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Vitamin D status was not associated with 'one-year' progression of coronary artery disease, assessed by coronary angiography in statin-treated patients

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Abstract

Background: Low vitamin D status is associated with increased risk of cardiovascular disease and may be involved in atherosclerosis. Our aim was to assess the association between vitamin D status and the progression of coronary artery disease (CAD).

Methods and Results: We measured 25-hydroxyvitamin D3 (25OHD3) by liquid chromatography tandem mass spectrometry (LC-MS/MS) in plasma from 348 participants with established CAD (84% males, mean ± standard deviation (SD) age 60 ± 10 years) of the Western Norway B-vitamin Intervention Trial (WENBIT, 1999–2006). The patients underwent invasive coronary angiography (CA) and percutaneous coronary intervention at baseline and a second CA after 302 ± 79 days of follow-up. From the angiograms, minimal lumen diameter (MLD) and diameter stenosis (DS) of atherosclerotic lesions were obtained. Significant CAD in non-intervened vessels was found in 309 coronary arteries from 183 participants either at baseline and/or at follow-up. To assess the association between levels of 25OHD3 and CAD progression in non-intervened vessels, we applied a linear quantile fitted mixed effects model with MLD or DS measured at follow-up as a function of continuous 25OHD3 concentrations.

There were no statistically significant associations between plasma 25OHD3 concentrations (median: 63.9, 95% confidence interval (CI): 48.1–78.5 nmol/l) measured at baseline and the follow-up measures of either MLD (estimated effect per 10 nmol/l increase of 25OHD3 and 95% CI: –0.015 (−0.032–0.002) mm, $p = 0.088$) or DS (0.225 (−0.354–0.804) percentage points, $p = 0.444$). Multivariate adjustment did not alter these results.

Conclusion: Plasma 25OHD3 levels were not associated with 'one-year' progression of CAD, assessed by CA in statin-treated patients.

Keywords

Vitamin D, 25-hydroxyvitamin D, calcifediol, coronary artery disease, atherosclerosis, disease progression, coronary angiography

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Introduction

Ischaemic heart disease has remained the leading cause of death and disability throughout the world for the past decades.[1,2] Observation data of seasonal variation of coronary artery events suggests that vitamin D status could be related.[3,4] Vitamin D status is best indicated by the concentration of the intermediate metabolite 25-hydroxyvitamin D (25OHD). 25OHD has longer half-life than the active metabolite 1,25 dihydroxyvitamin D

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and reflects endogenous synthesis due to ultraviolet B exposure and also dietary intake.[5] Based on its role in skeletal health, vitamin D status is commonly regarded as deficient if below 30 nmol/l (12 ng/ml), sufficient if serum levels exceed 50 nmol/l (20 ng/ml) and inadequate when between 30–50 nmol/l (12–20 ng/ml).[5] Vitamin D inadequacy is common in Norway, although to a lesser extent than that reported in central Europe.[6]

Today there is epidemiological evidence linking lower levels of 25OHD with increased risk of cardiovascular disease (CVD) and mortality.[7–9] Vitamin D activity is dependent on the vitamin D receptor (VDR), found to be distributed in different cells types involved in atherogenesis (macrophages, vascular smooth muscle cells, endothelial cells).[10–12] Various cell types carrying the VDR are directly involved in the atherosclerotic process in the arterial wall, thus implicating vitamin D status in atherosclerosis by the regulation of differentiation, proliferation and apoptosis of these cells.[13–15] Reduced activity of VDR can induce deleterious effects on cardiovascular health, as observed in the VDR-deficient mouse.[16]

We aimed to investigate whether vitamin D status, measured as plasma 25OHD3 concentrations, was related to progression of coronary artery disease (CAD), as measured by repeated conventional coronary angiography (CA), after nearly one year of follow-up.

Methods

Study population

The population in this study is a selection of patients from the Western Norway B-Vitamin Intervention Trial (WENBIT; ClinicalTrials.gov number NCT00354081) who underwent repeat CA during the trial. The WENBIT (1999–2006) was a randomised, double-blind placebo controlled trial conducted at two university hospitals in western Norway. A total of 3090 adult (79.5% men) patients undergoing elective CA due to suspected CAD were recruited and randomized to four groups, A–D (A: folic acid, vitamin B12 and vitamin B6; B: folic acid and vitamin B12; C: vitamin B6; D: placebo). The objective was to assess the effect of homocysteine-lowering B-vitamins on cardiovascular events, using a composite endpoint of all-cause death, nonfatal acute myocardial infarction, acute hospitalisation for unstable angina pectoris and nonfatal thromboembolic stroke. The exclusion criteria in the original cohort were suspected alcohol abuse, mental illness and known active malignant disease, as well as inability or reluctance to participate in the prospective longitudinal design. There was no overall effect of B-vitamins on cardiovascular risk.[17] WENBIT and the reangiography substudy were conducted according to the Declaration of Helsinki and approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Medicines Agency and the Data Inspectorate, and all participants provided written consent.

The reangiography substudy was primarily designed to provide information on atherosclerosis progression and the effect on progression by B-vitamin intervention. No overall effects of the homocysteine-lowering B-vitamins were found in the substudy, but a post-hoc test showed that treatment with folic acid in combination with vitamin B12 was associated with more rapid progression of atherosclerosis.[18] The substudy population was recently also used to observe associations between atherosclerosis progression and plasma levels of methylated amino acids.[19] In the present study, we have assessed the relation between atherosclerotic progression and vitamin D status in atherosclerotic lesions not treated with percutaneous coronary intervention (PCI).

Of the 3090 patients in WENBIT, 1359 (44%) underwent PCI. Among PCI-treated patients, 894 (66%) were treated at Haukeland University Hospital, Bergen and 465 (34%) at Stavanger University Hospital. The reangiography substudy of patients treated with PCI was performed at Haukeland University Hospital between October 2001–May 2004. The time frame for enrolment in the substudy was somewhat shorter compared to that of the main WENBIT trial (1999–2004). Accordingly, there were 570 patients treated with PCI at Haukeland within this time frame. A flow chart of the present study is included (Supplementary Material, Figure 1). Of the 570 patients, 199 patients were not eligible for a second CA according to predefined criteria or due to unacceptable high risk of complications associated with re-examination. Among the 371 eligible for a follow-up CA, 342 participants completed the examination, which coincided with the follow-up of the main WENBIT trial, approximately 10–11 months after inclusion. A total of 29 patients had an angiography before the scheduled re-examination due to clinical indications. Participants with a follow-up of less than 90 days ($n = 13$), with follow-up angiograms considered unsuitable for quantitative analysis ($n = 9$) or who withdrew consent ($n = 1$) were excluded, leaving a total of 348 participants eligible. Participants scheduled for the follow-up substudy provided additional written consent.

CA

A total of 16 coronary segments from the baseline and follow-up angiograms were analysed using quantitative

coronary angiography (QCA), including the 15 segments supported by the American Heart Association[20] and the right atrioventricular branch. The analysis was performed by two trained technicians who were blinded to the randomisation. Images were selected from end-diastole using digitalized QCA (Quantacor QCA, CAAS II V 5.0, Pie Medical Imaging, The Netherlands). If the characteristic of an individual stenosis differed between projections, the most severe was selected for analysis. The use of computer-defined obstruction analysis without manual contour correction was used as needed, with the exception of ostial stenosis and branched artery segments which required manually-defined obstruction analysis and manual correction of vessel contour, respectively. Calibration was performed with the tip of the catheter filled with contrast. To fulfil QCA analysis criteria, a single lesion had to be adequately visualised on baseline and follow-up angiograms. This is a prerequisite for measuring progression of CAD from baseline to follow-up. Furthermore, the lesions had to be non-PCI treated, as the progression of treated lesions might differ from non-treated. Finally, we only included lesions that either at the baseline or the follow-up angiography had a reference diameter of $\geq$2 mm and resulted in a diameter reduction of $\geq$30% compared to a healthy section of the same segment. The final criteria allowed the inclusion of new lesions that developed during follow-up. Reanalysis of a lesion by both technicians were performed if they disagreed on the eligibility of a lesion.

Outcome

The selected endpoints were minimum lumen diameter (MLD) and diameter stenosis (DS). MLD is the smallest measurable diameter of the lumen of the coronary artery at the site of atherosclerosis. DS is the degree of intrusion of the atherosclerotic lesion in to the lumen when using a healthy part of the same coronary segment as reference. Details on the methodological aspects of the angiographic measurements and inter-observer reliability are provided elsewhere.[18]

Laboratory assessment

The baseline samples were drawn 1–3 days prior to the CA at Haukeland University Hospital, Bergen. Venous blood samples were handled straightaway and stored in 2 ml Vacutainer tubes (Becton, Dickinson and Company, USA) at −80°C. Samples taken at baseline were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS)[21] in the laboratory of Bevital AS (www.bevital.no). Ethylenediaminetetraacetic acid (EDTA) plasma was analysed for 25OHD2 and 25OHD3. Only three participants had plasma 25OHD2 concentrations above the lower limit of detection of 6.6 nmol/l (2.6 ng/ml). Therefore, we did not aggregate the 25OHD2 and 25OHD3 concentrations, but included only 25OHD3 concentrations as a measure of vitamin D status. Bevital is certified by the Vitamin D External Quality Assessment Scheme (DEQAS, www.deqas.org). Plasma and serum 25OHD are stable at −20°C for at least 10 years and stable in room temperature to the extent that samples may be shipped by ordinary post between laboratories.[22]

The estimated glomerular filtration rate (eGFR) was calculated using the formula suggested by the Chronic Kidney Disease Epidemiology Collaboration.[23] An ultrasensitive immunoassay was used to measure serum C-reactive protein (CRP) on the Behring nephelometer II system (Detection limit of 0.17 mg/l and coefficient of variation of 8.1–11.4%; N Latex CRP mono, Behring Diagnostics, Marburg, Germany). Measurements of other biochemical variables were done with standard methods and are described elsewhere.[17]

Covariates

Self-administered questionnaires were administrated at enrolment and gave information regarding medical history, lifestyle factors and were compared to hospital records if possible. Measurements of anthropometry, blood pressure and collection and handling of blood samples were performed by study personnel. Smoking status was based on self-report and current smoker defined as smoker or stopped smoking less than 90 days ago. Left ventricular ejection fraction (LVEF) was measured by echocardiography or by ventriculography during cardiac catheterization.

Statistical analysis

MLD or DS from the second CA were both selected as primary endpoints for the purpose of this study. Due to outliers and non-normal distributions, we chose linear quantile mixed models, which provide a non-parametric approach to regression analysis, to assess the relationship between endpoints and explanatory variables. The characteristics of participants represented by multiple atherosclerotic lesions could be given more weight in the regression analysis than patients represented by only one lesion. We therefore adjusted for within-person clustering of lesions by modelling this as a random effect. In model 1, we applied the linear quantile mixed model (LQMM) with median MLD or DS measured at follow-up as the response variable, adjusting for the baseline values of MLD or DS, 25OHD3, and within-person clustering of

atherosclerotic lesions. In model 2, we further adjusted for factors known to correlate with 25OHD3 concentrations, including gender, age, smoking (current smoker or smoker quitting ≤90 days ago), body mass index (kg/m^2), CRP, glomerular-filtration rate, systolic blood pressure, ejection fraction (%) and season of blood drawing (January–March, April–June, July–September, October–December).[5] These factors are to various degrees also associated or established risk factors of CAD and might be considered as potential confounders of any relationship between 25OHD3 concentrations and CAD. We also included factors that could be related to disease progression regardless of vitamin D status, including the number of days of follow-up, diabetes mellitus (type I and II) and the B-vitamin supplementation used in WENBIT, which was shown to associate with more rapid progression of CAD in a post-hoc test.[18] The central tendency and distribution are reported with mean ± SD or median (interquartile range (IQR)) as appropriate for continuous variables and numbers (percentages) for categorical variables. The level of significance was set to $\alpha = 0.05$. All analyses were conducted in R, version 2.15.2.[24] For linear quantile mixed models we used the lqmm package, version 1.02.[25] Figures were created with the ggplot2 package, version 0.9.2.1.[26]

Results

After QCA analysis of coronary angiograms from 348 participants, significant atherosclerosis at the baseline or/and at the follow-up angiography was identified in 309 coronary segments from 183 participants. The remaining 165 participants did not have significant atherosclerosis beyond lesions treated with PCI at baseline and were therefore not included in further analyses of atherosclerosis progression.

Baseline characteristics

The median (IQR) 25OHD3 concentration was 63.9 (48.1–78.5) nmol/l in the total population. When comparing the concentration of 25OHD3 in blood samples collected during winter, spring, summer and autumn, we observe significant variation ($p < 0.001$) (Supplementary Material, Figure 2).

Table 1 shows important cardiovascular characteristics among the 183 patients with significant atherosclerosis beyond lesions treated with PCI. Three-quarters of the participants were diagnosed with stable angina pectoris (SAP) ($n = 137$) and one-quarter with acute coronary syndrome (ACS) ($n = 46$) constituting both ST-segment elevated and non-ST-elevated segment myocardial infarction as well as unstable angina pectoris. Of the 183 participants, 106 (58%) were represented by more than one atherosclerotic lesion.

Table 2 shows baseline demographics, the use of medication and laboratory characteristics of the participants. They received established medical treatment with aspirin, statins and beta blockers with few exceptions. There was a difference ($p = 0.002$) in median (IQR) 25OHD3 levels for the participants with SAP (64.1 (52.0–81.5) nmol/l) and the participants with ACS (53.9 (40.5–65.0) nmol/l), respectively. The patients with ACS did also have more ($p < 0.001$) inflammation than the SAP patients as measured by CRP (1.44 (0.83–3.28) mg/l and 12.6 (3.67–32.6) mg/l, respectively.

Table 1. Cardiovascular characteristics at baseline.

Variable	SAP ($n = 137$)	ACS[a] ($n = 46$)	p-value[b]
Previous cardiovascular history			
Previous acute myocardial infarction, *n* (%)	44 (32.1)	6 (13.0)	0.076
Previous percutaneous coronary intervention, *n* (%)	30 (21.9)	4 (9.09)	0.020
Previous coronary artery bypass surgery, *n* (%)	6 (4.38)	0 (0.00)	0.335
Extracardial vascular disease, *n* (%)[c]	11 (8.03)	3 (6.52)	0.990
Extent of coronary artery disease			
One-vessel disease, *n* (%)	62 (45.3)	15 (32.6)	0.183
Two-vessel disease, *n* (%)	54 (39.4)	17 (37.0)	0.903
Three-vessel disease, *n* (%)	21 (15.3)	14 (30.4)	0.042
Ejection fraction, (%)[d]	65.3 ± 9.02	58.0 ± 8.26	<0.001

ACS: acute coronary syndrome; SAP: stable angina pectoris; Data are presented by mean ± standard deviation for continuous variables and numbers (%) for categorical variables; [a]ACS constituting a composite syndrome of myocardial infarction (ST-segment elevated and non-ST-segment elevated) and unstable angina pectoris; [b]differences between the groups were tested with Students t-test for continuous variables and Pearson's Chi-squared test for categorical variables; [c]any previous diagnosis of peripheral or cerebrovascular disease; [d]left ventricle ejection fraction was measured during ventriculography or with ultrasound echocardiography.

Table 3 shows baseline angiographic characteristics. There were no statistically significant differences in the degree of atherosclerosis at baseline between patients with SAP or ACS as measured by MLD (1.94 ± 0.57 and 1.87 ± 0.51 mm, $p = 0.340$) or DS (38.2 ± 9.43 and 36.1 ± 10.1 percentage points, $p = 0.107$), respectively.

Progression of CAD

We have previously observed progression of CAD over time in this study cohort.[18] From baseline to follow-up there was a decrease ($p < 0.001$) in mean ± SD MLD from 1.92 ± 0.55 to 1.75 ± 0.51 mm and an increase in DS ($p < 0.001$) from 37.6 ± 9.64 to 42.0 ± 10.4 percentage points for all lesions.

Table 4 shows the linear quantile regression analysis for all lesions combined. The median of MLD or DS measured at follow-up were modelled as dependent variables, while adjusting for the baseline values of MLD or DS, baseline 25OHD3 concentration and known covariates. Neither in model 1 nor model 2 did we observe any statistical significant effects of 25OHD3 concentrations on DS or MLD (Figure 1(a) and (b)). No interacting effects on follow-up values of

Table 2. Demographics, clinical characteristics and laboratory data at baseline

Variable	SAP ($n = 137$)	ACS[a] ($n = 46$)	p-value[b]
Demographics			
Age (years)	61.0 ± 9.96	58.3 ± 9.99	0.117
Male sex, *n* (%)	113 (82.5)	41 (89.1)	0.404
Follow-up time (days)	303 ± 75.0	300 ± 89.8	0.825
Current smoker, *n* (%)[c]	41 (29.9)	20 (43.5)	0.132
Body mass index (kg/m^2)	26.9 ± 3.36	27.6 ± 3.25	0.232
Systolic blood pressure, mm Hg	145 ± 22.3	136 ± 22.6	0.025
Diastolic blood pressure, mm Hg	81.4 ± 11.0	76.1 ± 13.4	0.018
Hypercholesterolemia, *n* (%)[d]	82 (63.6)	26 (59.1)	0.727
Hypertension, *n* (%)	61 (44.5)	18 (39.1)	0.640
Diabetes mellitus, *n* (%)[e]	14 (11.4)	3 (6.52)	0.650
Medication			
Statins, *n* (%)	125 (91.2)	46 (100)	0.083
β-Adrenergic receptor antagonists, *n* (%)	101 (73.7)	40 (87.0)	0.100
Calcium antagonists, *n* (%)	28 (20.4)	5 (10.9)	0.215
Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, *n* (%)	45 (32.8)	15 (32.6)	0.904
Acetylsalicylic acid, *n* (%)	132 (96.4)	46 (100)	0.429
Adenosine 5'-diphosphate receptor antagonists, *n* (%)	75 (54.7)	45 (97.8)	<0.001
Laboratory findings			
25-hydroxyvitamin D3 level, nmol/l[f]	64.1 (52.0–81.5)	53.9 (40.5–65.0)	0.001
Deficiency (<30 nmol/l), *n* (%)	4 (2.92)	3 (6.52)	0.511
Inadequacy (30-50 nmol/l), *n* (%)	27 (19.7)	17 (37.0)	0.030
Sufficiency (>50 nmol/l), *n* (%)	104 (75.9)	26 (56.5)	0.020
Very high (≥125 nmol/l), *n* (%)	2 (1.46)	0 (0.00)	0.996
Low density lipoprotein cholesterol (mmol/l)	3.00 (2.48–3.63)	3.20 (2.63–3.78)	0.533
High-density lipoprotein cholesterol (mmol/l)	1.30 (1.10–1.50)	1.00 (0.90–1.20)	<0.001
Triglycerides (mmol/l)	1.50 (1.07–2.07)	1.69 (1.26–2.19)	0.621
C-reactive protein (mg/l)	1.44 (0.83–3.28)	12.6 (3.67–32.6)	<0.001
Glomerular filtration rate (ml/min/1.73 m^2)	93.0 (82.0–101)	97.0 (88.0–102)	0.116

ACS: acute coronary syndrome; SAP: stable angina pectoris; Data are presented by mean ± standard deviation for continuous variables, numbers (%) for categorical variables and median (inter quartile range) for biochemical parameters; [a]ACS constituting a composite syndrome of myocardial infarction (ST-segment elevated and non-ST-segment elevated) and unstable angina pectoris; [b]differences between the groups were tested with Students *t*-test for continuous variables and Pearson's Chi-squared test for categorical variables; [c]defined as self-reported current smoker or smoker quitting ≤90 days ago; [d]history of untreated total serum cholesterol ≥251.4 mg/dl (6.5 mmol/l) or familial hypercholesterolemia; [e]type I and type II diabetes mellitus; [f]to convert nmol/l to ng/ml, divide by 2.496.

Table 3. Baseline characteristics of 309 coronary lesions from 183 participants

Variable	SAP ($n = 229$)	ACS[a] ($n = 80$)	p-value[b]
Lesion morphology			
Length analysed segment, mm	21.7 ± 7.21	19.8 ± 5.41	0.017
Length stenotic segment, mm	10.4 ± 4.50	9.39 ± 3.43	0.037
Reference diameter, mm	3.13 ± 0.76	2.95 ± 0.73	0.057
Minimum lumen diameter areal, mm^2	4.17 ± 2.65	3.73 ± 2.08	0.133
Volume stenosis, mm^3	30.0 ± 11.5	28.4 ± 13.0	0.325
Minimum lumen diameter, mm	1.94 ± 0.57	1.87 ± 0.51	0.340
Diameter stenosis, %	38.2 ± 9.43	36.1 ± 10.1	0.107

ACS: acute coronary syndrome; SAP: stable angina pectoris; Data are presented by mean ± standard deviation for continuous variables, numbers (%) for categorical variables and median (inter quartile range) for biochemical parameters; [a]ACS constituting a composite syndrome of myocardial infarction (ST-segment elevated and non-ST-segment elevated) and unstable angina pectoris; [b]differences between the groups were tested with Students t-test for continuous variables and Pearson's Chi-squared test for categorical variables.

Table 4. Associations between baseline 25-hydroxyvitamin D3 (25OHD3) concentration and atherosclerosis progression

Variable	Estimated effect of 25OHD3[a]	p-value
Median minimum lumen diameter		
Model 1[b]	−0.015 (−0.032–0.002)	0.088
Model 2[c]	−0.006 (−0.029–0.015)	0.542
Median diameter stenosis		
Model 1 [b]	0.225 (−0.354–0.804)	0.444
Model 2 [c]	0.115 (−0.492–0.722)	0.708

[a]Variation in the median follow-up measurement of minimum lumen diameter (MLD) or diameter stenosis (DS) per 10 nmol/l increase of 25-hydroxyvitamin D3 concentration by linear quantile regression of 309 lesions from 183 participants; [b]model 1 was adjusted for the baseline measurement of MLD or DS and within-person clustering of atherosclerotic lesions; [c]model 2 was adjusted for the same as in model 1 and age, sex, smoking (self-reported), season of blood draw, body mass index, glomerular filtration rate, C-reactive protein, systolic blood pressure, days of follow-up, diagnosis of diabetes mellitus (type I and type II), folic acid treatment and ejection fraction.

MLD or DS were found between season of blood drawing and 25OHD3 concentration when an interaction term was added to either the crude or to the fully adjusted models. There were no associations between 25OHD3 concentrations and CAD progression when examining participants with SAP or ACS separately (Supplementary Material, Table 1) or those treated with folic acid or not separately (results not shown).

Discussion

In this study of patients with established CAD, plasma concentrations of 25OHD3 were not associated with atherosclerosis progression after approximately one year of follow-up, when measured as MLD or DS using serial QCA on 309 atherosclerotic lesions from 183 patients. Adjustment for seasonal variation, age, sex, severity of disease, treatment with folic acid, inflammation, and other coronary risk factors did not alter these results. We observed a difference in the baseline concentration of 25OHD3 between the patients with SAP and ACS that might be explained by higher levels of inflammation in the ACS patients at the time of blood collection.[27] We did not observe any differences in the degree of CAD at baseline between the SAP and ACS patients nor any associations between 25OHD3 concentration and CAD progression in stratified analyses.

There are many studies that have investigated the relationship between vitamin D status and CVD in observational cohort studies, but mainly in cohorts free of CAD at baseline.[28] Most of these studies found a significant increased risk of incident CVD at low 25OHD concentrations. Studies investigating cohorts with established CAD at baseline, however, show mixed results. Grandi et al.[29] and Bittner et al.[30] did not observe any relationship between 25OHD concentrations and cardiovascular events or mortality in patients with stable angina pectoris. In contrast, Naesgaard et al.[31] and Dobnig et al.[8] observed an association of 25OHD with cardiovascular mortality, investigating participants with both stable and unstable cardiovascular phenotype, respectively.

Cardiovascular events represent hard endpoints of the atherosclerotic process. However, the atherosclerotic process involves multiple stages before an event occurs that can be measured by different techniques, such as angiography or computed tomography. These and other measurements allow assessment of the

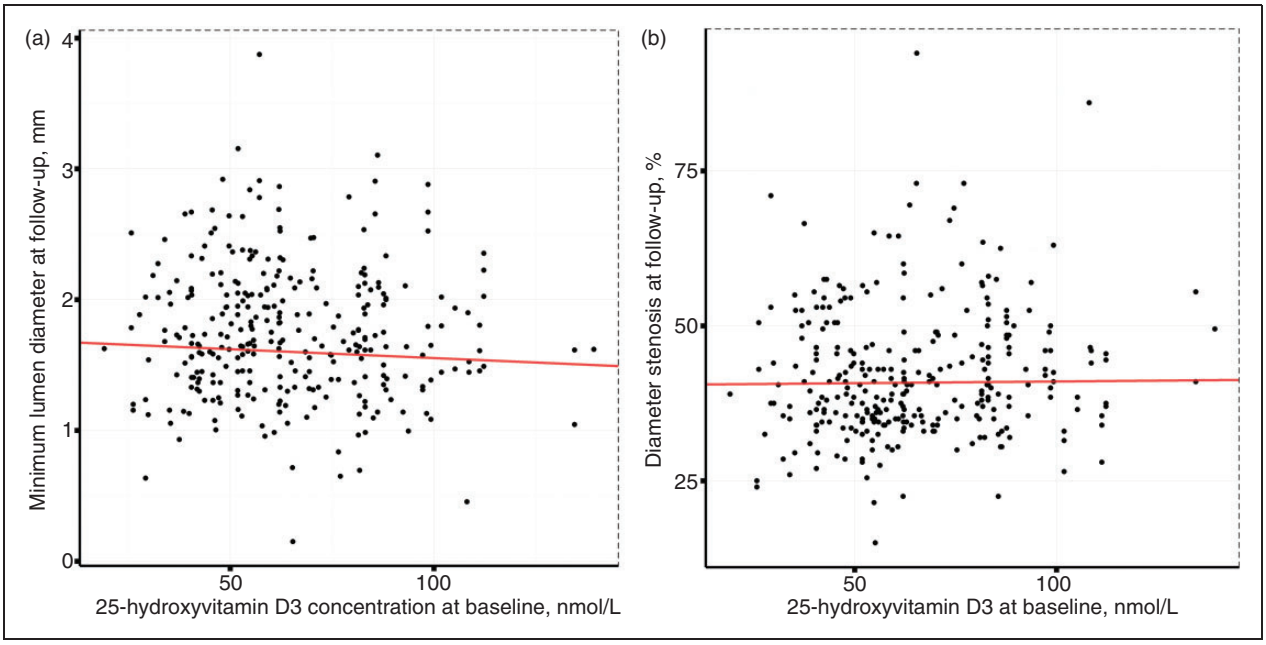

Figure 1. Graphs showing the relationship between plasma concentration of 25-hydroxyvitamin D3 at baseline and (a) minimum lumen diameter and (b) diameter stenosis measured after (mean ± standard deviation (SD)) 302 ± 79 days of follow-up. The regression line represents the variation in the median follow-up measurement of minimum lumen diameter (MLD) or diameter stenosis (DS) per 10 nmol/l increase of 25-hydroxyvitamin D3 concentration by linear quantile regression of 309 lesions from 183 participants, while adjusting for the baseline measurement of MLD or DS, the within-person clustering of atherosclerotic lesions, age, sex, smoking (self-reported), season of blood draw, body mass index, glomerular filtration rate, C-reactive protein, systolic blood pressure, days of follow-up, diagnosis of diabetes mellitus (type I and type II), folic acid treatment and ejection fraction.

progression of disease before an event occurs and allow studying the course of the disease over a shorter period.

Cross-sectional studies have reported an association between 25OHD levels and the prevalence of coronary artery stenosis.[32,33] Our prospective study, however, did not find an association of 25OHD with progression of coronary artery stenosis measured as DS and MLD. In addition to our study, three recent studies showed no association between vitamin D status and progression of carotid intima-media thickness or plaque burden[34–36] and three studies did not find any association with coronary artery calcification by computed tomography.[36–38] The conflicting findings from cross-sectional and prospective studies may be due to treatment and changes in lifestyle, as cardiovascular events or hospital visits can alter medical treatment and be incentives for diet and lifestyle changes. Different findings from cohorts studying healthy people at baseline compared to studies including CAD patients may be explained by significant improvements in risk management that may mask any potential effect of vitamin D status in patients with CAD. In our group of patients for instance, the statin and acetylsalicylic acid coverage was 93.4% and 97.2% at discharge after the first CA, respectively. In contrast, the coverage was 66.9% for acetylsalicylic acids prior to the first angiography.

Strengths and limitations

One of the strengths of this study is the methodological assessment of CAD and vitamin D status. Invasive CA is an established method for assessing CAD, and LC-MS/MS the future 'gold-standard' for assessment of 25OHD concentration.[5] Our participants received adequate medication and were thoroughly described in terms of cardiovascular risk factors that might confound the relationship between vitamin D status and atherosclerosis progression. In our study population, higher 25OHD3 concentration was associated with lower body mass index, but not with blood pressure or plasma concentration of CRP (data not shown). Unfortunately, data related to physical activity and diet was not available for the entire population investigated in the present work. The participants and the atherosclerotic lesions included in the final analysis went through an extensive selection process. The criteria induced by the study design might have inferred selection bias in the resulting population by including

participants who were healthier and characterised by less aggressive progression of atherosclerosis. The length of follow-up was on average less than one year and might have been too short to detect a relation between atherosclerosis progression and vitamin D status. However, we observe significant progression of atherosclerosis over time in this cohort and have previously associated atherosclerosis progression in this subgroup with the folic acid treatment[18] and plasma levels of methylated amino acids.[19]

The average 25OHD3 concentration was 64 nmol/l (26 ng/ml) and only 3.8% and 24% of the participants had concentrations below suggested cut-offs for deficiency (30 nmol/l) and inadequacy (50 nmol/l), respectively. If progression of atherosclerosis is more pronounced at low or very low levels of 25OHD3, we might have had too few participants within these categories to detect an association. However, these cut-offs are exclusively based on the relationship between vitamin D status and bone health. The available data on the relationship between vitamin D status and CVD was deemed inconclusive in a thorough review by the Institute of Medicine.[5] This conclusion and the established cut offs were criticised and a 25OHD concentration of 75 nmol/l suggested as the cut off for adequacy.[39] Regardless, it is relevant to investigate atherosclerosis progression in different populations covering the full range of 25OHD concentrations.

On the other hand, there are also studies suggestive of adverse effects of high 25OHD concentrations on CVD risk.[7,40] However, due to the limited number of patients with very high 25OHD3 levels, we were not able to detect any potential relation between high 25OHD3 concentrations and progression of atherosclerosis.

Conclusion

In conclusion, we did not find an association between vitamin D status measured by plasma 25OHD3 levels (median: 63.9, 95% CI: 48.1–78.5 nmol/l) and 'one-year' progression of CAD assessed by CA in statin-treated patients.

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Conflict of interest

The authors declare that there is no conflict of interest.

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