
There may be differences between this version and the published version. You are advised to consult the publisher’s version if you wish to cite from it.

http://eprints.gla.ac.uk/172900/

Deposited on: 9 November 2018
Vitamin K Status, Supplementation and Vascular Disease: A Systematic Review and Meta-Analysis

Lees JS\textsuperscript{a,b}, Chapman FA\textsuperscript{b}, Witham MD\textsuperscript{c}, Jardine AG\textsuperscript{a,b}, Mark PB\textsuperscript{a,b}

Author affiliations:

a. Institute of Cardiovascular and Medical Sciences, University of Glasgow
b. Glasgow Renal and Transplant Unit, NHS Greater Glasgow and Clyde
c. Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne

Corresponding author:

Dr Jennifer S Lees. Tel: +44 141 330 2723. Email: jennifer.lees2@nhs.net. BHF GCRC, Institute of Cardiovascular and Medical Sciences, University of Glasgow, 126 University Avenue, Glasgow, G12 8TA.

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in HEART editions and any other BMJPGL products to exploit all subsidiary rights.

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 health. 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 uremic 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.
Funding statement

JSL is funded by a Kidney Research UK Training Fellowship (TF_013_20161125).

Competing interests

MDW and PBM acknowledge project grant funding from British Heart Foundation (PG/14/75/31083) to support the K for Kidneys trial: ISRCTN21444964. The above Kidney Research UK Training Fellowship was awarded to JSL (supervised by PBM) for the ViKTORIES trial: ISRCTN22012044.

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
References


Tsugawa N. Cardiovascular diseases and fat soluble vitamins: Vitamin D and vitamin K. N. Tsugawa, Department of Hygienic Sciences, Kobe Pharmaceutical University, Kobe 658-8558, Japan. E-mail: tsugawa@kobepharma-u.ac.jp, Japan: Center for Academic Publications Japan (E-mail: mi@capj.or.jp) 2015. doi:10.3177/jnsv.61.S170


Yeap BB, Alfonso H, Chubb SAP, et al. Proportion of Undercarboxylated Osteocalcin
and Serum P1NP Predict Incidence of Myocardial Infarction in Older Men. *J Clin Endocrinol Metab* 2015;**100**:3934–42. doi:10.1210/jc.2015-1899


Moher D, Liberati A, Tetzlaff J, *et al.* Systematic Reviews and Meta-Analyses: The
doi:10.1371/journal.pmed1000097

doi:10.1001/jama.283.15.2008


doi:10.3945/jn.111.139634.the

doi:10.1210/jc.2007-2490


<table>
<thead>
<tr>
<th>Population risk</th>
<th>Author</th>
<th>Yr</th>
<th>Country</th>
<th>N=</th>
<th>Population</th>
<th>VK form</th>
<th>Dose (mcg/day)</th>
<th>Control</th>
<th>Dur*</th>
<th>Outcome measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Shea[7]</td>
<td>2009</td>
<td>USA</td>
<td>295</td>
<td>Older adults</td>
<td>K1</td>
<td>500</td>
<td>Multivitamin (including D)</td>
<td>36</td>
<td>Coronary artery calcification score</td>
</tr>
<tr>
<td></td>
<td>Shea[38]</td>
<td>2011</td>
<td>USA</td>
<td>374</td>
<td>Older adults</td>
<td>K1</td>
<td>500</td>
<td>No treatment</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fulton[8]</td>
<td>2016</td>
<td>Scotland</td>
<td>80</td>
<td>Older adults, vascular disease</td>
<td>K2-MK7</td>
<td>100</td>
<td>Placebo</td>
<td>6</td>
<td>Pulse wave velocity (SphygmoCor); dp-ucMGP (pmol/l)</td>
</tr>
<tr>
<td></td>
<td>Brandenburg[12]</td>
<td>2017</td>
<td>Germany</td>
<td>72</td>
<td>Aortic stenosis or sclerosis</td>
<td>K1</td>
<td>2000</td>
<td>Placebo</td>
<td>12</td>
<td>Aortic valve calcification score; dp-ucMGP (pmol/l)</td>
</tr>
<tr>
<td>Standard</td>
<td>Braam[9]</td>
<td>2004</td>
<td>Netherlands</td>
<td>121</td>
<td>Healthy</td>
<td>K1</td>
<td>100</td>
<td>Multivitamin (including D)</td>
<td>36</td>
<td>Compliance coefficient (mm²/kPa)</td>
</tr>
<tr>
<td></td>
<td>Booth[39]</td>
<td>2008</td>
<td>USA</td>
<td>452</td>
<td>Healthy men and postmenopausal women</td>
<td>K1</td>
<td>500</td>
<td>Multivitamin and calcium /vitamin D</td>
<td>36</td>
<td>ucOC (%)</td>
</tr>
<tr>
<td></td>
<td>Binkley[40]</td>
<td>2009</td>
<td>USA</td>
<td>381</td>
<td>Postmenopausal women</td>
<td>K1 &amp; K2-MK4</td>
<td>1000 K1 15000 K2</td>
<td>Calcium /vitamin D</td>
<td>12</td>
<td>ucOC (%)</td>
</tr>
<tr>
<td></td>
<td>Dalmeijer[19]</td>
<td>2012</td>
<td>Netherlands</td>
<td>38</td>
<td>Healthy</td>
<td>K2-MK7</td>
<td>360</td>
<td>Placebo</td>
<td>3</td>
<td>dp-ucMGP (pmol/l)</td>
</tr>
<tr>
<td></td>
<td>Theuwissen[41]</td>
<td>2012</td>
<td>Netherlands</td>
<td>24</td>
<td>Healthy</td>
<td>K2-MK7</td>
<td>360</td>
<td>Placebo</td>
<td>3</td>
<td>dp-ucMGP (pmol/l)</td>
</tr>
<tr>
<td></td>
<td>Knapen[10]</td>
<td>2015</td>
<td>Netherlands</td>
<td>244</td>
<td>Postmenopausal women</td>
<td>K2-MK7</td>
<td>180</td>
<td>Placebo</td>
<td>36</td>
<td>Pulse wave velocity (SphygmoCor); dp-ucMGP (pmol/l)</td>
</tr>
</tbody>
</table>
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: duration of study in months.
Table 2

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

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted Coefficient</th>
<th>95% CI</th>
<th>P</th>
<th>Tau²</th>
</tr>
</thead>
<tbody>
<tr>
<td>No covariate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of publication</td>
<td>6.39</td>
<td>-1.24, 14.02</td>
<td>0.10</td>
<td>232.7</td>
</tr>
<tr>
<td>Duration of follow-up</td>
<td>-0.82</td>
<td>-1.86, 0.22</td>
<td>0.12</td>
<td>185.3</td>
</tr>
<tr>
<td>Vitamin K form</td>
<td>23.64</td>
<td>-13.33, 60.60</td>
<td>0.21</td>
<td>278.0</td>
</tr>
<tr>
<td>Dose</td>
<td>-0.01</td>
<td>-0.04, 0.02</td>
<td>0.42</td>
<td>366.1</td>
</tr>
</tbody>
</table>

#### 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>95% CI</td>
</tr>
<tr>
<td>No covariate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of publication</td>
<td>15.24</td>
<td>11.34-19.14</td>
</tr>
<tr>
<td>Duration of follow-up</td>
<td>-0.64</td>
<td>-0.80,-0.47</td>
</tr>
<tr>
<td>Vitamin K form</td>
<td>9.93</td>
<td>-9.86,29.72</td>
</tr>
<tr>
<td>Dose</td>
<td>0.001</td>
<td>-0.001,0.002</td>
</tr>
</tbody>
</table>

**Model 1***

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of publication</td>
<td>Redundant – dropped from model</td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Duration of follow-up</td>
<td>-0.64</td>
<td>-0.80,-0.47</td>
<td>&lt;0.001</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**Model 2**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of publication</td>
<td>15.08</td>
<td>11.07,19.08</td>
<td>&lt;0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Dose</td>
<td>0.00</td>
<td>0.00,0.00</td>
<td></td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Model 3**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of follow-up</td>
<td>-0.63</td>
<td>-0.80,-0.46</td>
<td>&lt;0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Dose</td>
<td>0.00</td>
<td>0.00,0.00</td>
<td></td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Model 4***

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of publication</td>
<td>Redundant – dropped from model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of follow-up</td>
<td>-0.63</td>
<td>-0.80,-0.46</td>
<td>&lt;0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Dose</td>
<td>0.00</td>
<td>0.00,0.00</td>
<td></td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Model 5***

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of publication</td>
<td>Redundant – dropped from model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of follow-up</td>
<td>-0.63</td>
<td>-0.80,-0.46</td>
<td>&lt;0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>Vitamin K form</td>
<td>0.32</td>
<td>-1.48,2.12</td>
<td></td>
<td>0.73</td>
</tr>
</tbody>
</table>
Legends

Figure 1

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

(A) Vitamin K clinical trials

5105 titles and abstracts screened

42 full texts inc. reviews reviewed

11 eligible articles

3279 abstracts ineligible

30 full texts ineligible:
- 13 review articles
- 5 no VK supplementation
- 5 no control group
- 4 wrong endpoint (no VC, VS, VKDP)
- 3 trial protocols
- 1 duplicate data

(B) Longitudinal studies

1850 titles and abstracts screened

34 full texts reviewed

14 eligible articles

1816 abstracts ineligible

19 full texts ineligible:
- 5 review articles
- 5 measured surrogate markers of vascular disease
- 7 not longitudinal
- 3 did not measure baseline VKDP
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 ($\beta = -0.82$, 95% CI -1.86 - 0.22, $p=0.12$) and (B) vitamin K dose ($\beta = -0.01$, 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.