Vitamin K supplementation to improve vascular stiffness in chronic kidney disease – the K4Kidneys randomised controlled trial

Running title: Vitamin K for vascular stiffness

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Significance Statement

Vascular calcification is common in patients with CKD and is an independent contributor to increased vascular stiffness and vascular risk in this patient group. Vitamin K is a cofactor for proteins that inhibit vascular calcification. In this parallel-group, double-blind randomised controlled trial, 12 months of vitamin K2 supplementation did not improve vascular stiffness measured by pulse wave velocity, despite improvements in desphospho-uncarboxylated matrix Gla protein (an inhibitor of vascular calcification). An updated meta-analysis including these new results confirms a lack of efficacy of vitamin K supplementation on these endpoints. Longer treatment periods, or therapies other than vitamin K, may be required to improve vascular calcification and hence reduce arterial stiffness and cardiovascular risk in patients with CKD.

Abstract

Background

Vitamin K is a cofactor for proteins involved in prevention of vascular calcification, a risk factor for cardiovascular disease. We tested whether vitamin K supplementation could improve arterial stiffness in patients with chronic kidney disease.

Methods

Parallel group, double-blind, randomised, placebo-controlled trial. Participants aged 18 and over with chronic kidney disease stage 3b or 4 (estimated GFR 15-45 ml/min/1.73m^2) were randomised to receive 400mcg oral vitamin K2 or matching placebo once daily for a year. The primary outcome was the adjusted between-group difference in carotid-femoral pulse wave velocity at 12 months. Secondary outcomes included augmentation index, abdominal aortic calcification, blood pressure, physical function and blood markers of mineral metabolism and vascular health. We included results in an updated meta-analysis of trials.

Results

159 randomised participants were included in the modified intention to treat analysis; 80 were allocated to vitamin K and 79 to placebo. Mean age was 66 (SD 12) years, 62 (39%) were female and 87 (55%) had CKD stage 4. No difference was seen in pulse wave velocity at 12 months (adjusted treatment effect -0.1m/s, 95%CI -0.9 to 0.7, p=0.77). No difference was seen in augmentation index at 12 months (adjusted treatment effect 0%, 95%CI -2 to 2, p=0.97), blood pressure, B-type natriuretic peptide or physical function. Updated meta-analysis showed no effect of vitamin K supplementation on vascular stiffness or measures of vascular calcification.

Conclusion

Vitamin K2 supplementation did not improve vascular stiffness or other measures of vascular health in this trial.

Trial Registration: ISRCTN21444964

Introduction

Cardiovascular disease is the major cause of morbidity and mortality in patients with chronic kidney disease (CKD).[1] Cardiovascular risk increases with worsening renal function, but risk is substantially increased even in those with moderate CKD; patients with stage 3b CKD have over twice the mortality rate of those without CKD.[2] This risk persists despite efforts to control conventional cardiovascular risk factors including blood pressure and lipids.[3]

Vascular calcification is common in patients with CKD, and the degree of calcification correlates with the severity of renal impairment.[4] Calcification of elastic arteries is associated with increased vascular stiffness,[5] an important risk factor for cardiovascular events[6], driven by a series of adverse consequences including increased blood pressure, decreased coronary artery perfusion, and the development of left ventricular hypertrophy. The potential importance of vascular calcification is reinforced by analyses showing that it is an independent risk factor for cardiovascular events; abdominal aortic calcification is associated with an odds ratio of 1.6 for vascular events after adjustment for conventional risk factors.[7]

It is now clear that vascular calcification is not a passive process, but is an active, regulated process akin to ectopic new bone formation.[8] Vitamin K-dependent proteins are integral to the regulation of this phenomenon. Two important proteins that regulate and prevent vascular calcification – matrix Gla protein (MGP) and Gla-rich protein – require vitamin K as a cofactor for gamma-carboxylation: an essential step in their activation.[9,10] Other proteins requiring vitamin K for activation, such as osteocalcin and Gas6[11,12] are also involved in bone and mineral metabolism. Vitamin K intake is low for a large proportion of people[13] and for patients with CKD, impaired vitamin K recycling potentially compounds this relative deficiency of vitamin K.[14]

A recent meta-analysis of observational studies confirmed that higher levels of inactive vitamin K-dependent proteins are associated with higher rates of cardiovascular events and death.[15] Few trials of vitamin K have examined the impact of treatment on vascular calcification or stiffness to date, however meta-analyses of those that have suggest a potential significant benefit on vascular calcification, and a non-significant reduction in vascular stiffness.[15] Only one trial to date has examined whether vitamin K supplementation can improve vascular calcification in patients with non-dialysis dependent CKD.[16] This trial did not find a significant difference in coronary artery calcification between the vitamin K and placebo groups, but the trial was small (n=42), and the dose of vitamin K2 was low (90mcg per day); vascular stiffness was not tested as an outcome. The aims of the current trial were therefore to provide a robust test of whether 1 year of vitamin K2 supplementation (400mcg per day) improved pulse wave velocity and other markers of cardiometabolic health and mineral metabolism compared to placebo in patients with CKD stages 3b and 4.

Methods

Trial design

We conducted a parallel-group, placebo-controlled, double-blind, randomised trial. Ethics approval was obtained from the East of Scotland NHS Research Ethics committee (approval number 13/ES/0085). The trial was registered at www.isrctn.com (ISRCTN21444964). Written informed consent was obtained from all participants at the screening visit, and the trial was conducted according to the principles of the Declaration of Helsinki. The trial Sponsors were the University of Dundee and NHS Tayside. Tayside Clinical Trials Unit provided trial management, and the Robertson Centre for Biostatistics (Glasgow) provided data management and statistical analyses.

Trial population and recruitment

Participants were eligible if aged 18 years or over, with CKD stage 3b or 4 (defined as an estimated glomerular filtration rate [eGFR] of >15 ml/min and <45 ml/min by CKD-EPI equation[17]). Potential

participants were excluded if they were unable to give written informed consent, were taking warfarin or had atrial fibrillation, were taking vitamin K or had a known contraindication to vitamin K therapy, were pregnant, intolerant to soya products, were currently enrolled in another trial, or were within 30 days of completing another trial. Participants were recruited via the electronic renal patient records at two large nephrology centres in Scotland and additionally using the NHS Research Scotland SHARE registry (www.registerforshare.org)

Intervention and comparator

Matching tablets containing either 400mcg of vitamin K2 (MK7 subtype), or placebo, were manufactured and bottled by Legosan AB (Kumla, Sweden), and distributed to sites by Tayside Pharmaceuticals (Dundee, Scotland). The trial product was provided in identical bottles with a unique trial identifier on each bottle to ensure masking to participants, clinicians and researchers. Participants were asked to take one tablet each day for the 12 months of the trial. No clear evidence exists to favour vitamin K1 or K2, or to favour a particular subtype of vitamin K2.[15] However, we used the MK7 subtype of vitamin K2 in a previous trial that suggested possible benefit on vascular stiffness[18] and so this subtype was selected for use in the current trial, but at a higher dose than used previously. The dose was selected based on previous work suggesting that vitamin K2 supplementation has dose-dependent effects on undercarboxylation of MGP in patients with CKD at least up to a level of 360mcg per day.[19] Adherence was checked by counting returned tablets at each visit, with percentage adherence calculated as (number of tablets actually ingested / number of tablets expected to be ingested) x 100.

Randomisation and allocation concealment

Randomisation was performed in a 1:1 ratio by a web-based randomisation system, run by the Robertson Centre for Biostatistics, University of Glasgow, to ensure allocation concealment. A minimisation algorithm with a small random element was used to ensure balance across key baseline

measures. Minimisation factors were: study centre (Tayside or Glasgow), age (>70 years or <=70 years), sex, CKD stage (3b or 4), and baseline pulse wave velocity (>9.5m/s or <=9.5m/s). During the course of the trial, a problem with the medication supply for one individual arose which called into question whether the contents of bottles allocated to other participants were correct. All participants who were taking study medication at the time had samples of their medication tested to confirm that the medication they were allocated matched the bottle content list held by the manufacturer. All tested participants except for the index individual were taking study tablets with the correct content. The index individual was removed from the trial and from analysis. For a further 28 individuals who had completed their first six month supply of medication, testing could not be carried out as no tablets remained to test; these individuals were removed from the trial and from analysis as it was not possible to know what treatment they had taken. All medication testing and comparison with manufacturing lists was conducted by teams separate from the investigators, research nurses, statisticians and clinical teams to preserve masking of participants, clinicians and the study team.

The primary outcome was the between-group difference in pulse wave velocity at 12 months, adjusted for baseline values. We measured pulse wave velocity using the Sphygmocor system (AtCor Medical, Sydney, Australia) using measurements of the pulse wave at the carotid and femoral sites.[20] We chose to measure pulse wave velocity rather than directly measuring calcification for several reasons. Any significant change in calcification would be expected to cause a change in arterial stiffness,[5] but pulse wave velocity, as a marker of arterial stiffness, provides not just structural but functional information on large arteries. It is an independent risk factor for future cardiovascular events[6,21] and requires fewer participants to demonstrate clinically important treatment effects than measures of vascular calcification.[22]

Secondary Outcomes

We measured a series of other markers of vascular health. Augmentation index was derived from applanation tonometry at the radial artery using the Sphygmocor system (AtCor Medical, Naperville, Sydney, Australia). We calculated augmentation index expressed as ratio of pressure increase to pulse pressure normalised for a heart rate of 75 beats per minute (AIx@75) using the internal Sphygmocor algorithm. Office blood pressure was measured three times in the recumbent position using an OMROM HEP-705 oscillometric device and was recorded as the average of the second and third readings. 24 hour blood pressure was recorded using the SpaceLabs system (SpaceLabs Healthcare, Snoqualmie, WA, USA), taking half-hourly readings during daytime and hourly reading at night-time. Mean values derived from the whole 24 hour record are reported.

Renal function was assessed using serum creatinine concentration measured as part of routine NHS clinical care, with estimated glomerular filtration rate derived using the CKD-EPI equation.[18] Urinary protein/creatinine ratio was measured from a spot urine sample at baseline and 12 months. Osteocalcin, Tartrate resistant acid phosphatase 5b, parathyroid hormone, fetuin, Fibroblast growth factor-23, 25-hydroxyvitamin D, and 1,25hydroxyvitamin D were measured as markers of bone and mineral metabolism, and N-terminal pro B-type natriuretic peptide (NT-pro-BNP) was measured as a marker of vascular risk. Insulin and glucose were measured and used to calculate insulin resistance using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).[23] Desphospho-uncarboxylated matrix Gla protein (dp-ucMGP) was measured as a biological marker of vitamin K repletion; this marker has previously been shown to correlate with vascular events and is related to the degree of vascular calcification.[15] Details of assay manufacturers and coefficients of variation for each blood and urine assay are given in Supplementary Material.

We performed lateral abdominal radiography to assess aortic calcification. The degree of calcification was assessed by two independent blinded observers, scoring the degree of calcification on a scale from 0 to 3 for the segments of the abdominal aorta adjacent to each of lumbar vertebrae L1 to L4;

this method has previously been shown to correlate with risk factors for uraemic calcification.[24] Anterior and posterior walls were summed separately, giving a total score from 0 to 24 points. Vitamin K-dependent proteins have also been implicated in maintenance of neuromuscular function and bone health, and a previous trial suggested a modest improvement in postural sway with vitamin K supplementation.[18] CKD is associated with worse physical performance and with high falls and fracture rates;[25,26] we therefore assessed markers of muscle function as part of the trial. The Short Physical Performance Battery (SPPB; a test of lower limb balance, strength and function that is a powerful predictor of future disability, falls, need for care and death) was performed,[27] along with maximal handgrip strength measured using a Jamar dynamometer.[28] Monthly self-completed falls diaries were issued to participants to prospectively record falls.[29] All new symptoms or unscheduled healthcare contacts for new problems were recorded as adverse events and coded using Medical Dictionary for Regulatory Activities (MedDRA) version 16.1. All measurements were conducted by trained research nurses masked to treatment allocation. All outcomes were measured at baseline, six and 12 months with the exception of vascular calcification, which was measured at baseline and 12 months.

Sample size calculation

We based our sample size calculation on detecting a 1.0 m/s improvement in pulse wave velocity in the intervention group relative to placebo at 12 months. This degree of improvement corresponds to a reduction in cardiovascular risk of between 6 and 12%.[6,21] Our previous data indicated a standard deviation of 2.1m/s for pulse wave velocity,[18] which is consistent with previously published values for pulse wave velocity in patients with CKD.[30-32] To achieve a power of 80% with alpha=0.05 given the above parameters requires 70 patients per group (140 in total). We assumed a dropout of 15% per 12 months, thus the initial target for recruitment was to randomise 166 participants. The sample size was recalculated during the trial to account for individuals withdrawn from the trial as a result of the medication issue discussed above. To ensure adequate power despite these withdrawals, a revised target of 190 randomised participants was set in January 2017.

Statistical analysis

All analyses were conducted according to a prespecified Statistical Analysis Plan. Analyses were conducted using the statistical package SAS (version 9.3). A two-sided p value of <0.05 was taken as significant for all analyses in line with the sample size calculation above. Descriptive statistics were generated for baseline characteristics and for the adverse event data. For the primary outcome, Analysis of Covariance (ANCOVA) was used to determine the between-group difference at 12 months. Adjustments were made for gender (male/female), continuous age at baseline, study centre (Glasgow/Tayside), baseline continuous eGFR, and the baseline primary outcome continuous value. This analysis was repeated for the 6 month data to determine the between-group difference at 6 months, making the same adjustments as for 12 months. To determine the average effect of the treatment over the duration of the study, repeated measures analyses were carried out adjusting for gender, age at baseline, site and baseline eGFR. For all analyses, the treatment effect estimates along with the 95% confidence intervals (CIs) were provided. For dp-ucMGP results, values below the threshold of reliable linear assay performance (900 pmol/L) were assigned a value of 900 pmol/L prior to log transformation and analysis.

For each of the secondary outcomes, the ANCOVA analyses conducted for the primary outcome were repeated to determine the between-group difference in each of the outcomes at 12 months and 6 months. Repeated measures analyses for the outcomes were also carried out to determine the average treatment effect over the duration of the study, adjusting for gender, age, study centre, baseline eGFR and the baseline value of the outcome being tested. As a number of the secondary outcomes were biomarkers, the residual distribution of each outcome was assessed and the outcome data were transformed where necessary before carrying out the analyses. The falls diary data was assessed by comparing the incidence rate of falls between the study arms using a Poisson regression model. A sensitivity analysis using a negative binomial regression model was also performed. The time

to first fall was then examined using a Cox Proportional Hazards model, adjusting for age, gender, study centre and baseline eGFR. Multiple imputation was conducted as a sensitivity analysis to determine the impact of missing data on the primary and secondary outcome analysis results. Ten imputed datasets were created, using the Markov Chain Monte Carlo method assuming multivariate normality, based on baseline pulse wave velocity, baseline eGFR, gender, and age at baseline. Thereafter the ten datasets were analysed separately using ANCOVA to determine the between-group differences at 12 months and the results were combined using SAS PROC MIANALYZE.

Updated meta-analysis

To assimilate our trial findings with other published trials examining the effect of vitamin K supplementation on vascular stiffness and calcification, we updated our recently published meta-analysis.[15] In line with this previous analysis, the percentage change in vascular stiffness score and vascular calcification score for each group was used and combined with scores from other trials using random effects models in R statistical software (R Studio version 1.0.136), with a sensitivity analysis performed using a fixed effects model. Revised searches were run until end December 2019, using the same search strategy as in our previously published analysis.

Results

A total of 189 participants were randomised between 19th January 2016 and 20th September 2017. Of these, 30 were withdrawn from further participation and from the analysis due to the medication issue described above. The analysis population therefore comprised 159 participants; 80 who received vitamin K and 79 who received placebo. Baseline details are given in Table 1 and participant flow through the trial is shown in Figure 1. The mean adherence to therapy was 91.3% (SD 11.5) in the vitamin K arm and 90.7% (SD 14.7) in the placebo arm. Mean log-transformed dp-ucMGP results fell between baseline and 12 months with vitamin K treatment (7.08 vs 6.89) but not in the placebo group

(7.01 vs 7.06; treatment effect -0.20 [95%CI -0.28 to -0.13]; p<0.001). Individual changes in dp-ucMGP are shown in Figure 2.

Table 2 shows the results of the primary outcome analyses. No significant treatment effect was evident at 12 months (the prespecified primary outcome timepoint), at six months, or by repeated measures. Multiple imputation of missing data at 12 months showed no significant treatment effect and therefore did not change the results of the analysis. There was no significant association between change in log-transformed dp-ucMGP between baseline and 12 months and change in pulse wave velocity between baseline and 12 months (Spearmans rho = 0.08 [95% CI -0.11 to 0.26], p=0.42).

Secondary vascular and physical performance outcomes are shown in Table 3. No effect of treatment was seen on office blood pressure, NT-pro-BNP or augmentation index. No significant treatment effect was seen for the SPPB or for grip strength. The number of participants with at least one fall in each treatment arm was similar; the rate of falls per unit time (the falls rate) was higher in the placebo group, but this was driven by two individuals with very frequent falls. The time to first fall was similar between the two groups: HR 0.79 (95% CI 0.37 to 1.69, p=0.54). A sensitivity analysis removing the two individuals with very high falls rates (74 and 30 falls in 12 months) showed an incident rate ratio for falls of 0.76 (95% CI 0.41 to 1.40, p=0.38); a further sensitivity analysis using a negative binomial regression model yielded an incident rate ratio of 0.80 (95% CI 0.34 to 1.87, p=0.60)

Table 4 shows the results of the other secondary outcomes. Osteocalcin levels were significantly lower in the vitamin K group at 12 months compared to placebo, as was insulin resistance. No other significant treatment effects for markers of renal function or bone and mineral metabolism were observed. Table 5 shows adverse events in each group. No difference was seen between vitamin K and placebo in overall numbers of adverse events. Only small numbers of participants died or commenced renal replacement therapy.

Finally, we combined the results from the current study with results for vascular stiffness and for vascular calcification in an update to our recent meta-analysis. Details of the ten (nine plus the current trial) included studies[16,18,33-39], are given in the Supplementary Material, and the results are shown in Figure 3 (vascular stiffness) and Supplementary Material (vascular calcification). Overall, vitamin K produced no significant reduction in vascular stiffness compared to placebo using a random-effects model: -3.1% [95%CI -6.5 to 0.3]; p=0.07), although a fixed-effects sensitivity analysis showed a marginally significant result (-3.2% [95%CI -5.7 to -0.7]; p=0.014). Meta-analysis of vascular calcification results (Supplementary Material) showed no reduction in vascular calcification score compared to placebo [-3.3% (95%CI -10.4 to 3.7]; p=0.37).

Discussion

There are extensive pre-clinical data suggesting that vitamin K-dependent proteins regulate vascular calcification and biological plausibility that vitamin K supplementation may prevent vascular calcification in patients with advanced CKD. As vascular calcification is integrally associated with arteriosclerosis characterised by increased pulse wave velocity, we assessed whether vitamin K supplementation reduced or attenuated pulse wave velocity over the course of 12 months in patients with stage 3b or 4 CKD. In summary, in this double-blind placebo controlled RCT, vitamin K2 had no effect on reducing pulse wave velocity compared to placebo. Furthermore, vitamin K2 therapy did not lead to any improvement in progression of vascular calcification as measured by aortic calcification on lateral abdominal X-ray compared to placebo. This is despite good adherence to therapy and evidence of lowering of dp-ucMGP levels – a key marker of vitamin K insufficiency that is associated with vascular calcification – in the vitamin K treatment group. An updated meta-analysis confirmed no significant improvement in either vascular stiffness or vascular calcification with vitamin K supplementation when results from the current trial were combined with previous trial results.

There are a number of possible reasons why vitamin K2 supplementation did not improve pulse wave velocity in this trial. It is possible that as arteriosclerosis is challenging to reverse, pulse wave velocity may not be easily amenable to intervention in advanced CKD. This is possible, but other trials have demonstrated that this surrogate parameter is amenable to modification in similar populations, for instance with vitamin D supplementation.[40,41] Pulse wave velocity is generally considered a reliable surrogate marker for arteriosclerosis. However, it is not a direct measure of vascular calcification. Although calcification on abdominal X ray (AXR) is less widely used as a marker of vascular calcification than coronary calcification, AXR calcification has been clearly associated with future cardiovascular events in the Framingham studies.[42] It is possible that treatment for longer than 12 months is necessary to produce a detectable impact on vascular calcification or on vascular stiffness, phenomena that often progress slowly over many years. A previous trial examining the effect of vitamin K supplementation in post-menopausal women found no effect on vascular stiffness until the third year of treatment.[35] However, in the current study vitamin K2 was not associated with improvement in other circulating markers of mineral metabolism such as Fetuin-A concentrations, a biomarker implicated in the prevention of vascular calcification.[43]. The exception was a change in osteocalcin levels in the vitamin K treatment group, but as osteocalcin is itself a vitamin K-dependent protein, the significance of this change in terms of future vascular calcification risk is difficult to interpret. The change in osteocalcin does however give additional evidence that vitamin K supplementation was producing actions in vivo consistent with the known biological roles of vitamin K.

A second possibility is that participants in our trial were vitamin K replete. Although the circulating dp-ucMGP levels in our trial population were lower than those seen in some other populations (e.g. those on haemodialysis[38]), the fact that dp-ucMGP levels fell further in the vitamin K supplementation group suggests that this group of patients were not replete in vitamin K, although they may still have had

levels of active MGP sufficient to inhibit vascular calcification to some extent. We did not attempt to select trial participants on the basis of their vitamin K repletion status; to do so would have required screening participants using the dp-ucMGP assay which is not widely available as a clinical test. It is possible that any treatment effect could have been diluted by recruiting a mixed population of replete and non-replete participants. Future studies could measure dietary vitamin K intake to select participants with particularly low vitamin K intakes. A third possibility is that the dose of vitamin K2 used in our trial was not sufficient to alter vascular calcification or vascular stiffness. We cannot discount this possibility, but we note that higher doses of vitamin K2 (equivalent to 860mcg per day) used in the recent multicentre VALKYRIE trial[38] also failed to improve measures of vascular calcification.

Other trials have tested vitamin K2 supplementation in patients with CKD, although the focus for most other trials has been patients undergoing haemodialysis. Recent open label clinical trials for patients undergoing haemodialysis have also not shown any benefit of vitamin K2 supplementation on aortic calcification in haemodialysis patients[37] and no benefit on coronary calcification in combination with rivaroxaban in haemodialysis patients previously treated with vitamin K antagonist anticoagulants.[38] One short (8 weeks) single arm non-randomised clinical study of vitamin K2 supplementation demonstrated improvement in PWV in renal transplant recipients.[44] Therefore, our results in advanced non-dialysis dependent CKD are in keeping with other similar trials in patients undergoing dialysis.

Few studies have examined the effect of vitamin K supplementation on measures of physical performance in any population. One small trial suggested a possible benefit on postural sway in older people with vascular disease[18] but a more recent trial, testing a similar dose of vitamin K2 to that used in the current trial over 1 year in older people with a history of falls, found no effect on postural sway, falls, or measures of physical performance.[45] Our findings are therefore in keeping with this more

recent trial. Controversy continues as to whether vitamin K supplementation has a significant impact on bone mineral density or fracture in people with osteoporosis; previous reviews have been clouded by the inclusion of fraudulent trial results. The most recent meta-analysis suggests no significant effect on bone mineral density from vitamin K supplementation but is unable to confirm or exclude a clinically important effect on fracture risk.[46]

Strengths of our trial include adequate power to detect a relatively small change in pulse wave velocity, the use of more than one centre to recruit participants, and the use of a placebo control to ensure masking of outcomes assessments and analyses. However, there are a number of limitations. Despite our trial population having a mean age of 66 years, this is still younger than most patients with CKD stages 3b and 4 seen in clinical practice. Similarly, more men than women took part in the trial, and the trial population were overwhelmingly white. Our results may not therefore be generalisable to older populations, centres with a non-white ethnic population, or patients with CKD stage 5. The duration of intervention was limited to 1 year, and it is possible that a longer intervention period would have produced a greater treatment effect; similarly a higher dose of vitamin K or targeting of those most deficient in vitamin K might have delivered greater improvements. Our method of measuring vascular calcification was relatively crude, and the length of our study is unlikely to have been long enough to demonstrate prevention of progression of calcification; our ability to detect reductions in calcification is limited by the fact that some participants did not have detectable calcification on plain abdominal radiographs. A range of different populations, interventions and methods of outcome measurement are included in the meta-analysis. This variation is likely to introduce substantial heterogeneity, and these results should be interpreted with caution. We chose to present results using a random-effects analysis for the main estimate, in part because of this expected heterogeneity, and because such analyses tend to the conservative in deriving broader confidence intervals than seen with fixed-effect analyses.

Based on data from our trial and other recent studies, it does not seem likely that vitamin K supplementation is an effective therapy to prevent or reverse vascular calcification. Alternative strategies to address vascular calcification have recently emerged including a recent clinical trial suggesting that SNF472 (intravenous myo-inositol hexaphosphate) which inhibits formation and growth of hydroxyapatite, attenuates progression of coronary artery calcification in haemodialysis patients.[47] Sodium thiosulfate is often advocated as a treatment for calciphylaxis as it is proposed to mobilise calcium from deposits and forms soluble calcium thiosulphate complexes.[48] It has been shown to have some benefits on vascular calcification in dialysis patients in some studies.[49] Other strategies are likely to emerge (for instance manipulating fetuin-A activity) as our understanding of the biology of vascular calcification continues to advance.[43]

In conclusion, based on our prior assumption that PWV is a meaningful surrogate for further cardiovascular events in patients with CKD, our results do not support the hypothesis that administration of vitamin K2 will reduce cardiovascular events in this population. Combining our results with other recent trials does not currently support the conduct of a large cardiovascular outcome trial using vitamin K2 therapy; instead, alternative methods to improve vascular stiffness in patients with CKD should be explored.

Author contributions

MDW, MB, MW, RLF, IF, RCL, ADS and PBM conceived and designed the trial. MW, SB, RLF, DMcG, AS, NT, PBM and JPT enrolled participants, collected data and interpreted clinical data. DJC, MP and GK analysed and interpreted laboratory analyses. JSL, MRR, ER and IVMcC analysed and interpreted radiological images. KW and IF led analysis of the trial data. JSL led the meta-analysis. MDW and PBM co-wrote the initial report draft; all authors contributed to interpretation of the results, critically revised the manuscript and approved the final submitted version.

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Disclosures

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Other conflicts of interest

PBM reports personal fees and non-financial support from Vifor, personal fees from AstraZeneca, grants from Boehringer Ingelheim, personal fees and non-financial support from Pharmacosmos, personal fees from Janssen, personal fees from Novartis, personal fees from Pfizer, personal fees from Bristol Myers Squibb, personal fees and non-financial support from Napp, outside the submitted work. JSL reports personal fees from Bristol-Myers Squibb, Pfizer and Astra-Zeneca. None of the authors have any other conflicts of interest to declare.

Supplemental material contents

1. Details of assays used in the K4Kidneys trial
2. Details of studies included in the meta-analysis
3. Time to first fall
4. Vascular calcification results
5. Forest plots for vascular calcification

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Table 1. Baseline characteristics of full analysis dataset

		Vitamin K (n=80)	*Placebo (n=79)*
Mean age (years) (SD)		67.3 (11.0)	65.7 (13.5)
Age >70 years (%)		35 (44)	37 (47)
Age <=70 years (%)		45 (56)	42 (53)
Female sex (%)		32 (40)	30 (38)
White ethnicity (%)		76 (95)	77 (98)
Cause of renal dysfunction (%)	Hypertension	18 (23)	13 (17)
	Diabetes mellitus	18 (23)	16 (20)
	Glomerulonephritis	10 (13)	8 (10)
	Polycystic kidney disease	7 (9)	5 (6)
	Vascular disease	5 (6)	7 (9)
	Other	29 (36)	32 (41)
	Not known	5 (6)	5 (6)
Cardiovascular comorbidity (%)	Ischaemic heart disease*	10 (13)	15 (19)
	Stroke	7 (9)	7 (9)
	Heart failure	1 (1)	4 (5)
	Peripheral vascular disease	6 (8)	4 (5)
	Hypertension	69 (86)	63 (80)
	Diabetes mellitus	26 (33)	27 (34)
Previous fragility fracture (%)		5 (6)	3 (4)
Walking aids (%)		19 (24)	12 (15)
Current smoker (%)		6 (8)	12 (15)

Median number of medications (IQR)		7 (4,10)	7 (4,10)
Medication use:	ACEi/ARB	49 (61)	50 (63)
	Phosphate binder	1 (1)	0 (0)
	Erythropoietin	4 (5)	5 (6)
	Iron	12 (15)	7 (9)
Mean eGFR (ml/min/1.73m^2) (SD)		29 (8)	30 (8)
Chronic kidney disease stage 3b vs 4 (%)		37 vs 43 (46% vs 54%)	35 vs 44 (44% vs 56%)
Mean pulse wave velocity (m/s) (SD)		11.9 (3.5)	11.4 (3.2)
Mean augmentation index (%)(SD)		26 (11)	25 (10)
Mean office blood pressure (mmHg) (SD)		147/78 (27/12)	137/74 (19/10)
Mean postural blood pressure drop (mmHg) (SD)		15/4 (18/8)	9/3 (18/9)
Mean 24 hour blood pressure (mmHg) (SD)		128/69 (16/11)	130/70 (17/10)
Mean Short physical performance battery score (SD)		9.1 (2.6)	9.4 (2.6)
Mean handgrip strength (kg) (SD)	Males	31.7 (12.1)	30.2 (9.8)
	Females	14.5 (6.1)	16.6 (6.1)
Median NT-pro-BNP (IQR) (pg/ml) (Q1, Q3)		1494 (415,4133)	2274 (585, 5801)
Median Fetuin (IQR) (ng/ml) (Q1, Q3)		1237 (1005,1535)	1359 (1090, 1847)
Median FGF-23 (IQR) (RU/ml) (Q1, Q3)		170 (126,255)	156 (113,218)
Median Parathyroid hormone (IQR) (pmol/L) (Q1, Q3)		14 (8,22)	13 (8,19)
Median TRACP-5b (IQR) (mIU/ml) (Q1, Q3)		0.41 (0.28, 0.88)	0.55 (0.30, 1.27)
Median Osteocalcin (ng/ml) (Q1, Q3)		37 (24, 62)	36 (24, 52)
Mean 25-hydroxyvitamin D (nmol/L) (SD)		48 (29)	44 (23)
Mean 1,25-hydroxyvitamin D (pmol/L) (SD)		74 (33)	69 (29)
Mean Adjusted calcium (mmol/L) (SD)		2.33 (0.11)	2.33 (0.14)

Mean Phosphate (mmol/L) (SD)	1.17 (0.19)	1.18 (0.20)
Median HOMA IR (Q1, Q3)	2.36 (1.46, 3.66)	2.02 (1.44, 3.78)
Median urinary protein/creatinine ratio (mg/mmol) (Q1, Q3)	36 (5,141)	40 (5,104)
Median aortic calcification score (Q1, Q3)	3.5 (0,9)	2 (0,6.5)

ACEi: Angiotensin converting enzyme inhibitor. ARB: Angiotensin receptor blocker. eGFR: estimated Glomerular Filtration Rate. NT-pro-BNP: N-terminal pro B-type natriuretic peptide. TRACP-5b: Tartrate-resistant acid phosphatase 5b. FGF: Fibroblast growth factor. HOMA-IR: Homeostatic assessment insulin resistance

*previous myocardial infarction, percutaneous coronary angioplasty, coronary artery bypass grafting or diagnosis of angina

Table 2. Primary outcome: Carotid-femoral Pulse Wave Velocity (m/s)

	n	Vitamin K Mean (SD)	n	Placebo Mean (SD)	Unadjusted Treatment effect (95% CI)	p	Adjusted Treatment effect* (95% CI)	p
6 months	57	11.7 (3.2)	63	11.1 (3.1)	0.56 (-0.58 to 1.70)	0.33	0.12 (-0.66 to 0.90)	0.76
12 months	55	11.7 (3.2)	59	11.7 (3.6)	-0.03 (-1.31 to 1.25)	0.96	-0.12 (-0.93 to 0.69)	0.77
Repeated measures	-	-	-	-	-	-	0.01 (-0.66 to 0.68)	0.98
Multiple imputation: 12 months	-	-	-	-	-	-	-0.11 (-0.89 to 0.68)	0.79
Sensitivity analysis** (6 months)	70	11.6 (3.3)	78	11.1 (3.1)	0.52 (-0.53 to 1.57)	0.33	0.20 (-0.51 to 0.90)	0.59

*Adjusted for age, sex, eGFR, site and baseline values

** includes participants removed from main analysis due to uncertainty about medication content

Table 3. Secondary outcomes – vascular and physical function

		Vitamin K Mean (SD)	Placebo Mean (SD)	Treatment effect* (95% CI)	p
Office systolic blood pressure (mmHg)	6 months	139 (22)	134 (18)	0 (-5 to 5)	0.96
	12 months	139 (23)	137 (21)	-2 (-8 to 4)	0.58
	Repeated measures			0 (-5 to 4)	0.86
Office diastolic blood pressure (mmHg)	6 months	76 (11)	74 (10)	1 (-2 to 3)	0.65
	12 months	75 (10)	74 (10)	-1 (-4 to 1)	0.32
	Repeated measures			0 (-3 to 2)	0.70
Augmentation index (%)	6 months	24 (10)	23 (11)	0 (-2 to 2)	0.72
	12 months	25 (10)	25 (9)	0 (-2 to 2)	0.97
	Repeated measures			0 (-2 to 2)	0.94
Log [NT-pro-BNP (pg/ml)]	12 months	7.46 (1.29)	7.47 (1.53)	0.10 (-0.20 to 0.39)	0.51
SPPB	6 months	9.3 (2.8)	9.6 (2.2)	0.1 (-0.5 to 0.7)	0.71
	12 months	9.1 (3.0)	9.8 (2.2)	-0.4 (-1.0 to 0.3)	0.29
	Repeated measures			-0.1 (-0.6 to 0.4)	0.74
Grip strength (kg)	6 months	24.0 (11.6)	25.5 (10.7)	-0.2 (-1.7 to 1.2)	0.75
	12 months	24.1 (11.9)	23.8 (10.6)	-0.2 (-2.1 to 1.8)	0.88
	Repeated measures			-0.4 (-1.9 to 1.1)	0.59
Number with at least one fall		13	15		
Falls / months of fall diary data (falls rate per month)		18/759 (0.024)	128/795 (0.161)	0.147** (0.090 to 0.241)	<0.001

SPPB: Short Physical Performance Battery. NT-pro-BNP: N terminal pro B-type natriuretic peptide

*Adjusted for age, sex, eGFR, site and baseline values

**incident rate ratio

Table 4. Secondary outcomes – blood and urine measures at 12 months

	Vitamin K Mean (SD)	Placebo Mean (SD)	Treatment effect* (95% CI)	p
eGFR (ml/min/1.73m^2)	29 (9)	29 (10)	0 (-2 to 2)	0.96
Log [HOMA-IR]	0.68 (0.79)	0.90 (1.01)	-0.32 (-0.57 to -0.08)	0.01
Log [Osteocalcin (ng/ml)]	2.70 (0.77)	3.62 (0.69)	-0.93 (-1.14 to -0.73)	<0.001
Log [TRACP-5b (mIU/mL)]	-0.88 (1.10)	-0.76 (1.38)	-0.18 (-0.53 to 0.18)	0.32
Log [PTH (pmol/L)]	2.46 (0.76)	2.48 (0.69)	-0.05 (-0.20 to 0.10)	0.50
Phosphate (mmol/L)	1.19 (0.19)	1.19 (0.21)	0.01 (-0.05 to 0.07)	0.83
Calcium (mmol/L)	2.33 (0.11)	2.32 (0.12)	0.01 (-0.02 to 0.04)	0.61
Log [Fetuin (ng/ml)]	7.14 (0.33)	7.22 (0.31)	0.01 (-0.07 to 0.09)	0.81
Log [FGF-23 (RU/ml)]	5.33 (0.71)	5.34 (0.83)	-0.10 (-0.28 to 0.07)	0.23
Log [25OHD (nmol/L)]	3.84 (0.63)	3.75 (0.54)	0.04 (-0.09 to 0.17)	0.57
Log [1,25OHD (pmol/L)]	4.18 (0.51)	4.14 (0.38)	0.06 (-0.08 to 0.19)	0.39
Log [Urine P/Cr ratio (mg/mmol)]	3.85 (1.12)	3.76 (1.54)	-0.02 (-0.49 to 0.46)	0.95

*Adjusted for age, sex, eGFR, site and baseline values

eGFR: estimated Glomerular Filtration Rate. HOMA-IR: Homeostatic Model Assessment – insulin resistance. TRACP: Tartrate-resistant acid phosphatase. PTH: Parathyroid hormone. FGF: Fibroblast growth factor. 25OHD: 25-hydroxyvitamin D. 1,25OHD: 1,25-dihydroxyvitamin D. P/Cr: Protein/Creatinine

Table 5. Number of participants* with adverse events by MedDRA System Order Class (SOC), number of deaths and participants who commenced renal replacement therapy.

	Vitamin K (n=95)	Placebo (n=94)
Any event (%)	69 (72)	68 (72)
Blood and lymphatic (%)	3 (3)	4 (4)
Cardiac (%)	5(5)	4 (4)
Congenital and genetic (%)	1 (1)	0 (0)
Ear and labyrinth (%)	0 (0)	1 (1)
Endocrine (%)	0 (0)	1 (1)
Eye (%)	0 (0)	1 (1)
Gastrointestinal (%)	13 (14)	12 (13)
General (%)	2 (2)	1 (1)
Hepatobiliary (%)	1 (1)	3 (3)
Immune system (%)	0 (0)	1 (1)
Infections and infestations (%)	29 (31)	30 (32)
Injury, poisoning and procedural complications (%)	19 (20)	14 (15)
Investigations (%)	3 (3)	9 (10)
Metabolism and nutrition (%)	9 (9)	4 (4)
Musculoskeletal (%)	8 (8)	13 (14)
Neoplasms (%)	2 (2)	1 (1)
Nervous system (%)	5 (5)	10 (11)
Psychiatric (%)	0 (0)	2 (2)
Renal and urinary (%)	9 (9)	6 (6)
Reproductive and breast (%)	0 (0)	1 (1)
Respiratory (%)	4 (4)	6 (6)
Skin (%)	11 (12)	4 (4)
Surgical/medical procedures (%)	11 (12)	9 (10)
Vascular (%)	3 (3)	3 (3)
Deaths (%)	2 (3)	1 (1)
Commenced renal replacement therapy** (%)	1 (1)	1 (1)

*All 189 randomised individuals included in adverse event analysis

**Haemodialysis, peritoneal dialysis or renal transplant

Figure 1. CONSORT flow diagram

FAS: Full analysis set

Figure 2. Individual change in dp-ucMGP levels between baseline and 12 month follow-up

Dp-ucMGP: Desphospho-uncarboxylated matrix Gla protein

Figure 3. Forest plot – vascular stiffness

a. **Without current trial**

b. **With current trial**