Vitamin K Status, Supplementation and Vascular Disease: A Systematic Review and Meta-Analysis

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Key words

Vitamin K, calcification, stiffness, cardiovascular

Abstract

Objectives:

Vascular stiffness (VS) and calcification (VC) are surrogate markers of vascular health associated with cardiovascular events. Vitamin K-dependent proteins (VKDP) are associated with VS and VC and require vitamin K for activity. We conducted a systematic review and meta-analysis of: (i) the effect of vitamin K supplementation on VS and VC, and (ii) association of inactive VKDP levels with incident cardiovascular disease and mortality.

Methods

Two authors searched Medline and Embase databases, Cochrane and ISRCTN registries for studies of vitamin K clinical trials which measured effects on VC, VS or VKDP, and longitudinal studies assessing effect of VKDP on incident CVD or mortality. Random effects meta-analyses were performed.

Results

Thirteen controlled clinical trials (n=2162) and 14 longitudinal studies (n=10,726) met pre-specified inclusion criteria. Vitamin K supplementation was associated with significant reduction in VC (-9.1% [95%CI -17.7; -0.5]; p=0.04) and VKDP (desphospho-uncarboxylated Matrix Gla Protein; -44.7% [-65.1; -24.3], p<0.0001) and uncarboxylated osteocalcin; -12.0% [-16.7; -7.2], p<0.0001) compared to control, with a non-significant improvement in VS. In longitudinal studies with median follow-up 7.8 (IQR 4.9-11.3) years, VKDP levels were associated with a combined endpoint of CVD or mortality (HR 0.45 [0.07 – 0.83], p=0.02).

Conclusions:

Supplementation with vitamin K significantly reduced VC, but not VS, compared to control. The conclusions drawn are limited by small numbers of studies with

substantial heterogeneity. VKDP was associated with combined endpoint of CVD or mortality. Larger clinical trials of effect of vitamin K supplementation to improve VC, VS and long term cardiovascular health are warranted.

Key questions

What is already known about this subject?

Vitamin K is essential for the activation of proteins that help maintain vascular health, including preventing vascular calcification and stiffness. Vascular stiffness and calcification are associated with cardiovascular risk and may be exacerbated in subclinical vitamin K deficiency. Vitamin K supplementation may improve markers of vascular health and long term cardiovascular risk.

What does this study add?

The existing clinical trial data describing the effect of vitamin K supplementation on vascular health and serum markers of vitamin K deficiency is summarised. The findings are encouraging and justify ongoing study of vitamin K supplementation to improve cardiovascular risk.

How might this impact on clinical practice?

Assessment of vitamin K status and offering supplementation has the potential to be a cheap and safe intervention to improve vascular health and cardiovascular risk.

Introduction

Older patients, and those with diabetes and chronic kidney disease (CKD) are at substantially increased risk of cardiovascular disease (CVD). Independent of traditional cardiovascular risk factors, increased vascular stiffness (VS) is associated with future cardiovascular events[1] and often associated with presence of vascular calcification (VC). There are currently no pharmacological means to improve VS and VC; a growing body of evidence supports beneficial effects of vitamin K on cardiovascular and bone health and may offer a cheap and safe therapeutic intervention.

Vitamin K is a fat-soluble vitamin that is predominantly found in the form of phylloquinone (vitamin K1) in the western diet, from green, leafy vegetables (including kale, broccoli and spinach), and from phylloquinone-rich oils (including rapeseed, sunflower and olive oils). Other forms of dietary vitamin K (menaquinones – vitamin K2) can be found but are more commonly produced by conversion from K1 in the intestine. Vitamin K deficiency is common in groups at risk of cardiovascular disease, particularly those with end-stage CKD[2], possibly due to the overlap with dietary potassium restrictions.

Vitamin K is essential for the activation of various proteins important in vascular and bone health. These vitamin K dependent proteins (VKDP) include Matrix Gla protein (a potent inhibitor of vascular calcification), osteocalcin (a pro-osteoblastic hormone important in bone mineralization) and PIVKA-II (protein induced by vitamin K absence-II, also known as des-gamma carboxyprothrombin, an abnormal form of prothrombin). These proteins contain Gla-domains which require activation (carboxylation) by gamma glutamyl carboxylase: a vitamin K-dependent process. The uncarboxylated forms of these VKDP are used as biomarkers of vitamin K deficiency and are

detectable before manifestations of severe vitamin K deficiency (including bone fracture and uncontrolled bleeding) become clinically apparent. It is known that high level of uncarboxylated VKDP (ucVKDP) is associated with surrogate markers of vascular health including VS and VC[3–6], but it is not clear whether ucVKDP are associated with hard endpoints, including cardiovascular events or mortality.

Vitamin K supplementation may provide a straightforward and low-risk intervention which may reduce the development or progression of VC and VS, particularly in groups at high risk of cardiovascular disease prone to vitamin K deficiency. The biological rationale is that vitamin K supplementation will saturate the gamma glutamyl carboxylase enzyme and maximise carboxylation (activation) of these VKDP. The fully active VKDP are then able to exert their biological effects including the prevention or slowing of development of VC and VS. Some trials of vitamin K supplementation have been conducted to assess effect on VC and VS, but have yielded inconsistent results[7–12]. We conducted a two-part systematic review and meta-analysis to explore our hypotheses that vitamin K supplementation improves markers of vascular disease and cardiovascular risk, specifically VC and VS, and that ucVKDP level is associated with incident cardiovascular disease and mortality.

Methods

Two investigators (JSL and FAC) independently searched Medline and Embase databases, Cochrane and ISRCTN registries from 1966 to 30/05/2017 using the following search terms for interventional studies relating to vitamin K (“vitamin K”, “menadiol”, “menadione”, “menaquinone”, “menatetrenone”, “phytonadione”, “methylphytyl”, “phylloquinone”, “phytomenadione”) and vascular health or ucVKDP (“cardiovascular”, “cardiac”, “coronary”, “vascular”, “vessel”, “artery”, “arterial”, “aorta”, “stiffness”, “distensibility”, “calcification”). For longitudinal studies, we used terms

relating to ucVKDP (“dp-ucMGP”, “ucMGP”, “matrix Gla protein”, “osteocalcin”, “PIVKA”, “vitamin K deficiency”) and vascular disease (“cardiovascular”, “coronary”, “cardiac”, “CV”, “mortality”, “death”). Both investigators reviewed titles and/or abstracts using Mendeley Desktop v1.17.12. Reference lists of included articles and appropriate reviews[4,13–16] were screened for additional studies. If eligibility was unclear, the full text article was obtained and screened against the inclusion/exclusion criteria and differences were resolved by discussion. No language restrictions were applied though all eligible articles were written in English. All relevant abstracts had subsequently been published as full reports. Data were then extracted independently by two investigators (JSL and FAC).

Clinical trials of Vitamin K supplementation

This study is registered on PROSPERO (CRD42017060344). PICOS (Population, Intervention, Comparison, Outcome, Setting) criteria for study inclusion are detailed in Supplemental Table S1. We included randomised or non-randomised controlled trials conducted in adult human participants that compared vitamin K supplementation with control (placebo or no-treatment control group) for a period of 4 weeks or more. Studies with co-interventions in both arms were permitted, but vitamin K plus co-intervention versus placebo or control group was not. Participants with any baseline level of VC or VS were considered eligible. Studies using any form of vitamin K supplementation were considered, but only those supplementing K1 (phytomenadione or phylloquinone) or K2 (menaquinone) were available (**Table 1**).

We analysed the effect of vitamin K supplementation on VC, VS and ucVKDP (dp-ucMGP, ucOC; no relevant studies measured PIVKA-II). We defined the following as appropriate measures to assess VC: plain lateral abdominal x-ray, computed tomography measuring coronary artery calcification or volume calcification scores].

The following were considered appropriate measures of VS: pulse wave velocity (carotid-femoral, carotid-radial or aortic using Doppler ultrasound or magnetic resonance imaging), compliance coefficient, distensibility coefficient or stiffness index.

We extracted mean difference and standard deviation in VS, VC and ucVKDP from treatment and control groups. Where mean change and standard deviation were not reported for outcome measures of interest[7,8,11,19], these were calculated using other available data. Specifically, the mean difference and standard deviation in VC were calculated from median, interquartile range and sample size[7] using a method described previously[20]. Standard deviation of VS or VKDP was calculated from mean and 95% confidence interval[8] or mean and P value[11,19] according to standard methods[21]. Percentage effect sizes in VC, VS and ucVKDP were calculated to account for heterogeneity of type and scale of outcome measures. I^2 was assessed for each outcome measure as an estimation of consistency across studies. Tau-squared, a point-estimate of the among-study variance, was expressed as a measure of true variance (heterogeneity) among included studies. Meta-regression models were used to assess vitamin K form and dose, duration of follow-up, year of publication and outcome score as potential sources of heterogeneity. Variables accounting for heterogeneity among studies were identified if their inclusion in the model resulted in a significant reduction in tau-squared.

Study quality was assessed independently by two authors (JSL and FAC). The Cochrane Risk of Bias tool[21] was used to assign a risk of bias score (Low, High or Unclear) for each of the following: random sequence generation, allocation concealment, blinding of participant and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting and other bias. Differences were

resolved by discussion. We sought evidence of publication bias for all outcome measures using Trim and Fill analysis and Funnel plots.

Meta-analyses were conducted according to a random effects model. Analyses were conducted using *meta* and *metafor* packages for R statistical software (R Studio version 1.0.136).

Vitamin K dependent proteins: longitudinal studies

PICOS (Population, Intervention, Comparison, Outcome, Setting) criteria for study inclusion are detailed in Supplemental Table S1. We included longitudinal adult human studies that assessed serum ucVKDP (desphopho-uncarboxyated Matrix Gla Protein (dp-ucMGP), uncarboxylated osteocalcin (ucOC) and PIVKA-II (protein induced by vitamin K absence-II)) at baseline and recorded incident cardiovascular events (fatal or non-fatal; myocardial infarction, other coronary heart disease, stroke) or mortality.

Statistical analysis was conducted in two ways: i) using ucVKDP as a continuous variable: we extracted hazard ratios with 95% confidence intervals for risk of incident CVD (fatal or non-fatal) or all-cause mortality for increase in ucVKDP by one standard deviation, and ii) using ucVKDP in binary form, i.e. high versus low. In studies reporting effect of baseline ucVKDP in quantiles of 3 or more, only the quantiles with the highest and lowest mean values of ucVKDP were included. Specific cut-points used are detailed in **Table 2**. We preferentially extracted hazard ratios adjusted for age and sex, or the closest approximation of this. Hazard ratios and 95% confidence intervals were log transformed for analysis. Regression analyses were used to assess factors associated with the study design or population that could account for heterogeneity in outcomes.

Results

Clinical trials of Vitamin K supplementation

We identified 5105 references, of which 11 studies were included in the meta-analysis (Figure 1a); characteristics of included studies can be found in **Table 1**. On random effects meta-analysis, there was a significant reduction in progression of VC with vitamin K supplementation versus control (3 studies, n=407; MD -9.14% [-17.8; -0.52], p=0.038; Figure 2a). There was a trend towards improvement in VS (3 studies, n=445; MD -3.70 [-7.77; 0.37]%, p=0.075; Figure 2b). dp-ucMGP was significantly reduced with vitamin K supplementation (7 studies, n=872; MD -44.7 [-65.1; -24.3]%, p<0.0001; Figure 2c), as was ucOC (4 studies, n=962; MD -12.0 [-16.7; -7.2]%, p<0.0001; Figure 2d).

Meta-regression showed no significant impact on VC or VS of vitamin K form or dose, year of publication, duration of follow-up or outcome score used on outcome score on univariate analysis [Supplementary Data: Table S2]. It was not possible to combine multiple variables for VC or VS analyses because of the small number of studies. In studies assessing effect of vitamin K on dp-ucMGP, none of vitamin K form or dose, year of publication or duration of follow-up showed significant association with outcome on univariate analysis, however, in a combined model, longer duration of follow-up and higher vitamin K dose were significantly associated with outcome favouring vitamin K and accounted for 100% of heterogeneity in this case (Table 3 and Figure 3). Earlier year of publication (β = 15.24, 95% CI 11.34-19.14, p<0.001) and longer duration of follow-up (β = -0.64, 95% CI -0.80 - -0.47, p<0.001) were significantly associated with reduction in ucOC in vitamin K groups, though the 4 included studies were published over 2 consecutive years and this may not be clinically significant; year of publication was automatically dropped from the meta-

regression models as a redundant variable due to perfect correlation with duration of follow-up (Table 4).

Random sequence generation and allocation concealment were adequate in 56% of studies, though 89% studies adequately blinded participants and personnel and 100% demonstrated blinding of outcome assessment (Supplementary Data: Table S3). The effect of vitamin K supplementation on calcification and ucVKDP was maintained on assessment of publication bias using the Trim and Fill method (Supplementary Data: Figures S3 and S4) but was diminished for vascular stiffness (Supplementary Data: Figure S5).

Vitamin K dependent proteins: longitudinal studies

Of 1850 screened abstracts, we found 14 longitudinal studies (n=10,726) that recorded ucVKDP at baseline and recorded prospectively CVD events, mortality or both. Twelve of 14 (85.7%) measured dp-ucMGP; 1 measured PIVKA-II and 1 measured ucOC. Study characteristics are detailed in **Table 2**; **Figure 2b** shows the flow chart of identified and excluded studies.

There were 8 reported hazard ratios for step-wise increase in ucVKDP and association with CVD or mortality (n=5413), with median follow-up of 11.1 (IQR 8.6 – 12.2) years. Six of 8 of these studies reported an increased risk of CVD or mortality with increase in ucVKDP. It was not possible combine these in a meta-analysis because of heterogeneity in reporting measures (see Supplementary Data: Table S4).

In 7626 participants across 12 studies reporting ucVKDP as high versus low, median follow-up was 5.6 (IQR 3.0-10.0) years. We combined only studies measuring dp-ucMGP (10 of 12) in a meta-analysis. High dp-ucMGP was associated with combined endpoint of CVD/mortality (log HR 0.45 [0.07 - 0.83], p=0.02); however,

when CVD and mortality were considered separately, there was no significant association with either outcome (log HR 0.26 [-0.13 - 0.66], p=0.20; log HR 0.64 [-0.02 - 1.29], p=0.06 Supplemental Figures 6a and 6b respectively). In a subgroup of studies containing high-risk groups (CKD, vascular disease or diabetes), high dp-ucMGP level was associated with mortality (log HR 0.87 [0.13 – 1.62], p=0.02). This effect was not maintained when assessed in 3 studies[6,22,23] with CKD only (log HR 0.11 [-0.44 – 0.67], p=0.69).

There was no association of PIVKA-II (HR 1.71 (0.79-3.7), p=0.173)[24] or ucOC (HR 1.13 (0.85 – 1.5))[25] with CVD events in two other studies.

Funnel plots and Trim and Fill analysis suggest publication bias in favour of positive results for those studies reporting ucVKDP as high versus low (Supplementary Data: Figure S7).

All studies were longitudinal cohort studies in design measuring baseline ucVKDP and assessing for incident CVD, mortality or both. The average duration of follow-up ranged from 1.9 – 14.1 years. The definition of high ucVKDP differed across studies. In 12 of 14 studies measuring dp-ucMGP at baseline, the cut-point for high dp-ucMGP varied from >400 to >1977 pmol/l depending on the population (Table 2), which may have confounded the results. Multiple regression analysis did not detect any significant association between reported hazard ratio of CVD or mortality and duration of follow-up (p=0.234), cut point used for dp-ucMGP (p=0.649) or high-risk versus standard-risk groups (p=0.815).

Discussion

Vitamin K supplementation significantly reduces ucVKDP in serum and improves VC with a trend towards improving VS in limited studies. We have shown that ucVKDP are not associated with CVD but may be associated with mortality or a

combined endpoint of CVD/mortality. Our results are in keeping with a recent review of the association between vitamin K status and cardiovascular health, which reported inconsistent association of dp-ucMGP concentrations with cardiovascular or all-cause mortality[26]. It is impossible to exclude other confounding variables contributing to both vitamin K deficiency and risk of mortality, such as malnutrition. Despite apparent sensitivity in detecting changes in vitamin K status, ucVKDP in this form are unlikely to be informative biomarkers in predicting vascular risk.

VC is associated with VS, and both are associated with mortality[27–29]. There has been increasing interest in the potential therapeutic ability of vitamin K to reduce progression of VC. There are only 3 completed studies available for analysis, and only one was placebo-controlled; the other 2 included co-interventions containing vitamin D. There is increasing evidence for a synergistic effect between vitamins D and K[30]: vitamin D is thought to influence production of ucVKDP. It is difficult to comment on the effect of vitamin K alone in the setting of co-administration with another biologically active compound, however, vitamin K+D groups showed greater changes in VC and VS than groups receiving co-interventions (including vitamin D) alone. The combined existing data are favourable in suggesting improved vascular health in a variety of patient populations treated with vitamin K compared with control. Given the limited data and weaknesses of the analysis described below, these results must be interpreted with caution, but we believe they support the case for conducting clinical trials in other population and disease groups to assess efficacy of vitamin K supplementation on cardiovascular heatlh. To date, we have identified 7 ongoing or unreported clinical trials of the effect of oral vitamin K1 or K2 supplementation on VS or VC (Supplementary Data: Table S5). Pending the outcome of these ongoing studies, larger Phase 3 trials may be warranted.

The weaknesses of the analysis of clinical trials lie in the heterogeneous nature of the studies, both in vitamin K formulation and dose and variability in the means used to assess VS and VC. Population-level analyses (in Europe and the USA) suggests "adequate" intakes of vitamin K are sub-optimal; all studies using vitamin K1 supplementation appear to have given doses greater than the dietary recommendations for adequate vitamin K1 (phylloquinone) intake of around 1 microgram/kg phylloquinone per day[31]. There is no available advice on recommended intake of K2, though K2 is considered a more potent form than is K1[32], and thus larger doses of K1 than K2 are likely to be required. The clinical trials were relatively small with variable duration and there was unknown risk of reporting bias. Our meta-regression models suggest longer duration of follow-up and possibly higher vitamin K dose is associated with a greater reduction in ucVKDP, but we were unable to confirm these associations with vascular calcification or stiffness. There was significant heterogeneity in the longitudinal studies in terms of the populations assessed, ucVKDP measured, cut-points used to define high ucVKDP, and the duration of follow-up. In both clinical trials and longitudinal analyses, the published studies report outcomes across a variety of populations, including healthy groups; it is difficult to know the 'at risk' populations.

This study was conducted and reported in accordance with recognised guidelines[33,34]. The longitudinal data are difficult to summarise because they are conducted in different populations with variable end-point definitions, though the data are abundant and clinically plausible, and therefore likely to be correct.

We have shown that vitamin K supplementation does reduce absolute level of ucVKDP. We were surprised to find a lack of association of ucVKDP, predominantly as dp-ucMGP, with cardiovascular morbidity or mortality. If we are to assume that

vitamin K is as important for vascular health as the published data suggest, there are a few possible explanations. First, we have reported only on association of absolute level of ucVKDP and their association with CVD or mortality. In a cohort of patients with advanced CKD requiring dialysis, patients with calcific uraemic arteriolopathy (CUA) had similar total levels of uncarboxylated MGP and carboxylated MGP, but a lower proportion of carboxylated:total MGP compared with controls matched for age, sex, race and use of warfarin[35]. The risk of CUA markedly increased with reduction in concentration of carboxylated MGP. Ratio of carboxylated:uncarboxylated MGP may be a more clinically informative biomarker. Second, serum dp-ucMGP has no known biological effect, but is thought to be associated with of level of available MGP in the vessel wall[36]. There are no commercially available assays to measure protein level or activity in the vessel wall itself; serum levels of MGP species may not associated with biological effect. Finally, in high-risk populations such as patients with CKD and/or diabetes, it is possible that death related to extensive vascular disease such as sepsis from an ischaemic limb may not actually be classified as cardiovascular death.

In 2941 participants in the Framingham Heart Study, high intake of vitamin K such as from green vegetables was associated with significantly higher intake of fruits, fish, fibre and dietary supplements, and significantly lower intake of red meat and saturated fat[37]. Those adopting a heart-healthy diet may also be more likely to undertake regular exercise. The observed effects of vitamin K status on cardiovascular morbidity or mortality may therefore serve as a more complex marker of healthy diet and lifestyle. Similarly, supplementation of vitamin K cannot replace the other benefits obtained by eating a health-balanced diet and undertaking regular exercise. Nevertheless, interest in vitamin K as a therapeutic option has been greatest

in populations at high risk of CVD, in whom vitamin K deficiency is prevalent and can be treated more readily with vitamin K supplementation than with lifestyle overhaul. When a satisfactory biomarker becomes routinely available, there may be an argument for testing vitamin K status in high-risk groups and supplementing accordingly. However, before this translates to clinical practice, the following steps are required. First, confirmation of the most clinically appropriate biomarker to measure vitamin K deficiency and specify the cut-point. Second, further trials of vitamin K on surrogate markers of vascular health and required to identify the optimum dose, preparation and duration of treatment. Finally, larger Phase 3 trials are necessary to establish the effect of vitamin K on hard endpoints including CVD and mortality.

In conclusion, this analysis provides some evidence of benefit of vitamin K supplementation on surrogate markers of vascular health. Further trials (both on surrogate markers of VS and VC, and large, cardiovascular outcome trials) are needed before supplementation can be recommended. Low dietary vitamin K intake is likely to be important particularly in higher risk groups such as older populations, and those with diabetes, vascular disease and CKD. Vitamin K supplementation may prove to be of benefit as a long-term strategy to improve vascular health and reduce cardiovascular risk.

Contributorship statement

JSL, MDW, AGJ and PBM designed the research; JSL and FAC conducted the research; JSL and MDW analysed the data; JSL, MDW and PBM wrote the paper; JSL had primary responsibility for final content. All authors read and approved the final manuscript.

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Competing interests

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Abbreviations:

CKD: chronic kidney disease

CUA: calcific uraemic arteriolopathy

CVD: Cardiovascular disease

dp-ucMGP: desphospho-uncarboxylated Matrix Gla Protein

ucOC: uncarboxylated osteocalcin

PIVKA-II: proteins induced by vitamin K absence

VC: Vascular calcification

VS: Vascular stiffness

VKDP: Vitamin K-dependent proteins

ucVKDP: Uncarboxylated (inactive) Vitamin K-dependent proteins

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Table 1

Population risk	Author	Yr	Country	N=	Population	VK form	Dose (mcg/day)	Control	Dur[n]	Outcome measure
High	Shea[7]	2009	USA	295	Older adults	K1	500	Multivitamin (including D)	36	Coronary artery calcification score
	Shea[38]	2011	USA	374	Older adults	K1	500	No treatment	36	dp-ucMGP (pmol/l)
	Kurnatowska[11]	2015	Poland	40	CKD	K2-MK7	90	Vitamin D	9	Coronary artery calcification score; dp-ucMGP (pmol/l)
	Fulton[8]	2016	Scotland	80	Older adults, vascular disease	K2-MK7	100	Placebo	6	Pulse wave velocity (SphygmoCor); dp-ucMGP (pmol/l)
	Brandenburg[12]	2017	Germany	72	Aortic stenosis or sclerosis	K1	2000	Placebo	12	Aortic valve calcification score; dp-ucMGP (pmol/l)
Standard	Braam[9]	2004	Netherlands	121	Healthy	K1	1000	Multivitamin (including D)	36	Compliance coefficient (mm^2/kPa)
	Booth[39]	2008	USA	452	Healthy men and postmenopausal women	K1	500	Multivitamin and calcium /vitamin D	36	ucOC (%)
	Binkley[40]	2009	USA	381	Postmenopausal women	K1 & K2-MK4	1000 K1 15000 K2	Calcium /vitamin D	12	ucOC (%)
	Dalmeijer[19]	2012	Netherlands	38	Healthy	K2-MK7	360	Placebo	3	dp-ucMGP (pmol/l)
	Theuwissen[41]	2012	Netherlands	24	Healthy	K2-MK7	360	Placebo	3	dp-ucMGP (pmol/l)
	Knapen[10]	2015	Netherlands	244	Postmenopausal women	K2-MK7	180	Placebo	36	Pulse wave velocity (SphygmoCor); dp-ucMGP (pmol/l)

Characteristics of clinical trials which compared vitamin K supplementation versus control on vascular calcification, vascular stiffness or serum level of vitamin K dependent protein. Population risk was considered high if conducted in the following populations: older patients (> 60 years), diabetes, pre-existing vascular disease or chronic kidney disease/dialysis/renal transplantation. Yr: year study published; VK form: form of vitamin K used in study; Dur^{n}: duration of study in months.

Table 2

Population risk	Author	Year	Country	N=	Population	FU (yrs)	VKDP measured	Change in VKDP for which HR given	Outcome
High	Schurgers[6]	2010	France	107	Chronic kidney disease	2.3	dp-ucMGP	Per 100pm log-transformed increase; >921 vs. ≤921 pmol/l	Mortality
	Ueland[42]	2010	Norway	118	Symptomatic aortic stenosis	1.9	dp-ucMGP	>950 vs. ≤950 pmol/l	Mortality
	Schlieper[23]	2011	Serbia	188	Haemodialysis versus normal renal function	3	dp-ucMGP	Higher than median versus lower (median value not reported)	CVD; mortality
	Ueland[43]	2011	Norway	179	Stable heart failure	2.9	dp-ucMGP	≥1977 vs. <1977 pmol/l	Mortality
	Dalmeijer[44]	2013	Netherlands	518	Type 2 diabetes	11.2	dp-ucMGP	Per one SD increase	CVD
	van den Heuvel[45]	2014	Netherlands	192	Older adults (LASA)	5.6	dp-ucMGP	Per 100pm log-transformed increase; highest versus lowest tertile (>400 vs <266 pmol/l)	CVD
	Mayer[46]	2014	Czech Republic	799	Coronary heart disease or ischaemic stroke	5.6	dp-ucMGP	≥977 vs. <977 pmol/l	CVD; mortality
	Keyzer[22]	2015	Netherlands	518	Renal transplant	9.6	dp-ucMGP	Per unit increase (log-transformed); highest vs. lowest quartiles (>1535 vs. <734 pmol/l)	Mortality
	Yeap[25]	2015	Australia	3389	Older men (70-89 years)	7	ucOC	>28.2 vs. <28.2 microgram/l	CVD
	Mayer[47]	2016	Czech Republic	799	Stable vascular disease	5.6	dp-ucMGP	≥977 vs. <977 pmol/l	Mortality
	Shea[48]	2017	USA	635	Older men and woman (Health ABC)	12.1	dp-ucMGP	≥574 vs. <574pmol/l	CVD
Standard	Dalmeijer[49]	2014	Netherlands	1406	Women undergoing breast cancer screening (EPIC-NL)	11.5	dp-ucMGP	Per one SD increase; highest vs. lowest quartiles (mean 348 vs. 47 pmol/l)	CVD
	Liu[50]	2015	Belgium	789	FLEMENGHO: no CVD at baseline	14.1	dp-ucMGP	Per unit increase of squared dp-ucMGP	CVD; mortality
	Danziger[24]	2016	USA	355	MESA	11	PIVKA-II	> 4.64 vs. <2.4 ng/ml	CVD

Characteristics of longitudinal studies measuring vitamin K dependent protein at baseline and recording incident cardiovascular disease/mortality. Population risk considered high if conducted in the following populations: older patients (>60 years), diabetes, pre-existing vascular disease or chronic kidney disease/dialysis/renal transplantation. dp-ucMGP: desphospho-uncarboxylated Matrix Gla protein. PIVKA-II: Protein induced by vitamin K absence - II. MESA: Multi-Ethnic Study of Atherosclerosis. Heart and Soul Study: history of ischaemic heart disease. EPIC-NL: Dutch contribution to European Prospective Investigation into Cancer and Nutrition cohort. FLEMENGHO: Flemish Study on Genes, Environment, and Health Outcomes (1985-2004). Health ABC: Prospective longitudinal cohort study to examine age-related changes in physical function and body composition in older black and white men and women. LASA: Longitudinal Aging Study Amsterdam. Health In Men Study: cohort study of community-dwelling older men from Perth, Western Australia.

Table 3

Meta-regression model with the mean difference (%) in dp-ucMGP as the dependent variable

Variable	Unadjusted			
	Coefficient	**95% CI**	**P**	**Tau^2**
No covariate				**451.1**
Year of publication	6.39	-1.24, 14.02	0.10	232.7
Duration of follow-up	-0.82	-1.86, 0.22	0.12	185.3
Vitamin K form	23.64	-13.33, 60.60	0.21	278.0
Dose	-0.01	-0.04, 0.02	0.42	366.1
	Adjusted			
Model 1				
Duration of follow-up	-0.82	-1.58, -0.05	0.04	66.7
Vitamin K form	19.72	-6.97, 46.42	0.15	
Model 2				
Duration of follow-up	-1.03	-1.45, -0.61	<0.001	0.0
Dose	-0.02	-0.04, 0.00	0.045	
Model 3				
Duration of follow-up	-0.51	-1.67,0.65	0.39	195.1
Year of publication	5.01	-2.99, 13.00	0.22	
Model 4				
Duration of follow-up	-0.70	-2.23, 0.83	0.37	339.5
Dose	-0.01	-0.06, 0.04	0.68	
Vitamin K form	6.74	-62.58, 76.05	0.85	
Model 5				
Duration of follow-up	-0.81	-1.31, -0.31	0.002	0.74
Dose	-0.02	-0.04, -0.001	0.04	
Year of publication	4.60	-0.93, 10.13	0.10	

Table 4

Meta-regression model with the mean difference (%) in ucOC as the dependent variable

*Redundant variable (year of publication) was dropped from Models 1, 4 and 5 due to perfect correlation with duration of follow-up.

Variable	**Unadjusted**			
	Coefficient	**95% CI**	**P**	**Tau^2**
No covariate				**20.3**
Year of publication	15.24	11.34-19.14	<0.001	0.00
Duration of follow-up	-0.64	-0.80, -0.47	<0.001	0.00
Vitamin K form	9.93	-9.86, 29.72	0.33	74.9
Dose	0.001	-0.001, 0.002	0.29	71.5
	Adjusted			
Model 1*				
Year of publication	Redundant – dropped from model			0.00
Duration of follow-up	-0.64	-0.80, -0.47	<0.001	0.00
Model 2				
Year of publication	15.08	11.07, 19.08	<0.001	0.00
Dose	0.00	0.00, 0.00	0.73	
Model 3				
Duration of follow-up	-0.63	-0.80, -0.46	<0.001	0.00
Dose	0.00	0.00, 0.00	0.73	
Model 4*				
Year of publication	Redundant – dropped from model			
Duration of follow-up	-0.63	-0.80, -0.46	<0.001	0.00
Dose	0.00	0.00, 0.00	0.73	
Model 5*				
Year of publication	Redundant – dropped from model			
Duration of follow-up	-0.63	-0.80, -0.46	<0.001	0.00
Vitamin K form	0.32	-1.48, 2.12	0.73	

Legends

Figure 1

Flow chart of included (A) clinical trials and (B) longitudinal studies

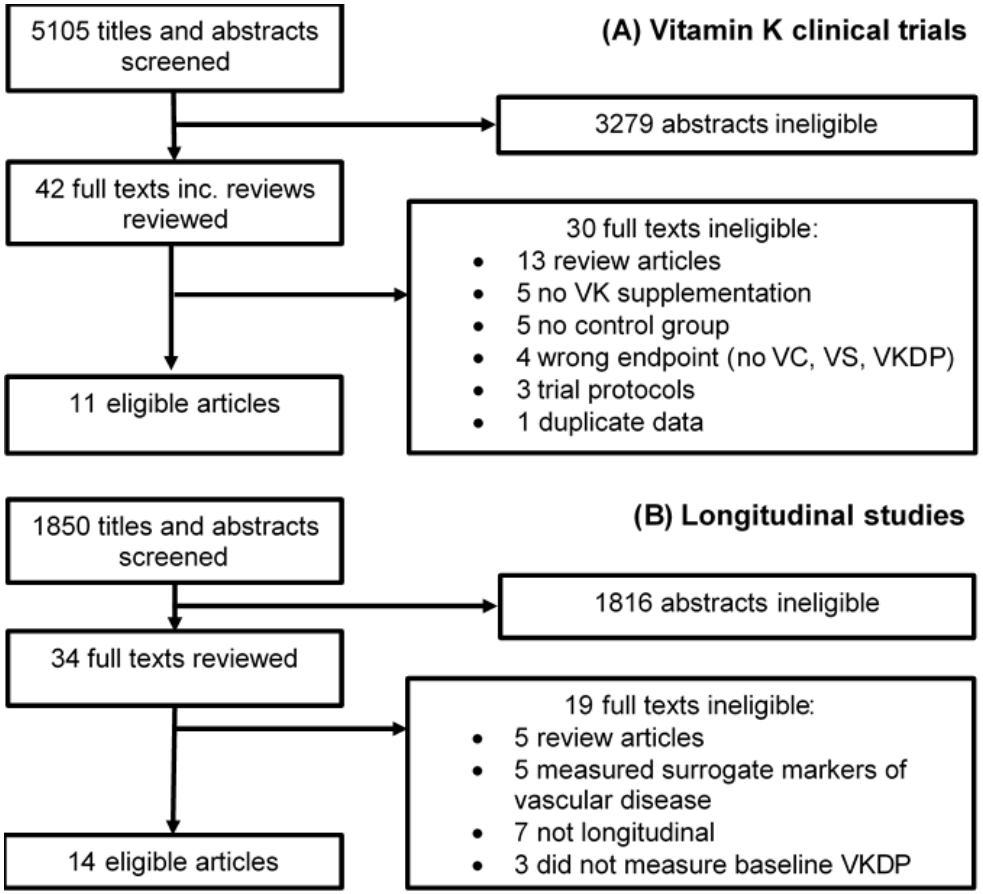

Figure 2

Forest plots showing the effect of vitamin K supplementation on % change in vascular calcification (A), vascular stiffness (B), dp-ucMGP (C) and ucOC (D). Random effects meta-analysis was used. Data are presented as mean % difference and 95% confidence interval.

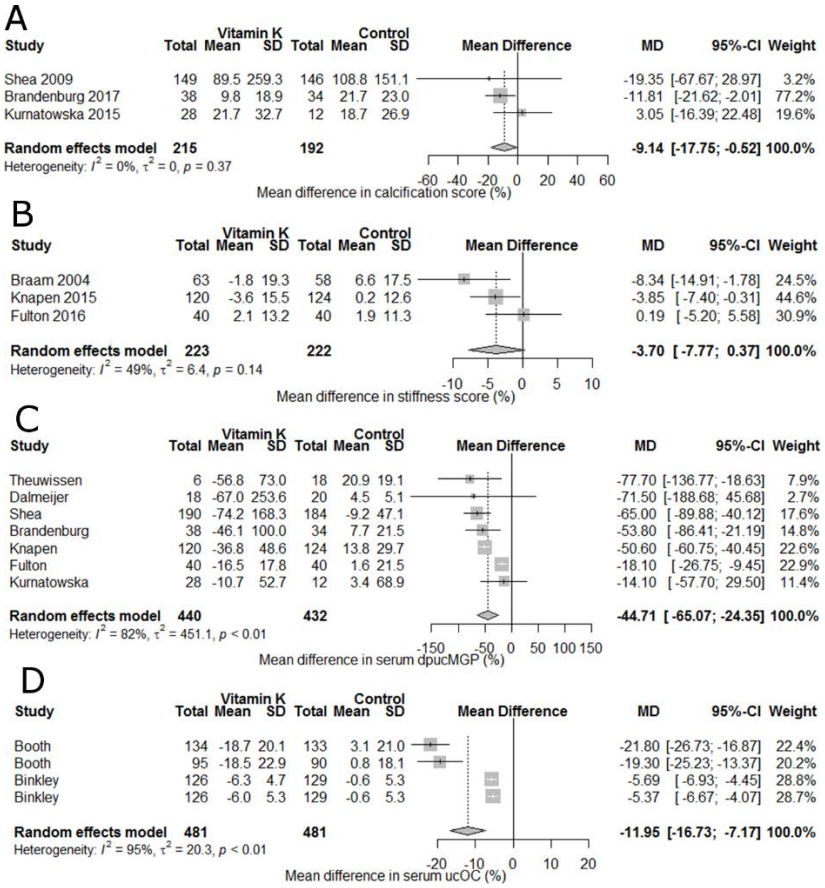

Figure 3

Meta-regression plot of mean difference (%) and (A) duration of follow-up (β = -0.82, 95% CI -1.86 - 0.22, p=0.12) and (B) vitamin K dose (β = -0.01, 95% CI -0.04 - 0.02, p=0.42). Negative values favour intervention and positive values favour control. Circles represent studies included in the meta-analysis. The size of the circle is inversely proportional to the variance of the estimated treatment effect. The solid line indicates a perfect fit.