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Effects of Vitamin D supplementation on markers of vascular function after myocardial infarction—A randomised controlled trial☆

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ABSTRACT

Background: Low vitamin D levels are common, and are associated with a higher incidence of future vascular events. We tested whether vitamin D supplementation could improve endothelial function and other markers of vascular function in patients with a history of myocardial infarction.
Methods: Parallel group, placebo-controlled, double-blind randomised trial. Patients with a history of myocardial infarction were randomised to receive 100,000 units of oral vitamin D3 or placebo at baseline, 2 months and 4 months. Outcomes were measured at baseline, 2 and 6 months. Reactive hyperaemia index on fingertip plethysmography was the primary outcome. Secondary outcome measures included blood pressure, cholesterol, C-reactive protein, von Willebrand factor, tumour necrosis factor alpha, E-selectin, B-type natriuretic peptide, thrombomodulin and 25-hydroxyvitamin D levels.
Results: 75 patients were randomised, mean age 66 years. 74/75 (99%) completed 6 month follow-up. 25 hydroxyvitamin D levels increased in the intervention group relative to placebo (+13 vs +1 nmol/L, p = 0.04). There was no between-group difference in change in reactive hyperaemia index between baseline and 6 months (−0.18 vs −0.07, p = 0.40). Of the secondary outcomes, only C-reactive protein showed a significant decline in the intervention arm relative to placebo at 6 months (−1.3 vs 2.0 mg/L, p = 0.03). Systolic blood pressure (+1.4 vs +2.3 mm Hg, p = 0.79), diastolic blood pressure (+2.0 vs +0.8 mm Hg, p = 0.54) and total cholesterol (+0.26 vs +0.24 mmol/L, p = 0.88) showed no between-group difference at 6 months.
Conclusions: Supplementation with vitamin D did not improve markers of vascular function in patients with a history of myocardial infarction.

1. Introduction

Low vitamin D levels are common in the general population and a large body of observational evidence shows associations between low vitamin D levels and future vascular events—in the healthy general population [1,2], in those undergoing investigation for suspected coronary artery disease [3] and in those with acute coronary syndrome [4]. Older age, obesity and ill-health are all contributory factors to low vitamin D levels [5], and the presence of these confounding relationships makes it difficult to show a causal relationship between low vitamin D levels and vascular disease. Interventional studies are therefore required to test whether vitamin D can affect the natural history of cardiovascular disease in a beneficial way.

Patients with previous myocardial infarction remain at increased risk of future events despite the widespread use of secondary prevention agents such as antiplatelet medications, statins, renin–angiotensin system blockers and beta-blockers. Reducing this increased risk remains an important priority in management. Recent evidence suggests a number of potential biological mechanisms whereby vitamin D might ameliorate one or more of the pathophysiological processes that underlie the persistent increased risk of vascular events in patients with a history of myocardial infarction. Vitamin D has a suppressant effect on the renin–angiotensin system [6,7], has anti-inflammatory actions [8,9], improves endothelial function [10,11], and may influence platelet function via effects on calcium influx [12].

In order to justify conducting large multicentre trials of vitamin D supplementation in this patient group, evidence is required that vitamin D supplementation can improve surrogate markers of vascular function and vascular outcome. We therefore performed a randomised controlled trial to test whether administration of high dose oral vitamin D could improve markers of vascular function in patients with a history of myocardial infarction.

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2. Methods

2.1. Study design and participants

We performed a double-blind, parallel group, placebo-controlled randomised trial. Participants were recruited via cardiology clinics, cardiovascular medicine clinics and via cardiac rehabilitation services in Tayside, Scotland. Participants were eligible for inclusion if they had a history of myocardial infarction as defined by the universal criteria for myocardial infarction diagnosis [13], and were at least 6 weeks post myocardial infarction.

Participants were excluded if they had albumin-adjusted calcium levels of >2.60 mmol/L or <2.15 mmol/L, liver function tests >3× upper limit of normal, estimated glomerular filtration rate by MDRD4 method of <40 ml/min, supine systolic blood pressure <80 mm Hg, metastatic malignancy, history of renal stones, or a previous clinical diagnosis of osteomalacia. Participants were also excluded if already taking pharmacological vitamin D preparations (fish oils were permitted), were unable to give written informed consent, were pregnant, lactating, or were women of childbearing age without taking reliable contraception. No restrictions were placed on baseline 25OHD levels for admission to the trial. Written informed consent was obtained from all participants; ethical approval was given by Fife and Forth Valley Research Ethics committee (ref: 09/S0501/53). The study conformed to the principles of the Declaration of Helsinki. Clinical trials authorisation was obtained from the UK Medicines and Healthcare Regulatory Authority (EuDRACT ref: 2009-010367-17) and the trial was prospectively registered with www.controlled-trials.com (ISRCTN32927244).

2.2. Intervention

After baseline outcomes assessment, subjects received vitamin D3 (Vigantol oil, Merck Serono) or an identical placebo oil (Mygliol oil, used as the base oil for Vigantol) as a 5 ml dose at baseline, 2 months and 4 months. Total dose was therefore 300,000 units of vitamin D3 or placebo. Randomisation was performed in balanced blocks of 20 on a 1:1 basis. Medications were overlabelled and prepared by Tayside Pharmaceuticals (Dundee, Scotland) and were supplied in identical bottles with study number but no indication of group allocation. Allocation was therefore concealed from researchers and subjects.

2.3. Outcomes

All outcomes were performed at 0, 2 and 6 months by a research nurse blinded to study allocation. The primary outcome was changed in reactive hyperaemia index (RHI) [14], measured using the EndoPAT system (Itamar Medical, Israel), between baseline and 6 months. Endothelial dysfunction is a key final common pathway leading to atherosclerosis, and integrates the effect of multiple noxious stimuli on vascular function. Reactive hyperaemia index as measured by EndoPAT was chosen as the measure of endothelial function as it closely parallels coronary artery endothelial function [14] and has been shown to predict future vascular events in patients with coronary artery disease [15]. Office blood pressure was measured in the seated position after 5 min of rest using an OMRON HEM-705CP automated blood pressure cuff. Three consecutive readings were taken; the mean of the second and third readings were taken as the outcome measure. A peripheral venous blood sample was drawn for measuring a panel of markers related to vascular health: C-reactive protein (CRP), known to correlate with both risk of vascular events and 25OHD levels [3], tumour necrosis factor alpha (TNFa), a marker of inflammation related to cardiovascular dysfunction [9], thrombomodulin and von Willebrand factor as markers of thrombosis, endothelial cell injury and dysfunction [16,17], E-selectin as a marker of endothelial cell activation and inflammation that is related to atherosclerotic progression [18], B-type natriuretic peptide (BNP), a marker of cardiovascular risk even in the absence of heart failure [19,20], and 25-hydroxyvitamin D. 25-hydroxyvitamin D was measured using the IDS radioimmunoassay, B-type natriuretic peptide (BNP) was measured using radioimmunoassay (Bachem UK, Merseyside, UK). Intraassay coefficient of variation was 14%. Thrombomodulin, E-selectin, von Willebrand factor and TNFa were measured by ELISA (coefficients of variation 7.5%, 7.1%, 8.4%, 5–11%) and CRP was measured using a high-sensitivity ELISA assay (Kalon Biologicals, Guildford, UK; coefficient of variation 14.7%).

Calcium, phosphate, PTH, creatinine, glucose, HbA1c, total cholesterol, HDL and triglycerides were measured as routine clinical samples according to standard protocols in the Dept. of Biochemical Medicine, NHS Tayside. 12-lead ECG recordings were taken and both corrected QT interval and QT dispersion were recorded via automated analysis.

2.4. Statistical analysis

On the basis of previously published results, and assuming a baseline RHI of 1.27 (SD 0.37) [14] 29 patients per group are needed to detect a 25% change in the RHI with 90% power at the 0.05 probability level. We therefore aimed to randomise 80 patients (40 per group), predicting a dropout rate of 25% by 6 months, which would have left a final evaluable sample of 60 participants.

Analyses were performed using SPSS version 18 (SPSS, Chicago, USA). We compared the change (baseline vs follow-up) in each outcome measure between groups. Comparisons between continuous variables were performed using ANOVA. Categorical variables were compared using Pearson's chi-squared, or using Fisher's exact test when the contents of any cell was 5 or less. For the primary outcome, ANOVA analyses were also performed with correction for baseline factors, including systolic blood pressure, 25OHD level and baseline RHI.

3. Results

139 potential participants expressed interest in the study and underwent prescreening of notes. 21 potential participants did not fulfil the inclusion or exclusion criteria. 43 potential participants were eligible to take part, but declined to do so after receiving the full study information. 75 patients underwent a screening visit; all 75 passed the screening visit and were randomised into the study. Recruitment ceased at 75 as the final evaluable sample size had been met due to lower than anticipated dropout. Baseline demographics for the 75 subjects randomised into the trial are shown in Table 1; the median time between the index infarction and enrolment in the study was 4.5 months (range of 1.5 to 74 months). 74/75 (99%) of participants underwent the 6 month visit; primary outcome data were available for all participants at baseline, 72/75 (96%) of patients at 2 months, and 70/75 (93%) of participants at 6 months. Information on participant flow through the trial is given in Fig. 1. One participant declined repeat EndoPAT testing; one died before the 6 month visit; other missing data were due to insufficient EndoPAT signal quality to permit accurate analysis. 66/75 (88%) of participants had baseline 25OHD levels of <75 nmol/L, the level suggested as optimal for health across a number of domains, and 53/75 (71%) had baseline levels of <50 nmol/L, the level more classically associated with vitamin D insufficiency.

Table 2 shows the effect of the intervention on the outcome measures, 25OHD levels and on calcium homeostasis. 25OHD levels rose significantly in the intervention group by 2 months and were still elevated at 6 months compared to placebo. Serum calcium rose by a small but significant amount relative to placebo, and PTH levels fell in the intervention arm relative to placebo. Comparisons of reactive

Table 1
Baseline demographics of randomised participants.

	Intervention (n = 39)	Placebo (n = 36)	p
Age (yrs) (SD)	64.3 (9.8)	67.5 (10.6)	0.18
Sex (male) (%)	28/39 (72)	24/36 (67)	0.63
GRACE score (% prob recurrent MI)	9.3	9.8	0.82
Anterior infarction (%)	22/39 (56)	21/36 (58)	0.87
ST elevation MI (%)	22/39 (56)	16/36 (44)	0.30
Successful reperfusion (thrombolysis, PCI or CABG) (%)	35/39 (90)	28/36 (78)	0.16
Any revascularisation (PCI or CABG) (%)	31/39 (79)	27/36 (75)	0.64
ACE inhibitor (%)	38/39 (97)	34/36 (94)	0.51
Statin (%)	38/39 (97)	35/36 (97)	0.95
Beta blocker (%)	34/39 (87)	29/36 (81)	0.43
Antiplatelets (%)	36/39 (92)	33/36 (92)	0.92
Hypertension (%)	23/39 (59)	19/36 (53)	0.59
Diabetes (%)	7/39 (18)	4/36 (11)	0.40
Chronic heart failure (%)	2/39 (5)	0/36 (0)	0.17
Baseline systolic BP (mm Hg) (SD)	127.5 (21.3)	127.5 (18.9)	0.99
Baseline diastolic BP (mm Hg) (SD)	71.6 (9.2)	71.8 (7.9)	0.92
Baseline Creatinine (umol/L) (SD)	84 (19)	90 (20)	0.18
Baseline adjusted calcium (mmol/L) (SD)	2.30 (0.07)	2.32 (0.08)	0.18
Baseline 25OHD level (nmol/L) (SD)	49 (20)	45 (16)	0.34
Baseline cholesterol (mmol/L) (SD)	3.6 (0.9)	3.6 (0.8)	0.86
Baseline Reactive Hyperemia Index (SD)	2.09 (0.73)	2.06 (0.55)	0.87
Median baseline BNP (pg/ml) (range)	36 (8 to 362)	46 (7 to 282)	0.10
Median baseline CRP (mg/L) (range)	1.5 (0.1 to 31.0)	0.9 (0 to 6.4)	0.63

25OHD: 25-hydroxyvitamin D.
BNP: B-type natriuretic peptide.
CRP: C-reactive protein.
PCI: Percutaneous coronary intervention.
CABG: Coronary artery bypass grafting.

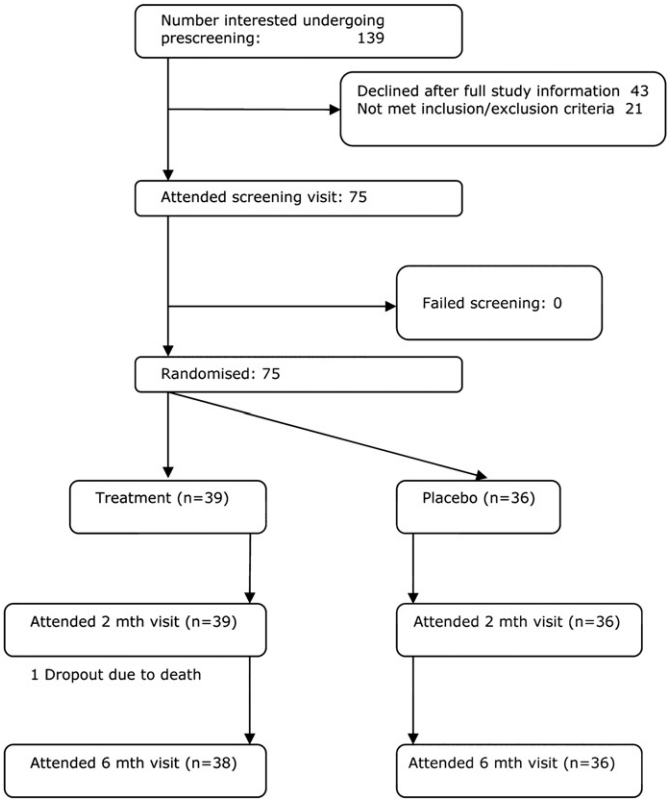

Fig. 1. CONSORT flow diagram.

hyperaemia index (RHI) values were analysable for 72 patients (38 in the treatment group, 34 in the placebo group) between baseline and 2 months, and for 70 patients (37 treatments, 33 placebos) between baseline and 6 months. No significant differences were seen between the groups, even after adjustment for baseline factors (25OHD level, blood pressure and baseline RHI) that could potentially affect RHI. The adjusted change in RHI at 6 months was −0.17 in the intervention group and −0.09 in the placebo group ($p = 0.50$ for difference). No significant effects were seen on secondary outcome measures with the exception of C-reactive protein, which showed a significant fall in the intervention group relative to placebo. Analysis of the interaction between baseline 25OHD level and response to intervention showed no effect of baseline 25OHD level on the degree of improvement in 25OHD level or RHI in the treatment arm when compared to placebo. Similarly, there was no significant correlation between the change in 25OHD levels and the change in RHI between baseline and 6 months ($r = -0.14$, $p = 0.27$, Pearson's test).

The intervention was well tolerated, with similar levels of adverse events in each study arm. A breakdown of study events is shown in Table 3. Only one participant in the intervention group had an elevated calcium level; this was asymptomatic. More participants in the intervention group had a rise in serum creatinine; this did not exceed 50% of baseline in any participant.

Table 2
Outcome measures.

Mean change		Intervention	Placebo	p
25OHD (nmol/L)	0 v 2 months	7	0	0.03
	0 v 6 months	13	1	0.04
PTH (pmol/L)	0 v 2 months	−0.35	0.58	0.002
	0 v 6 months	−0.11	0.86	0.01
Adjusted calcium (mmol/L)	0 v 2 months	0.01	−0.04	0.04
	0 v 6 months	0.00	−0.03	0.09
Reactive Hyperemia Index	0 v 2 months	0.03	−0.10	0.34
	0 v 6 months	−0.18	−0.07	0.40
Systolic BP (mm Hg)	0 v 2 months	−2.0	−1.0	0.73
	0 v 6 months	1.4	2.3	0.79
Diastolic BP (mmHg)	0 v 2 months	0.4	−1.2	0.23
	0 v 6 months	2.0	0.8	0.54
Thrombomodulin (U/L)	0 v 2 months	0.22	−0.14	0.06
	0 v 6 months	0.24	−0.04	0.17
C-reactive protein (mg/L)	0 v 2 months	−0.9	0.8	0.11
	0 v 6 months	−1.3	2.0	0.03
E-Selectin (U/L)	0 v 2 months	1.1	2.1	0.44
	0 v 6 months	2.7	1.6	0.42
Von Willebrand Factor (U/L)	0 v 2 months	−9.8	7.6	0.29
	0 v 6 months	6.3	12.4	0.77
TNF-alpha (U/L)	0 v 2 months	0.50	−0.13	0.09
	0 v 6 months	0.49	0.44	0.95
Total cholesterol (mmol/L)	0 v 2 months	0.10	0.14	0.73
	0 v 6 months	0.26	0.24	0.88
BNP (pg/ml)	0 v 2 months	3.3	−5.8	0.44
	0 v 6 months	1.6	−7.5	0.49
Corrected QT interval (ms)	0 v 2 months	0.9	−4.1	0.43
	0 v 6 months	−2.8	2.8	0.26
QT dispersion (ms)	0 v 2 months	−1.7	−2.5	0.76
	0 v 6 months	−1.8	0.7	0.41

TNF: Tumour necrosis factor.
BNP: B-type natriuretic peptide.

4. Discussion

Despite the administration of relatively high doses of oral vitamin D (equivalent to 1800 units/day), we failed to demonstrate any improvement in endothelial function as measured by peripheral artery tonometry, blood pressure, or soluble markers of endothelial function and thrombotic risk. C-reactive protein fell significantly, but the improvement in this inflammatory marker was not supported by improvement in tumour necrosis factor alpha.

Several possibilities merit discussion to explain our findings. It is possible that the dose of vitamin D was insufficient to produce the required biological effect. In support of this is the relatively modest increase in 25OHD levels seen in the intervention group. However, similar increments in 25OHD level associated with similar doses have produced improvements in both blood pressure [10,21] and endothelial function [10,22] in previous studies, although the technique for measuring endothelial function in these previous studies (flow-mediated dilatation of the brachial artery) differed from the current study. Our previous work in patients with type 2 diabetes mellitus showed no evidence of a threshold 25OHD level which needs to be reached in order to produce beneficial vascular effects; indeed patients with 25OHD levels of >50 nmol/L in this previous study showed similar improvements in blood pressure with supplementation to those with lower baseline 25OHD levels [21]. The majority of participants in this study had baseline 25OHD levels well below the 75–80 nmol/L value suggested to be optimal by extrapolation from observational studies [23,24], and subgroup analysis in the current study did not reveal a greater improvement in RHI in those with the lowest baseline 25OHD levels.

Previous studies using the EndoPAT system to measure endothelial function have found a mean RHI rather lower than that seen in the

Table 3
Adverse events.

	Intervention	Placebo
Cardiovascular	5	4
Musculoskeletal	2	3
Urological	1	0
Infection	3	7
Other adverse events	2	4
Calcium >2.65 mmol/L	1	0
Creatinine rise of >20%	4	1
Death	1	0

current study; RHI levels of <1.35 were associated with coronary endothelial dysfunction in patients without obstructive coronary artery disease [14] and indices below approximately 1.5 were associated with increased risk of cardiovascular adverse events over a 7 year follow-up [15]. The higher RHI seen in our study suggests that despite our attempts to select a population at increased risk of vascular events, endothelial function of our participants was relatively good, and thus improvements with intervention might be difficult to demonstrate. This may in part have been due to the high levels of statin and angiotensin-system blockers used in our study participants; these treatments are known to have beneficial effects on endothelial function [25]. Although we did not measure components of the renin–angiotensin–aldosterone system in this study, our previous studies of vitamin D supplementation [10,21,22] have shown conflicting effects on this system, explicable in part by the use of other agents acting on the renin–angiotension axis.

Another possibility is that only selected groups of patients at risk of vascular disease benefit from vitamin D supplementation. The effect of vitamin D on blood pressure is seen only in those studies where blood pressure is elevated at baseline [26] and patients with type 2 diabetes mellitus appear to benefit more in terms of blood pressure reduction. Participants in this study had well controlled blood pressure at baseline, and thus may not have been able to benefit from supplementation at the doses we used. It is also possible that vitamin D supplementation does not in fact produce clinically important effects in many patients; recent meta-analyses from trials of vitamin D in osteoporosis have failed to show any reduction in cardiovascular deaths from lower-dose vitamin D therapy [27].

Strengths of our study include the randomised, blinded design and the assessment of a range of markers of vascular health. We were able to enrol a relatively unselected population of patients with a history of myocardial infarction, which improves the generalisability of our results. Limitations include the relatively small study size, modest increase in 25OHD levels, and comparatively high RHI indices at baseline.

Future work in this area should focus on patients with coronary artery disease and significantly impaired reactive hyperaemia indices. Larger doses of vitamin D may be required to produce benefit, and given the current uncertainty over which of the possible biological mechanisms of vitamin D are clinically important for vascular health, a focus on alternative outcomes, e.g. vascular stiffness or platelet function, may be merited. Whilst our results do not exclude a clinically important effect of vitamin D supplementation on cardiovascular disease in this patient group, further studies examining surrogate vascular markers are needed to better define both the optimum dosing schedule and target patient subgroup before committing to large-scale cardiovascular outcome trials with vitamin D.

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Conflict of interest

The authors have received funding for research into vitamin D from Chest Heart and Stroke Scotland, Diabetes UK, Heart Research UK, Chief Scientist Office Scottish Government, Tenovus Scotland and ME Research UK.

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