI: −3.08 to −0.43, $P = 0.009$; $I^2 = 89.2\%$, $P < 0.001$).

Visual inspection of funnel plots suggested the absence of publication bias and of small-study effect (Supplemental Fig. 2), confirmed by the Egger test ($P = 0.258$). Meta-regression models showed that the difference in the prevalence of male sex between patients with PAD and control subjects was associated with a higher difference in vitamin D levels (Z-value: −2.338, $P = 0.019$) (Supplemental Fig. 3), whereas no effect for all the other clinical and demographic variables on the outcome was found (Supplemental Table 3). Thus, the analysis was repeated including only the three studies (27, 28, 33) that enrolled control subjects matched to patients with PAD, and significantly lower vitamin D levels were confirmed (MD: −4.204 ng/mL, 95% CI: −6.460 to −1.947, $P < 0.001$) without heterogeneity among studies ($I^2 = 0\%$, $P = 0.951$).

**Table 1. Demographic and Clinical Data of Subjects Enrolled in Studies Evaluating Levels of Vitamin D in Patients With PAD and Control Subjects**

| Author | Study Design | Patients With PAD (n) | Non-PAD Control Subjects (n) | Age (y) | Male (%) | BMI (kg/m$^2$) | Hypertension (%) | Smoker (%) | Hyperlipidemia (%) | Diabetes (%) | PAD Definition |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fahrleitner *et al.* (27) | Prospective | 161 | 45 | 66 | 53.5 | NR | NR | NR | NR | NR | Angiography |
| Fahrleitner *et al.* (28) | Prospective | 95 | 44 | 65.5 | 54 | NR | NR | NR | NR | NR | Angiography |
| Li *et al.* (29) | Prospective | 207 | 821 | 64.5 | 39.4 | 24.45 | 61.95 | 13.1 | NR | 100 | ABI <0.9 |
| Liew *et al.* (30) | Prospective | 75 | 300 | 66.0 | 75 | 28.4 | 61 | 54 | 69 | 24 | ABI <0.9 |
| McDermott *et al.* (19) | Prospective | 402 | 305 | 71.8 | 51.1 | NR | 68.15 | 11.4 | NR | 27.8 | ABI <0.9 |
| Melamed *et al.* (31) | Retrospective | 406 | 4433 | 56.2 | 48.2 | 28.4 | 43.7 | 21.2 | NR | 8.3 | ABI <0.9 |
| Oh *et al.* (32) | Prospective | 272 | 8688 | 67.55 | 45.9 | 24.35 | 45.05 | 39.7 | 8.85 | 18.4 | ABI <0.9 |
| Pasqualini *et al.* (33) | Prospective | 49 | 94 | 76.15 | 60 | NR | 80 | 25.5 | 28.5 | 34 | ABI <0.9 |
| Reis *et al.* (34) | Retrospective | 334 | 3429 | 56.03 | 49.2 | 28.72 | 50.76 | 23.76 | 41.56 | 11.24 | ABI <0.9 |
| Zagura *et al.* (35) | Retrospective | 78 | 74 | 62 | 53.5 | 26.2 | 38.5 | 62 | NR | 0 | ABI <0.9 |

Abbreviations: ABI, ankle brachial index; BMI, body mass index; DM, diabetes mellitus; NR, not reported.

**Table 2. Demographic and Clinical Data of Subjects Enrolled in Studies Evaluating the Prevalence of PAD in Different Vitamin D Categories**

| Author | Study Design | Vitamin D <20 ng/mL | Vitamin D 20–30 ng/mL | Vitamin D >30 ng/mL | Age (y) | Male (%) | BMI (kg/m$^2$) | Hypertension (%) | Smoker (%) | Hyperlipidemia (%) | Diabetes (%) | PAD Definition |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Anderson *et al.* (37) | Retrospective | 7478 | 19,474 | 15,121 | NR | 25.1 | NR | 37.6 | NR | 45.1 | 19.9 | ICD-9 |
| Kim *et al.* (17) | Retrospective | 6909 | 2031 | 967 | NR | NR | NR | 35.8 | 25.2 | NR | 8.9 | ABI <0.9 |
| Melamed *et al.* (31) | Retrospective | 2737 | 1153 | 949 | 56.2 | 48.2 | 28.4 | 43.7 | 21.2 | NR | 8.3 | ABI <0.9 |
| Rapson *et al.* (38) | Prospective | 1820 | 5260 | 2739 | 56.8 | 43.4 | 27.9 | 32.3 | 21.5 | NR | 14.1 | ABI <0.9 |
| Steinvil *et al.* (36) | Retrospective | 2737 | 20,013 | 7383 | 55 | 23.4 | NR | 24 | 6.8 | 14.5 | 11.1 | ICD-9 |
| Veronese *et al.* (20) | Prospective | 437 | 380 | 751 | 74 | 34 | 27.91 | 70.2 | 8.5 | NR | 13.0 | ABI <0.9 |

Abbreviations: ABI, ankle brachial index; BMI, body mass index; ICD-9, International Classification of Diseases, Ninth Revision; NR, not reported.

### Prevalence of PAD according to vitamin D status

The six studies (17, 20, 31, 36–38) showed that the absolute risk of PAD was 5.4% (95% CI: 2.3% to 11.9%) among the 48,311 subjects with vitamin D insufficiency (vitamin D from 20 to 30 ng/mL) as compared with 4.6% (95% CI: 1.9% to 10.5%) among 27,910 subjects with normal levels of vitamin D (vitamin D >30 ng/mL) with a corresponding OR of 1.098 (95% CI: 1.010 to 1.195, $P$ = 0.029) (Fig. 2) with no heterogeneity among studies ($I^2$: 0%, $P$ = 0.600) and with an attributable risk of 14.8%. A higher prevalence of PAD was also found when comparing the 23,171 subjects with vitamin D deficiency (vitamin D <20 ng/mL), with 27,910 subjects with normal levels of vitamin D (vitamin D >30 ng/mL) (6.9%, 95% CI: 3.5% to 13.1% vs 4.6%, 95% CI: 1.9% to 10.5%, OR: 1.484, 95% CI: 1.348 to 1.635, $P$ < 0.001) (Fig. 2) with a nonsignificant heterogeneity among studies ($I^2$: 7.65%, $P$ = 0.367) and with an attributable risk of 33.3%. When including studies using ABI <0.9 as criteria to define PAD (17, 20, 31, 38), the increased prevalence of PAD was consistently confirmed in subjects with vitamin D from 20 to 30 ng/mL (OR: 1.138, 95% CI: 1.009 to 1.284, $P$ = 0.036; $I^2$: 0%, $P$ = 0.734) and in subjects with vitamin D <20 ng/mL (OR: 1.435, 95% CI: 1.242 to 1.660, $P$ < 0.001; $I^2$: 22.9%, $P$ = 0.273). When analyzing only prospective studies (20, 38), the increased prevalence of PAD was confirmed for subjects with vitamin D <20 ng/mL (OR: 1.327, 95% CI: 1.155 to 1.525, $P$ < 0.001; $I^2$: 0%, $P$ = 0.779) but not for those with vitamin D from 20 to 30 ng/mL (OR: 1.096, 95% CI: 0.954 to 1.258, $P$ = 0.196; $I^2$: 0%, $P$ = 0.963).

To further adjust for potential confounders, we analyzed the four studies (five datasets) (17, 31, 36, 38), providing data derived from multivariate analysis on the cumulative risk of PAD according to vitamin D levels. As compared with those with vitamin D >30 ng/mL, an increased risk of PAD was consistently found in patients with vitamin D insufficiency and in those with vitamin D deficiency, without heterogeneity among studies (Supplemental Fig. 4) and with results similar to the primary analysis (Supplemental Fig. 5).

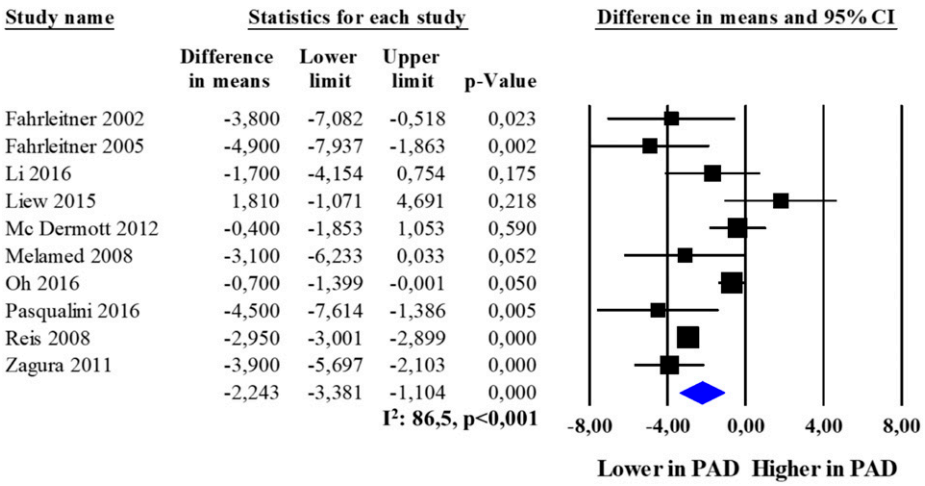


**Figure 1.** Vitamin D levels (ng/mL) in patients with PAD and control subjects.

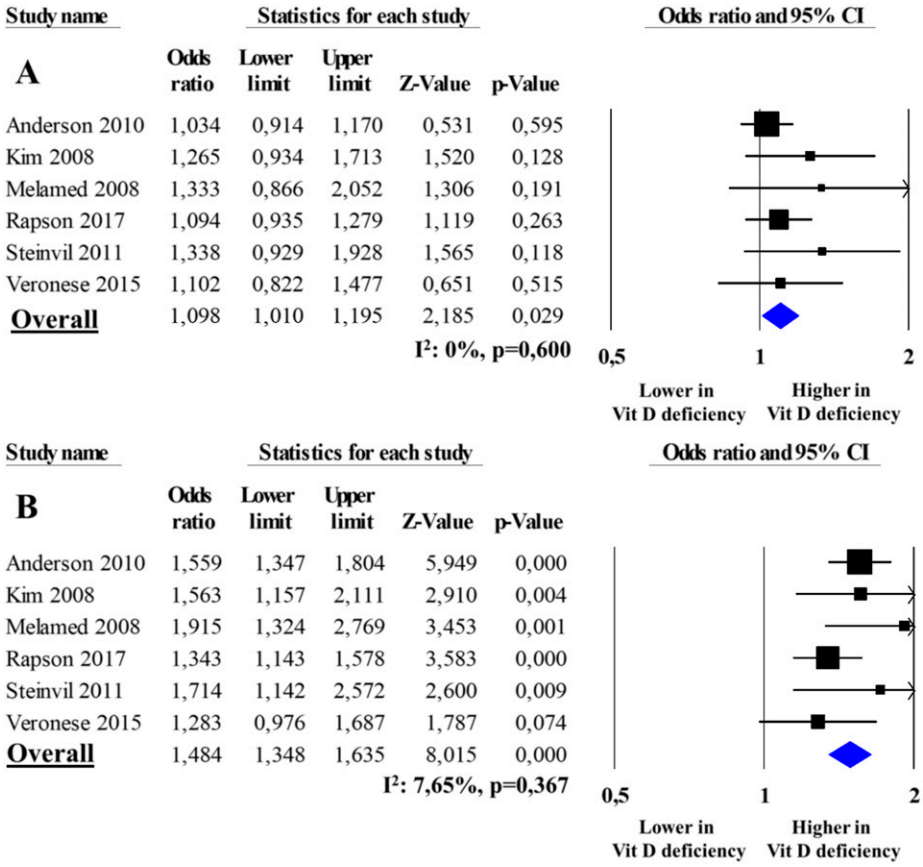


**Figure 2.** Prevalence of PAD (A) in patients with vitamin D levels from 20 to 30 ng/mL vs those with vitamin D >30 ng/mL and (B) in patients with vitamin D levels <20 ng/mL vs those with vitamin D >30 ng/mL.

All studies included in this analysis had an NOS for quality ≥6; thus, no sensitivity analysis was needed according to the quality of studies.

Visual inspection of funnel plots and the Egger test suggested the absence of publication bias and of small-study effect for studies comparing subjects with vitamin D <20 ng/mL with those with vitamin D >30 ng/mL (Egger $P$ = 0.431) (Supplemental Fig. 6). In contrast, a significant publication bias was found for studies comparing subjects with vitamin D from 20 to 30 ng/mL with subjects with vitamin D >30 ng/mL (Egger $P$ = 0.009) (Supplemental Fig. 6). The Duval and Tweedie trim and fill analysis (Supplemental Fig. 6) showed that, after adjusting for publication bias, a higher prevalence of PAD was confirmed in patients with vitamin D <20 ng/mL (OR: 1.46, 95% CI: 1.31 to 1.62) but not in those with vitamin D from 20 to 30 ng/mL (OR: 1.06, 95% CI: 0.98 to 1.15). Meta-regression models (Supplemental Table 4) showed that none of evaluated clinical and demographic characteristics influenced the outcome of interest.

## Discussion

In the present meta-analysis, we have investigated the association between vitamin D status and PAD. The only previous meta-analysis available on this topic (21) only included ~50% of available studies, was affected by publication bias, and did not provide an analysis of the prevalence of PAD in different vitamin D levels categories.

Results of our study consistently support the association between vitamin D status and PAD, also providing separate analyses for vitamin D deficiency and vitamin D insufficiency.

In two independent analyses performed pooling 15 studies, we found significantly lower vitamin D levels in patients with PAD as compared with non-PAD control subjects, and we documented an increasing risk of PAD for decreasing levels of vitamin D. We found that patients with vitamin D insufficiency and those with vitamin D deficiency had a 10% and 48% higher prevalence of PAD, respectively, as compared with those with normal levels of vitamin D.

These findings are confirmed and extended by the sensitivity analyses and regression models that consistently confirmed results of primary analyses and showed no confounding effect of major CV risk factors on the outcomes of interest. This suggests that the association between PAD and vitamin D levels can be considered independent by concomitant CV risk factors. The only variable influencing the difference in vitamin D levels

between patients with PAD and control subjects was the difference in the prevalence of male sex. Because this is a potential source of heterogeneity, we repeated the analysis specifically including studies with control subjects matched to patients with PAD, and the results were confirmed without heterogeneity among studies. Moreover, the significant association between vitamin D deficiency/insufficiency and PAD was consistently confirmed after adjusting for potential confounders by means of multivariate analysis.

Previously published studies reported an increased risk of major CV events and increased CV mortality in patients with vitamin D deficiency (39), suggesting that low vitamin D levels can be considered an independent CV risk factor (40, 41). In the current study, we have further supported this association by showing that reduced vitamin D levels are associated with PAD, which is widely recognized as clinical condition associated with CV and all-cause mortality, coronary heart disease, and stroke, independently of traditional CV risk factors (42, 43).

By a pathophysiological point of view, vitamin D deficiency exerts its effects on several phases of the atherosclerotic process, with direct effects on vessel wall cells and indirect effects through its association with proatherosclerotic risk factors such as hypertension, dyslipidemia, and insulin resistance (44–46). Vitamin D promotes the expression of antiatherogenic monocyte/macrophage subtypes and decreases production by endothelial and vascular smooth cells of several molecules involved in atherogenesis (*e.g.*, type I collagen, vascular endothelial growth factor, matrix metalloproteinases, elastin) (47). This evidence helps explain why reduced vitamin D levels are associated with increased formation and progression of atherosclerosis (41). Among its vascular protective effects, vitamin D has been shown to upregulate nitric oxide and to inhibit platelet aggregation (9, 41). In addition, vitamin D influences vascular remodeling and atherothrombosis by upregulating thrombomodulin expression (48) and by downregulating expression of plasminogen activator inhibitor-1 (49) and matrix metalloproteinase-2 and -9 (50). Moreover, recent data has shown that high interleukin-23 and low interleukin-10 levels are associated with PAD (51, 52). Vitamin D decreases the synthesis of IL-23 and increases the production of IL-10 (53), resulting in a decrease in Th1 cell responses and in a higher production of Tregs, thereby reducing inflammatory status (53). The experimental evidence reported herein strongly supports the biological plausibility of our findings, confirming the hypothesis that vitamin D deficiency may be a potential risk factor for PAD. However, whereas vitamin D supplementation was associated with a reduction of all-cause mortality (8), no effect on CV event reduction was documented (54). Moreover, some data suggested a favorable effect of vitamin D supplementation on endothelial dysfunction and arterial stiffness, but nothing is known about a potential effect on the prevention of PAD (55–57). Thus, in subjects with vitamin D deficiency, besides suggesting vitamin D supplementation, we should plan a strict monitoring of CV risk factors and of subclinical signs of atherosclerosis.

Some potential limitations of our study need to be discussed. First, the difference in vitamin D levels between patients with PAD and non-PAD subjects, although statistically significant, seems to be quite limited. However, it is relevant to highlight that vitamin D affects a wide variety of biological processes by influencing the genetic transcriptional profile (58, 59) without a linear relationship between circulating vitamin D levels and biological response (60).

Studies included in our meta-analysis have different inclusion and exclusion criteria, and most of the patients included in the analysis had concomitant CV risk factors. In the current study, we found that the difference in the prevalence of male sex between patients with PAD and control subjects was associated with a higher difference in vitamin D levels. Based on this result, we repeated the analysis including only studies with control subjects matched to patients with PAD, and all results were confirmed without heterogeneity among studies. However, because meta-analysis is performed on aggregate data and because some information is missing in each study, the meta-regression approach allowed for the adjustment for some, but not all, potential confounders. To overcome this potential limitation, we analyzed data derived from multivariate analysis on the cumulative risk of PAD according to vitamin D levels. The significant association between vitamin D deficiency/insufficiency and PAD was consistently confirmed after adjusting for potential confounders.

We have to take into account heterogeneity among studies. Substantial heterogeneity was documented among studies reporting vitamin D levels in patients with PAD and control subjects but not for studies reporting on the prevalence of PAD in different categories of vitamin D levels. Moreover, although it was not possible to conclusively ascertain the sources of heterogeneity, the presence of publication bias has been excluded for most of the outcomes assessed, and, when present, results were adjusted by means of the Duval and Tweedie trim and fill analysis. We have also tried to reduce the heterogeneity by performing sensitivity analyses that confirmed results of the primary analysis. When analyzing only prospective studies, we consistently confirmed reduced vitamin D levels in patients with PAD as compared with non-PAD control subjects, whereas the increased prevalence of

PAD was confirmed only in subjects with vitamin D deficiency (<20 ng/mL) and not in those with vitamin D insufficiency (20 to 30 ng/mL).

A further source of heterogeneity might be represented by the different criteria used to define PAD. As highlighted in Table 1, not all included studies defined PAD as ABI <0.9. To exclude this potential source of heterogeneity, the analyses were repeated including only studies using ABI to define PAD, and results were consistently confirmed.

In summary, our meta-analysis showed that patients with PAD have lower vitamin D levels than control subjects and that both vitamin D deficiency and vitamin D insufficiency are significantly associated with an increased prevalence of PAD. Thus, reduced vitamin D levels might represent an independent risk factor for PAD, and patients with impaired vitamin D status may benefit from a more meticulous screening for CV risk factors and more specific CV prevention strategy. However, additional and specifically designed studies are needed to establish the optimal management of these patients.

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*Author Contributions:* M.N.D.D.M. conceived and designed the study, performed statistical analysis, interpreted results, and drafted the manuscript. R.L. reviewed literature data, interpreted the results, drafted the manuscript, and performed critical revisions. G.I. and F.F. acquired clinical data and drafted the manuscript. All authors read and approved the final version of the manuscript. G.I. and F.F. had full access to all data in the study and take responsibility for the integrity of the original data.

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