Vitamin K supplementation and progression of coronary artery calcium in older men and women[1–4]

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ABSTRACT

Background: Coronary artery calcification (CAC) is an independent predictor of cardiovascular disease. A preventive role for vitamin K in CAC progression has been proposed on the basis of the properties of matrix Gla protein (MGP) as a vitamin K–dependent calcification inhibitor.

Objective: The objective was to determine the effect of phylloquinone (vitamin K1) supplementation on CAC progression in older men and women.

Design: CAC was measured at baseline and after 3 y of follow-up in 388 healthy men and postmenopausal women; 200 received a multivitamin with 500 μg phylloquinone/d (treatment), and 188 received a multivitamin alone (control).

Results: In an intention-to-treat analysis, there was no difference in CAC progression between the phylloquinone group and the control group; the mean ($\pm$SEM) changes in Agatston scores were 27 $\pm$ 6 and 37 $\pm$ 7, respectively. In a subgroup analysis of participants who were $\geq$85% adherent to supplementation (n = 367), there was less CAC progression in the phylloquinone group than in the control group (P = 0.03). Of those with preexisting CAC (Agatston score $>$ 10), those who received phylloquinone supplements had 6% less progression than did those who received the multivitamin alone (P = 0.04). Phylloquinone-associated decreases in CAC progression were independent of changes in serum MGP. MGP carboxylation status was not determined.

Conclusions: Phylloquinone supplementation slows the progression of CAC in healthy older adults with preexisting CAC, independent of its effect on total MGP concentrations. Because our data are hypothesis-generating, further studies are warranted to clarify this mechanism. This trial was registered at clinicaltrials.gov as NCT00183001. *Am J Clin Nutr* 2009;89:1799–807.

INTRODUCTION

Coronary artery calcification (CAC) is an independent predictor of cardiovascular disease (CVD) and CVD-related mortality (1–3). Matrix Gla protein (MGP) is a vitamin K–dependent protein that functions as a calcification inhibitor (4) and may be integral in the regulation of human vascular mineralization (5, 6). Vitamin K is required for the function of MGP through its role as an enzyme cofactor in the γ-carboxylation of the protein. Vitamin K antagonism with warfarin inhibits the vitamin K–dependent carboxylation of MGP, which leads to arterial calcification in rats (7). Furthermore, diets high in vitamin K have been shown to reverse aortic calcification and improve arterial elasticity in warfarin-treated rats (8).

Our current understanding of the potential role of vitamin K intake in protecting against vascular calcification in humans is limited. An inverse cross-sectional association between menaquinone-4 (MK-4, or vitamin K2) intake and arterial calcification was reported (9), whereas no associations between intake of phylloquinone, the primary dietary source of vitamin K, and abnormal calcification were noted (9, 10). In a single randomized controlled trial that assessed the effect of phylloquinone on vascular health in postmenopausal women, supplementation with phylloquinone, calcium, and vitamin D for 3 y improved elasticity and compliance in the common carotid artery compared with supplementation without phylloquinone (11). The authors speculate that the improvement resulted from an increase in the vitamin K–dependent carboxylation of MGP, which leads to a decrease in vascular calcium deposition. However, neither MGP nor vascular calcification was measured directly (11).

The purpose of this prospective randomized controlled trial was to determine whether supplementation with 500 μg/d phylloquinone for 3 y would decrease the progression of age-related CAC in older men and women. We hypothesized that the putative effect of phylloquinone supplementation on CAC would be associated with a concomitant change in serum MGP concentrations. Given the potential influence of vitamin K on inflammation,

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independent of its role as an enzyme cofactor for the γ-carboxylation of MGP (12), we also examined the associations between CAC and serially measured proinflammatory markers, C-reactive protein (CRP), interleukin-6 (IL-6), and osteoprotegerin (OPG) in the same cohort of older men and women.

SUBJECTS AND METHODS

Study participants

The participation criteria of this study were described elsewhere (13). Ambulatory men and postmenopausal women aged 60–80 y were recruited for the purpose of this study. All participants completed a detailed medical history questionnaire before enrollment. Exclusion criteria included the diagnosis of a kidney stone in the past 5 y; hyperthyroidism; bilateral hip surgery; therapy with a bisphosphonate, calcitonin, estrogen, tamoxifen, testosterone, or warfarin in the previous 6 mo; known coronary heart disease; prior open heart surgery; atrial fibrillation; pacemaker; femoral neck bone mineral density >1.8 SD below the mean for subjects of the same age and sex; laboratory evidence of kidney or liver disease; and inability to provide informed consent. All participants signed a written informed consent, and this study was approved by the Institutional Review Board at Tufts University. Of the 452 participants who enrolled, there were 421 whites, 14 blacks, 4 Hispanics, 11 Asians, and 2 Native Americans.

Study design

In this 3-y, double-blind, randomized controlled trial, equal numbers of men and women were randomly assigned to receive either a daily multivitamin with 500 μg phylloquinone (phylloquinone treatment group; $n = 229$) or a daily multivitamin without phylloquinone (control group; $n = 223$) to study the effect of phylloquinone supplementation on age-related bone loss and progression of vascular calcification (**Figure 1**). Of the 401 subjects who completed the intervention, the 388 subjects [200 in the treatment group (117 women) and 188 in the control group (117 women)] who underwent measurement of CAC at both baseline and year 3 were used in the primary intention-to-treat analyses. The participants for whom CAC measures were not available at baseline and year 3 ($n = 13$) had lower mean (±SD) serum MGP (166 ± 30 ng/mL compared with 200 ± 48 ng/mL; $P < 0.01$) and lower total cholesterol (180 ± 34 mg/dL compared with 205 ± 38 mg/dL; $P = 0.02$) concentrations than did the 388 subjects who had valid CAC measures at both time points. Otherwise, there were no differences in baseline characteristics. The subgroup of 295 participants who consistently took the supplements throughout the study period (predefined as ≥85% adherence, according to direct pill counts over 3 y) for whom measures of CAC, MGP, and inflammation were available were included in the secondary analysis [149 in the phylloquinone treatment group (84 women) and 146 in the control group (88 women)]. The adherent participants used in the secondary analysis had statistically significantly higher CAC at baseline than did those who were nonadherent; the median [interquartile range (IQR)] Agatston scores (AS) were 32 (195) and 7 (73) for the adherent and nonadherent groups, respectively ($P = 0.03$). All other baseline characteristics were not significantly different between the 2 groups.

Supplements

The phylloquinone treatment group received 500 μg phylloquinone as part of a daily effervescent multivitamin formulation (1 tablet), whereas the control group received the multivitamin formulation without phylloquinone (1 tablet). The basic effervescent multivitamin tablet contained thiamine (1.6 mg), riboflavin (1.8 mg), vitamin B-6 (2.1 mg), vitamin B-12 (3 μg), vitamin C (75 mg), vitamin E (12 mg), pantothenic acid (6 mg), niacin (20 mg), folate (160 μg), and biotin (30 μg). All study participants also received a second daily effervescent tablet that contained 600 mg elemental calcium in the form of calcium carbonate and 10 μg (400 IU) vitamin D in the form of cholecalciferol. Subjects were instructed to take the calcium and vitamin D supplement at the same time as the multivitamin tablet. The supplement manufacturer (Hermes Arzeneimittel GmbH, Munich, Germany) produced a 12-mo supply on an annual basis. To verify stability of the phylloquinone, a tablet from 10% of the tubes containing phylloquinone was analyzed on receipt and every 4–5 mo. Tablets containing phylloquinone contained a mean (±SD) of 564 ± 77 μg phylloquinone on receipt; at 19 mo, the final content was 428 ± 32 μg phylloquinone.

CAC measurement

At baseline and after 3 y of follow-up, imaging was conducted on each participant by using an 8-slice multidetector computed tomography (MDCT) scanner (LightSpeed Ultra; General Electric, Milwaukee, WI) with prospective electrocardiogram triggering during a single breath hold in midinspiration (typically 18 s) by using sequential data acquisition to image each participant. Scans were prospectively initiated at 50% of the RR interval, which is commonly used for MDCT-based measurements of CAC and has been shown to provide the best average image quality (14). Forty-eight contiguous 2.5-mm thick slices (120 kVp, 320 mA, gantry rotation time of 500 ms, temporal resolution of 330 ms) were acquired (14). The effective radiation exposure was 1 mSv. Images were reconstructed by using a field of view of 35 cm. Each participant was repositioned and sequentially scanned a second time.

CAC measurement and definition of thresholds

CT scans were assessed for the presence of CAC by an experienced radiologist. A calcified lesion was identified as an area of ≥3 connected pixels with a CT attenuation >130 Hounsfield units (HU) applying 3-dimensional connectivity criteria (6 points). The modified AS was calculated as previously described (15); the area of each calcified lesion was multiplied by a weighted CT attenuation score dependent on the maximal CT attenuation (HU) within the lesion. The number of pixels (P_N) >130 HU was multiplied by the pixel area (P_A) in mm^2 by using isotrophic interpolation to determine the area of each calcified lesion. If a particular lesion was observed in multiple CT cross sections, the AS for each scan was defined as the sum of the AS from each individual cross section, and the mean AS from both chest scans was used. The 3-y change in CAC was calculated by subtracting the mean baseline AS from the mean year 3 AS for each study participant.

Biochemical measurements

All blood samples were drawn after a 12-h fast, and dedicated aliquots were stored at −80°C until the time of analysis. Serum

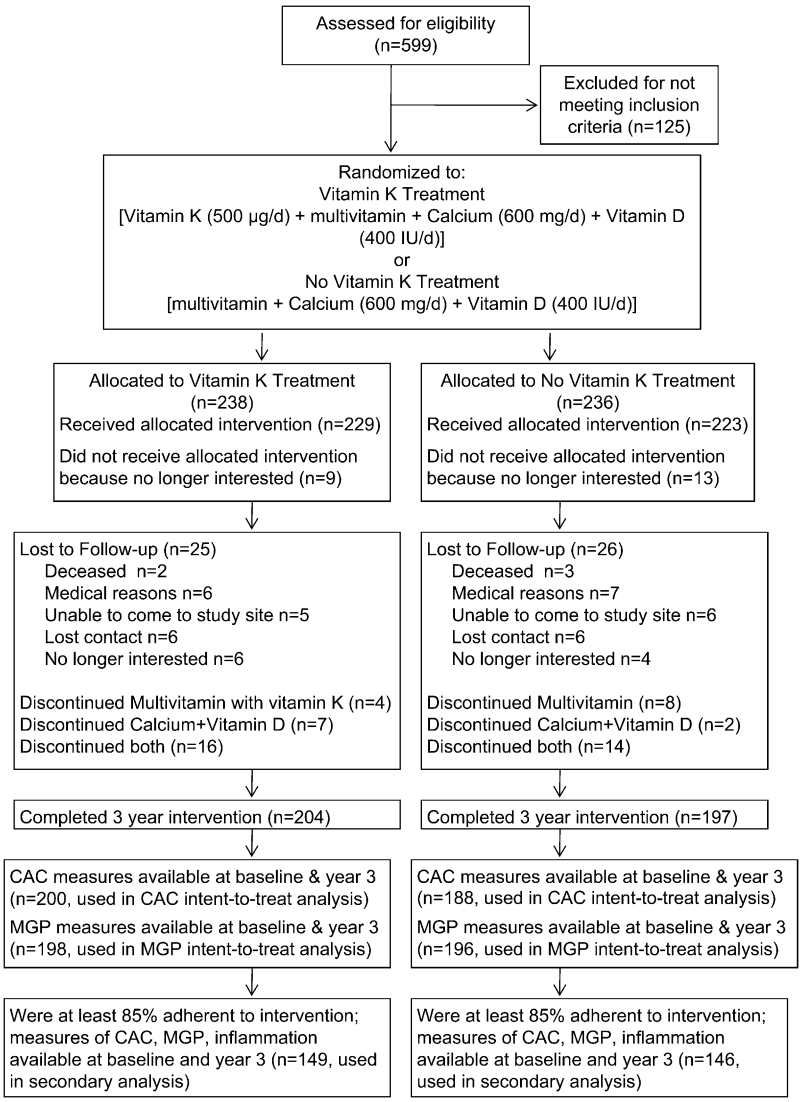

FIGURE 1. Flow chart of study design and subject participation throughout the study. CAC, coronary artery calcification; MGP, matrix Gla protein.

MGP was assayed by radioimmunoassay (16, 17). Plasma concentrations of phylloquinone were measured by HPLC (18). Plasma OPG and IL-6 were assayed by enzyme immunoassay with commercially available high-sensitivity kits (Immunodiagnostics from Biomedica, Vienna, Austria, and R&D Systems, Minneapolis, MN) (19). Plasma CRP was assayed by using high-sensitivity Immulite CRP kits (Diagnostic Products Corporation, Los Angeles, CA) and the COBAS MIRA (Roche Instruments, Belleville, NJ) (19).

Covariates

Information was collected at baseline and year 3 regarding medical history, medication use, smoking status, and physical activity (20). Measurements of weight and height were taken, and body mass index (BMI; in kg/m^2) was calculated.

Statistical analyses

A logarithmic transformation was applied to plasma concentrations of phylloquinone, IL-6, and CRP and to AS to reduce skewness in formal analyses. Student's t test for independent samples (for normally distributed continuous outcomes), Wilcoxon's sign-rank test (for nonnormally distributed continuous outcomes), and the chi-square test for homogeneity of proportions (for categorical outcomes) were used to test for baseline differences between treatment groups. Student's t test for paired samples and McNemar's test for correlated proportions for categorical outcomes were used to examine differences from baseline to year 3. All participants with measures of CAC at baseline and year 3 were used in primary intention-to-treat analyses, regardless of adherence ($n = 200$ in the treatment group and 188 in the control group). Secondary analyses were limited to those who were $\geq$85% adherent to treatment of whom all covariate data were available ($n = 149$ in the treatment group and 146 in the control group). Because CAC progresses more rapidly in individuals who already have CAC present, as a hypothesis-generating subgroup analysis, we examined the effect of vitamin K supplementation on CAC progression and serum MGP in individuals who had at least mild CAC at baseline (defined as AS > 10) [81 in the treatment group (32 women) and 89 in the control group (44 women)]. An AS > 10

is predictive of increased risk of CAC progression and for CVD and mortality (21, 22). Interactions between sex and treatment and between sex and baseline Agatston score with respect to the 3-y change in CAC were not significant, so all analyses were sex-pooled and adjusted for sex.

In intention-to-treat and secondary analyses, an independent-samples *t* test was used to compare the 3-y change in AS (which was normally distributed) between the phylloquinone-treated group and the control group. Analysis of covariance (ANCOVA, using *proc glm*; SAS version 9.1, SAS Institute, Cary, NC) was subsequently used to adjust for baseline AS and for known determinants of CAC progression: sex, age, smoking status (yes or no), statin use (yes or no), prevalent diabetes (yes or no), triglycerides, LDL cholesterol, systolic blood pressure, BMI, and physical activity (23). Because a natural log transformation was applied to baseline and year 3 AS for formal analyses, we repeated these analyses using the ratio of the natural log of the AS at year 3 to the natural log of the AS at baseline as the outcome.

A similar approach was used to examine for the effect of phylloquinone supplementation on change in serum MGP. An independent-samples *t* test was used to compare the 3-y change in serum MGP between treatment groups by using all participants with measures of MGP at baseline and year 3 of follow-up ($n =$ 198 in the treatment group and 195 in the control group), which was followed by ANCOVA to adjust for baseline MGP and for sex, age, smoking status (yes or no), statin use (yes or no), triglycerides, LDL cholesterol, systolic blood pressure, BMI, and physical activity. Secondary analyses were repeated to include only those participants who were adherent to treatment and with measures of all covariates ($n = 149$ in the treatment group and 146 in the control group). To determine whether the phylloquinone treatment influenced a change in CAC by altering serum MGP, we included the change in MGP as a covariate in the models used to assess the influence of vitamin K supplementation on change in AS (24). To provide insight into the associations between vitamin K status, MGP, and CAC, we used partial correlation coefficients between plasma phylloquinone, serum MGP, and AS, as measured at baseline and adjusted for triglycerides, age, and sex.

To examine differences in measures of MGP, OPG, IL-6, and CRP across AS category at baseline (25), we used ANCOVA with AS category as the outcome and baseline biochemical measure as the primary exposure, adjusted for the same covariates in previous statistical models. As previously reported, there was no effect of phylloquinone treatment on change in circulating OPG, IL-6, and CRP (19). To determine whether phylloquinone treatment influenced a change in AS by influencing changes in serum OPG, IL-6, and CRP, we included the 3-y change in OPG, IL-6, or CRP as a covariate in the models we used to assess the effect of vitamin K supplementation on change in AS (24). For all analyses, statistical significance was set at $P \leq 0.05$.

RESULTS

Characteristics of the 388 study participants (154 men, 234 women) included in the intention-to-treat analyses, and of the 295 adherent participants (123 men, 172 women) used in the secondary analyses, are presented according to treatment group in **Table 1**. As expected, plasma phylloquinone concentrations increased in the group that received the phylloquinone supplement ($P < 0.001$), but did not change in the control group ($P = 0.79$) (13). Statin medication use increased equally in both groups ($P < 0.001$), although there was no between-group difference in statin use at year 3 ($P =$ 0.92). There was also an equal, but nonsignificant decrease, in antiinflammatory medication use observed in both groups.

Although the median AS at baseline was higher in the control group, the difference in baseline AS between the 2 groups was not significant ($P = 0.59$, Wilcoxon's rank-sum test). Of the adherent participants, the median (IQR) baseline AS did not differ between treatment groups [phylloquinone treatment group: 18 (189); control group: 40 (199); $P = 0.35$], nor did other baseline characteristics (all $P \geq 0.06$). Of the adherent participants with AS $>$ 10 at baseline (81 in the treatment group and 89 in the control group), those in the control group had a significantly higher median (IQR) CRP concentration [2.0 (4.1)] than did those in the phylloquinone treatment group [1.2 (2.4); $P = 0.02$], but there were no significant differences between treatment groups in any other baseline characteristic, including median (IQR) AS [phylloquinone treatment group: 145 (395); control group: 146 (325); $P = 0.90$; all other $P \geq 0.10$].

In an intention-to-treat unadjusted analysis, there was no difference in the progression of CAC between the phylloquinone treatment group and the control group (**Table 2**). As shown in **Table 3**, when secondary analyses were restricted to those who were ≥85% adherent to the intervention, those in the phylloquinone treatment group had less progression of CAC than did those in the control group ($P = 0.03$). Similar decreases in CAC progression were observed when statistical analysis was limited to individuals who had at least mild CAC at baseline (defined as an AS $>$ 10) ($P = 0.03$). The effect of phylloquinone supplementation on CAC progression was not appreciably changed when baseline AS or other known determinants of CAC progression were held constant.

Of the adherent subjects with AS $>$ 10 at baseline, those in the phylloquinone treatment group had 6% less progression than did those in the control group, based on the analysis of the ratio of the natural log of the AS at year 3 to natural log of the AS at baseline as the outcome (data not shown; $P = 0.04$). Of those individuals with no CAC at baseline ($n =$ 65 and 61 in the phylloquinone and control groups, respectively), 9 in the phylloquinone treatment group and 8 in the control group had new CAC at year 3. There were no differences in the incidence of CVD events, defined as diagnosed coronary heart disease, myocardial infarction, stroke, angioplasty, angina, atrial fibrillation, or heart failure.

In both the intention-to-treat and secondary analyses, serum MGP increased in the phylloquinone treatment group and decreased in the control group (treatment effect: $P \leq 0.03$ in all analyses) (Tables 2 and 3). However, when the change in MGP was included as a covariate in the models to assess the influence of phylloquinone supplementation on change in CAC, the significance of the treatment effect did not change in the adherent participants or in the adherent participants with a baseline AS $>$ 10. Neither baseline nor change in MGP concentrations predicted the change in CAC, which suggested that any putative effect of phylloquinone treatment on progression of CAC was independent of changes in MGP. There was also no correlation between baseline plasma phylloquinone and baseline serum MGP ($P = 0.23$), even though each measure was correlated with baseline AS (phylloquinone and AS: partial $r = -0.12$, $P = 0.02$; MGP and AS: partial $r = 0.10$, $P = 0.06$).

TABLE 1
Participant characteristics at baseline and year 3[1]

Characteristic	All participants with CAC measures used in intention-to-treat analysis[2]		Adherent participants used in secondary analysis[2,3]	
	Vitamin K (n = 200)	Control (n = 188)	Vitamin K (n = 149)	Control (n = 146)
Female sex (%)	59	62	56	60
Age (y)				
Baseline	68 ± 6[4]	68 ± 5	68 ± 6	68 ± 5
Year 3	71 ± 6[5]	71 ± 6[5]	71 ± 6[5]	71 ± 5[5]
BMI (kg/m^2)				
Baseline	28.4 ± 5.4	27.4 ± 4.7	28.4 ± 5.0	27.4 ± 4.6
Year 3	28.2 ± 5.3	27.2 ± 4.7	28.1 ± 5.0	27.2 ± 4.6
Phylloquinone (nmol/L)				
Baseline	0.8 (1.2)[6]	0.8 (1.1)	0.8 (1.1)	0.8 (1.2)
Year 3	2.4 (2.7)[5]	0.8 (1.1)	2.7 (2.6)[5]	0.8 (1.2)
MGP (ng/mL)				
Baseline	200 ± 48	200 ± 48	201 ± 478	200 ± 49
Year 3	207 ± 63	192 ± 59	208 ± 63	183 ± 52[§]
Triglycerides (mg/dL)				
Baseline	113 ± 71	121 ± 70	116 ± 79	115 ± 64
Year 3	110 ± 66	108 ± 51[5]	110 ± 68	105 ± 50[5]
LDL cholesterol (mg/dL)				
Baseline	124 ± 31	126 ± 32	124 ± 31	129 ± 33
Year 3	119 ± 31[5]	125 ± 32	117 ± 30[5]	125 ± 32
Total cholesterol (mg/dL)				
Baseline	203 ± 38	207 ± 38	205 ± 39	208 ± 38
Year 3	196 ± 38[5]	200 ± 36[5]	194 ± 37[5]	200 ± 36[5]
Systolic blood pressure (mm Hg)				
Baseline	132 ± 17	131 ± 18	133 ± 17	132 ± 17
Year 3	128 ± 15[5]	129 ± 15[5]	128 ± 15[5]	129 ± 14[5]
Diastolic blood pressure (mm Hg)				
Baseline	76 ± 9	75 ± 9	76 ± 9	75 ± 9
Year 3	75 ± 8	75 ± 8	75 ± 9	75 ± 8
Framingham 10-y CHD risk score				
Baseline	12 ± 7	12 ± 8	12 ± 7	12 ± 8
Year 3	11 ± 8	12 ± 8	11 ± 7	12 ± 8
Agatston score				
Baseline	19 (174)[6]	34 (189)	18 (190)	40 (303)
Year 3	30 (209)[5]	53 (230)[5]	22 (208)[5]	57 (231)[5]
CRP (mg/dL)				
Baseline	1.3 (2.8)	1.7 (3.4)	1.3 (2.5)	1.8 (3.4)
Year 3	1.2 (3.0)	1.8 (2.9)	1.1 (2.7)	1.8 (3.1)
IL-6 (pg/mL)				
Baseline	1.5 (1.2)	1.4 (1.2)	1.5 (1.1)	1.3 (1.0)
Year 3	1.6 (1.1)	1.9 (1.5)	1.5 (1.1)	1.4 (1.2)
OPG (pmol/L)				
Baseline	4.9 ± 1.7	4.8 ± 1.8	4.9 ± 1.7	4.8 ± 1.7
Year 3	5.2 ± 1.9[5]	5.1 ± 1.8[5]	5.2 ± 1.9[5]	5.2 ± 1.7[5]
PASE				
Baseline	127 ± 59	128 ± 57	125 ± 62	131 ± 57
Year 3	136 ± 66	130 ± 56	133 ± 66	134 ± 56
Statin use (%)				
Baseline	25	27	26	27
Year 3	35[5]	36[5]	39[5]	36[5]
Antiinflammatory medication use (%)				
Baseline	18	19	20	20
Year 3	12	14	12[5]	16[5]
Current smoker (%)				
Baseline	6	4	5	5
Year 3	7	5	6	7

(Continued)

TABLE 1 (*Continued*)

Characteristic	All participants with CAC measures used in intention-to-treat analysis[2]		Adherent participants used in secondary analysis[2,3]	
	Vitamin K ($n = 200$)	Control ($n = 188$)	Vitamin K ($n = 149$)	Control ($n = 146$)
Diabetes (%)				
Baseline	6	6	5	6
Year 3	7	9	6	7

[1] CAC, coronary artery calcification; CHD, coronary heart disease; MGP, matrix Gla protein; PASE, Physical Activity Scale for Elderly; CRP, C-reactive protein; IL-6, interleukin-6; OPG, osteoprotegerin.
[2] No participant characteristics were significantly different between treatment groups at baseline.
[3] Adherence to supplement use was predefined as ≥85% adherence, as determined by direct pill counts.
[4] Mean ± SD (all such values).
[5] Significant 3-y change within treatment group, $P \leq 0.05$ (paired-samples *t* test for continuous outcomes or McNemar's test for correlated proportions for dichotomous outcomes).
[6] Median; interquartile range in parentheses (all such values due to nonnormal distribution); between-group comparison of median values based on Wilcoxon's rank-sum test.

When the change in circulating OPG, IL-6, or CRP was included as a covariate in the models that assessed the influence of vitamin K supplementation on change in CAC, the significance of the treatment effect was not appreciably changed in the adherent participants or in the adherent participants with a baseline AS > 10. There was a trend for increasing serum OPG concentrations across AS categories at baseline, although it was not significant (P for trend = 0.051). Baseline OPG concentrations were positively predictive of change in CAC ($P = 0.004$ in intention-to-treat, adjusted for treatment). In contrast, there were no significant differences between baseline CRP or IL-6 concentrations and CAC.

DISCUSSION

In this 3-y, double-blind, randomized controlled trial, daily supplemental vitamin K in amounts achievable by high dietary intake of green, leafy vegetables resulted in less progression of CAC in older men and women who were adherent to the treatment and took vitamin K than in those who did not take vitamin K. Of those individuals with preexisting CAC, we estimated that those who received phylloquinone had 6% less progression than did those who did not. In contrast, vitamin K supplementation did not reduce the development of new CAC, of which there was an annual increase of ≈5% in both groups. These estimates are consistent with other studies, which estimated an annual 6% increase in new CAC among asymptomatic older adults (23, 26).

Although a role for vitamin K in the regulation of vascular calcification was proposed >30 y ago (27–29), the evidence in humans to date has been limited. High phylloquinone intake has not been consistently associated with a low risk of CVD in population studies (30). However, 3 y of supplementation with 1000 μg/d phylloquinone improved carotid elasticity among postmenopausal women (11). Similar to our study, supplemental calcium and vitamin D was provided with phylloquinone in that study, and it is not known whether similar findings would be noted if phylloquinone was provided without calcium and vitamin D.

We hypothesized that any protective effect of vitamin K on progression of CAC would be associated with a concomitant decrease in serum MGP concentrations. Elevated serum concentrations of MGP have been reported in patients with severe atherosclerosis (31). We previously reported that serum MGP

TABLE 2
Effect of phylloquinone supplementation on the mean (95% CI) 3-y change in Agatston score (AS) and serum matrix Gla protein (MGP) in the intention-to-treat analyses

	Intention-to-treat analyses		
	Vitamin K	Control	P for treatment effect[1]
Change in AS			
n	200	188	
Unadjusted[2]	27 (15, 38)	37 (24, 50)	0.26
Adjusted for baseline[3]	28 (17, 39)	35 (24, 47)	0.36
Fully adjusted[4]	28 (16, 39)	36 (27, 47)	0.34
Change in MGP (ng/mL)			
n	198	195	
Unadjusted[2]	7.2 (−1.2, 15.5)	−7.3 (−16.5, 1.8)	0.02
Adjusted for baseline[3]	7.2 (−0.7, 15.2)	−7.4 (−15.4, 0.5)	0.01
Fully adjusted[4]	7.4 (−0.2, 15.0)	−7.4 (−15.1, 0.3)	0.01

[1] $P > 0.05$ indicates no treatment effect.
[2] Based on independent samples *t* test
[3] Values are least-squares means, based on ANCOVA, adjusted for baseline AS or MGP. Least-squares means represent the mean change in outcome when covariates are held constant.
[4] Additionally adjusted for age, sex, prevalent diabetes, triglycerides, LDL cholesterol, systolic blood pressure, BMI, smoking, physical activity, and statin use.

TABLE 3
Effect of phylloquinone supplementation on the mean (95% CI) 3-y change in Agatston score (AS) and serum matrix Gla protein (MGP) in participants adherent to treatment[1]

	Vitamin K	Control	*P* for treatment effect[2]
Change in AS			
Adherent			
n	149	146	
Unadjusted[3]	17 (4, 29)	37 (24, 50)	0.03
Adjusted for baseline[4]	18 (6, 30)	35 (23, 49)	0.05
Fully adjusted[5]	18 (5, 30)	36 (24, 49)	0.04
Adherent with baseline AS > 10			
n	81	89	
Unadjusted[3]	25 (3, 47)	59 (38, 80)	0.03
Adjusted for baseline[4]	26 (4, 47)	58 (38, 78)	0.03
Fully adjusted[5]	24 (2, 46)	59 (39, 80)	0.02
Change in MGP (ng/mL)			
Adherent			
n	149	146	
Unadjusted[3]	6.5 (−3.2, 16.2)	−17.8 (−27.6, −8.0)	<0.001
Adjusted for baseline[4]	6.7 (−1.9, 15.4)	−18.0 (−26.8, −9.2)	<0.001
Fully adjusted[5]	5.6 (−2.7, 13.8)	−16.8 (−25.0, −8.5)	<0.001
Adherent with baseline AS > 10			
n	81	89	
Unadjusted[3]	4.2 (−9.3, 17.6)	−16.8 (−29.6, −4.0)	0.03
Adjusted for baseline[4]	5.3 (−6.9, 17.5)	−17.9 (−29.4, −6.3)	0.01
Fully adjusted[5]	4.4 (−7.2, 16.1)	−17.0 (−28.2, −6.0)	0.01

[1] Adherence to supplement use was predefined as ≥85% adherence, as determined by direct pill counts.
[2] $P > 0.05$ indicates no treatment effect.
[3] Based on independent-samples *t* test.
[4] Data are least-squares means (95% CI), based on ANCOVA, adjusted for baseline AS or baseline MGP. Least-squares means represent the mean change in outcome when covariates are held constant.
[5] Additionally adjusted for age, sex, baseline statin use, diabetes, triglycerides, LDL cholesterol, systolic blood pressure, BMI, smoking status, and physical activity.

concentrations are significantly higher among those adults with a higher Framingham Coronary Heart Disease Risk Score, but are not consistently associated with higher CAC (32). Similarly, there were only nonsignificant trends between baseline serum MGP and CAC in this study, which lends further support to previous conclusions that serum MGP concentrations are not a robust predictor of CAC (16). In contrast, serum MGP concentrations were higher in this study among the phylloquinone treatment group than in the control group, which was unexpected because the phylloquinone treatment group had less progression of CAC. Controlling for the 3-y change in serum MGP did not attenuate the significance of the treatment effect with respect to change in AS, which suggested that any effect of phylloquinone treatment on CAC progression was independent of the change in serum MGP. The assay used to measure MGP did not differentiate between the γ-carboxylated and uncarboxylated forms of MGP (17). It is assumed that only the carboxylated form of MGP is functional as an inhibitor of calcification, so interpretation of serum total MGP concentrations is problematic. Novel assays that purportedly measure the uncarboxylated form of MGP may be used in the future to elucidate the role of functional forms of MGP in response to vitamin K supplementation and CAC (33). Alternatively, it is plausible that serum MGP concentrations, regardless of γ-carboxylation status, do not reflect MGP expression in arterial walls, as observed in mice lacking MGP (34).

We also examined associations between changes in proinflammatory markers and changes in calcification as an alternative mechanism by which vitamin K may protect against progression of coronary artery calcium. Baseline serum OPG concentrations are higher among those individuals with higher calcification scores. However, there was no influence of phylloquinone treatment on change in circulating OPG, IL6, and CRP (19), and controlling for the 3-y change in cytokines did not alter the significance of the treatment effect on change in AS. This suggests that any putative effect of phylloquinone treatment on progression of CAC was independent of changes in individual cytokines.

The amount of CAC is a well-known independent predictor of CVD risk (34). In several population-based studies, CAC was incrementally predictive of cardiovascular events beyond traditional risk factors or the Framingham Risk Score (1, 35). Thus, slowing down the progression of CAC, which leads to lower absolute CAC, may constitute a favorable result. However, progression of CAC has not yet been established as a surrogate marker of therapy success or as an indicator of cardiovascular event risk. Intervention studies that have assessed the efficacy of standard cardiovascular therapies, such as lipid-lowering medications or other supplementation with other nutrients, such as vitamins C and E, on minimizing the progression of CAC have thus far reported no effect (36, 37).

Certain limitations of this study should be acknowledged. Known CVD was an exclusion criterion for our study, and our follow-up was limited to 3 y; therefore, we were not able to show that slowing down the progression of CAC with vitamin K supplementation reduced cardiovascular event risk, and longitudinal studies are warranted to test this hypothesis. Although differences in baseline CAC between treatment groups were not significant, the

CAC was not equally matched between groups, despite the use of a double-blind randomized controlled study design. However, the difference was controlled for in our statistical analyses. Ethnic variability in CAC progression is reported (24). Because our sample was >90% white, our findings cannot be generalized to other ethnic groups. Because vitamin K supplementation reduced the progression of CAC among men and women who adhered to our intervention, overall and in those with preexisting CAC, our results should be considered hypothesis-generating. Given the variability in CAC measures, our results would be strengthened by larger confirmatory studies and an investigation of the effect of vitamin K supplementation on CAC among those at greater risk of progressive CAC.

In conclusion, vitamin K supplementation reduced the progression of existing CAC in asymptomatic older men and women when taken with recommended amounts of calcium and vitamin D. The mechanisms by which vitamin K conferred a protective role are still uncertain. Larger studies in other populations are needed to confirm these findings, and to assess the risks and benefits of vitamin K supplementation on clinical CVD.

The authors' responsibilities were as follows— SLB: designed the study and contributed to the design of the analyses, interpretation of the data, and writing of the manuscript; MKS: performed the statistical analyses and drafted the manuscript; GED, BD-H, CJO, and UH: contributed to the design of the analyses, the interpretation of the data, and the writing of the manuscript; JMO: contributed to the design of the analyses and interpretation of the data; and PAP and MKW: contributed to the laboratory analyses and the writing of the manuscript. All authors reviewed the final manuscript. None of the authors had a conflict of interest to declare.

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