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Effect of a Single, Oral, High-dose Vitamin D Supplementation on Endothelial Function in Patients with Peripheral Arterial Disease: A Randomised Controlled Pilot Study

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WHAT THIS PAPER ADDS

- In this study, although underpowered for definite conclusions, high-dose vitamin D substitution did not alter parameters of arterial stiffness, microcirculation, inflammation and haemostasis in patients with peripheral arterial disease, adding more data to other studies that did not confirm a causal role of vitamin D in cardiovascular disease. Another result is a striking difference in the individual response of serum vitamin D levels after a single, oral supplementation.

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ABSTRACT

Objective: Apart from its role in bone metabolism, vitamin D may also influence cardiovascular disease. The objective of this study was: (1) to determine the effect of a single, oral, high-dose vitamin D supplementation on endothelial function and arterial stiffness in patients with peripheral arterial disease (PAD) and (2) to investigate the impact of this supplementation on coagulation and inflammation parameters.

Methods: In this double-blind, placebo-controlled, interventional pilot study, we screened 76 Caucasian patients with PAD for vitamin D deficiency. Sixty-two were randomised to receive a single, oral supplementation of 100 000 IU vitamin D3 or placebo. At baseline and after 1 month, we measured serum vitamin D and parathormone levels, and surrogate parameters for cardiovascular disease.

Results: Sixty-five of 76 patients (86%) had low 25-hydroxyvitamin D levels (<30 ng ml^{-1}); of those, 62 agreed to participate in the study. At baseline, only parathormone was related to vitamin D. In supplemented patients, vitamin D levels increased from 16.3 ± 6.7 to 24.3 ± 6.2 ng ml^{-1} ($P < 0.001$), with wide variations between single patients; in the placebo group vitamin levels did not change. Seasonal factors accounted for a decrease of vitamin D levels by 8 ng ml^{-1} between summer and winter. After 1 month, none of the measured parameters was influenced by vitamin substitution.

Conclusion: In this pilot study, most patients with PAD were vitamin D deficient. Vitamin D supplementation increased serum 25-hydroxyvitamin D without influencing endothelial function, arterial stiffness, coagulation and inflammation parameters, although the study was underpowered for definite conclusions.

Peripheral arterial disease (PAD), a clinical manifestation of atherosclerosis, is associated with a high risk of morbidity and mortality from cardiovascular disease.[1] The development and progression of PAD is mediated by pathophysiological mechanisms involving atherogenesis and thrombogenesis, leading to endothelial damage and dysfunction, increased arterial stiffness and acute thrombotic complications.[2] Traditional risk factors for PAD, similarly to other manifestations of atherosclerosis, include diabetes

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mellitus, smoking, hypertension and dyslipidaemia,[3] and other parameters such as C-reactive protein (CRP) and homocysteine were shown to be associated with PAD.[4,5]

Apart from its well-known role in bone and calcium metabolism, vitamin D deficiency has recently received widespread attention for its potential role as a risk factor in cardiovascular disease[6] and for its association with traditional cardiovascular risk factors.[7] Particularly, low serum 25-hydroxyvitamin D (25-OH vitamin D) levels were associated with a higher prevalence of PAD.[8]

The serum 25-OH vitamin D concentration is accepted as the nutritional parameter of vitamin D status, but controversy exists about the definition of vitamin D deficiency. A recent recommendation defined the optimal vitamin D concentration to prevent osteoporosis to be at least 30 ng ml^{-1}.[9]

In contrast with the positive results from cross-sectional or observational studies, data from randomised controlled trials are limited and conflicting[10,11] or did not show an association of low vitamin D levels with cardiovascular mortality.[12] In a recent statement, therefore, it was felt that the evidence for vitamin D to reduce the risk of extra skeletal disease was as yet inconsistent.[13]

We performed a randomised, double-blind, placebo-controlled interventional pilot study in patients with PAD and vitamin D deficiency with the objective to determine the effect of a single, oral, high-dose vitamin D supplementation on surrogate markers of endothelial function and arterial stiffness, known to correlate with cardiovascular outcome[14] and to investigate the impact of this supplementation on coagulation and inflammation parameters.

Materials and Methods

Subjects

The study was conducted at the regional hospital of Locarno between May 2010 and February 2011. The protocol was approved by the local Ethics Committee. Caucasian patients with chronic PAD (defined as an ankle–brachial index <0.9 plus a duplex or angiographically based verification of a $>50\%$ stenosis or occlusion in a leg artery) were screened for vitamin D deficiency. Inclusion criteria were: (1) a serum 25-hydroxyvitamin D level <30 ng ml^{-1}, (2) unchanged medication in the 6 weeks prior to the study, (3) no vascular interventions in the 2 months preceding the study and (4) informed written consent. Exclusion criteria were: (1) acute intercurrent illness, (2) chronic critical ischaemia, (3) thromboangiitis obliterans, (4) renal insufficiency defined as serum creatinine >130 μmol l^{-1}, (5) acute myocardial infarction or stroke within 2 months, (6) current oral anticoagulation medication, (7) liver cirrhosis and (8) presence of an active malignant tumour. Furthermore, patients were excluded if their medication was changed during the study period.

At baseline and at the end of the study after 1 month, clinical examination, blood collection and specific tests (see below) were performed in all patients. All participants were instructed not to expose themselves to sunlight during the study month. The study had a double-blinded, randomised, parallel group design. After randomisation in blocks of four (http://www.randomization.com), half of the patients were given a vial containing 100 000 IU of a single dose of an oily solution of vitamin D3 (cholecalciferol), thus ensuring 100% compliance, the other half placebo (triglyceride and ethylis gallas), both provided by Streuli Pharma AG (Uznach, Switzerland). The vials were stored in the hospital's pharmacy; patients were allocated by the pharmacist to vitamin D or placebo following the randomisation list, which was concealed to physician and patient.

Haemodynamic measurements

Endothelial function was assessed by measuring skin blood flow after ischaemic challenge using a laser Doppler flowmeter (PF 3, Perimed AB, Sweden), according to the method of Binggeli et al.[15] Fasting patients were examined between 8:30 a.m. and 9:30 a.m. in a room with constant ambient temperature, after a 15-min resting period in a supine position. Reactive hyperaemia in skin microcirculation has been validated as a measure related to other established methods of endothelial function testing.[15] Baseline blood flow measured as arbitrary units of laser Doppler flux (LDF) was registered for 5 min using a probe fixed by a probe-holder close to the wrist. After 5 min, a cuff placed around the arm was left inflated for 4.5 min. After release of the cuff, reactive hyperaemic blood flow was registered, and the difference of blood flow between baseline and the mean flow between 30 and 60 s after release was registered.

Central blood pressure using radial artery applanation tonometry and sphygmocardiography (SphygmocCor version 7.1, AtCor, Sydney, Australia) was measured according to the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) protocol, by using a validated, semiautomatic oscillometric device (Omron 705CP, Omron, Amsterdam, the Netherlands) for brachial blood pressure. After a 5-min rest, blood pressure over the brachial artery was measured 3 times at 5-min intervals in seated patients, using the mean of the last two measures. Hence, radial artery pressure waveforms of the same arm were recorded over 10 s with a Millar tonometer (SPC-301, Millar Instruments, Houston, TX, USA), and waveforms were processed with the SygmoCor software to obtain the corresponding central aortic-pressure waveform. From the parameters provided by the software, augmentation index, a parameter of arterial stiffness (AIx; the ratio of augmentation to central pulse pressure, which is considered a marker of arterial stiffening),[14] corrected for heart rate, was obtained. The mean of at least two consecutive radial pressure wave samplings were used for calculating central pressure.

Laboratory analysis

At baseline and at the end of the study, blood was drawn from an antecubital vein between 8 a.m. and 9 a.m. after 15 min of rest in a supine position. Blood was collected in a 10-ml plastic syringe (Monovette, Sarstedt, Nümbrecht, Germany) containing 1 ml 0.106 M trisodium citrate. Blood samples were immediately placed on melting ice. Plasma was separated by cold centrifugation, and aliquots were stored at -70 °C until processing.

F1+2 (Enzygnost F1+2; Dade Behring, Marburg, Germany), thrombin–antithrombin complex (TAT) (Enzygnost TAT micro, Dade Behring), D-dimer (miniVidas by bioMérieux, Lyon, France), plasminogen activator inhibitor-1 (PAI-1) (Biopool, Umea, Sweden) and Soluble CD40L (sCD40L) (Biosource, Camarillo, CA, USA) were determined by enzyme linked immunosorbent assay (ELISA) according to the protocols of the manufacturers. Plasma homocysteine (Hcy) was determined by high-performance liquid chromatography and fluorescence detection. Ultrasensitive CRP (hs-CRP) was measured by nephelometry (Dade Behring, Marburg, Germany). Total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol as well as triglyceride (TG) were measured with standard colourimetric methods (Roche Diagnostics, Zug, Switzerland). Ionised calcium, phosphorus, and creatinine were measured by standard methods.

25-OH vitamin D was measured in serum by a radioimmunoassay (RIA) double antibody assay (DiaSorin, Saluggia, Italy), which has an excellent agreement with high-performance liquid chromatography. Plasma intact parathormone (iPTH) was

measured by paramagnetic particle, chemiluminescent immunoassay (Access Intact PTH, Beckman Coulter, Inc., Fullerton, CA, USA).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). For baseline variables, continuous variables were compared using an independent *t*-test and categorical variables were compared using the chi-square test. Variables before and 1 month after substitution were compared using a paired *t*-test. Multiple linear regression analysis was performed when looking for parameters influenced by vitamin D concentration. Furthermore, one-way analysis of variance (ANOVA) was used to assess the relationship between quartiles of vitamin D concentration at baseline and their respective values of the measured parameters. Finally, a power analysis based on the results of this study was done to look for the number of patients to be included into a study to detect a change in augmentation index. Statistic was performed with Statistical Package for Social Sciences (SPSS) version 19.

Results

From a total of 76 consecutive patients with PAD, 65 (86%) resulted vitamin D deficient (25-OH vitamin D <30 ng ml^{-1}), with 20 patients (31%) showing a 25-OH vitamin D level <15 ng ml^{-1}. Three patients did not give consent for the study, leaving 31 subjects to be included for each group (Fig. 1). Thirty-two patients were included in June and August, the other 30 from September to February. The baseline details are presented in Tables 1–3. At baseline, no significant differences were found in 25-OH vitamin D and iPTH levels, haemodynamic parameters including peripheral and central blood pressure, endothelial function (LDF variation after ischaemic challenge), parameters of coagulation and inflammation, medications as well as cardiovascular risk factors. At baseline, 25-OH vitamin D levels correlated only with iPTH ($r = -0.4$, $P = 0.001$) and not with other parameters, including calcium and phosphate.

Single, oral, vitamin D administration of 100 000 IU significantly increased 25-OH vitamin D levels compared with placebo by about 50% (+8 vs. +0.5 ng ml^{-1}, $P < 0.001$), but individual responses greatly varied (Fig. 2). In nine patients (5 (17%) from the vitamin D group and 4 (13%) from the placebo group), 25-OH vitamin D levels were in the normal range (i.e., >30 ng ml^{-1}) after 1 month; all patients were recruited during summer. In a multivariate regression model, serum 25-OH vitamin D concentration at 1 month was related to its baseline level, to vitamin D substitution and to seasonal factors, which accounted for a decrease of 8 ng/ml between summer and winter, for an R^2 of 0.55 in this model ($P < 0.0001$).

There was no other significant correlation with 25-OH vitamin D levels when dividing the parameters in quartiles (data not shown).

Metabolic parameters associated with vitamin D effects, surrogates of inflammation and haemostasis and haemodynamic parameters did not change significantly after vitamin D substitution between the two groups (Tables 4 and 5). However, there was a trend for microcirculatory improvement after vitamin D supplementation with a post-ischaemic increase of LDF from 13.5 ± 16.8 to 20.6 ± 16.0 AU ($P = 0.097$) (difference of LDF flux after ischaemic challenge between groups: 8.0 AU, confidence interval (CI): −1.1 to 17.2, $P = 0.06$, Table 4). No side effects were observed during the study in either group of patients. A calculation of the sample size necessary to have an 80% power to detect a difference in the means of the augmentation index yielded 253 subjects per group, for a total sample size of about 500 patients.

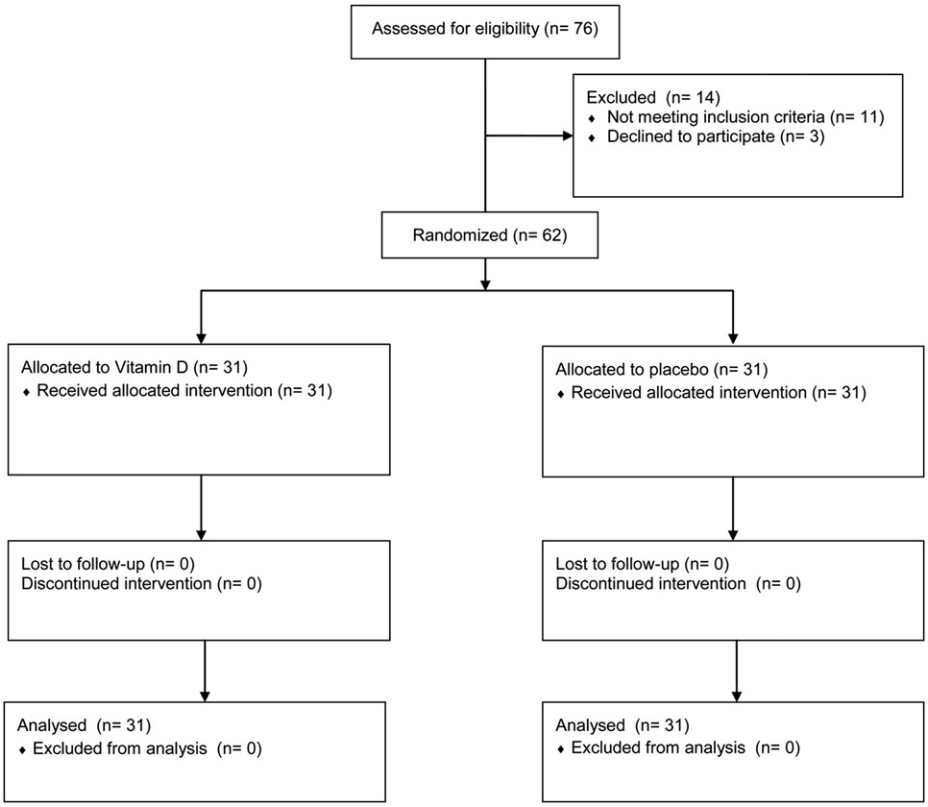

Fig. 1. Flow diagram.

Table 1
Baseline demographic characteristics.

	Control group ($n = 31$)	Vitamin D supplementation group ($n = 31$)	*P* value
Age (years)	74.8 ± 14.6	72.9 ± 8.7	0.5
Male sex (%)	19/31 (61)	19/31 (61)	0.94
Smoker (yes/no/ex)	14/9/8	8/9/14	
Diabetes mellitus (%)	7 (23)	10 (32)	0.57
Hypertension (%)	23 (74)	24 (77)	0.77
Aspirin /Clopidogrel (%)	31 (100)	31 (100)	1
Statins (%)	28 (90)	23 (74)	0.18
ACE-I/Sartane (%)	19 (61)	18 (53)	1
b-Blocker (%)	12 (39)	12 (39)	1
Ca-antagonist (%)	6 (19)	7 (23)	1
Insulin (%)	1 (3)	3 (10)	0.6
Oral antidiabetics (%)	3 (10)	7 (23)	0.3

Baseline demographic characteristics (mean ± SD) in the control ($n = 31$) and the vitamin D supplementation group ($n = 31$).

Table 3
Baseline laboratory characteristics.

	Control group ($n = 31$)	Vitamin D supplementation group ($n = 31$)	*P* value
25-OH-vitamin D (ng/ml)	17.0 ± 5.5	16.3 ± 6.7	0.63
iPTH (pmol/L)	5.5 ± 2.1	5.4 ± 3.2	0.92
Calcium, ionized (mmol/L)	1.2 ± 0.05	1.19 ± 0.04	0.55
Phosphorus (mmol/L)	1.13 ± 0.17	1.09 ± 0.17	0.32
Creatinine (μmol/L)	79 ± 23.7	82.5 ± 22.1	0.56
LDL-cholesterol (mmol/L)	2.62 ± 1.0	2.85 ± 1.13	0.4
hs-CRP (mg/L)	3.7 ± 5.2	2.7 ± 2.8	0.35
Homocysteine, total (μmol/L)	15.3 ± 5.8	14.1 ± 5.1	0.4
TAT (μg/L)	6.76 ± 3.76	6.95 ± 5.79	0.88
F1+2 (pmol/L)	230 ± 94.7	251 ± 132.9	0.49
D-dimer (ng/ml)	0.81 ± 0.51	1.0 ± 1.25	0.44
PAI-1 (pg/ml)	663 ± 431.1	653 ± 552.0	0.93
sCD40L (ng/ml)	0.22 ± 0.22	0.27 ± 0.26	0.5

Baseline laboratory characteristics (mean ± SD) in the control ($n = 31$) and the vitamin D supplementation group ($n = 31$). iPTH denotes intact parathormone; TAT, thrombin antithrombin complex; F1+2, prothrombin fragment 1 + 2; PAI-1, plasminogen activator inhibitor-1.

Discussion

In observational studies, reviews and meta-analysis, vitamin D deficiency was associated with cardiovascular outcomes and mortality,[6,11,16] venous thrombo-embolism,[17] arterial stiffness and vascular dysfunction[14,18] as well as inflammatory parameters.[19] However, observational studies might be subject to bias, and randomised, controlled trials are felt to provide more reliable data by reducing the possibility of confounding or selection bias.[20]

In this double-blinded, randomised, interventional pilot study addressing elderly patients with PAD, a single, oral supplementation with 100 000 IU of vitamin D3 significantly increased serum vitamin concentration by 50%, interestingly without a decrease in iPTH concentration. Although we used a high-dose supplementation, only 17% of the patients (5 of 31) reached the target concentration of 30 ng ml^{-1}, and all of them were randomised during summer.

The main objective of this study was to assess the impact of vitamin D substitution on cardiovascular surrogate parameters. Moreover, in the case of insignificant variations of these markers, a power calculation based on our data would have permitted estimating the number of patients to be included in future trials. Based on a previous study, where endothelial function, measured by flow-mediated vasodilation, and blood pressure improved in 17 diabetic, vitamin D-deficient patients substituted with a single dose of 100 000 U of vitamin D2,[21] we felt that the number of patients included in this study would have been adequate. AIx, among other parameters, is considered to be a complex and composite marker of arterial stiffening; furthermore, endothelial dysfunction and arterial stiffness have been shown to precede and contribute to the development of cardiovascular disease.[14] AIx measurement is less operator-dependent compared to the assessment of endothelial function and has shown excellent inverse correlation with vitamin D concentration,[14] which prompted us to use AIx for power calculation. Based on these premises and on our data, we could not demonstrate a decrease of central and peripheral blood pressure by vitamin D supplementation, nor could we show an improvement in arterial stiffness and in endothelial function, with unchanged values for AIx and difference in LDF, respectively. We feel that the necessity for a study with a surrogate marker to include >500 subjects, as resulted from our data, may point to a potentially low impact of vitamin D substitution on clinical outcomes.

A second objective of our study was to assess the impact of a vitamin D supplementation on coagulation and inflammation, by measuring TAT and F1+2 (markers for thrombin generation), D-dimer and PAI-1 (markers for fibrinolysis), sCD40L (marker for platelet activation), hsCRP (marker for inflammation) and Hcy. We

Table 2
Baseline haemodynamic characteristics.

	Control group ($n = 31$)	Vitamin D supplementation group ($n = 31$)	*P* value
P-SBP (mmHg)	140 ± 18.7	133 ± 18.5	0.16
P-DBP (mmHg)	76 ± 8.0	73 ± 8.2	0.17
Heart rate (bpm)	65 ± 10	67 ± 11	0.38
C-SBP (mmHg)	132 ± 17.1	124 ± 17.1	0.11
C-DBP (mmHg)	77 ± 8.0	74 ± 8.3	0.18
AIx (%)	39.5 ± 7.1	38.6 ± 7.3	0.77
Δ LDF (AU)	15.2 ± 14.0	13.5 ± 16.8	0.66

Baseline haemodynamic characteristics (mean ± SD) in the control ($n = 31$) and the vitamin D supplementation group ($n = 31$). P-SBP denotes peripheral systolic blood pressure; P-DBP, peripheral diastolic blood pressure; C-SBP, central (aortic) systolic blood pressure; C-DBP, central diastolic blood pressure; AIx, aortic augmentation index; Δ LDF, postischemic increase of laser Doppler flux.

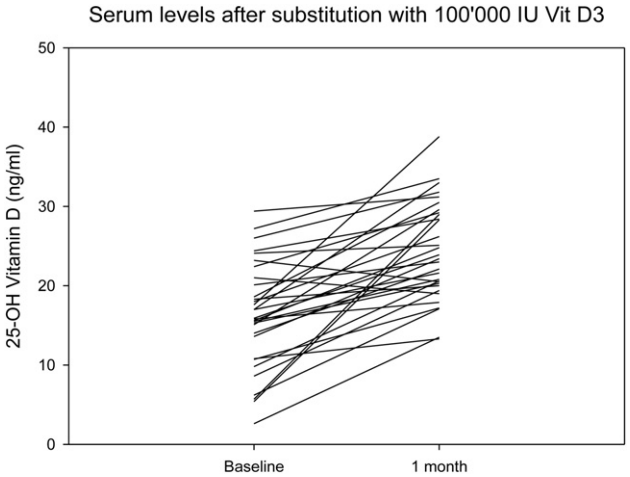

Fig. 2. Individual serum levels of 25-OH vitamin D ad baseline and 1 month after supplementation with 100 000 IU of oral vitamin D3.

Table 4
Blood pressure, aortic stiffness and endothelial function before and after supplementation.

	Control group			Vitamin D group		
	Baseline	After 1 month	*P* value	Baseline	After 1 month	*P* value
P-SBP (mmHg)	140 ± 18.7	143 ± 18.2	0.27	133 ± 18.5	136 ± 18.7	0.24
P-DBP (mmHg)	76 ± 8.0	76 ± 10.1	1	73 ± 8.2	73 ± 8.1	0.74
C-SBP (mmHg)	132 ± 17.1	134 ± 17.8	0.28	124 ± 17.1	127 ± 18.0	0.37
C-DBP (mmHg)	77 ± 8.0	77 ± 10.3	0.99	74 ± 8.3	74 ± 8.3	0.61
AIx (%)	39.5 ± 7.1	38.6 ± 7.2	0.65	38.6 ± 7.3	38.5 ± 8.7	0.93
Δ LDF (AU)	15.2 ± 14.0	14.3 ± 10.8	0.77	13.5 ± 16.8	20.6 ± 16.0*	0.097

Haemodynamic parameters at baseline and 1 month after supplementation with 100 000 IU of oral vitamin D3 or placebo. P-SBP denotes peripheral systolic blood pressure; P-DBP, peripheral diastolic blood pressure; C-SBP, central (aortic) systolic blood pressure; C-DBP, central diastolic blood pressure; AIx, aortic augmentation index; Δ LDF, postischemic increase of laser Doppler flux.
*P value = 0.06 for difference between groups.

could not demonstrate a significant change in these parameters. We are aware of only one study where the influence of vitamin D supplementation on parameters of thrombosis was analysed; no change of plasma levels of PAI-1, tPA Ag and thrombin generation was observed.[22]

These findings are in contrast with previous studies.[21,23] In a similar study, Pfeifer et al. demonstrated a 9% decrease in systolic blood pressure 6 weeks after giving vitamin D supplements to severely vitamin-D-deficient women whose serum vitamin level increased to 26 ng ml^{-1}.[23]

Other studies, however, support our results. In a randomised, controlled interventional trial, a 4-monthly oral 100 000 IU vitamin D3 substitution during 5 years in subjects more than 65 years old had no effect on total or cardiovascular mortality but prevented fractures.[24] In the RECORD (Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia in Diabetes) trial, 5292 patients aged at least 70 years with osteoporotic fractures were randomised to 800 IU vitamin D3 daily, 100 mg of calcium, both, or placebo. No effect on mortality or vascular disease was detected after a 3 years' follow-up.[25] In a recent systematic review and meta-analysis of 51 randomised interventional trials, Elamin et al. did not find a significant effect of vitamin D supplementation on death, stroke, myocardial infarction, lipids and blood pressure.[26]

Our negative result deserves some comments. First, our patients were well treated for their cardiovascular risk factors with 82% of the whole population taking statins and a majority of the hypertensive patients treated with angiotensin-converting enzyme (ACE) inhibitors, sartanes or calcium antagonists, which may have left no space for further improvements of surrogate parameters. Second, established and widespread cardiovascular disease could have led to irreversible alterations of the vascular wall, which could have hampered a positive response to develop. Third, the short follow-up period could have blunted a possible response, but a longer observation period would have raised concerns about confounding factors such as sun exposure, and previous studies found a reducing effect on blood pressure by vitamin D substitution after a similar period of time.[21] Taken together, our findings do not support the hypothesis that vitamin D substitution has a decisive effect on cardiovascular risk factors, although the study was underpowered to definitively exclude such an influence.

In this study, a single, oral dose of 25-OH cholecalciferol increased serum vitamin D levels by 50%, and seasonal factors accounted for a decrease of about 8 ng/ml between summer and winter, which may cause a 30–50% variation in individual serum vitamin levels throughout a year. Vitamin D deficiency was frequently observed in our patients suffering from PAD, with 86% of serum levels below 30 ng/ml. Interestingly, individual responses to supplementation greatly varied (Fig. 2). The factors known to influence vitamin D levels in healthy subjects include age, body weight, race, sun exposure and probably also genetic factors.[27]

There are several strengths in our study. In this well-defined subset of cardiovascular patients with PAD, we did an interventional study with a single, oral dose of vitamin D, which circumvents problems with patient adherence regularly found in long-term studies. Although a single dose often fails to attain a desired serum 25-OH vitamin D concentration,[28] even in longer-range studies serum levels did not increase by more than 40%.[24] We acknowledge, however, that a concentration-driven supplementation of vitamin D, by obtaining serum levels above a pre-specified threshold, would have resulted in a more irreprehensible interpretation of our data.

Seasonal influences on sun exposure of the patients is a weakness in this trial and could have diluted our results, but the absence largely found of an association between vitamin D levels and cardiovascular surrogate parameters make this a very remote hypothesis.

In summary, although a single, high-dose oral supplementation with 25-OH cholecalciferol increased serum vitamin levels by 50% after 1 month, there was no change in cardiovascular surrogate parameters in vitamin D-deficient patients with PAD. Individual response of serum concentrations greatly varied. A sufficiently powered study is needed to definitely exclude an influence of vitamin D on cardiovascular surrogate parameters such as arterial stiffness. The final answer whether substitution of vitamin D has an impact on cardiovascular disease must attend the results of the eagerly awaited ongoing large-scale interventional trials.

Table 5
Coagulation and inflammation parameters before and after supplementation.

	Control group			Vitamin D group		
	Baseline	After 1 month	*P* value	Baseline	After 1 month	*P* value
TAT (μg/L)	6.76 ± 3.76	9.0 ± 6.2	0.1	6.95 ± 5.8	7.1 ± 3.5	0.94
F1+2 (pmol/L)	230 ± 94.7	229 ± 100.4	0.97	251 ± 132.9	277 ± 191.6	0.54
D-Dimer (ng/ml)	0.81 ± 0.51	0.81 ± 0.45	0.97	1.0 ± 1.25	0.89 ± 1.22	0.94
PAI-1 (pg/ml)	663 ± 431.1	630 ± 408.5	0.76	653 ± 552.0	567 ± 341.7	0.47
sCD40L (ng/ml)	0.22 ± 0.22	0.2 ± 0.19	0.68	0.27 ± 0.26	0.3 ± 0.28	0.66
Hcy (μmol/L)	15.3 ± 5.2	15.7 ± 5.7	0.71	14.2 ± 5.1	13.8 ± 4.8	0.64
hsCRP (mg/L)	3.7 ± 5.2	2.8 ± 2.8	0.42	2.7 ± 2.8	3.0 ± 2.3	0.61

Coagulation and inflammation parameters at baseline and 1 month after supplementation with 100 000 IU of oral vitamin D3 or placebo. TAT denotes thrombin antithrombin complex; F1+2, prothrombin fragment 1 + 2; PAI-1, plasminogen activator inhibitor-1; Hcy, total homocysteine.

Conflict of Interest

All authors declare no conflicts of interest.

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