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EFFECT OF VITAMIN K ON VASCULAR HEALTH AND PHYSICAL FUNCTION IN OLDER PEOPLE WITH VASCULAR DISEASE – A RANDOMISED CONTROLLED TRIAL

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Abstract: *Background and Aims:* Vitamin K insufficiency is common and linked to an increased risk of cardiovascular disease and osteoporotic fractures. The aim of this study was to examine whether daily supplementation with oral vitamin K could improve vascular health and physical function in older people with established vascular disease. *Methods and results:* A double blind, randomised, placebo-controlled trial. Participants aged ≤ 70 years with a history of vascular disease were randomised to receive 6 months of daily oral 100mcg vitamin K2 (MK7 subtype) or matching placebo with outcomes measured at 0, 3 and 6 months. The primary outcome was between-group difference in endothelial function assessed using flow-mediated dilatation of the brachial artery at 6 months. Secondary outcomes included carotid-radial pulse wave velocity, augmentation index, blood pressure, carotid intima-media thickness, C-reactive protein, B-type natriuretic peptide, cholesterol and desphospho-uncarboxylated matrix Gla protein levels. Handgrip strength and the Short Physical Performance Battery assessed physical function, while postural sway was measured using a 3-dimensional force platform. *Results:* 80 participants were randomised, mean age 77 (SD 5) years; 44/80 were male. Vitamin K levels rose in the intervention arm compared to placebo (+48 pg/ml vs -6 pg/ml, p=0.03) at 6 months. Desphospho-uncarboxylated Matrix Gla protein levels fell in the intervention group compared to placebo at 6 months (-130 [SD 117] pmol/L vs +13 [SD 180] pmol/L, p<0.001). No change was seen in endothelial function (between group difference -0.3% [95%CI -1.3 to 0.8], p=0.62). A modest, non-significant improvement in pulse wave velocity was seen in the vitamin K group (-0.8m/s [95%CI -1.8 to 0.3], p=0.15) while all other vascular and physical function outcomes unchanged. *Conclusions:* Six months of vitamin K2 supplementation did not improve markers of vascular health or physical function in older patients with vascular disease.

Key words: Vitamin K, flow mediated dilatation, vascular health, physical function, older people.

Abbreviations: BNP: B-type natriuretic peptide; CRP: C-reactive protein; Dp-ucMGP: Desphospho-uncarboxylated Matrix Gla Protein; ECG: Electrocardiogram; FMD: Flow mediated dilatation; HPLC: High performance liquid chromatography; IMT: intima-media thickness; MGP: Matrix Gla Protein; PWV: Pulse wave velocity; RDI: Recommended daily intake; SBP: Systolic blood pressure; SPPB: Short physical performance battery

Introduction

Vitamin K intake is below currently recommended daily levels in 60% of adults in the UK (1). While observational evidence suggesting that low dietary intake of vitamin K2 (2) correlates with an increased incidence of coronary artery disease even after adjustment for other factors, high levels of circulating vitamin K were associated with lower levels of inflammatory markers, in the Framingham cohort (3) suggesting a possible role in suppression of the chronic inflammation known to accompany vascular disease. Studies of biochemical vitamin K status, using circulating desphospho-uncarboxylated Matrix Gla Protein (dp-ucMGP) levels show that functional vitamin K insufficiency is associated with an increased risk of cardiovascular events even after adjustment for conventional risk factors in healthy middle aged people (4); similar results were seen in cohorts with type 2 diabetes mellitus (5) and end-stage renal disease (6).

Such associations are biologically plausible. More than a dozen non-coagulation vitamin K dependent proteins have been identified, including osteocalcin, involved in bone growth and glucose homeostasis, Matrix Gla Protein (MGP), a powerful inhibitor of vascular calcification (7), and Gas6, implicated in control of inflammatory response as well as protection from apoptosis in nerve and smooth muscle tissue. Preliminary evidence from animal studies suggests that vitamin K supplementation may be able to reverse calcification of arteries, and a 3 year randomised controlled trial in middle-aged women found that the addition of vitamin K to vitamin D therapy, but not vitamin D alone, arrested decline in carotid artery elasticity compared with placebo (8). Few intervention studies have attempted to evaluate whether vitamin K supplementation can improve markers of vascular health, particularly in older people and those with established vascular disease. This group

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is not only at high risk of future vascular events; they often have extensive vascular calcification and significantly impaired endothelial function – the final common pathway leading to atherosclerosis and a key independent risk marker for future vascular events.

Physical function is closely linked to vascular health in older people; up to half the decline in physical function with age may be attributable to vascular disease (9). While existing concerns that some established cardiovascular interventions might increase the risk of falls in older people evidence from the ECKO osteoporosis trial (10) suggests that vitamin K may in fact reduce fracture rates despite no change in bone mineral density. These findings, along with the possible role of vitamin K dependent proteins on nerve function suggest that vitamin K may be able to exert beneficial effects on neuromuscular as well as vascular function. We therefore carried out the current study to ascertain if daily vitamin K2 supplementation produced beneficial effects on established markers of vascular health and physical function in older people with established vascular disease.

Methods

Study Design & Sample Population

The study was a parallel group, double blind, placebo controlled, randomised trial. Participants were enrolled between September 2011 and September 2012, with the last participant visit in April 2013. Written informed consent was obtained from all participants. The trial was approved by Tayside Committee on Medical Research Ethics (ref number 11/ES/0009); and Chest, Heart & Stroke Scotland provided funding (reference R11/A137). The study was registered with www.controlled-trials.com (ISRCTN93213492) and conformed to the principles of the Declaration of Helsinki.

Community dwelling people aged 70 and over with a history of hypertension, diabetes mellitus or previously diagnosed vascular disease (defined as documented myocardial infarction, coronary revascularisation, previous confirmed ischaemic stroke, or peripheral vascular disease defined as claudication symptoms with angiographically proven arterial stenosis or ankle-brachial pressure index of <0.7) were recruited from two primary care practices within Tayside, Scotland. Exclusion criteria were: a history of atrial fibrillation (AF), taking warfarin or other coumadin derivatives, taking vitamin K supplements, inability to give informed consent or inability to walk without human assistance. All study visits were carried out in study rooms within Tayside Institute for Cardiac Research, Ninewells Hospital Dundee, Scotland

Intervention

Participants were randomised to receive one capsule per day of either vitamin K2 (MK7 subtype) 100mcg (NattoPharma ASA, Hovik, Norway) or matching placebo (manufactured by Legosan SA, Kumla, Sweden), with both intervention and placebo medications being over encapsulated to give an identical appearance. This dose was selected to ensure that all participants would achieve at least the current daily intake recommendations without the burden of completing daily food intake diaries, while use of this dose and has shown biological effects on markers of vitamin K metabolism in previous studies. Previous epidemiological studies have suggested that vitamin K2 intake may be more closely correlated with future vascular events than vitamin K1 intake (2), thus we selected vitamin K2 for this trial. The MK7 subtype is readily available and has been shown to reduce dp-ucMGP levels previously (11, 12). Randomisation was performed by Tayside Pharmaceuticals; a 1:1 allocation ratio was used with no stratification or minimisation. After completion of baseline assessments, participants were allocated the next sequential study medication bottle; the bottles bore no indication of group allocation. Allocation was therefore concealed from both researchers and participants.

Medication adherence was assessed by a tablet count at 3 and 6 months following baseline visit. Adherence was calculated as the proportion of tablets actually taken over the 6 month period divided by the amount which should have taken. Adherence of 80% or more of the initial tablets supplied at baseline was regarded as medication adherent.

Outcome Measurement

The primary outcome was the change in flow mediated dilatation (FMD) of the brachial artery at 6 months. FMD of the brachial artery, assessed according to standard guidelines as described by Coretti et al, (13) is a non-invasive reproducible technique used to assess endothelial function and has been identified as a marker for future cardiovascular events (14-16). Brachial artery diameter and velocity were determined by M-mode and Doppler ultrasound using a Philips iE33 ultrasound machine (Philips Medical Systems, Reigate, Surrey, UK). Concurrent electrocardiogram (ECG) monitoring enabled the resulting image to be gated to the R wave of the QRS complex. The brachial artery was longitudinally imaged above the elbow 5 to 10 cm above the antecubital fossa using an 11.3-MHz probe. The image was recorded for 2 min, followed by induction of forearm ischemia. This was induced by inflating a cuff below the elbow to 200 mmHg (or 50 mmHg above systolic blood pressure, whichever was higher) for 5 min and deflating rapidly (Hokanson Rapid Cuff Inflator; Hokanson Inc., Belleview, WA, USA). The brachial artery diameter was recorded for a further 2 minutes post cuff deflation and the maximum vessel diameter achieved during this period was calculated using Brachial Tools edge detection software (Medical Imaging Applications, Iowa City, USA).

Secondary outcomes

Carotid intima-media thickness (IMT) of the common carotid artery was measured via B-mode scanning of one of the carotid arteries performed using Siemens Acuson Sequoia 512

using an 8 MHz transducer. The participant's carotid artery was identified initially with the transducer in a transverse plane and then studied longitudinally. The common carotid artery was examined with three measurements taken over a 1cm section at a point on the far wall of the artery approximately 10mm proximal to the carotid bulb using electronic calipers and the average measurement calculated.

Lying and standing blood pressures were recorded using an OMRON HEP-705 oscillometric machine prior to recording arterial stiffness using the SphygmoCor system version 7.1 (AtCor Medical) which records waveforms from the radial and carotid artery by applanation tonometry. From the central aortic pulse wave derived via the internal SphygmoCor algorithm, the augmentation index was calculated expressed as ratio of pressure increase to pulse pressure normalised for a heart rate of 75 beats per minute (AIx@75). Pulse wave velocity was derived by measuring the pulse waveform sequentially at the radial & carotid artery and comparing time of arrival with the time of the ECG R-wave.

Fasting blood was drawn for measuring total, serum C-reactive protein (CRP), plasma B-type natriuretic peptide (BNP), desphospho-uncarboxylated Matrix Gla Protein (dp-ucMGP) and cholesterol. All serum samples were kept for a minimum of 10 minutes while plasma citrate and EDTA samples were placed on ice immediately after collection with all samples centrifuged at1500×g in a cold centrifuge for 10 min. All samples were aliquoted and 1ml samples frozen at -80 °C until assayed in subject sets. CRP was analysed using a microplate-based EIA (ELISA) double antibody sandwich assay for C-reactive protein from Kalon Biological Ltd. BNP reflects myocardial wall stress, myocardial ischemia, and was measured using a radioimmunoassay (Bachem UK, Merseyside, UK).

Dp-ucMGP was measured using a pre-commercial dual-antibody ELISA (InaKtif MGP IDS-iSYS assay) which is based on the previously described sandwich ELISA developed by VitaK, Maastricht University, The Netherlands (17). In this assay, the capture antibody is directed against the non-phosphorylated sequence 3–15 of human MGP, and the detection antibody is directed against the uncarboxylated MGP sequence 35–49, as described previously. Total cholesterol was measured using a Roche multichannel analyser as part of routine clinical workflow by National Health Service Tayside department of biochemical medicine.

Participants completed the short physical performance battery (SPPB) test at both baseline and 6 month visits. This three-part test focuses on lower limb function using tasks that mimic daily activities. It includes a timed 4 metre walk to measure gait speed; one chair stand followed by 5 timed chair stands, if the first is successfully completed; and balance stands with the feet held in 3 different positions for 10 seconds each. The SPPB is an objective assessment tool for evaluating lower extremity functioning in older persons and has been designed to measure physical performance and decline over time (18). Handgrip dynamometry was used to directly measure muscle strength. All participants' handgrip strength was assessed using the non-dominant hand at both baseline and the 6 month follow-up visit using a hand held dynamometer, the T.K.K 5001 Grip – (Takei Scientific Instruments Company Ltd, Japan). The best of three recordings was used at each timepoint. Data were also collected on age, sex, medical history, current medication use and place of residence.

Balance is an essential component of physical function (19). Postural sway was measured at baseline and at the 6 month follow up visit using a force platform (AMTI model BP400600, Advanced Mechanical Technologies Inc. Watertown, MA, USA) to provide 3-dimensional pattern of weight changes defining the centre of pressure. Centre of pressure recordings were taken over a 30 second trial, measured at a high frequency of 1000 Hz. The mean of two trials was used as the final measure. Sway was assessed with eyes open, looking straight ahead, and then again with eyes closed. Assessments were conducted barefoot, on the force platform, without the use of walking aids. No consensus exists as to the optimum measure of sway (20), thus five commonly-used variables were derived from the final measure– the sum of anteroposterior sway excursions, the sum of mediolateral sway excursions, the total sway path length, the root-mean-square excursion from the mean centre of pressure, and the ellipse area circumscribing 95% of measures.

Statistical Analysis

All data were analysed using SPSS statistical package (SPSS, Chicago, USA Version 21.0). Analyses of the primary and secondary outcomes were performed prior to breaking the treatment codes. A 2-sided p value of < 0.05 was taken to be significant for all analyses. Differences between treatment groups for outcome measures were assessed. Baseline data were compared between groups using an independent samples t-test for all normally distributed data, and Mann Whitney test for non-normally distributed data. Categorical variables were compared at baseline using Pearson's Chi-squared test.

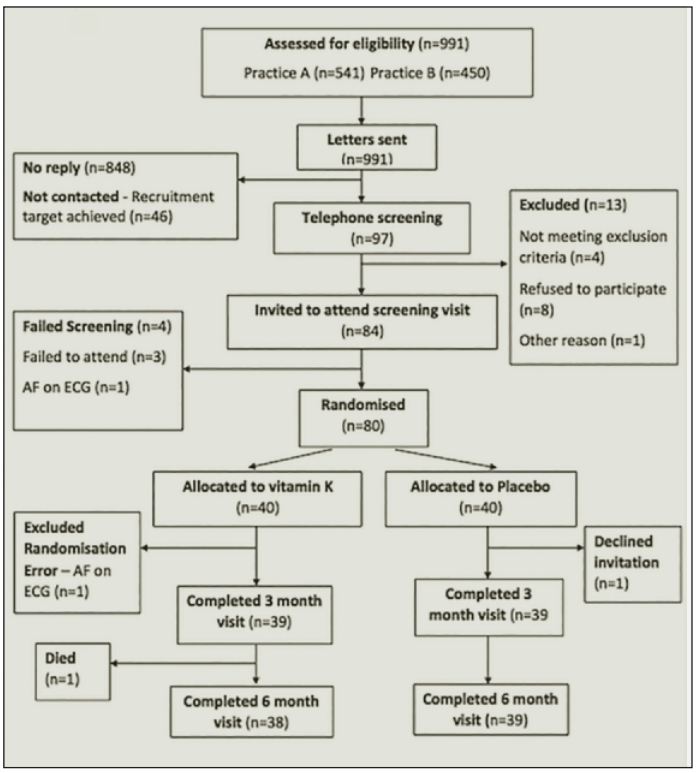
All analyses of treatment effect were conducted according to the principles of intention to treat analysis. For each outcome, change scores (follow-up minus baseline) were calculated for each follow-up timepoint. Change scores were tested for normal distribution, and compared between groups for each follow-up time point using analysis of covariance (ANCOVA) models. Unadjusted analyses were calculated, and adjusted analyses were performed, adjusting for age, baseline values (e.g. for the analysis of FMD outcomes, adjustment was made for baseline FMD values), and for vascular outcomes only we also adjusted for baseline systolic blood pressure. For the primary outcome of change in FMD, multiple imputation was performed in SPSS using age, sex, baseline SBP, 6 month SBP, baseline FMD and 6 mo FMD measures. Five sets of imputations were combined in SPSS and analysed to produce a pooled estimate of treatment effect.

Based on previous work we calculated in anticipation of a 10% dropout rate that a final sample size of 72 (36 per arm) would give 80% power at alpha = 0.05 to detect a 2% absolute change in FMD (given a standard deviation of change of 3% as seen in previous studies). This change is similar to that seen with exercise and statin therapy (21, 22). We therefore aimed to randomise 80 participants to allow for 10% dropout over the course of the trial.

Results

143 potential participants contacted via primary care expressed interest in the study, of whom 97 were contacted and underwent telephone screening. 80 participants were randomised and participant flow through the trial is shown in Figure 1. 77/80 (96%) of participants underwent the 6 month visit. Baseline details for the 80 randomised participants are shown in Table 1. Participants in the vitamin K group had significantly higher baseline pulse wave velocity, were more likely to live alone and had a borderline higher prevalence of osteoarthritis than those in the placebo group.

Figure 1



Adherence and effects of intervention on vitamin K metabolism

Of the 78 participants who returned for the 6 month visit 35/38 (92%) of the vitamin K group and 37/39 (95%) of the placebo had taken >80% of the dispensed medication, based on tablet counts. Dp-ucMGP levels fell significantly in the intervention group compared to placebo at six months (-130 [SD 117] pmol/L vs +13 [SD 180] pmol/L, p<0.001).

Table 1
Baseline characteristics of randomised participants

	Vitamin K (n=40)	Placebo (n=40)	p
Age (years) (SD)	76.0 (4.4)	77.1 (4.8)	0.31
Male sex (%)	21 (53)	23 (58)	0.37
Weight (kg) (SD)	79 (15)	77 (13)	0.64
Hypertension (%)	36 (90)	36 (90)	1.00
Previous stroke or TIA (%)	8 (20)	9 (23)	0.79
Previous myocardial infarction (%)	7 (18)	8 (20)	0.28
Angina (%)	11 (28)	7 (18)	0.28
Coronary revascularisation (%)	7 (18)	5 (13)	0.76
Diabetes Mellitus (%)	9 (23)	9 (23)	1.00
Peripheral vascular disease (%)	5 (13)	3 (8)	0.71
Osteoarthritis (%)	16 (40)	9 (23)	0.09
Walking aid (%)	10 (25)	4 (10)	0.17
Lived alone (%)	18 (45)	10 (25)	0.006
Home help (%)	4 (10)	4 (10)	1.0
Current smoker (%)	3 (8)	7 (18)	0.39
Antiplatelet medications (%)	25 (63)	18 (45)	0.12
ACEi / ARB (%)	30 (75)	25 (63)	0.23
Beta blocker (%)	8 (20)	10 (25)	0.59
Calcium channel blockers (%)	20 (50)	14 (35)	0.18
Other anti-anginals (%)	7 (18)	3 (8)	0.81
Statins (%)	26 (65)	27 (68)	0.31
Median total no. of medications (IQR)	8 (5)	5 (4)	<0.001
Creatinine (umol/L) (SD)	79 (22)	83 (18)	0.16
dp-ucMGP levels (pmol/L) (SD)	789 (363)	823 (360)	0.68

Values are means unless otherwise specified; List of abbreviations: TIA: transient ischemic attack. ARB: Angiotensin receptor blocker. ACEi: Angiotensin Converting Enzyme Inhibitor. dp-ucMGP – desphospho-uncarboxylated Matrix Gla protein

Effects of intervention on vascular outcomes

Table 2 shows the effect of the intervention on the primary outcome of brachial artery FMD and the secondary outcome measures markers of vascular risk. The unadjusted treatment effect for FMD at 3 months was 0.9% (-0.7 to 2.5; p=0.26) and at 6 months was 0.1% (-1.0 to 1.2; p=0.87). No significant effect of treatment was noted after adjustment for baseline variables, as shown in Table 2. Using multiple imputation to account for missing FMD data, the adjusted between group difference in FMD at 6 months was -0.3% (95%CI -1.4 to 0.8). No significant effects were seen on blood pressure, carotid IMT thickness, cholesterol, BNP or CRP levels, as shown in

Table 2
Vascular outcomes

	Group	Baseline (SD) [IQR]	3 mo (SD) [IQR]	6 mo (SD) [IQR]	Unadjusted Baseline vs 3 mo (95% CI)	Adjusted Baseline vs 3 mo (95% CI)	Unadjusted Baseline vs 6 mo (95% CI)	Adjusted Baseline vs 6 mo (95% CI)
FMD (%)	I	6.3 (2.7)	7.1 (2.5)	7.6 (2.7)	0.6 (-1.5, 0.7)	0.4 (-0.6, 1.4)	1.5 (0.8, 2.3)	1.4 (0.6, 2.1)
	P	7.3 (2.4)	7.0 (3.0)	8.6 (2.4)	-0.3 (-1.4, 0.8)	-0.1 (-1.0, 0.9)	1.5 (0.7, 2.2)	1.6 (0.9, 2.3)
	TE	-	-	-	0.9 (-0.7, 2.5)	0.4 (-0.9, 1.8)	0.1 (-1.0, 1.2)	-0.3 (-1.3, 0.8)
Systolic BP (mmHg)	I	144 (17)	141 (16)	140 (17)	-3 (-9, 3)	-4 (-8, 1)	-4 (-10, 1)	-5 (-10, 0)
	P	148 (20)	144 (17)	140 (18)	-3 (-9, 3)	-2 (-7, 2)	-7 (-13, -1)	-6 (-11, -1)
	TE	-	-	-	0 (-8, 9)	-1 (-8, 6)	3 (-5, 11)	1 (-6, 8)
Diastolic BP (mmHg)	I	81 (11)	82 (9)	80 (9)	0 (-3, 3)	0 (-3, 2)	-1 (-4, 2)	-2 (-4, 1)
	P	83 (10)	82 (9)	80 (9)	-1 (-5, 2)	-1 (-4, 2)	-3 (-6, 0)	-2 (-4, 0)
	TE	-	-	-	2 (-2, 6)	1 (-3, 4)	2 (-2, 6)	1 (-3, 5)
Carotid IMT (mm)	I	0.077 (0.015)	0.078 (0.013)	0.076 (0.020)	0.002 (-0.003, 0.007)	0.001 (-0.002, 0.004)	0.000 (-0.006, 0.006)	-0.001 (-0.006, 0.004)
	P	0.080 (0.021)	0.078 (0.010)	0.080 (0.010)	-0.002 (-0.007, 0.002)	-0.002 (-0.005, 0.001)	-0.001 (-0.007, 0.005)	0.000 (-0.005, 0.005)
	TE	-	-	-	0.004 (-0.003, 0.011)	0.003(-0.002, 0.007)	0.001(-0.008, 0.009)	-0.001 (-0.008, 0.006)
Total Cholesterol (mmol/L)	I	4.7 (1.1)	-	4.6 (1.1)	-	-	-0.1 (-0.3, 0.1)	-0.1 (-0.3, 0.1)
	P	4.7 (1.1)	-	4.5 (1.1)	-	-	-0.1 (-0.3, 0.1)	-0.1 (-0.3, 0.0)
	TE	-	-	-	-	-	0.0 (-0.3, 0.3)	0.1 (-0.2, 0.3)
HDL Cholesterol (mmol/L)	I	1.5 (0.5)	-	1.4 (0.5)	-	-	0.0 (-0.1, 0.0)	0.0 (-0.1, 0.0)
	P	1.5 (0.6)		1.4 (0.4)	-	-	-0.1 (-0.1, 0.0)	-0.1 (-0.1, 0.0)
	TE	-	-	-	-	-	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)
LDL Cholesterol (mmol/L)	I	3.3 (1.0)	-	3.3 (0.9)	-	-	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.2)
	P	3.3 (1.1)		3.4 (1.0)	-	-	0.1 (-0.2, 0.3)	0.1 (-0.2, 0.3)
	TE	-	-	-	-	-	-0.1 (-0.4, 0.3)	0.0 (-0.3, 0.3)
BNP (pg/ml)	I	33 [29]	-	40 [24]	-	-	11 (0, 22)	11 (0, 22)
	P	33 [22]	-	37 [29]	-	-	3 (-8, 14)	3 (-8, 14)
	TE	-	-	-	-	-	8 (-8, 23)	8 (-8, 24)
CRP (mg/L)	I	1.8 [2.1]	-	1.8 [3.5]	-	-	0.0 (-4.6, 4.5)	-1.1 (-3.7, 1.4)
	P	1.8 [2.1]	-	1.8 [2.6]	-	-	-1.8 (-6.4, 2.7)	-0.7 (-3.3, 1.8)
	TE	-	-	-	-	-	1.8 (-4.7, 8.3)	-0.4 (-4.0, 3.2)
PWV (m/s)	I	9.7 (2.1)	10.0 (3.1)	9.9 (1.4)	0.1 (-0.9, 1.2)	-0.2 (-1.1, 0.7)	-0.2 (-1.0, 0.7)	-0.4 (-1.1, 0.4)
	P	10.7 (2.3)	10.2 (1.7)	10.9 (3.0)	-0.4 (-1.5, 0.6)	-0.1 (-1.0, 0.9)	0.2 (-0.6, 1.0)	0.4 (-0.4, 1.1)
	TE	-	-	-	0.6 (-0.9, 2.1)	-0.1 (-1.4, 1.3)	-0.3 (-1.5, 0.8)	-0.8 (-1.8, 0.3)
Augmentation index (%)	I	28 (10)	26 (9)	25 (8)	-1 (-4, 2)	-1 (-3, 1)	-2 (-5, 1)	-2 (-4, 0)
	P	28 (7)	28 (6)	27 (6)	0 (-3, 3)	0 (-2, 3)	0 (-3, 2)	0 (-2, 2)
	TE	-	-	-	-1 (-5, 3)	-1 (-5, 2)	-1 (-5, 3)	-2 (-5, 2)

I: Intervention group. P: Placebo group. TE: Treatment effect; IMT: Intima-media thickness. PWV: Pulse wave velocity. BNP: B-type natriuretic peptide. CRP: C-reactive protein; Adjusted for baseline age, systolic BP and baseline value of outcome; Blood pressure: n=40,39,38 for intervention group and n=40,39,39 for placebo group. Cholesterol, CRP and BNP: n= 40, 38 for intervention group and placebo group. PWV and AIx: n=37,38,37 for intervention group and n=38,33,37 for placebo group. FMD: n=38,37,36 for intervention group and n=39,35,36 for placebo group. Carotid IMT: n=39,38,38 for intervention group and 40,39,39 for placebo group; Baseline, 3 month and 6 month values expressed as mean (SD) or median [IQR]. Change scores between baseline and follow up calculated by ANCOVA, expressed as mean (95% CI)

Table 3
Physical function measures

Outcome		Baseline [IQR]	6 mo [IQR]	Unadjusted Baseline vs 6 mo (95% CI)	Adjusted Baseline vs 6 mo (95% CI)
Grip strength (Kg)	I	25.8 [13.0]	26.7 [12.8]	0.4 (-0.3, 1.2)	0.4 (-0.4, 1.2)
	P	27.3 [13.3]	29.0 [10.5]	0.3 (-0.4, 1.0)	0.3 (-0.4, 1.1)
	TE	-	-	0.1 (-0.9, 1.2)	0.1 (-1.0, 1.2)
Short Physical Performance Battery	I	9 [4]	9 [3]	0.0 (-0.4, 0.5)	0.0 (-0.4, 0.4)
	P	10 [3]	10 [4]	0.4 (0.0, 0.8)	0.4 (0.0, 0.9)
	TE	-	-	-0.3 (-0.9, 0.3)	-0.5 (-1.1, 0.2)
Sway measures - Eyes open					
A-P sway (mm)	I	31.1 [9.5]	27.6 [14.0]	-1.6 (-4.4, 1.2)	-1.1 (-3.8, 1.6)
	P	28.6 [10.8]	27.2 [13.2]	0.2 (-2.7, 3.2)	-0.3 (-3.2, 2.6)
	TE	-	-	-1.8 (-5.9, 2.3)	-0.8 (-4.8, 3.2)
M-L sway (mm)	I	21.6 [13.0]	15.2 [9.3]	-7.2 (-10.9, -3.5)	-7.6 (-10.8, -4.4)
	P	23.7 [17.8]	16.3 [11.0]	-8.0 (-11.9, -4.0)	-7.5 (-10.9, -4.1)
	TE	-	-	0.8 (-4.6, 6.2)	-0.1 (-4.7, 4.6)
Path length (mm)	I	2388 [600]	2494 [556]	-128 (-329, 72)	-84 (-209, 40)
	P	2426 [582]	2484 [683]	-21 (-233, 192)	-70 (-202, 62)
	TE	-	-	-108 (-400, 185)	-14 (-198, 169)
RMS (mm)	I	7.3 [2.3]	6.5 [2.9]	-1.1 (-1.9, -0.3)	-1.1 (-1.8, -0.4)
	P	7.7 [3.5]	6.4 [4.0]	-0.8 (-1.7, 0.1)	-0.8 (-1.6, -0.1)
	TE	-	-	-0.3 (-1.5, 0.9)	-0.2 (-1.2, 0.8)
Ellipse (mm2)	I	905 [730]	322 [291]	-963 (-1385, -540)	-931 (-1058, -804)
	P	1075 [1070]	329 [491]	-867 (-1314, -420)	-902 (-1037, -767)
	TE	-	-	-95 (-710, 519)	-29 (-216, 159)
Sway measures - Eyes closed					
A-P sway (mm)	I	43.9 [22.2]	38.7 [19.6]	-5.0 (-10.4, 0.3)	-3.9 (-8.4, 0.5)
	P	41.3 [13.7]	36.8 [15.2]	-2.7 (-8.2, 2.8)	-3.9 (-8.4, 0.7)
	TE	-	-	-2.3 (-10.0, 5.4)	-0.1 (-6.5, 6.3)
M-L sway (mm)	I	38.3 [22.2]	22.1 [16.5]	-15.4 (-20.1, -9.8)	-13.9 (-18.0, -9.8)
	P	38.1 [24.3]	21.9 [17.5]	-9.3 (-15.0, -3.5)	-10.8 (-15.1, -6.6)
	TE	-	-	-6.1 (-14.1, 1.9)	-3.1 (-9.0, 2.8)
Path length (mm)	I	2661 [694]	2588 [701]	-256 (-505, -7)	-201 (-334, -68)
	P	2670 [732]	2579 [585]	-146 (-402, 111)	-204 (-341, -68)
	TE	-	-	-110 (-468, 247)	3 (-188, 194)
RMS (mm)	I	10.6 [5.3]	8.5 [4.5]	-2.2 (-3.5, -0.9)	-1.8 (-2.7, -0.8)
	P	10.2 [4.7]	8.3 [5.1]	-1.1 (-2.4, 0.3)	-1.5 (-2.5, -0.6)
	TE	-	-	-1.1 (-3.0, 0.8)	-0.3 (-1.6, 1.1)
Ellipse (mm2)	I	1034 [1010]	542 [648]	-652 (-1009, -295)	-519 (-697, -341)
	P	968 [1001]	547 [813]	-341 (-709, 27)	-481 (-665, -298)
	TE	-	-	-311 (-824, 201)	-37 (-295, 220)

SPPB: Short physical performance battery. A-P: Antero-posterior. M-L: Medio-lateral; Adjusted for baseline age and baseline value of outcome; For SPPB and grip strength, N=38 for intervention group and n=39 for placebo group at baseline and 6 months. For sway measures, n=35 for intervention group and n=30 for placebo group at baseline and 6 months; Baseline and 6 month values expressed as median [IQR]. Change scores between baseline and follow up calculated by ANCOVA, expressed as mean (95% CI)

Table 2. Pulse wave velocity was not significantly different in the treatment group compared to placebo at 6 months after adjustment for baseline values, and AIx@75 did not change significantly with treatment.

Effects of intervention on physical function

Table 3 gives the outcomes for the physical function measures. No significant treatment effect was shown for either the SPPB or for handgrip strength. Results are given for five measures of sway, measured with eyes open and eyes closed. None of the measures showed significant differences between groups after adjustment for baseline values.

Adverse events

Table 4 shows adverse events. An excess of falls and of gastrointestinal side effects were seen in the vitamin K group compared to placebo, but no difference in serious adverse events or deaths was noted.

Table 4
Adverse events by group

	Vitamin K (n=40)	Placebo (n=40)
Cardiovascular	6	5
Pain	4	3
Gastrointestinal disturbances	10	5
Postural symptoms	2	2
Falls / musculoske-letal	17	7
Infections	6	5
TOTAL Events:	45	27
Hospitalisations	3	2
Deaths	1	0

Discussion

To our knowledge this is the first randomised control trial to examine the effects of vitamin K on vascular health in older people with established vascular disease. The study found that 6 months of vitamin K supplementation did not improve endothelial function of the brachial artery measured by FMD when compared to placebo administration. In addition no significant differences were seen in PWV, carotid IMT, BNP, CRP or in physical function as measured by hand grip strength and SPPB between the two groups. This was despite excellent adherence to medication and a low dropout rate from the study which involved older people with significant co-morbidity.

There are several potential explanations as to why no significant effects were seen. The study participants received 100mcg of vitamin K2 daily, a dose just above the UK RDI for vitamin K currently set at 80 mcg, which is based purely on the role vitamin K has on the clotting mechanism. Although we administered a dose reflective of the current RDI a larger dose may be required to produce significant effects on the markers we assessed. It is possible that a degree of resistance to the effects of vitamin K could be present; such resistance is seen in patients with chronic kidney disease (23), and in this group, greater effects on uncarboxylated MGP level were evident with 360mcg of vitamin K2 per day than at 135mcg per day (12). No tolerable upper limit for vitamin K has been set (24) with no known toxicity or adverse effects in healthy individuals associated with intake of the recommended dose. While some participants recorded episodes of GI upset these were mild and would be unlikely to effect the feasibility of any future trials aiming to investigate the effects of these higher doses.

Over the 6 month study period our results showed a trend towards modest improvement in pulse wave velocity in the treatment group compared to placebo. This may be consistent with the postulated effect of vitamin K retarding or reversing arterial calcification; it is perhaps to be expected that changing vascular calcification with vitamin K2 may take longer than the 6 month study window. Our choice of a 6 month study period was linked to our decision to use endothelial function as our primary outcome; previous trials have shown changes in endothelial function within a few weeks of other types of therapeutic intervention. Effects might also be more evident in the aorta and lower limb arteries (which are more prone to calcification) than the brachial artery, hence carotid-femoral arterial stiffness measures may show greater changes. Similar arguments also apply to the carotid intima-media thickness, which may take many months to change, even with therapies such as statins (25). It is also possible however that the relationship between low vitamin K levels and vascular disease is not causal; disease states may perturb vitamin K metabolism (as is seen in chronic kidney disease (23)) leading to confounding by reverse causality. We cannot exclude the possibility that vitamin K1 might be more efficacious than K2, although our current understanding is that they possess similar biological effects.

The lack of effect on endothelial function seen in this trial is unlikely to be due to short follow up, as many therapies (including statins, allopurinol and vitamin D) improve endothelial function within a few weeks (21, 26, 27). The lack of effect may be due to an insufficiently large dose, but might also be because vitamin K is not acting on pathways that improve endothelial function.

Despite their burden of comorbid disease, the study population did not have severely impaired physical performance. It is therefore likely that ceiling effects contributed to the lack of improvement seen in physical function measures. Falls were more common in the intervention group, but this may be due to the intervention group having a number of baseline attributes that denoted higher risk of falls, including lower strength, lower SPPB score, and greater use of walking aids.

The study had several strengths including the successful recruitment of older patients with a mean age in the high-70's, a very low dropout rate, and good adherence to the study intervention. Studying typical older patients with vascular disease and few exclusion criteria is likely to enhance the generalizability of our findings. Despite the randomised nature of the study, important differences existed between the groups for some key baseline factors, including FMD and arterial stiffness, which complicates the interpretation of the results. For patient comfort and to reduce the physical burden on the older patients being recruited the researchers choose to measure carotid-radial PWV, which may not be as sensitive as carotid-femoral measurement for detecting large-artery stiffness changes as it encompasses the different physiology of large and medium sized arteries in the measurement. The relatively short follow up and single, relatively low dose of vitamin K2 used limits our ability to exclude potentially beneficial effects over the longer term. Future trials examining the effect of vitamin K2 on physical function may need to enrol frailer people, and further trials examining arterial stiffness with longer follow up and higher vitamin K2 doses would seem merited.

Conclusions

This double blind randomised controlled trial found that supplementation of the current RDI of vitamin K for a six month period did not improve endothelial function of the brachial artery measured by FMD when compared to placebo administration. In addition, no significant differences were seen between the two groups for any of the secondary vascular outcomes or in physical function. Adherence to the study medication was high, and dropout rates were low despite the study group comprising older people with significant multimorbidity.

Although not significant, a trend towards improvement in arterial stiffness was noted at 6 month suggesting that treatment with vitamin K over a longer period of time might indeed provide further benefit. These results could still be consistent with a biological effect of vitamin K acting to inhibit of calcification, rather than directly improving endothelial function. Those who volunteered for the study were relatively fit given their age and comorbid disease; the findings therefore may not be generalizable to either younger patients or frailer patients and further trials in these groups, testing different doses and types of vitamin K supplementation are required.

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