Vitamin K supplementation and arterial calcification in dialysis: results of the double-blind, randomised, placebo-controlled RenaKvit trial

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Running title: Vitamin K and arterial calcification in CKD
ABSTRACT

**Background.** Arterial calcification is associated with cardiovascular mortality in dialysis patients. Active matrix Gla-protein (MGP) is a vitamin K-dependent inhibitor of arterial calcification. Elevated plasma concentrations of inactive MGP, i.e. dephosphorylated-uncarboxylated MGP (dp-ucMGP), is prevalent in dialysis patients. MGP inactivity might contribute to arterial calcification. We investigated if vitamin K supplementation had an effect on arterial calcification in chronic dialysis patients.

**Methods.** In a two-year double-blind, placebo-controlled intervention trial, 48 dialysis patients were randomised to vitamin K (menaquinone-7 (MK-7), 360 µg daily), or placebo. MK-7 in serum and dp-ucMGP in plasma were used to assess vitamin K status. Carotid-femoral pulse wave velocity (cfPWV) and scores of coronary arterial calcification (CAC) and abdominal aorta calcification (AAC) were used to assess arterial calcification.

**Results.** Thirty-seven participants completed year one, 21 completed year two. At year two, serum MK-7 was 40-fold higher, and plasma dp-ucMGP 40% lower after vitamin K supplementation compared with placebo (mean dp-ucMGP difference: -1380 pmol/L (95% CI: -2029;-730)). There was no significant effect of vitamin K supplementation on cfPWV (mean difference at year two: 1.2 m/s (95% CI: -0.1; 2.4)). CAC Agatston score increased significantly in vitamin K supplemented participants, but not significantly different from placebo (mean difference at year two: 664 (95% CI: -554; 1881)). AAC scores increased in both groups, significantly so within the placebo group at year 1, but with no significant between-group differences.

**Conclusion.** Vitamin K supplementation improved vitamin K status, but did not hinder or modify the progression of arterial calcification in dialysis patients.

**Keywords:** chronic kidney disease, coronary arterial calcification, menaquinone-7, pulse wave velocity
INTRODUCTION

Patients with chronic kidney disease have extensive arterial calcification contributing to cardiovascular disease, their most frequent cause of premature death [1-4]. Compared to the general population, death due to cardiovascular disease is 10-20 times more frequent and accounts for more than 50% of the total death toll in dialysis patients [3-5]. In chronic kidney disease, calcification of the arterial tunica media leads to stiffening of the arterial wall and a rising pulse wave velocity (PWV) [6, 7]. In human observational studies, increasing PWV, coronary arterial calcification (CAC), and abdominal aortic calcification (AAC) all increase the risk of cardiovascular disease [8, 9].

Arterial calcification is influenced by matrix Gla protein (MGP). In their landmark study from 1997, Luo et al. observed that MGP knockout mice died prematurely from aortic rupture due to extensive calcification within 6 weeks from birth [10]. MGP is produced by osteoclasts, chondrocytes, and vascular smooth muscle cells in an inactive form (dephosphorylated-uncarboxylated MGP; dp-ucMGP). dp-ucMGP is activated by phosphorylation and vitamin K-dependent carboxylation [11]. In observational studies of chronic kidney disease patients, higher concentrations of inactive dp-ucMGP were associated with more extensive arterial calcification, and increased cardiovascular, and all-cause mortality [12-16]. Chronic kidney disease patients, in particular dialysis patients, have strongly elevated plasma levels of dp-ucMGP reflecting vascular vitamin K deficiency [17]. It has been hypothesized that vitamin K supplementation might improve MGP activation and lead to less arterial calcification in dialysis patients. Previous studies have indeed demonstrated that vitamin K supplementation reduces plasma dp-ucMGP levels. However, only two studies on the effect of vitamin K supplementation on arterial calcification in dialysis patients have been published so far. Both were unblinded and did not include placebo treatment [18, 19]. The present trial examined the effect of vitamin K supplementation on dp-ucMGP and arterial calcification in dialysis patients in a randomised, double-blind, and placebo-controlled setting.
MATERIALS AND METHODS

The RenaKvit study is a two-year randomised, double-blind, and placebo-controlled intervention trial of vitamin K supplementation in dialysis patients and examines the effect of vitamin K on bone quality and arterial calcification. The present manuscript reports findings on arterial calcification.

Based on separate statistical power calculations for bone and artery primary outcomes made a priori (see below, section "Statistical methods"), 123 participants were enrolled. All participated in the bone arm of the study (to be published separately). A subgroup of 48 individuals participated in the present arterial calcification study arm. PWV was the primary study outcome. PWV was chosen as the primary outcome because we expected the chance of identifying PWV changes to be higher than changes in any of the calcification scores, since calcification scores represent the final and probably less dynamic stage of the calcification process. Secondary outcomes were changes in CAC scores, AAC score, and blood markers of vitamin K status. The RenaKvit study was conducted at four different nephrology units in Denmark (Zealand University Hospital Roskilde, Holbæk Hospital, Aarhus University Hospital and Aalborg University Hospital) in the period November 2016-June 2020. The study was approved by The Scientific Ethics Committee for the Region of Zealand, The Danish Data Protection Agency, all participating sites and registered at ClinicalTrial.gov (NCT02976246). The supporting CONSORT checklist is available as Supplemental Table 1S.

Study population

Eligible participants were patients with a dialysis vintage of more than three months, age ≥ 18 years, and a life expectancy of at least two years. All participants provided their informed written consent.

Exclusion criteria in the arterial calcification arm of RenaKvit were: Ongoing treatment with vitamin K or vitamin K-antagonists, atrial fibrillation or other cardiac arrhythmias, severe aortic valve stenosis, bilateral arteriovenous fistulas of upper extremities, amputations of lower extremities above metatarsal level, chronic gastrointestinal malabsorption, ongoing malignancy, any kind of alcohol/drug abuse, former partial or total parathyroidectomy, and ongoing treatment with recombinant parathyroid hormone,
bisphosphonates or other anti-osteoporotic drugs. Fertile women who were without contraception, pregnant or breastfeeding were also excluded. During follow-up, participants were excluded if they received a kidney transplant, initiated vitamin K-antagonist treatment, had unacceptable side effects of study tablets, or were obviously study non-compliant.

**Intervention and Measurements**

**Intervention**

Participants were randomised to either one daily tablet containing 360 µg vitamin K in the form of menaquinone-7 (MK-7), or a visually identical placebo tablet. Tablets had to be taken every morning with some dairy fat. Synthetic MK-7 (K2VITAL®Delta) was produced by Kappa BioScience AS, Oslo, Norway, according to the HACCP (Hazard Analysis Critical Control Point) principles. ORKLA AS, Ishøj, Denmark, produced all tablets. The 360 µg dose was chosen on the basis of one published study demonstrating a dose-dependent decline in dp-ucMGP with a 60% decline at the maximum dose of 360 µg daily [20], and another study reporting a maximum decline in dp-ucMGP of 46% at a maximum dose of 1080 µg thrice weekly (equivalent to 463 µg daily) [21]. Taken together these data suggested that 360 µg daily was a potent dosage. We were reluctant to go too high in dosing due to a fear of unknown side effects in our long-term trial (the dose-finding studies were of few weeks's duration).

**Adherence and adverse events**

Tablet containers were handed out at baseline, and at three, six, 12 and 18 months. Tablet adherence was checked after three, six, 12, 18 and 24 months by counting left-over tablets in returned tablet containers. Patient records were examined for adverse events including death after 12 and 24 months.

**Randomization**

Blocked randomisation of four participants (2:2) was performed by the local statistical department. Each block consisted of patients attending the same dialysis center and being treated with the same dialysis
modality. Coded labelling of tablet containers was carried out by the Clinical Pharmaceutical Service at Herlev University Hospital, Copenhagen, Denmark. Randomisation codes were blinded to participants, hospital staff, and investigators until all data harvesting and data spreadsheet input had been finished.

Measurements

Biochemical samples were collected, radiographic imaging, carotid-femoral pulse wave velocity (cfPWV) and 24-hour ambulatory blood pressure (BP) measurements performed, and clinical data harvested from patient records at baseline, after one, and after two years.

Carotid-femoral Pulse Wave Velocity

Changes in cfPWV after one year was the primary study endpoint. cfPWV is defined as the distance between the two recording sites divided by the difference in pulse wave travel time and expressed in meters per second (m/s). All pulse wave measurements were performed in double under standardised examination conditions according to guidelines [22] by the same trained research nurse or trial investigator at each study site to minimize inter-observer variation. Methodology details are presented in Supplemental material 1S.

Radiographic imaging

Computed tomography (CT) of the heart were performed using a 256-slice Philips iCT scanner (Philips Healthcare, Amsterdam, Netherlands) or a Siemens 128-slice CT scanner (Siemens Healthcare GmbH, Erlangen, Germany). Recordings were synchronized with ECG and participants were instructed to lie supine, still and intermittently hold their breath. Quantification of calcification was estimated both as two-dimensional scoring ad modum Agatston and three-dimensional volume scoring using IntelliSpace software (Philips Healthcare, Cleveland, OH, USA) [23]. Calcification scores were obtained from the coronary arteries (coronary arterial calcification; CAC), and from the aortic and mitral valves (cardiac valve calcification; CVC).
All heart CT scans from all participants were reviewed separately by two experienced cardiologists. If calcification estimates differed more than five percent, the cardiologists reviewed the scan together.

Abdominal aortic calcification (AAC) score was quantified using a laterally exposed conventional X-ray of the lumbar spine and abdominal aorta according to Kauppila et al [24]. All X-rays from all participants were reviewed by the same experienced radiologist to eliminate inter-observer variation.

24-hour Ambulatory Blood Pressure

A 24-hour BP measurement was performed using the validated BP device Mobil-O-Graph® (IEM-HMS, Stolberg, Germany) as recommended [25] (details presented in Supplemental material 4S).

Biochemical measurements

Fasting blood samples were taken from the patient outlet line in haemodialysis patients at the initiation of a dialysis session, and from a peripheral vein in peritoneal and hybrid dialysis patients. The blood samples were centrifuged for 10 minutes at 3000 g within 30-60 minutes and were immediately stored at -80°C until analysis as a single batch up to 32 months later.

Serum Vitamin K1 and MK-7 were analysed by mass spectrometry according to Boegh et al with modifications for MK-7 analysis (Supplemental Tables 2S-3S) [26]. Protein Induced by Vitamin K Absence-II (PIVKA II) was measured in plasma using a commercially available electrochemiluminescence immunoassay (ECLIA) according to the manufacturer’s instructions (Cobas e602 analyser, Roche Diagnostics, Denmark). The intermediary precision was <7%. Dp-ucMGP was measured in plasma using a commercially available ECLIA according to the manufacturer’s instructions (ImmunoDiagnostic Systems Holdings PLC, East Boldon, United Kingdom). The intermediary precision was <10%. 1,25-(OH)2 vitamin D was measured in serum using a chemiluminescence immunoassay (CLIA) in the IDS-iSYS automated analyser (ImmunoDiagnostic Systems Holdings PLC, East Boldon, United Kingdom). Intermediary precision was <17%. Routine blood analyses were performed at local certified laboratories.
Statistical Methods

Based on previous studies, our study was designed to give 80% power to detect a minimal relevant difference in cfPWV of 1 m/s after one year at a significance level of 5% at an assumed standard deviation (SD) of 0.95 m/s [6, 27]. We expected a yearly drop-out rate of 20%. Based on these assumptions, we aimed at 2 x 16 participants completing year one, and therefore aimed at including at least 2 x 20 participants.

Maximum likelihood estimates of treatment effects on levels as well as change of biological markers were estimated using linear mixed effects regression models including a random intercept for each patient. The analyses were adjusted for baseline measures by constraining the baseline means of the treatment groups to be equal as suggested by Twisk et al [28]. Examination of the underlying model assumptions were performed by visual inspection of QQ-plots of residual errors and of random effects. Nonparametric bootstrapping estimation with 1000 replications was implemented if non-normality of residuals or random effects was observed. Severe deviation from homoscedasticity was observed for MK-7 due to increasing variation in the K-vitamin group at follow-up measurements. The analysis for this particular outcome was modified allowing for separate residual variance estimates at follow-up, but only for the treatment group. With the applied methodology missing values were handled and list exclusion avoided, meaning that analyses were intention to treat analyses. Linear mixed effects models were implemented in Stata Statistical software, version 16, 2019 (Statacorp LCC, College Station, TX, USA). A P value of < 0.05 was considered statistically significant.
RESULTS

A total of 689 dialysis patients were screened, and 48 patients enrolled. Participants were randomised to either vitamin K (n = 24), or placebo (n = 24). Eleven patients (23%) dropped out during year one. An additional 16 participants (33%) dropped out during year two of the trial, leaving 21 completing participants (Figure 1). Baseline characteristics of all 48 randomised participants are shown in Table 1.

Biochemical outcomes (Table 2)

Individual serum MK-7 and plasma dp-ucMGP values are shown in Figure 2.

Serum MK-7 increased progressively over the 2-year period in the vitamin K supplemented group and was 40-fold elevated compared with placebo at year two.

Plasma dp-ucMGP decreased significantly around 30% within the vitamin K group during year one, but then rose during year two. In the placebo group, a steady increase in dp-ucMGP over both years was observed. The treatment effect was almost identical at years one and two with the vitamin K group having dp-ucMGP concentrations 40-45% lower than the placebo group.

For plasma concentrations of PIVKA-II, vitamin K supplementation caused a 45% lowering after one and two years compared with placebo.

cfPWV (Table 3)

Individual cfPWV measurements are presented in Supplemental Figure 4S.

Mean cfPWV declined during year one in both groups, significantly so in the placebo group. The cfPWV changes during intervention did not differ significantly between groups, but there was a slight trend towards a higher cfPWV with vitamin K supplementation at the 2-year follow-up. cfPWV results remained unaltered after adjustment for mean arterial pressure obtained just prior to PWV measurements (mean difference in cfPWV at year two after adjustment: 1.1 m/s (95% CI: -0.1;2.2, P = 0.07)).

CAC, CVC and AAC (Table 3)
Individual CAC Agatston, CVC Agatston, and AAC scores are shown in Supplemental Figure SS4.

CAC Agatston and volume scores increased in both groups over the 2-year intervention period, but the increments at year two in the vitamin K supplemented group were the only significant within-group changes. In the between-group comparisons, we observed no significant differences.

For CVC Agatston and volume scores, a progressive increase was seen over the 2-year intervention period in both groups, significantly so at year two in the placebo group only. Again, there were no significant differences in the between-group comparisons.

AAC scores also increased with time in both groups. The within-group increment at year two was significant in the placebo group, but in between-group comparisons differences were far from significant.

Adherence and adverse events

In the vitamin K group, mean adherence was 99% ranging from 97 to 103% at year one (few individuals had ingested more than one tablet daily on average). Completers of year two had a 2-year mean adherence of 99% ranging from 96 to 100%. In the placebo group, the corresponding adherence at year one was 91% ranging from 60 to 100%, and 92% ranging from 93 to 100% at two years.

We observed no significant between-group differences in adverse events over the two-year intervention period. Five participants in the vitamin K group versus four in the placebo group experienced a thromboembolic event, and four participants died versus six in the placebo group.
DISCUSSION

The RenaKvit study was a two-year randomised, double-blind, placebo-controlled trial investigating the effect of vitamin K supplementation on arterial calcification in dialysis patients. Vitamin K supplementation (MK-7 360 µg/day) caused marked improvements in vitamin K status as assessed from blood concentrations of MK-7, dp-ucMGP and PIVKA-II. Nonetheless, arterial calcification assessed from cfPWV, CAC, CVC, and AAC scores was not significantly affected compared with placebo treatment. In both groups, arterial calcification actually progressed over the two-year intervention period.

A few earlier trials have consistently reported a decrease in plasma dp-ucMGP with MK-7 supplementation in dialysis patients [20, 21, 29]. These studies reported a dose-dependent 17-86% reduction in dp-ucMGP after only 4-8 weeks of MK-7 supplementation in daily dosages of 45-463 µg. In our study, vitamin K supplementation led to 40-45% lower dp-ucMGP concentrations at years one and two compared with placebo. Our data correspond quite closely to the results obtained in the shorter-termed studies by Caluwé et al [21] and Westenfeld et al [20] and illustrate that MK-7 effects on plasma dp-ucMGP concentrations are maintained over longer terms with continuing MK-7 supplementation. Our long-term follow-up further demonstrated that the decline in dp-ucMGP from baseline observed at year one was followed by an increase during year two despite continued vitamin K supplementation, so that dp-ucMGP at baseline and year two did not differ in the vitamin K supplemented group. This observation shows that vitamin K supplementation markedly reduces dp-ucMGP levels, but cannot inhibit a subsequent gradual rise in dp-ucMGP over years. It should also be noted, that vitamin K supplementation at no stage normalised dp-ucMGP concentrations in our study. The reference interval for dp-ucMGP in healthy individuals is < 532 pmol/L [30]. At year one, our vitamin K supplemented participants had a mean dp-ucMGP of 1290 pmol/L.

Some earlier trials in non-dialysis patients indicated that vitamin K supplementation might have a beneficial effect on cfPWV and arterial calcification [31-33]. However, other studies were negative [34, 35], and most recently, Witham et al reported an absence of vitamin K2 effect on cfPWV and AAC in a one-year double-blind placebo-controlled trial of patients with chronic kidney disease stage 3b-4 [36].
Only two published studies investigated the effect of MK-7 supplementation on arterial calcification in dialysis patients. Oikonomaki et al randomised 102 hemodialysis patients to +/- MK-7 supplementation (200 µg daily) for one year in an open label trial published in 2019 [19]. Fifty-two patients were available for analysis (drop-out rate 51%). Arterial calcification assessed from abdominal aortic Agatston scores did not differ between groups despite a 50% decrease in uncarboxylated MGP (ucMGP) among MK-7 supplemented participants. In 2020, De Vriese et al published their study of 132 hemodialysis patients randomised to either vitamin K-antagonist, rivaroxaban, or rivaroxaban + MK-7 in an open label, non-placebo-controlled study [18]. After 18 months, 77 participants remained in the study reflecting a drop-out rate of 42%, primarily due to deaths. cfPWV and calcifications scores were not significantly affected. Accordingly, the authors concluded that MK-7 had no significant favourable effect on arterial calcification progression. Our present results are fully in line with those of Oikonomaki and De Vriese. All three studies found arterial calcification progression in dialysis patients despite high-dosed MK-7 supplementation for 1-2 years.

As of December 2020, the present study is the first randomised, double-blind, placebo-controlled trial to report on the effects of vitamin K supplementation on arterial calcification in dialysis patients to our knowledge. As a strength, we excluded patients with cardiac arrhythmias, bilateral arteriovenous fistulas of the upper extremities, severe aortic valve stenosis, and major amputations of lower extremities in order to obtain reliable cfPWV measurements - our primary outcome - according to current method guidelines [22, 37]. The validity of our findings was supported by the demonstration of a high degree of study adherence, not only from tablet counting, but also from actual measurements of MK-7 serum concentrations. Also, our limited 1-year drop-out rate of 23% close to the expected 20% drop-out rate on which we based our power calculation may be considered a strength. The number of participants with available cfPWV measurements at year one was sufficient to give the study the planned power of 80% to detect changes of 1 m/s in cfPWV. A change in cfPWV of 1 m/s may translate into a clinically significant 15% change in cardiovascular risk, and therefore it was important for us to ensure that our study had the power to detect a treatment effect of that size [38]. Also noteworthy is the fact that our study had
sufficient power to demonstrate significant increases in calcification scores in both the vitamin K and the placebo group.

In the interpretation of our study, it should be noted that our participants belonged to the more healthy part of a dialysis population due to our study exclusion criteria. Thus, our findings might not apply to less healthy dialysis patients. However, we consider it unlikely that they would respond more favourably to vitamin K supplementation than our participants. A study duration of two years might be considered too short and a study weakness, but the few earlier trials in advanced chronic kidney disease patients in this field had shorter observation periods of 12-18 months [18, 19, 36]. Furthermore, it should be taken into account that arterial calcification effects of vitamin K supplementation should probably appear within a year or so if they should have any chance to impact the survival of dialysis patients who are known to have a high mortality rate. Our study comprised both haemodialysis and peritoneal dialysis patients. In the hypothetical case that the arterial calcification process responds differently on vitamin K supplementation depending on dialysis modality, the inclusion of patients on both modalities might weaken the power to detect any effects. However, we consider it less likely that it could be the case. Finally, it should be noted that we did not use very low CAC Agatston scores (< 30) as a study exclusion criterion. The seminal study by Block et al demonstrated that individuals on haemodialysis with very low CAC Agatston scores were unlikely to have arterial calcification progression over 18 months [39]. In our study, a small minority of patients (around 10%) had very low CAC Agatston scores at baseline, and they did indeed have no or very small changes in CAC scores over time. It is evident that the inclusion of these patients may have reduced over our study power modestly regarding CAC scoring, but our data demonstrate that it did not prevent us from demonstrating significant increments in CAC Agatston score within the vitamin K supplemented group.

Despite lege artis methodology with respect to study design, study execution, and arterial calcification assessments, we did not find any signs of vitamin K supplementation having an essential role in preventing the progression of arterial calcification in dialysis patients. However, due to the limited number of study participants, a minor, but still relevant effect of vitamin K on arterial calcification in
dialysis patients cannot be excluded. Larger studies of vitamin K supplementation in dialysis patients are underway, and their results are eagerly awaited.
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CONFLICT OF INTEREST STATEMENT

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AUTHORS’ CONTRIBUTIONS

K.L.-S., M.F.-M., D.H., N.E.F, and P.M. designed the study. K.L.-S., C.S., J.D.J., K.D.K, and C.D.P. collected the clinical data. A.S., C.L.B., J.S.M., and N.R.J. were responsible for the specialized biochemical analyses. J.B.F., H.E., C.T.L., and H.S. led the radiological investigations, and the images were analysed by H.E., C.T.L., and H.S. K.L.-S. and M.F.-M. analysed the cfPWV measurements. K.L.-S. and I.P. did the statistical analyses and drafted all tables and figures. K.L.-S. and P.M. drafted the paper. All authors revised the paper and approved the final version.
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## DATA AVAILABILITY STATEMENT

| Question                                                                 | Answer                                                                 |
|-------------------------------------------------------------------------|------------------------------------------------------------------------|
| Will individual participant data be available?                          | Yes                                                                    |
| What data in particular will be shared?                                 | Individual participant’s deidentified data that underlie the results reported in this article (text, tables, and figures). |
| What other documents will be available?                                 | Study protocol (in Danish), Participants information (in Danish), and Informed consent form (in Danish). |
| When will data be available?                                            | Beginning immediately following publication and ending 36 months following article publication. |
| With whom?                                                              | Researchers who provide a methodologically sound proposal.             |
| For what types of analyses?                                             | To achieve aims in the approved proposal.                              |
| By what mechanism will data be available?                               | Proposals should be directed to karinschousboe@dadlnet.dk.             |
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Table 1. Baseline characteristics of 48 randomised dialysis patients

|                                | Vitamin K | Placebo |
|--------------------------------|-----------|---------|
| No (total)                     | 24        | 24      |
| Males                          | 19 (79)   | 18 (75) |
| Age (years)                    | 62 (±11)  | 66 (±11) |
| Height (cm)                    | 174 (±10) | 174 (±7) |
| Body Mass Index (BMI; kg/m²)   | 26.5 (±5.8) | 29.2 (±6.1) |
| Cardiovascular calcification risk factors |           |         |
| - Systolic blood pressure (mmHg) | 134 (±19) | 137 (±17) |
| - Diastolic blood pressure (mmHg) | 80 (±12)  | 82 (±15) |
| - P-LDL Cholesterol (mmol/L)   | 2.0 [1.4;2.8] | 2.4 [2.2;3.5] |
| - P-Phosphate (mmol/L)         | 1.64 [1.38;1.75] | 1.45 [1.25;1.93] |
| - P-Ionized calcium (mmol/L)   | 1.19 (±0.10) | 1.20 (±0.07) |
| - P-PTH (pmol/L)               | 25 [19;42]  | 18 [13;34] |
| - P-1,25-OH2 vitamin D (pmol/L) | 53 (±33)   | 44 (±24) |
| - P-25OH vitamin D (nmol/L)    | 69 (±39)   | 58 (±30) |
| Smokers                        |           |         |
| - Active                       | 7 (29)    | 6 (25)  |
| - Former                       | 13 (54)   | 9 (38)  |
| Comorbidity                    |           |         |
| - Cerebral stroke              | 3 (13)    | 0 (0)   |
| - Peripheral arterial disease (PAD) | 4 (17)    | 1 (4)   |
| - Ischemic heart disease       | 5 (21)    | 1 (4)   |
| Dialysis modality              |           |         |
| - Haemodialysis                | 16 (67)   | 11 (46) |
| - Peritoneal dialysis          | 8 (33)    | 10 (42) |
- Hybrid dialysis\textsuperscript{a} & 0 (0) & 3 (12) \\

| Dialysis vintage (months) | 28 [7;48] & 22 [12;45] |
|---------------------------|-------------|-------------|
| Former kidney transplant  | 4 (17)      | 4 (17)      |

**Medication**

- **Antidiabetic** & 7 (29) & 7 (29) \\
- **Lipid lowering** & 9 (38) & 6 (25) \\
- **Antihypertensive** & 21 (88) & 24 (100) \\
  - Betablockers & 14 (58) & 16 (67) \\
  - Calcium channel blockers & 14 (58) & 16 (67) \\
  - Diuretics & 16 (67) & 14 (58) \\
  - Aldosteron antagonists & 1 (4) & 3 (13) \\
  - RAS blockers (ACEI/ARB) & 6 (25) & 14 (58) \\
  - Others (Moxonidin, Minoxidil) & 4 (17) & 4 (17) \\

- **Vitamin D**
  - Native & 20 (83) & 14 (58) \\
  - Activated & 17 (71) & 18 (75) \\

- **Phosphate binders**
  - Calcium-containing & 11 (46) & 14 (58) \\
  - Non-calcium-containing & 21 (88) & 18 (75) \\
  - Calcimimetics & 4 (17) & 8 (33) \\

Categorical data are presented as number and percentage (n (%)). Continuous variables are presented as mean and standard deviation (mean (± SD)), or median and interquartile range (median, [IQR]).

**Abbreviations:** MK-7, menaquinone-7; P, plasma; LDL, low-density lipoprotein; PTH, parathyroid hormone; RAS, Renin-Angiotensin System; ACEI, ACE-inhibitor; ARB, Angiotensin-II Receptor Blockers.

\textsuperscript{a} Hybrid dialysis; treatment consisting of both haemo- and peritoneal dialysis.
Table 2. Effect of vitamin K supplementation (MK-7 360 µg daily) or placebo in dialysis patients: Biochemical outcomes estimated from linear mixed models with random intercepts.

| Outcome       | Time   | Mean levels (95% CI) | Mean change within group (95% CI) | Mean difference between groups (95% CI) |
|---------------|--------|----------------------|----------------------------------|---------------------------------------|
|               |        | Vitamin K            | Placebo                          | Vitamin K P Placebo P P                     |
| S-vitamin K1  |        | 0.6 (0.4 ; 0.9), n=48| 0.6 (0.2 ; 1.1), n=17            | 0.1 (-0.4 ; 0.6) 0.66 -0.0 (-0.5 ; 0.5) 0.94 0.1 (-0.5 ; 0.7) 0.69 |
| (nmol/L)      | Year 1 | 0.7 (0.3 ; 1.2), n=19| 0.6 (0.2 ; 1.1), n=17            | 0.3 (-0.3 ; 0.8) 0.40 0.4 (-0.3 ; 1.0) 0.27 -0.1 (-0.9 ; 0.7) 0.76 |
|               | Year 2 | 0.9 (0.3 ; 1.4), n=12| 1.0 (0.4 ; 1.6), n=9             | 14.4 (1.4 ; 27.4) 0.03 0.0 (-0.3 ; 0.4) 0.80 14.4 (1.4 ; 27.4) 0.03 |
|               | Year 2 | 15.0 (8.1 ; 21.9), n=19| 14.4 (1.4 ; 27.4) 0.03 0.0 (-0.3 ; 0.4) 0.80 14.4 (1.4 ; 27.4) 0.03 |
| S-MK-7        | Baseline| 0.6 (-1.2 ; 5.0), n=48| 0.6 (-1.2 ; 5.0), n=48           | 29.5 (20.9 ; 38.1), n=12 0.7 (-9.2 ; 10.6), n=9 29.2 (12.9 ; 45.6) <0.001 0.2 (-0.3 ; 0.6) 0.46 29.1 (12.7 ; 45.5) <0.001 |
| (nmol/L)      | Year 2 | 29.5 (20.9 ; 38.1), n=12| 29.5 (20.9 ; 38.1), n=12 0.7 (-9.2 ; 10.6), n=9 29.2 (12.9 ; 45.6) <0.001 0.2 (-0.3 ; 0.6) 0.46 29.1 (12.7 ; 45.5) <0.001 |
| P-dp-ucMGP    | Baseline| 2104 (1793 ; 2415), n=48| 2104 (1793 ; 2415), n=48         | 1952 (1472 ; 2432), n=12 0.7 (-9.2 ; 10.6), n=9 29.2 (12.9 ; 45.6) <0.001 0.2 (-0.3 ; 0.6) 0.46 29.1 (12.7 ; 45.5) <0.001 |
| (pmol/L)      | Year 1 | 1290 (883 ; 1696), n=20| 1290 (883 ; 1696), n=20          | 152 (-588.83 ; 285) 0.49 1228 (729 ; 1726) <0.001 -1380 (-2029 ; -730) <0.001 |
|               | Year 2 | 1952 (1472 ; 2432), n=12| 1952 (1472 ; 2432), n=12 0.7 (-9.2 ; 10.6), n=9 29.2 (12.9 ; 45.6) <0.001 0.2 (-0.3 ; 0.6) 0.46 29.1 (12.7 ; 45.5) <0.001 |
| P-PIVKA-II    | Baseline| 41.8 (35.4 ; 48.1), n=47| 41.8 (35.4 ; 48.1), n=47         | 17.2 (7.5 ; 26.9), n=20 31.3 (20.8 ; 41.8), n=17 -24.6 (-36.1 ; -13.0) <0.001 -10.4 (-22.7 ; 1.8) 0.10 -14.1 (-28.4 ; 0.1) 0.05 |
| (ng/ml)       | Year 1 | 17.2 (7.5 ; 26.9), n=20| 17.2 (7.5 ; 26.9), n=20          | 22.4 (9.9 ; 34.8), n=12 39.3 (24.9 ; 53.7), n=9 -19.4 (-33.4 ; -5.4) 0.01 -2.5 (-18.2 ; 13.3) 0.76 -16.9 (-36.0 ; 2.2) 0.08 |
|               | Year 2 | 22.4 (9.9 ; 34.8), n=12| 22.4 (9.9 ; 34.8), n=12          | 22.4 (9.9 ; 34.8), n=12 39.3 (24.9 ; 53.7), n=9 -19.4 (-33.4 ; -5.4) 0.01 -2.5 (-18.2 ; 13.3) 0.76 -16.9 (-36.0 ; 2.2) 0.08 |

Abbreviations: MK-7, menaquinone-7; S, serum; P, plasma; dp-ucMGP, dephosphorylated-uncarboxylated matrix Gla protein; PIVKA-II, Protein induced by vitamin K absence-II.
Table 3. Effect of vitamin K supplementation (MK-7 360 µg daily) or placebo in dialysis patients: Arterial outcomes estimated from linear mixed models with random intercepts.

| Outcome | Time  | Mean levels (95% CI) | Mean change within group (95% CI) | Mean difference between groups (95% CI) |
|---------|-------|----------------------|------------------------------------|----------------------------------------|
|         |       | Vitamin K            | Placebo                           | Vitamin K P | Placebo P | P       |
|         |       | 9.8 (9.0 ; 10.5), n=47 |                                    | 0.34 0.04   | 0.5 (0.6 ; 1.5) 0.37 |
| cfPWV   | Baseline | 9.4 (8.5 ; 10.4), n=18 | 9.0 (7.9 ; 10.0), n=14 | -0.3 (-1.0 ; 0.4) | -0.8 (-1.6 ; -0.0) | 0.04 |
|         | Year 1  | 10.5 (9.4 ; 11.5), n=11 | 9.3 (8.2 ; 10.4), n=9 | 0.7 (-0.2 ; 1.6) | -0.5 (-1.4 ; 0.5) | 0.35 |
|         | Year 2  | 10.5 (9.4 ; 11.5), n=11 | 9.3 (8.2 ; 10.4), n=9 | 0.7 (-0.2 ; 1.6) | -0.5 (-1.4 ; 0.5) | 0.35 |
|         | Year 2  | 10.5 (9.4 ; 11.5), n=11 | 9.3 (8.2 ; 10.4), n=9 | 0.7 (-0.2 ; 1.6) | -0.5 (-1.4 ; 0.5) | 0.35 |
| CAC     | Baseline | 2034 (1650 ; 2419), n=48 |                                    | 0.06 0.11   | -151 (-1154 ; 852) 0.77 |
| Agatston | Year 1  | 2573 (2034;3111), n=19 | 2723 (1923;3524), n=17 | 538 (-32 ; 1109) | 689 (-149 ; 1527) | 0.11 |
|         | Year 2  | 3253 (2285;4222), n=11 | 2590 (1913;3266), n=9 | 1219 (196 ; 2242) | 555 (-87 ; 1197) | 0.09 |
|         | Year 2  | 3253 (2285;4222), n=11 | 2590 (1913;3266), n=9 | 1219 (196 ; 2242) | 555 (-87 ; 1197) | 0.09 |
| CAC     | Baseline | 1710 (1405 ; 2015), n=48 |                                    | 0.08 0.13   | -102 (-893 ; 689) 0.80 |
| Volume  | Year 1  | 2118 (1691;2546), n=19 | 2220 (1590;2850), n=17 | 408 (-44 ; 860) | 510 (-148 ; 1168) | 0.13 |
| Agatston | Year 2  | 2678 (1883;3466), n=11 | 2125 (1569;2682), n=9 | 968 (134 ; 1802) | 415 (-119 ; 949) | 0.13 |
|         | Year 2  | 2678 (1883;3466), n=11 | 2125 (1569;2682), n=9 | 968 (134 ; 1802) | 415 (-119 ; 949) | 0.13 |
| CVC     | Baseline | 1111 (801 ; 1421), n=48 |                                    | 0.43 0.07   | -580 (-1444 ; 284) 0.19 |
| Agatston | Year 1  | 1238 (861 ; 1614), n=19 | 1818 (1068 ; 2567), n=17 | 127 (-192 ; 446) | 707 (-71 ; 1484) | 0.07 |
|         | Year 2  | 1527 (892 ; 2162), n=11 | 1830 (1317 ; 2342), n=9 | 416 (-213 ; 1045) | 719 (226 ; 1212) | <.001 |
|         | Year 2  | 1527 (892 ; 2162), n=11 | 1830 (1317 ; 2342), n=9 | 416 (-213 ; 1045) | 719 (226 ; 1212) | <.001 |
| CVC     | Baseline | 914 (668 ; 1161), n=48 |                                    | 0.19 0.47   | -303 (-1117 ; 512) 0.47 |
| Volume  | Year 1  | 1013 (717 ; 1308), n=19 | 1451 (870 ; 2031), n=17 | 98 (-150 ; 347) | 536 (-66 ; 1139) | 0.08 |
|         | Year 1  | 1013 (717 ; 1308), n=19 | 1451 (870 ; 2031), n=17 | 98 (-150 ; 347) | 536 (-66 ; 1139) | 0.08 |

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|                  | Year 2 | Baseline                        | Year 1 | Baseline                        | Year 2 | Baseline                        |
|------------------|--------|---------------------------------|--------|---------------------------------|--------|---------------------------------|
| **24H SysBP** (mmHg) |        |                                 |        |                                 |        |                                 |
|                  |        |                                 |        |                                 |        |                                 |
| Baseline         | 1261 (746 ; 1776), n=11 | 9.1 (8.0 ; 10.2), n=43 | 1498 (1092 ; 1903), n=9 | 10.2 (8.5 ; 11.9), n=16          | 135 (129 ; 141), n=45 | 131 (122 ; 140), n=17 |
|                  | 346 (-165 ; 857) | 10.3 (9.0 ; 11.6), n=16         | 10.0 (8.3 ; 11.8), n=8 | 1.1 (-0.5 ; 2.7)               | 137 (127 ; 146), n=15 | 135 (125 ; 147), n=10 |
|                  | 0.18   | 1.2 (0.2 ; 2.2)                 | 0.16   | 0.9 (-0.7 ; 2.6)                | 0.77   | 0.92 |
|                  | -237 (-890 ; 416) | 1 (-8 ; 11)                     | 1 (-11 ; 12) | 0.9 (-0.5 ; 2.7)               | 0.41   | 0.92 |
|                  | 0.48   | 0.77                           | 0.91   | 1 (-13 ; 12)                    | 0.41   | 1 (-15 ; 17) |

|                  |        |                                 |        |                                 |        |                                 |
| **24H DiaBP** (mmHg) |        |                                 |        |                                 |        |                                 |
|                  |        |                                 |        |                                 |        |                                 |
| Baseline         | 1261 (746 ; 1776), n=11 | 10.3 (9.0 ; 11.6), n=16         | 1498 (1092 ; 1903), n=9 | 1.1 (-0.5 ; 2.7)               | 346 (-165 ; 857) | 10.3 (9.0 ; 11.6), n=16 |
|                  | 0.18   | 1.2 (0.2 ; 2.2)                 | 0.16   | 0.9 (-0.7 ; 2.6)                | 0.48   | 1 (-8 ; 4) |
|                  | -237 (-890 ; 416) | 1 (-8 ; 11)                     | 1 (-11 ; 12) | 0.9 (-0.5 ; 2.7)               | 0.41   | 1 (-8 ; 4) |
|                  | 0.48   | 0.77                           | 0.91   | 1 (-13 ; 12)                    | 0.41   | 0.77 |
|                  | -237 (-890 ; 416) | 0 (-6 ; 6)                      | 1 (-11 ; 12) | 0.9 (-0.5 ; 2.7)               | 0.41   | 0 (-6 ; 8) |
|                  | 0.48   | 1.00                           | 0.41   | 4 (-6 ; 15)                     | 0.41   | 1.00 |

**Abbreviations:** MK-7, menaquinone-7; cfPWV, carotid-femoral pulse wave velocity; CAC, Coronary arterial calcification; CVC, Cardiac valve calcification; AAC, Abdominal Aortic calcification; 24H, 24 hours; Sys, systolic; Dia, diastolic; BP, blood pressure; PP, pulse pressure.

**a** CAC scores include the left anterior descending coronary artery (LAD), the right coronary artery (RCA) and the left circumflex coronary artery (LCX).

**b** CVC scores include the aortic and mitral valves.

*Estimated using nonparametric bootstrapping with 1000 replications.*
Legends to figures.

FIGURE 1: CONSORT flow diagram. MK-7, menaquinone-7.

FIGURE 2: Effect of vitamin K supplementation (MK-7 360 µg daily) or placebo in dialysis patients on 2A) serum menaquinone-7 (MK-7) (nmol/L), and 2B) plasma dephosphorylated-uncarboxylated matrix Gla protein (dp-ucMGP) (nmol/L).
Assessed for eligibility (n = 689)

Excluded (n = 641)
- Not meeting inclusion criteria (n = 53)
- Meeting exclusion criteria (n = 247)
- Declined to participate (n = 215)
- Other reasons (n = 121)

Randomised (n = 48)

Allocation

Vitamin K, MK-7, 380 μg daily (n = 24)

Discontinued intervention (n = 4)
- Death (n = 3)
- Exclusion (n = 1)

Analysed (n = 24)

Placebo (n = 24)

Discontinued intervention (n = 7)
- Death (n = 3)
- Consent withdrawn (n = 1)
- Exclusion (n = 3)

Analysed (n = 24)

Year 1

Discontinued intervention (n = 8)
- Death (n = 1)
- Consent withdrawn (n = 2)
- Exclusion (n = 5)

Analysed (n = 24)

Year 2

Discontinued intervention (n = 8)
- Death (n = 3)
- Exclusion (n = 5)

Analysed (n = 24)
Assessed for eligibility (n = 689)

Randomised (n = 48)

Allocation

Vitamin K, MK-7, 360 µg daily (n = 24)

Discontinued intervention (n = 4)
  • Death (n = 3)
  • Exclusion (n = 1)

Year 1

Discontinued intervention (n = 7)
  • Death (n = 3)
  • Consent withdrawn (n = 1)
  • Exclusion (n = 3)

Analysed (n = 24)

Analyses

Placebo (n = 24)

Discontinued intervention (n = 8)
  • Death (n = 1)
  • Consent withdrawn (n = 2)
  • Exclusion (n = 5)

Year 2

Discontinued intervention (n = 8)
  • Death (n = 3)
  • Exclusion (n = 5)

Analysed (n = 24)

Excluded (n = 641)
  • Not meeting inclusion criteria (n = 58)
  • Meeting exclusion criteria (n = 247)
  • Declined to participate (n = 215)
  • Other reasons (n = 121)
