ORIGINAL ARTICLE

Vitamin K1 and progression of cardiovascular calcifications in hemodialysis patients: the VitaVasK randomized controlled trial

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ABSTRACT

Background. Cardiovascular calcifications are prevented by matrix Gla protein (MGP), a vitamin K–dependent protein. Haemodialysis patients exhibit marked vitamin K deficiency. The randomized, prospective, open-label, multicentre VitaVasK trial analysed whether vitamin K1 supplementation reduces progression of coronary artery calcifications (CACs) and thoracic aortic calcifications (TACs).

Methods. Patients with pre-existing CACs were randomized to continue on standard care or to additionally receive 5 mg of vitamin K1 orally thrice weekly. Hierarchically ordered primary endpoints were progression of TAC and CAC in computed tomography scans at 18 months. Linear mixed effects models with repeated measures at baseline and 12 and 18 months assessed treatment effects after adjusting for study site.
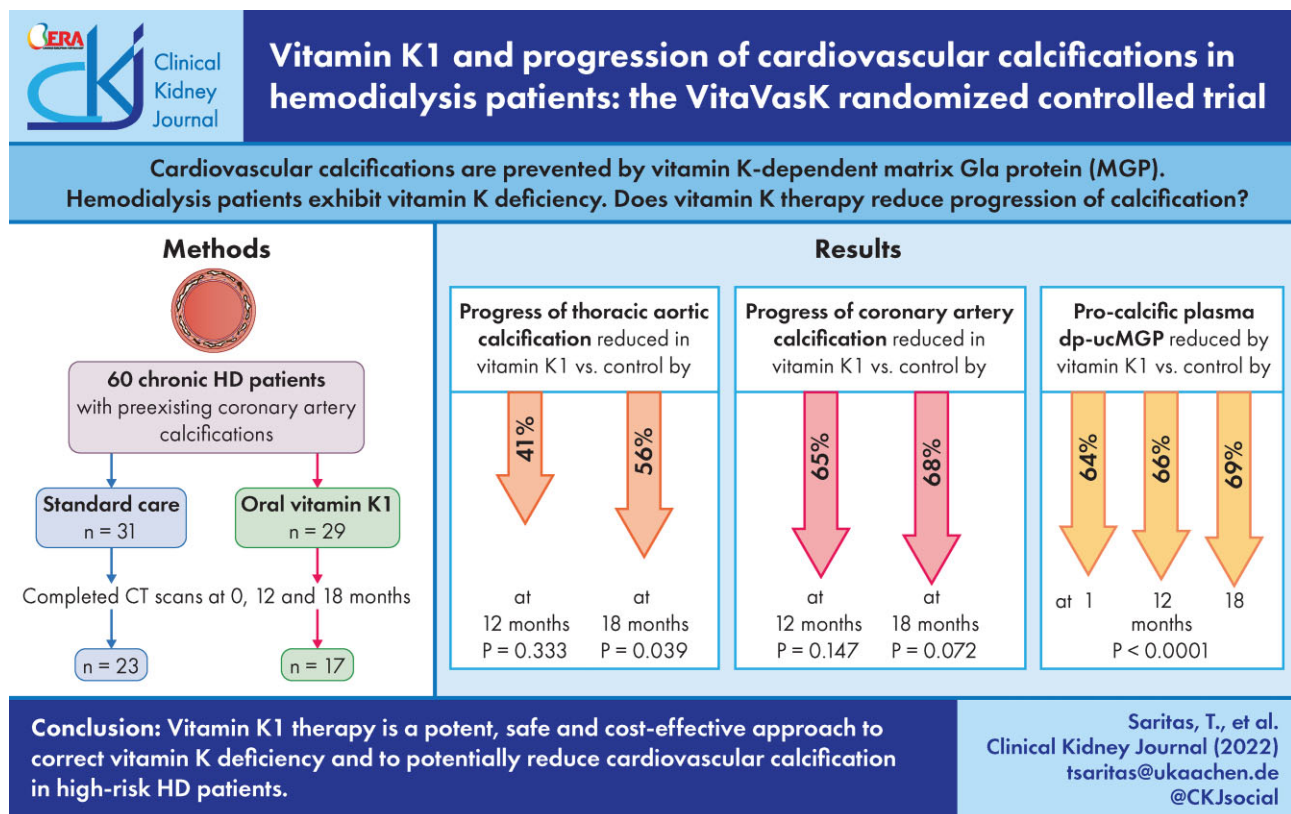
Results. Of 60 randomized patients, 20 dropped out for reasons unrelated to vitamin K1, resulting in 23 control and 17 vitamin K1 patients. The trial was stopped early due to slow recruitment. At 18 months, the average TAC progression was 56% lower in the vitamin K1 compared with the control group (p = .039). CAC significantly progressed within the control group, but not within the vitamin K1 group. Average progression at 18 months was 68% lower in the vitamin K1 compared to the control group (P = .072). Vitamin K1 reduced plasma levels of pro-calcific uncarboxylated MGP by 69% at 18 months. No treatment-related adverse events were noted.

Conclusion. Vitamin K1 intervention is a potent, safe and cost-effective approach to correct vitamin K deficiency and to potentially reduce cardiovascular calcification in this high-risk population.

LAY SUMMARY

Patients on chronic dialysis exhibit extensive cardiovascular calcifications and vitamin K deficiency. The vitamin K–dependent matrix Gla protein (MGP) is a potent inhibitor of vascular calcification. The multicentre, randomized, open-label, controlled VitaVasK trial showed marked attenuation of cardiovascular calcification progression in chronic haemodialysis patients treated with vitamin K1. Vitamin K1 supplementation greatly increased the serum vitamin K concentration and reduced inactive MGP levels. The treatment was safe, as no adverse advents were noted. Larger randomized controlled trials are needed to confirm vitamin K1 therapy as a safe, potent and cost-effective treatment option to reduce the progression of vascular calcification in haemodialysis patients.

GRAPHICAL ABSTRACT

Vitamin K1 and progression of cardiovascular calcifications in hemodialysis patients: the VitaVasK randomized controlled trial

Cardiovascular calcifications are prevented by vitamin K-dependent matrix Gla protein (MGP). Hemodialysis patients exhibit vitamin K deficiency. Does vitamin K therapy reduce progression of calcification?

Methods

60 chronic HD patients with preexisting coronary artery calcifications

Standard care n = 31 — Oral vitamin K1 n = 29

Completed CT scans at 0, 12 and 18 months

n = 23 — n = 17

Results

Progress of thoracic aortic calcification reduced in vitamin K1 vs. control by
41% at 12 months P = 0.333
56% at 18 months P = 0.039

Progress of coronary artery calcification reduced in vitamin K1 vs. control by
65% at 12 months P = 0.147
68% at 18 months P = 0.072

Pro-calcific plasma dp-ucMGP reduced by vitamin K1 vs. control by
64% at 1 month
66% at 12 months
69% at 18 months
P < 0.0001

Conclusion: Vitamin K1 therapy is a potent, safe and cost-effective approach to correct vitamin K deficiency and to potentially reduce cardiovascular calcification in high-risk HD patients.

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INTRODUCTION

Patients on maintenance haemodialysis (HD) exhibit a greatly increased cardiovascular mortality associated with cardiovascular calcifications [1]. Cardiovascular calcifications result from a positive phosphate and calcium balance, as well as reduced activity of inhibitors of calcification [e.g. matrix Gla protein (MGP)]

[2, 3]. MGP is an arterial wall- and cardiac valve-based inhibitor of calcification [4]. MGP requires post-translational modification by vitamin K–dependent gamma-glutamyl carboxylation to be fully active. In vitamin K deficiency or antagonism, uncarboxy-lated, inactive MGP (ucMGP) accumulates. Therapeutic vitamin K antagonism accelerates the development of cardiovascular cal-cifications and the risk of calciphylaxis in HD patients [5–7].

Vitamin K encompasses two forms, namely vitamin K1 (phylloquinone) and a number of K2 species [menaquinones (MK) 4–13] [5]. In humans, the major forms of vitamin K2 that have been investigated are MK-4 and MK-7, as well as menaquinones derived from intestinal bacterial synthesis. MK-4 can also be generated enzymatically from vitamin K (all forms) by UbiA prenyltransferase domain-containing protein 1 (UBIAD1) [8].

Hepatic vitamin K status is adequate in most populations, as indicated by normal coagulation parameters. However, most healthy individuals have detectable levels of circulating dephos-phorylated (dp)-ucMGP, suggesting a suboptimal vascular supply of vitamin K [5]. In chronic dialysis patients, circulating levels of dp-ucMGP are markedly higher than in the normal popula-tion in about two-thirds of patients and predict both calcifica-tion and mortality [5, 7, 9]. This subclinical vitamin K deficiency results from dietary restrictions [10], possibly impaired vitamin K recycling in uraemia [11] and uraemia-associated alterations of vitamin K uptake and transport in lipoproteins [12].

The above observations, combined with the lack of any known human toxicity of dietary vitamin K supplementa-tion, have laid the basis for a number of intervention trials targeting cardiovascular calcification in HD patients. Supple-mentation of vitamin K2, specifically MK-7, at doses of up to 860 µg/day (recommended daily vitamin K intake ranges from 75 to 120 µg/day) rapidly lowered serum levels of dp-ucMGP by up to 50% [13–18]. Despite this biochemical benefit of MK-7 supple-mentation, several intervention trials in advanced chronic kid-ney disease (CKD) or HD patients consistently failed to note any benefit for the cardiovascular system, such as calcification pro-gression, pulse wave velocity, blood pressure or cardiovascular events [13, 15–19].

In 2010, a pathway was discovered whereby vitamin K1 was converted into K2 (MK-4, but not MK-7) [8], which allowed us to use high-dose, drug-grade vitamin K1 for an intervention. To show that vitamin K1 therapy at 15 mg/week added to standard care reduces the progression of cardiovascular cal-cification compared with standard care alone, we conducted the randomized multicentre VitaVasK trial [20]. The primary endpoints are the progression of thoracic aortic and coronary artery calcification [CAC; calculated as absolute changes in the volume scores at the 18-month multislice computed tomogra-phy (MSCT) versus the baseline MSCT]. Secondary endpoints comprise changes in the Agatston score, mitral and aortic valve calcification as well as major adverse cardiovascular events (MACEs) and all-cause mortality.

MATERIALS AND METHODS

Trial design, population and intervention

VitaVasK is an investigator-initiated, randomized (1:1 in two par-allel groups), controlled, prospective, fixed sample, open-label but assessor-blinded clinical trial conducted in four sites in Germany, two sites in Belgium and one site in Sweden (see Acknowledgements). The study protocol was approved by the ethics committees of all participating sites. The study was reg-

istered on ClinicalTrials.gov (NCT01742273) and EudraCT (2010-021264-14). Written informed consent was obtained from all pa-tients. There were no changes in the protocol during the conduct of the trial.

The rationale and design of VitaVasK has been described pre-viously [20]. We calculated a total sample size of 348 patients in order for the trial to have 80% power at a two-sided type 1 error of 0.05, based on the assumptions that the mean differ-ence in the increase in the thoracic aortic calcification (TAC) score will be 30% lower in the vitamin K1 group {absolute differ-ence ~90 [standard deviation (SD) 235]} after 18 months and the dropout rate will be 37.5% due to comorbid conditions in dialysis patients [20].

Adults on chronic maintenance HD for at least 6 months with a baseline CAC volume score >100 mm^3 were eligible for inclu-sion. Key exclusion criteria included intake of vitamin K, history of thrombosis [except arteriovenous (AV) shunt occlusion], in-take of vitamin K antagonists at baseline or in the 3 months prior to baseline, inflammatory bowel disease, short-bowel syn-drome, significant liver dysfunction, any condition likely to im-pair vitamin K absorption (i.e. chronic pancreatitis), malignancy and more than one stent in one coronary artery plus one or more stents in an additional artery (Supplementary Table S1). Randomization lists were generated by the Institute of Medi-cal Statistics using the randomization tool RITA (version 1.5.0; Evidat, Germany). The randomization procedure utilized, strati-fied by centre, was Efron's biased coin, whereby the probability was chosen as two-thirds. Participants were randomly assigned following simple randomization procedures (computerized ran-dom numbers) to one of two treatment groups. Whereas patients and physicians allocated to the intervention group were aware of the allocated arm, outcome assessors and data analysts were kept blinded to the allocation.

Prohibited medications during the trial were only vitamin K antagonists and supplements containing vitamin K. Patients were encouraged to maintain their usual diet during the trial.

Eligible patients were randomized to receive 5 mg of vitamin K1, provided in liquid form at a concentration of 10 mg/ml (Ka-Vit, Infectopharm, Heppenheim, Germany), given orally thrice weekly under observation during HD in addition to usual stan-dard care. This dose was chosen based on our prior trial in which 2 mg/day of vitamin K1 reduced valvular progression in patients with an estimated glomerular filtration rate >60 ml/min/1.73 m^2 [21]. VitaVasK control patients continued to receive usual stan-dard care. Participants were recruited from October 2013 to Jan-uary 2019. Clinical, serum-based laboratory and MSCT data were obtained at baseline and at 12 and 18 months. Additional bio-chemical data were obtained at 4 weeks after randomization. Adverse events were collected by questioning the patients or the treating physicians as well as by monitoring vital signs and rou-tine laboratory tests.

Imaging

To assess cardiovascular calcifications, unenhanced, electrocardiogram-synchronized MSCT of the chest, extending from the aortic arch to the oesophageal hiatus of the diaphragm were performed. To homogenize image quality between study sites, calibration scans with incremental current time products at 120-kV tube voltage were conducted using an anthropomor-phic coronary calcification phantom [22]. Two phantom belts simulating different obese conditions were additionally used to enable three study protocols on each scanner for small, medium and obese patients. Image noise was evaluated as the SD of
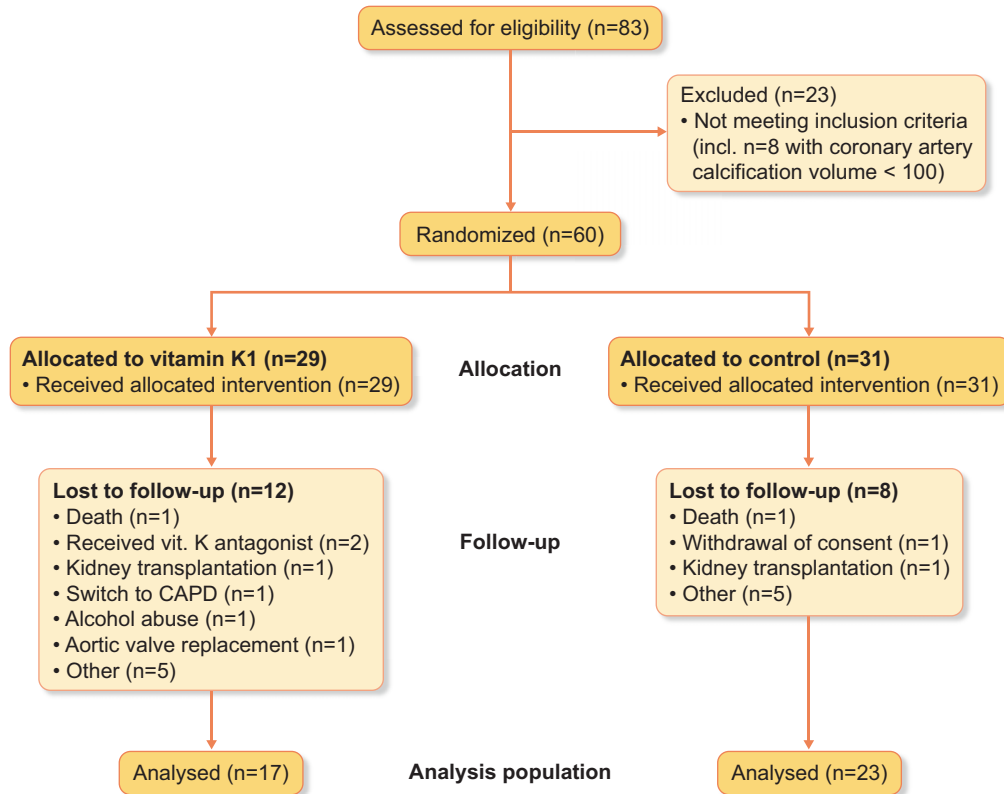
Figure 1: Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

the density value determination of a measurement area (field of view >1 square centimetre) in a water-isodense area of the phantom. For each MSCT scanner and each of the three patient sizes, the protocol with image noise <25 HU that required the lowest X-ray tube current was selected to minimize radiation exposure and to provide consistent image quality.

MSCT scans were performed at baseline and at 12 and 18 months. In all datasets, the Agatston score, the volume score and the mass score [23] were determined for the four locations of calcifications: thoracic aorta, aortic and mitral valves and the coronary arteries. The software Syngo.Via VA20 (Siemens Healthineers, Forchheim, Germany) was used for quantification purposes. The Agatston score was determined using a 3 mm CT slice thickness with an increment of 3 mm (non-overlapped). A detection threshold of ≥130 HU involving ≥1 mm^2 area/lesion (3 pixels) was applied. The mass scores at baseline are reported in Supplementary Table S2.

All scans were evaluated independently and in a blinded fashion by two experienced radiologists (SR and RS). Relevant deviations between the two readers were countered by a joint evaluation in which the relevant measured values were determined by mutual agreement and consistently across all measurement time points. In case of coronary intervention during the study period, the affected area of the coronary artery was excluded from the evaluation and the previous or subsequent examinations were consistently evaluated in the same way.

Biochemical measurements

Fasting blood samples were obtained prior to dialysis, centrifuged for 10 min at 3000 g within 30–60 min and immediately stored at −80°C until dry ice shipment for centralized analysis. Serum levels of vitamin K were measured using liquid chromatography tandem mass spectroscopy (LC-MS/MS) consisting of an initial sample purification step prior to tandem MS detection (Magtivio, Nuth, The Netherlands). The assay performance was evaluated through participation in the international Vitamin K External Quality Assurance Scheme. Specific measurement of MK-4 in serum was not considered, since this is only detectable after very high oral MK-4 doses [24].

Circulating dp-ucMGP was quantified in plasma using the commercially available In Vitro Diagnostic CE-marked chemiluminescent InaKtif MGP assay on the IDS-iSYS system (IDS, Boldon, UK). In brief, samples were exposed to magnetic particles coated with monoclonal antibodies against dp-MGP and ucMGP. Trigger reagents were then added, resulting in light emission that was directly proportional to the level of dp-ucMGP in the sample. The within-run and total variations of this assay are 0.8–6.2% and 3.0–8.2%, respectively. The assay quantitation range was between 50 and 12 000 pmol/l and was linear up to 11 651 pmol/l.

Routine blood analyses were performed at locally certified laboratories.

Table 1: Baseline characteristics of all participants included in the analysis of the VitaVasK study.

Characteristics	Controls (n = 23)	Vitamin K1 (n = 17)
Female, n (%)	6 (26)	6 (38)
Age (years)	64.4 ± 13.3	62.5 ± 12.3
Dialysis vintage (months)	82.1 ± 65.6	65.2 ± 56.6
Body mass index (kg/m^2)	27.4 ± 6	26.7 ± 4.9
Smoking status,[a] n (%)		
Current or ex-smoker	12 (55)	11 (69)
Non-smoker	10 (46)	5 (31)
Systolic blood pressure[b] (mmHg)	141.9 ± 18.4	138.9 ± 19.7
Diastolic blood pressure[b] (mmHg)	69.6 ± 13.6	75.0 ± 16.7
Diabetes mellitus, n (%)	8 (34.8)	2 (11.8)
Haemoglobin A1c (%)	5.5 ± 0.8	5.5 ± 1
International normalized ratio	0.98 ± 0.09	0.96 ± 0.07
Haemoglobin (g/l)	111.5 ± 8.6	109.9 ± 9.9
Serum ionized calcium (mmol/l)	1.1 ± 0.1	1.1 ± 0.2
Serum phosphate (mmol/l)	1.8 ± 0.5	1.9 ± 1.2
Serum intact parathyroid hormone (ng/l)	265.3 ± 306.7	536.1 ± 426.8
Serum alkaline phosphatase (U/I)	93 ± 52	93 ± 48
Bone-specific alkaline phosphatase (U/I)	27 ± 23	36 ± 33
25-hydroxyvitamin D3 (μg/l)	45.2 ± 20.2	31.7 ± 19.7
Serum total magnesium (mmol/l)	0.9 ± 0.1	1 ± 0.2
Total cholesterol (mg/dl)	169.4 ± 41.3	166.7 ± 34.3
C-reactive protein (mg/l)	5.5 ± 8.6	4.7 ± 4.3
Statin therapy, n (%)	11 (47.8)	8 (47.0)
Native vitamin D therapy, n (%)	18 (85.7)	14 (93.3)
Vitamin D receptor agonist therapy, n (%)	15 (71.4)	8 (53.3)
Calcium-free phosphate binder therapy, n (%)	12 (65.2)	13 (82.4)
Calcium-containing phosphate binder therapy, n (%)	15 (71.4)	9 (60.0)
Calcimimetic therapy, n (%)	9 (42.9)	7 (46.7)
Months from baseline CT to planned 12-month CT	12.6 ± 1.3	12.7 ± 1.4
Months from baseline CT to planned 18-month CT	19.6 ± 1.4	18.3 ± 1.1
Coronary artery disease, n (%)	11 (34.8)	8 (41.8)
TAC (mm^3)	4864.6 ± 4792.0	4830.3 ± 10 621.3
Aortic valve calcification (mm^3)	148.2 ± 246.3	133.5 ± 171.0
CAC (mm^3)	1886.9 ± 1143.1	1550.5 ± 1419.7
Mitral valve calcification (mm^3)	1063.1 ± 2237.1	936.6 ± 1764.8

Values are presented as mean ± SD unless stated otherwise. All calcification values are volume scores.
[a]Information on smoking status was missing for one person in each group.
[b]Pre-dialysis blood pressure after long interdialytic interval.

Outcomes

The two hierarchical co-primary endpoints were progression of TAC (i.e. the absolute change in the volume score at the 18-month MSCT versus the baseline MSCT) as well as progression of CAC (i.e. the absolute change in the volume score at the 18-month MSCT versus the baseline MSCT). Secondary endpoints included progression of TAC or CAC assessed via the Agatston score method (see below), progression of aortic and mitral valve calcification and mortality from any cause or MACE within 18 months after the start of treatment (Supplementary Table S1). VitaVasK was stopped early in January 2019 because of slow recruitment.

Statistical methods

Statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC, USA) and graphical presentations were done using R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). For the first primary endpoint analysis of the TAC (volume score), we applied a linear mixed effects model with repeated effect for the time points baseline, 12 months and 18 months (proc MIXED in SAS). We modelled treatment and centre as fixed effects and also included the interaction between treatment and time point. We adjusted for baseline calcification, as repeated mixed effects models were used, and the changes from baseline status were used for interpretation. The spatial power covariance structure was used and residual plots were examined visually to assess the model fit (Supplementary Fig. S1 and S2). Thus extreme outliers (n = 1 per primary endpoint or 1–2 per secondary endpoints) based on the restricted likelihood distance were excluded. Group differences in the changes of TAC from baseline to 18 months were computed with linear contrasts. As the trial was stopped prematurely, the p-values are explorative and were denoted as statistically significant if they fell below the usual significance level of 5%. Applying the significance level to the hierarchical decision rule, as planned in the protocol, the second primary endpoint, CAC (volume score), was analysed using the same statistical model.

For the first and second primary endpoints, two sensitivity analyses were performed. As the randomization was not stratified by gender, we accounted for this confounder in the analysis. For this purpose we first included the confounders gender and age and in the second we included further confounders gender,

age, smoking status and diabetes mellitus as fixed effects. For the secondary endpoints TAC (Agatston score), CAC (Agatston score), aortic valve calcification (volume and Agatston scores) and mitral valve calcification (volume and Agatston scores), the statistical models were fitted analogously to the primary endpoints and sensitivity analyses were performed accordingly. For the secondary endpoints all-cause mortality and major cardiovascular events, we performed Fisher exact tests to investigate the occurrence between the two treatment groups. Further analyses were performed for serum vitamin K concentrations and dp-ucMGP levels, applying a linear mixed effects model with repeated effect for the time points baseline, 1 month, 12 months and 18 months and with the same specifications as before.

The analysis population was the full analysis set following International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use E9 guidelines [25].

RESULTS

Trial participants and baseline parameters

Of the 60 patients randomized into the trial, 20 patients dropped out over the subsequent 18 months, 12 of 29 patients in the K1 group and 8 of 31 in the standard care group [odds ratio 2.0 (95% confidence interval 0.68–6.05)]. This is expected in a multimorbid population [20], and there were no obvious differences in causes for dropping out dominating in either group (Fig. 1).

The final analysis population consisted of 40 patients, mostly men, mostly non-diabetic and on chronic HD for an average of 75 months (Table 1). There were numerically more smokers or ex-smokers in the vitamin K1 group and more diabetic patients in the control group, with no differences in current haemoglobin A1c levels.

Times from baseline MSCT to the 12-month MSCT were similar between the two groups, whereas MSCTs for the primary endpoint at 18 months were performed on average 1.3 months later in the controls compared with the vitamin K1 group. The mean baseline calcification volume scores of the thoracic aorta were very similar in the two groups, whereas mean coronary calcification scores at baseline were ~20% higher in the control group compared with the vitamin K1 group (Table 1).

Primary endpoint

As shown in Fig. 2A and Table 2, TAC progressed significantly between baseline and 12 and 18 months in both study populations. However, the progression of TAC was significantly reduced by an average of 56% {892.1 mm^3 [standard error (SE) 423.0], model 1]} in the vitamin K1 group compared with the control group at 18 months (P =.039). Progression at 12 months was reduced by a mean of 41% [325.8 mm^3 (SE 334.0), model 1] in the vitamin K1 group compared with the control group, but this difference failed to reach statistical significance (P = .333). Of the various parameters potentially affecting calcification progression, only age at baseline exerted a significant effect on the endpoint (Supplementary Table S3). Consistent with this, multivariate adjustment in the different models did not lead to any qualitative change in the above statements (Table 2).

The extent of CAC also increased significantly in both groups between baseline and 18 months (Fig. 2B, Table 3). Progression at 18 months was lower by an average of 68% [412.7 mm^3 (SE 225.4), model 1] in the vitamin K1 group compared with the control group (P = .072). Progression at 12 months was reduced by a mean of 65% [265.7 mm^3 (SE 180.8), model 1] in the vitamin K1 group compared with the control group (P = .147). Sensitiv-
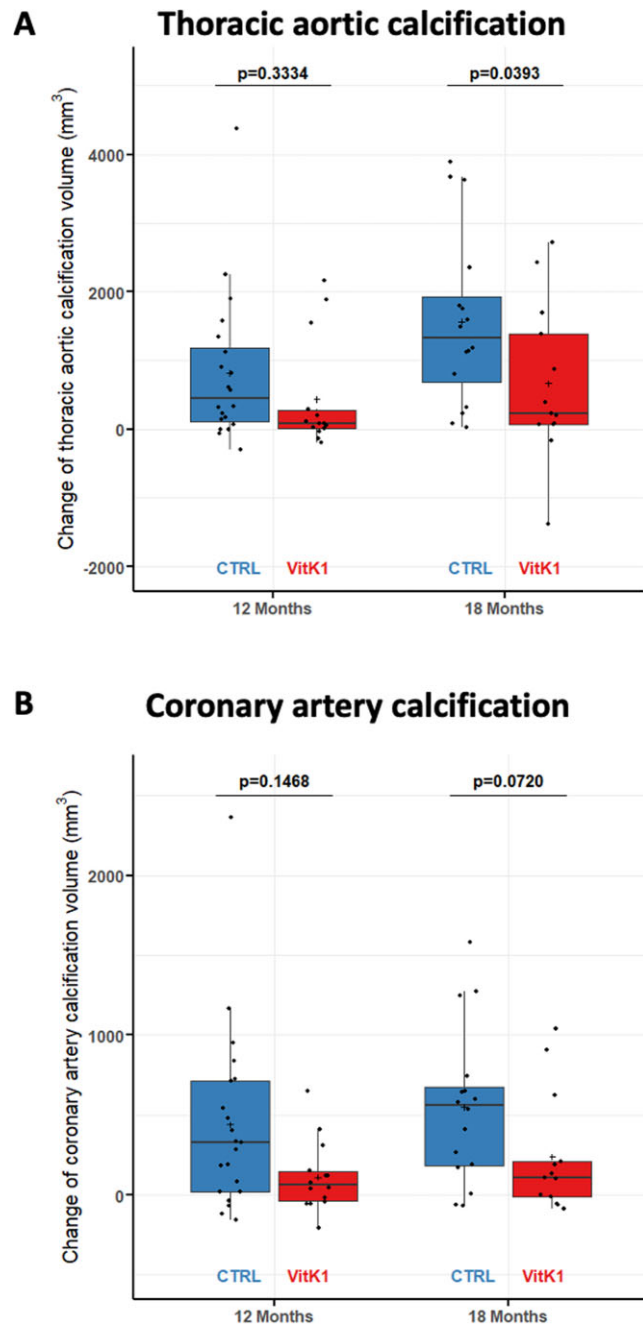

Figure 2: Primary endpoints of the VitaVasK trial: **(A)** progression of TAC (i.e. the absolute change of the volume score at the 12- and 18-month MSCT versus the baseline MSCT) as well as **(B)** progression of CAC (i.e. the absolute change in the volume score at the 12- and 18-month MSCT versus the baseline MSCT). Individual data, means and SDs are shown.

ity analyses yielded qualitatively similar data (Supplementary Table S3).

Secondary endpoints

When progression of TAC was assessed using the Agatston score instead of the volume score, qualitatively similar results were obtained: progression was significantly reduced in the vitamin K1 group at 18 months but not at 12 months (Fig. 3A, Supplementary Table S4). Progression at 18 months was reduced by

Table 2: Primary endpoint TAC score: changes in volume scores (mm^3) between baseline and 12 and 18 months in participants of the VitaVasK study.

		Model 1	Model 2	Model 3
TAC volume estimates (SE)				
Vitamin K	Baseline	3273.6 (1328.6)	4729.3 (1313.4)	1409.6 (1402.5)
	12 months	3752.5 (1329.9)	5200.7 (1314.1)	1880.8 (1403.2)
	18 months	3983.1 (1332.3)	5430.6 (1318.2)	2115.4 (1406.2)
Control	Baseline	5231.7 (1066.3)	5971.6 (1026.3)	3794.7 (1075.9)
	12 months	6036.4 (1067.3)	6775.8 (1027.3)	4615.4 (1077.2)
	18 months	6833.3 (1071.0)	7578.3 (1032.9)	5413.0 (1080.1)
Changes in volume score versus baseline within groups				
Vitamin K	Baseline versus 12 months	478.9 (256.4), 0.0668	471.4 (263.6), 0.0791	471.2 (265.9), 0.0820
	Baseline versus 18 months	709.5 (322.6), 0.0318	701.3 (336.8), 0.0418	705.7 (339.6), 0.0424
Control	Baseline versus 12 months	804.7 (214.1), 0.0004	804.1 (215.9), 0.0004	820.7 (228.4), 0.0007
	Baseline versus 18 months	1601.6 (273.6), <0.0001	1606.7 (275.5), <0.0001	1618.3 (285.0), <0.0001
Changes in volume score versus baseline between groups				
Vitamin K versus control	Baseline versus 12 months	325.8 (334.0), 0.3334	332.8 (340.7), 0.3329	349.5 (350.6), 0.3231
	Baseline versus 18 months	892.1 (423.0), 0.0393	905.4 (435.1), 0.0420	912.6 (443.4), 0.0443

Data are linear mixed model estimates (SE) and P-value.
Model 1: adjusted for centre; model 2: adjusted for centre, gender and age; model 3: adjusted for centre, gender, age, smoking status and diabetes mellitus. All models automatically adjust for baseline calcification as repeated mixed effects models were used.

Table 3: Primary endpoint CAC score: changes in volume scores (mm^3) between baseline and 12 and 18 months in participants of the VitaVasK study.

		Model 1	Model 2	Model 3
CAC volume estimates (SE)				
Vitamin K	Baseline	1045.6 (347.8)	1237.0 (400.4)	1381.2 (400.4)
	12 months	1187.4 (349.9)	1378.1 (401.7)	1521.8 (401.7)
	18 months	1242.2 (351.5)	1433.1 (404.5)	1580.0 (403.9)
Control	Baseline	1388.4 (286.5)	1422.3 (322.5)	1517.8 (313.0)
	12 months	1796.0 (287.4)	1829.8 (323.2)	1904.2 (313.9)
	18 months	1997.8 (291.8)	2032.6 (328.6)	2109.8 (317.4)
Changes in volume score versus baseline within groups				
Vitamin K	Baseline versus 12 months	141.8 (138.3), 0.3092	141.1 (141.9), 0.3241	140.7 (143.4), 0.3308
	Baseline versus 18 months	196.6 (169.5), 0.2505	196.2 (176.8), 0.2716	198.8 (178.1), 0.2690
Control	Baseline versus 12 months	407.5 (116.4), 0.0009	407.5 (117.4), 0.0010	386.4 (124.3), 0.0029
	Baseline versus 18 months	609.3 (148.6), 0.0001	610.3 (149.9), 0.0001	592.0 (154.9), 0.0003
Changes in volume score versus baseline between groups				
Vitamin K versus control	Baseline versus 12 months	265.7 (180.8), 0.1468	266.4 (184.2), 0.1534	245.7 (189.8), 0.2006
	Baseline versus 18 months	412.7 (225.4), 0.0720	414.2 (231.7), 0.0790	393.2 (236.0), 0.1012

Data are linear mixed model estimates (SE) and P-value.
Model 1: adjusted for centre; model 2: adjusted for centre, gender and age; model 3: adjusted for centre, gender, age, smoking status and diabetes mellitus. All models automatically adjust for baseline calcification as repeated mixed effects models were used.

an average of 57% [1166.1 (SE 551.4), model 1; $P = .039$; Supplementary Table S4]. With respect to progression of CAC, Agatston score progression was significantly decreased in the vitamin K1 group at 18 months [average reduction 72%; 565.8 (SE 252.0), model 1; $P = .028$] but not at 12 months (Fig. 3B, Supplementary Table S5). Multivariate adjustment with the different models did not lead to any qualitative change in the above statements (Supplementary Tables S4 and S5).

Progression of aortic valve calcification measured by volume scores was on average 76% lower at 18 months in the vitamin K1 group, but this result failed to reach statistical significance (Fig. 3C, Supplementary Table S6). Similar findings were obtained using the Agatston score (data not shown). Calcification progression of the mitral valve at 18 months measured by the volume score was reduced by a mean of 38% in the vitamin K1 group; again, this was not statistically significant (Fig. 3D, Supplemen-

tary Table S7). Assessment via the Agatston score yielded similar findings (data not shown).

All-cause mortality at 18 months did not differ between the two groups (i.e. all 60 randomized patients) ($P = .703$) (Table 4). Five major cardiovascular events (myocardial infarction, stroke, acute coronary syndrome, embolism, symptom-driven revascularization, death from cardiovascular cause) were observed in the control group and three in the vitamin K1 group (Table 4), a non-significant difference ($P = .751$).

Biochemical measurements

Fig. 4A and Supplementary Table S8 show that serum vitamin K concentrations progressively increased in the vitamin K1 cohort (+590% at 18 months), but not in the control group (−4% at 18 months). Circulating plasma dp-ucMGP showed a rapid,
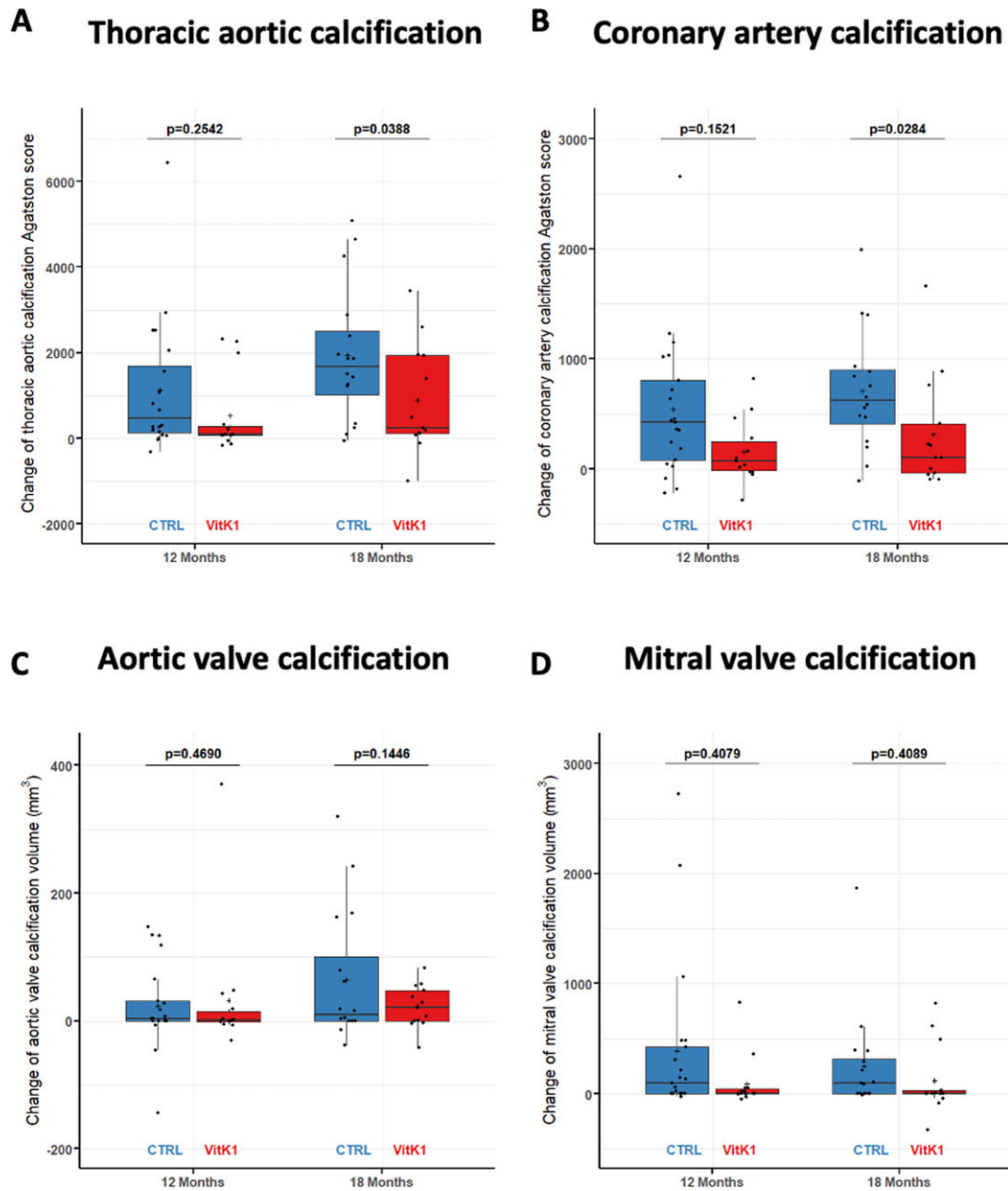
Figure 3: Secondary endpoints of the VitaVasK trial: **(A)** progression of TAC (i.e. the absolute change in the Agatston score at the 12- and 18-month MSCT versus the baseline MSCT), **(B)** progression of CAC (i.e. the absolute change in the Agatston score at the 12- and 18-month MSCT versus the baseline MSCT), **(C)** progression of aortic valve calcification (i.e. the absolute change in the volume score at the 12- and 18-month MSCT versus the baseline MSCT) and **(D)** progression of mitral valve calcification (i.e. the absolute change in the volume score at the 12- and 18-month MSCT versus the baseline MSCT). Individual data, means and SDs are shown.

pronounced reduction of the baseline level to $36.5 \pm 2.0\%$ at the week 4 visit, $33.9 \pm 35.2\%$ at the 12-month visit and $30.9 \pm 64.2\%$ at the 18-month visit in the vitamin K1 group as opposed to $109.1 \pm 5.6\%$ (week 4), $115.6 \pm 4.7\%$ (12 months) and $101.7 \pm 20.9\%$ (18 months), respectively, in the control group (Fig. 4B and Supplementary Table S9).

Vitamin K uptake is potentially affected by sevelamer via intestinal binding [26]. However, when comparing patients receiving sevelamer versus non-sevelamer phosphate binders, there were no significant differences in vitamin K1 or dp-ucMGP levels or progression of calcification over 18 months (data not shown). Serum concentrations of phosphate, intact parathyroid

hormone and bone-specific alkaline phosphatase remained relatively constant during the study period with no obvious trends in either group (Supplementary Table S9).

Adverse events

In the 60 patients allocated to the treatment groups, no new safety signal evolved in the group receiving vitamin K1 (Table 4). In particular, we noted no increase in thromboembolic events in this group, but rather a trend towards lower event rates of AV fistula thrombosis (20 cases in the control group versus 6 cases in the vitamin K1 group; $P = .185$).

Table 4: Number of participants with adverse events according to the Medical Dictionary for Regulatory Activities System Order Class version 23.1 and number of deaths in the VitaVasK study.

Event	Controls (n = 32)	Vitamin K1 (n = 30)
Any adverse event	91	55
Any serious adverse event	64	53
Deaths	3	4
Death from cardiovascular cause within 18 months after start of treatment	1	0
Blood and lymphatic	2	1
Myocardial infarction	3	1
Acute coronary syndrome	2	2
Other cardiac[a]	13	4
Congenital and genetic	0	0
Ear and labyrinth	0	0
Endocrine	1	1
Eye	2	2
Gastrointestinal	10	14
General	14	7
Hepatobiliary	1	1
Immune system	0	0
Infections and infestations	10	8
Injury, poisoning and procedural complications	8	2
Investigations	8	5
Metabolism and nutrition	0	0
Musculoskeletal	9	6
Neoplasms	0	3
Nervous system	6	2
Stroke	1	1
Pregnancy	0	0
Product issues	0	0
Psychiatric	3	1
Renal and urinary	0	0
Reproductive and breast	0	0
Respiratory	11	14
Skin	2	1
Social	0	0
Surgical/medical procedures	17	18
Embolism	0	0
Symptoms-driven revascularization	0	1
AV fistula thrombosis	20	6
Other vascular	10	3

[a]Arrhythmia, hypertensive crisis, hypotension, stable angina pectoris.

DISCUSSION

Our multicentre, randomized controlled VitaVasK trial is the first to document a marked attenuation of aortic calcification progression in chronic HD patients with vitamin K1 therapy. We noted a similar trend towards less rapid progression of CAC in the group of patients receiving vitamin K1, which reached statistical significance if evaluated using the Agatston score.

A number of recent randomized controlled trials have failed to observe any cardiovascular benefit from vitamin K supplementation in HD patients or patients with advanced CKD [13, 15–18]. The central difference between these earlier trials and our VitaVasK trial is that we administered 15 mg of vitamin K1 per week, which is ∼29 times higher than the daily recommended vitamin K intake of 75 μg. Previous trials in dialysis patients used 200–400 μg of MK-7 [13, 16, 17], i.e. 2.7–5.3-fold higher than the recommended intake, or 90–400 μg daily in non-dialysis CKD patients [15, 18]. Second, all other trials focused on vitamin K2, specifically MK-7. Long-chain menaquinones such as MK-7 are believed to act in non-hepatic tissues, exhibit a

long half-life in healthy subjects [24, 27] and, among the vitamin K2 species, MK-7 is available in synthetic form. While this renders MK-7 attractive in cardiovascular intervention trials, we recently reported that in chronic dialysis patients, the uraemic high-density lipoprotein particles, i.e. important transport vehicles of MK-7 in healthy individuals, hardly incorporate any MK-7 [12]. Thus our very high vitamin K1 dosage, the thrice weekly administration under observation and the altered biodistribution of MK-7 in uraemia can explain why we found a rapid and sustained ∼75% reduction in ucMPG levels with vitamin K1, which can convert into the vitamin K2 species MK-4. In the earlier trials, correction of vitamin K deficiency was less potent and dp-ucMGP plasma levels decreased either slowly and maximally by 47% [17], transiently by 39% [16] or to a small extent only in warfarin-naïve patients [13] by 11% [15] or by 17% [18].

Other aspects may also account for the discrepant observations of benefit versus no benefit from vitamin K therapy. Thus several of the above-mentioned trials lasted only 9–12 months [15, 17, 18], which would not have yielded a significant
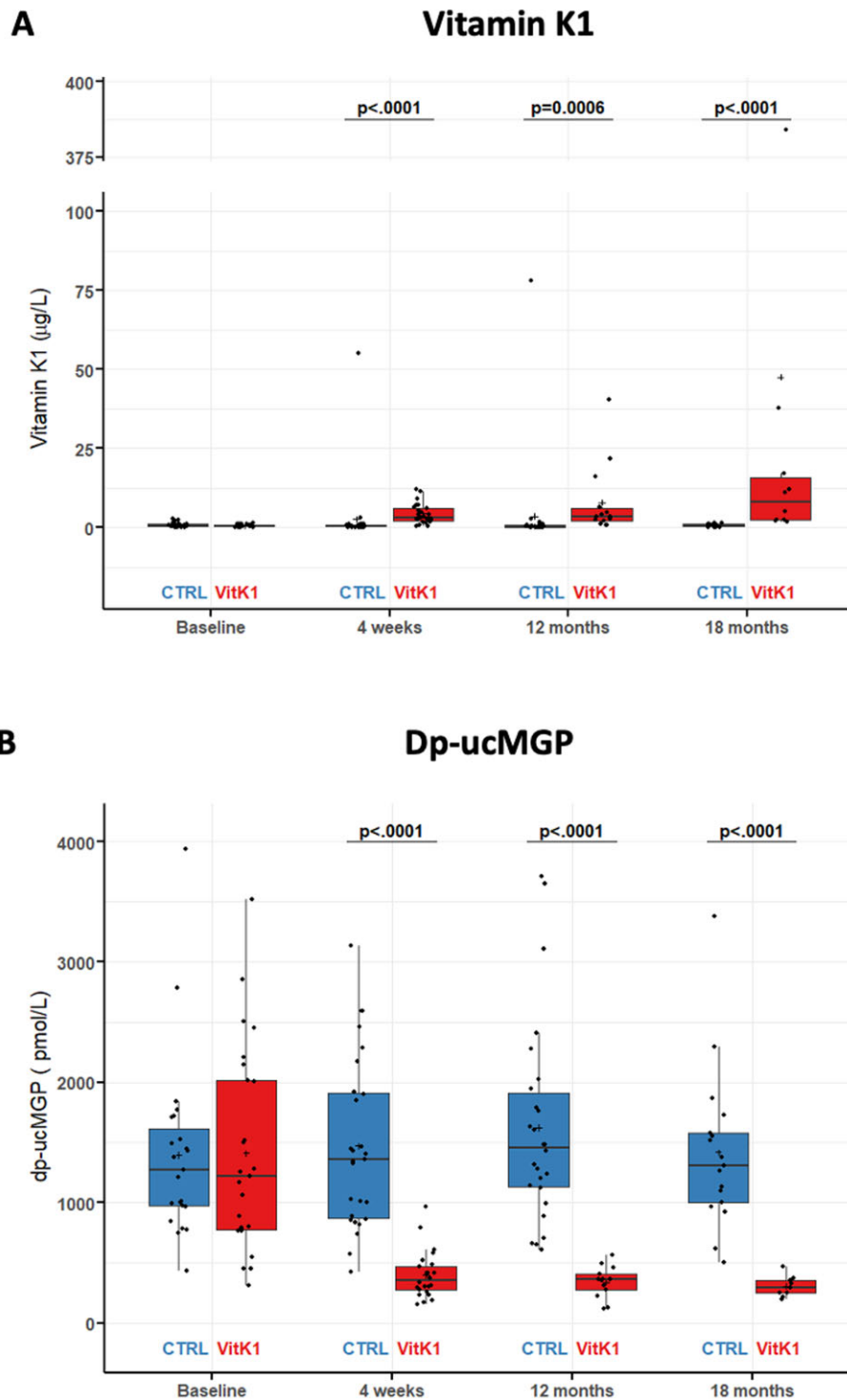
Figure 4: Biochemical endpoints of the VitaVasK trial: **(A)** change in serum vitamin K1 concentration versus baseline at follow-up visits of 4 weeks, 12 months and 18 months and **(B)** change in plasma ucMGP concentration versus baseline at follow-up visits of 4 weeks, 12 months and 18 months. Individual data, means and SDs are shown.

benefit in VitaVasK in any case, even though a trend towards lesser calcification progression with vitamin K was apparent at this time point. In addition, whereas in VitaVasK, 40 of 60 randomized patients were available for analysis at the 18-month study endpoint, other trials had dropout rates of ∼50% at 12 months [17] or 56% over 24 months [16]. Important differences also relate to the Valkyrie trial, which, like VitaVasK, lasted 18 months. Valkyrie included a very particular HD population, notable for an average age of ∼80 years, non-valvular atrial fibrillation in all patients and prior vitamin K antagonist

therapy in the majority of patients [13]. Not unexpectedly, the extent of baseline calcification, e.g. in the thoracic aorta, was therefore ~1.5–2-fold higher in Valkyrie compared with VitaVasK. Nevertheless, a trend towards less calcification progression in the thoracic aorta with MK-7 supplementation was present in Valkyrie as well [13].

The key finding of the multivariate analysis in VitaVasK, namely slower progression of calcification in vitamin K1–treated patients, is consistent with a number of studies in patients without or with only modest CKD. Healthy individuals with CACs experienced slower progression if they received 500 µg vitamin K1 per day for 36 months [28]. In our prior trial in patients with calcific aortic valve stenosis and CKD stages 0–3, daily administration of 2 mg of vitamin K1 for 12 months led to less progression of radiographic calcification and better haemodynamic outcomes than in controls [21]. However, in non-CKD populations there are also clinical trials in which vitamin K1 or MK-7 failed to affect the progression of CAC or haemodynamic changes such as pulse wave velocity [29, 30].

While experimentally the combination of any phosphate binder with vitamin K2 was more potent than either measure alone to retard progression of calcification [31], there are also data indicating that sevelamer in particular may interfere with vitamin K absorption in dialysis patients [26, 32]. However, within the limitations of our trial, we found no evidence for any significant effect of sevelamer on the efficacy of our vitamin K1 therapy.

A major limitation of our trial is that it had to be stopped early, given the slow recruitment, most likely mainly due to the lack of financial incentives and competing trials with better financial rewards. However, our final analysed population is in a similar range compared with previous vitamin K studies in the dialysis population [15–17]. Our power calculation was largely based on the study by Mazzaferro et al. [33] and the ADVANCE trial [34], which focused on progression of calcification in dialysis patients, as these were the only available studies when we designed VitaVasK. However, the cohorts in both studies are different from that studied in ViKaVasK, in particular younger and ~40–50% less calcified at baseline. Both of these are likely to result in much slower progression of calcification. In contrast, VitaVasK may have a much higher power to detect changes in calcification. The open-label nature of the trial does not appear to be a major limitation, given that all radiographic and biochemical endpoints were obtained and analysed in a blinded fashion. In our partially unblinded design, patients might have changed their dietary habits (Hawthorne effect), but stable dp-ucMGP levels in control patients argue against major changes in dietary vitamin K intake. Further, as the RITA software was used for randomization, which was organized centrally, predictability, and thus allocation bias, was mitigated. An imbalance among the covariates of both groups was addressed by the adjusted statistical models and analyses chosen, as adjustment for these covariates was implemented. Missing data are another problem that we dealt with in the statistical analysis model. A central strength of VitaVasK is the administration of vitamin K1 under observation during dialysis, which allows for very high patient adherence compared with daily oral regimens, and thus mitigates compliance bias [16–18].

In summary, despite early termination, this trial identifies vitamin K1 as a promising drug for correcting vitamin K deficiency and decreasing the progression of vascular calcification in HD patients. Vitamin K1 therapy is a potent, safe and cost-effective approach to reducing cardiovascular calcification in this high-risk population.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

J.F., L.S., R.D.H., T.K. and S.R. were responsible for the research idea and study design. T.S., S.R., T.K., M.K., O.L., L.L., P.S., C.K., R.W., P.E., R.S. and J.F. were responsible for data acquisition. R.S. and S.R. were responsible for the quantification of cardiovascular calcifications. L.S. measured vitamin K and uncarboxylated MGP. S.W. and R.D.H. were responsible for the statistical analysis. T.S. and J.F. were responsible for original draft preparation and editing. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

DATA AVAILABILITY STATEMENT

Public posting of individual-level participant data is not covered by the informed patient consent form.

CONFLICT OF INTEREST STATEMENT

The authors have no financial conflicts of interest to disclose.

APPENDIX

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