Title

Circulating Vitamin K Is Inversely Associated with Incident Cardiovascular Disease Risk among Those Treated for Hypertension in the Health, Aging, and Body Composition Study (Health ABC).

Journal

Journal of Nutrition, 147(5)

ISSN

0022-3166

Authors

Shea, M Kyla
Booth, Sarah L
Weiner, Daniel E
et al.

Publication Date

2017-05-01

DOI

10.3945/jn.117.249375

Peer reviewed

The Journal of Nutrition
Nutritional Epidemiology

Circulating Vitamin K Is Inversely Associated with Incident Cardiovascular Disease Risk among Those Treated for Hypertension in the Health, Aging, and Body Composition Study (Health ABC)[1–3]

M Kyla Shea,[4]* Sarah L Booth,[4] Daniel E Weiner,[5] Tina E Brinkley,[6] Alka M Kanaya,[7] Rachel A Murphy,[8] Eleanor M Simonsick,[9] Christina L Wassel,[10] Cees Vermeer,[11] and Stephen B Kritchevsky[6] for the Health ABC Study

[4]USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA; [5]Division of Nephrology, Department of Medicine, Tufts Medical Center, Boston, MA; [6]Sticht Center on Aging, Wake Forest School of Medicine, Winston-Salem, NC; [7]University of California–San Francisco, San Francisco, CA; [8]School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada; [9]National Institute on Aging/NIH, Baltimore, MD; [10]Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; and [11]VitaK, University of Maastricht, Maastricht, Netherlands

Abstract

Background: A role for vitamin K in coronary artery calcification (CAC), a subclinical manifestation of cardiovascular disease (CVD), has been proposed because vitamin K–dependent proteins, including the calcification inhibitor matrix Gla protein (MGP), are present in vascular tissue. Observational studies found that low circulating phylloquinone (vitamin K-1) was associated with increased CAC progression, especially in persons treated for hypertension. It is unknown whether hypertension treatment modifies this putative role of vitamin K in clinical CVD risk.

Objective: We determined the association between vitamin K status and incident clinical CVD in older adults in the Health ABC (Health, Aging, and Body Composition Study) and whether the association differed by hypertension treatment status.

Methods: Plasma phylloquinone was measured in 1061 participants free of CVD (70–79 y of age, 58% women, 39% black). Plasma uncarboxylated MGP [(dp)ucMGP] was measured in a subset of 635 participants. Multivariate Cox models estimated the HR for incident CVD over 12.1 follow-up years. Effect modification by hypertension was tested with the use of interaction terms.

Results: Neither low plasma phylloquinone (<0.2 nmol/L) nor elevated (dp)ucMGP (≥574 pmol/L) was significantly associated with incident CVD [respective HRs (95% CIs): 1.27 (0.75, 2.13) and 1.02 (0.72, 1.45)]. In participants treated for hypertension (n = 489; 135 events), low plasma phylloquinone was associated with higher CVD risk overall (HR: 2.94; 95% CI: 1.41, 6.13). In those with untreated hypertension (n = 153; 48 events) and without hypertension (n = 418; 92 events), low plasma phylloquinone was not associated with incident CVD. The association between high (dp)ucMGP did not differ by hypertension treatment status (P-interaction = 0.72).

Conclusions: Vitamin K status was not significantly associated with CVD risk overall, but low plasma phylloquinone was associated with a higher CVD risk in older adults treated for hypertension. Additional evidence from larger clinical studies is needed to clarify the importance of vitamin K to CVD in persons treated for hypertension, a segment of the population at high risk of clinical CVD events. *J Nutr* 2017;147:888–95.

Keywords: vitamin K, phylloquinone, matrix Gla protein, cardiovascular disease, hypertension

Introduction

Cardiovascular disease (CVD)[12] is a leading cause of disability, morbidity, and mortality in older adults (1, 2). A protective role for vitamin K against CVD has been proposed on the basis of the presence of vitamin K–dependent proteins in vascular tissue (3). The most studied vitamin K–dependent protein in vascular tissue is matrix Gla protein (MGP), which, when carboxylated, inhibits calcification (4, 5). The carboxylation of MGP requires vitamin K; thus, uncarboxylated MGP increases when vitamin K status is low. Vitamin K–supplemented diets reduced aortic calcification and improved arterial stiffness in rats treated with warfarin, a vitamin K antagonist (6). In a randomized controlled

Manuscript received February 13, 2017. Initial review completed February 23, 2017. Revision accepted March 3, 2017.
First published online March 29, 2017; doi:10.3945/jn.117.249375.

trial in 388 older adults, vitamin K (phylloquinone) supplementation reduced the progression of coronary calcium (CAC), a marker of subclinical CVD, over 3 y (7). Interestingly, growing evidence suggests that the role of vitamin K in CVD may be particularly important for certain high-risk subgroups.

We previously analyzed the association between serum phylloquinone (vitamin K-1, the primary circulating form of vitamin K) and CAC progression in the MESA (Multi-Ethnic Study of Atherosclerosis) and detected a significant interaction between low serum phylloquinone and hypertension status. Participants treated for hypertension who had low serum phylloquinone had >2-fold higher odds of CAC progression over 2.5 y than did those treated for hypertension without low serum phylloquinone (OR: 2.37; 95% CI: 1.38, 4.09). No association between serum phylloquinone and CAC progression was detected in those not treated for hypertension (8). We replicated this finding in a post hoc analysis of the vitamin K supplementation randomized trial in older adults described above (8). Although these findings suggest a role for vitamin K in reducing CVD, evidence of the relation between vitamin K status and clinical CVD is limited to the indirect measurement of CAC. The association between plasma phylloquinone and clinical CVD has not been reported. However, elevated plasma uncarboxylated MGP [(dp)ucMGP] was associated with an elevated risk of clinical CVD in some (9, 10), but not all (11), European population–based cohorts. Because there is not currently a single biomarker of vitamin K status that is used clinically, studies linking vitamin K status to clinical outcomes are strengthened when 2 biomarkers are used (12). However, studies of the association between vitamin K status and incident CVD that use multiple biomarkers are lacking.

The present study aimed to determine the association between plasma phylloquinone and (dp)ucMGP with the risk of incident CVD in community-dwelling adults aged 70–79 y over 12 y of follow-up. On the basis of our previous findings (8), we also examined whether the association differed in those treated for hypertension.

Methods

Participants were drawn from the Health, Aging, and Body Composition Study (Health ABC; n = 3075), a prospective longitudinal cohort study designed to examine age-related changes in physical function and body composition in older black and white men and women. Health ABC recruitment has been well described (13). When enrolled, all participants were free of disability in activities of daily living and reported no difficulty walking 0.25 mile or up 10 steps. All of the participants provided written informed consent, and the institutional review boards at both study sites approved all protocols. Plasma phylloquinone was measured from samples obtained at the year 2 clinic visit in 1127 participants in the Knee Osteoarthritis (Knee OA) substudy (14, 15) and in 731 randomly selected participants who were not included in the Knee OA substudy, for a total of 1858 (**Figure 1**). Plasma (dp)ucMGP was only measured in the Knee OA substudy due to sample availability and funding (n = 1127). Plasma samples were obtained after an overnight fast and stored at −70°C until analysis. After excluding those with prevalent CVD, those taking warfarin, and those missing complete covariate data, 1061 participants were available for the analysis of plasma phylloquinone and incident CVD and 635 participants were available for the analysis of plasma (dp)ucMGP and incident CVD.

Those with plasma phylloquinone measures had higher LDL cholesterol (123 compared with 119 mg/dL; $P < 0.01$), less exposure to cigarette smoking (2 compared with 6 pack-years; $P < 0.01$), and were more likely to be black, female, and have knee pain (all $P < 0.01$) than participants whose plasma phylloquinone was not measured. Similar differences were noted between participants whose plasma (dp)ucMGP was measured and Health ABC participants in whom it was not. In addition, those with (dp)ucMGP measured had a higher BMI (in kg/m^2; 28.0 compared with 26.7; $P = 0.03$) and reported less walking per week (30 compared with 45 min/wk; $P < 0.01$) than the remaining Health ABC participants. Compared with the Knee OA substudy participants, the 789 randomly selected participants who were not in the Knee OA substudy whose plasma phylloquinone was measured had a lower BMI (26.4 compared with 27.5; $P < 0.01$), lower systolic blood pressure (SBP; 131 compared with 133 mm Hg; $P = 0.04$), lower circulating IL-6 (2.3 compared with 2.5 pg/mL; $P = 0.03$), better lower-extremity function [Short Physical Performance Battery score (on a 0–4 scale): 2.3 compared with 2.2 (16); $P < 0.01$], and were less likely to have knee pain ($P < 0.01$), take anti-inflammatory medication ($P < 0.01$), be female ($P < 0.01$), or black ($P < 0.01$). Participants excluded for incomplete covariate information did not differ from those with complete covariate data with respect to plasma phylloquinone or (dp)ucMGP. Those with complete data had a longer median follow-up time than those excluded for incomplete data (12.1 compared with 9.4 y; $P < 0.01$).

Incident CVD. CVD event adjudication has been described (17). Incident CVD was defined as newly diagnosed myocardial infarction, angina, ischemic heart disease, stroke, or CVD death between the year 2 clinic visit and 31 August 2014, the censoring date for those who did not have incident CVD [median (IQR) follow-up = 12.1 (8.9) y]. Only confirmed and/or definite events were included.

Vitamin K status biomarkers. Plasma phylloquinone was measured by using reverse-phase HPLC at the USDA Human Nutrition Research Center on Aging at Tufts University (18). This laboratory currently participates in the international vitamin K external quality assurance scheme, KEQAS (19). The limit of detection for circulating phylloquinone for this assay with the use of the sample volume available was <0.2 nmol/L (18). Low and high control specimens had average values of 0.56 and 3.1 nmol/L, with intra- and interassay CVs of 4.2% and 4.9% respectively. The concentration of circulating phylloquinone considered to be deficient is not defined. However, circulating concentrations <0.2 nmol/L are generally achieved when dietary intakes are restricted to ≤20% of current recommendations in metabolic feeding studies (20). In a previous analysis of Health ABC, we found that participants with low plasma phylloquinone (defined as <0.2 nmol/L, the lower limit of assay detection by using the sample volume available) were more likely to have knee osteoarthritis progression (14). Because the vitamin K–dependent proteins implicated in osteoarthritis are also implicated in CVD (3, 21), and osteoarthritis disability is associated with CVD (22), we likewise defined low plasma phylloquinone as <0.2 nmol/L, which is

[1] Supported in part by the Intramural Research Program of the NIH, National Institute on Aging, and contracts N01-AG-6-2101, N01-AG-6-2103, and N01-AG-6-2106; National Institute on Aging (R01-AG028050); the National Institute of Nursing Research (R01-NR012459); the National Institute of Arthritis Musculoskeletal and Skin Diseases (R21AR062284 and K01AR063167); and a New Investigator Grant from the Arthritis Foundation and the USDA, Agricultural Research Service, under Cooperative Agreement 58-1950-7-707.

[2] Author disclosures: MK Shea, SL Booth, DE Weiner, TE Brinkley, AM Kanaya, RA Murphy, EM Simonsick, CL Wassel, and SB Kritchevsky, no conflicts of interest. VitaK is a research and development organization owned by Maastricht University. At the time of this study, C Vermeer was an advisor to VitaK but had no financial interests in this organization.

[3] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

*To whom correspondence should be addressed. E-mail: kyla.shea@tufts.edu.

[12] Abbreviations used: CAC, coronary artery calcium; CVD, cardiovascular disease; DBP, diastolic blood pressure; (dp)ucMGP, (desphospho)uncarboxylated matrix Gla protein; Health ABC, Health, Aging, and Body Composition Study; Knee OA, Knee Osteoarthritis (substudy); MESA, Multi-Ethnic Study of Atherosclerosis; MGP, matrix Gla protein; SBP, systolic blood pressure.

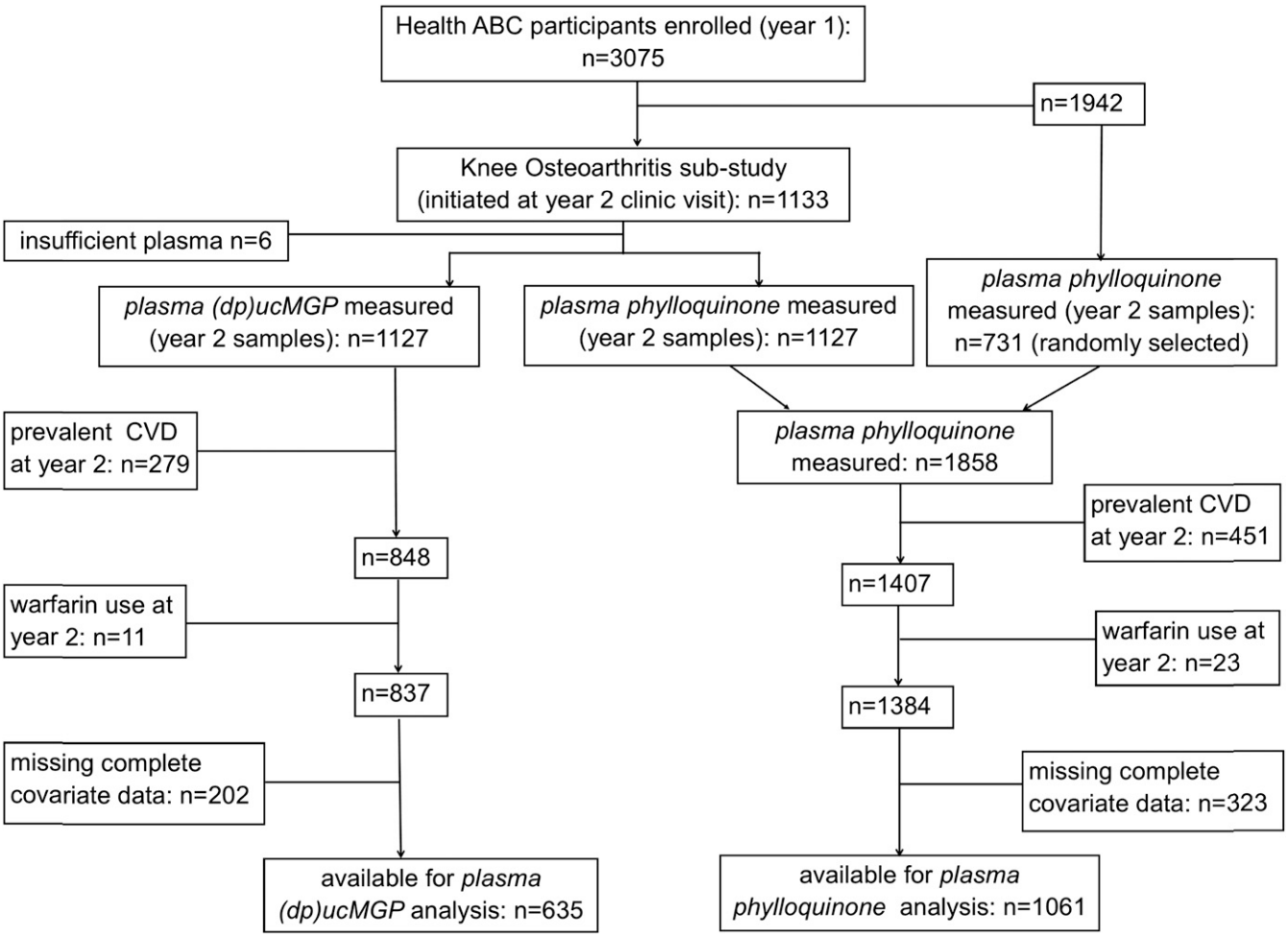

FIGURE 1 Diagram of Health ABC participants included in this analysis. CVD, cardiovascular disease; (dp)ucMGP, (desphospho)uncarboxylated matrix Gla protein; Health ABC, Health, Aging, and Body Composition Study.

commensurate with the concentrations achieved with dietary phylloquinone restriction.

Plasma (dp)ucMGP, the circulating MGP form that is reduced in response to vitamin K supplementation (23), was measured by using a sandwich ELISA at VitaK (University of Maastricht), which uses 2 monoclonal antibodies directed against the nonphosphorylated amino acid sequence 3–15 and the noncarboxylated amino acid sequence 35–49 in human MGP (24). The reported intra- and interassay CVs for this assay were 5.6% and 9.9%, respectively (24). Plasma (dp)ucMGP is a novel biomarker without clinical correlates nor validation with the use of metabolic feeding studies. In lieu of this, we used the highest distribution tertile (≥574 pmol/L) to define lower vitamin K status according to (dp)ucMGP. We secondarily defined high plasma (dp)ucMGP as the highest 5% of the distribution (≥1012 pmol/L).

Hypertension. Treated hypertension was defined by using self-reported diagnosis of hypertension ascertained from medical history questionnaires with the use of specific medications at the year 2 clinic visit (e.g., diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium-channel blockers, β-blockers). Participants brought all medications taken within the past 2 wk to the clinic visit. Medication use was recorded and coded by using the Iowa Drug Information system. Participants not treated for hypertension were classified as having untreated hypertension if they had SBP ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg based on readings obtained at the year 2 clinic examination. Blood pressure was measured from the seated position after a 5-min rest by using a conventional mercury sphygmomanometer 3 times. The average of the last 2 measures was used. Participants who had an SBP <140 mm Hg, a DBP <90 mm Hg, and were not taking hypertension medication were considered free of hypertension.

Covariates. At the baseline clinic visit, demographic and lifestyle characteristics including age, sex, race, education, alcohol use, and smoking status were ascertained by using interviewer-administered questionnaires. TGs, total and HDL cholesterol, and creatinine were measured in fasting serum on a commercially available analyzer (Vitros 950; Johnson & Johnson). LDL cholesterol was calculated by using the Friedewald equation (25), and estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease equation (26). IL-6 was measured in fasting serum in duplicate by ELISA (Quantikine HS; R&D Systems, Inc.). BMI was calculated as weight divided by squared height (kg/m^2). Plasma glucose was measured by using an automated assay (YSI 2300 Glucose Analyzer; Yellow Springs Instruments), and serum insulin was determined by using a commercially available RIA (Pharmacia) (27). Lower extremity function was assessed by using a modified Short Physical Performance Battery, which consisted of the following tasks scored on a 0–4 scale: standing balance (side-by-side, semi-tandem, full-tandem, single-leg for 30 s), time to complete 5 chair stands, and 6-m walk and narrow 6-m walk (13).

At the year 2 clinic visit, dietary intake over the previous year was estimated by using an interviewer-administered 108-item FFQ developed specifically for Health ABC. The FFQ food list was derived by using 24-h recall data obtained from the NHANES III for non-Hispanic black and white adults aged ≥65 y residing in the northeast or southern United

States. The Health ABC FFQ was analyzed for micronutrient and macronutrient content by using the Block Dietary Data System (28). Serum 25-hydroxyvitamin D was determined by using a 2-step RIA (DiaSorin, Inc.) (13). The Healthy Eating Index was calculated as described (29). Physical activity was based on the reported time spent in walking for exercise or in other walking over the previous week (28). The season during which the blood sample was obtained was included to account for seasonal effects on vitamin K. The use of lipid-lowering, anti-inflammatory, and diabetes medications was identified following the same approach as hypertension medication use.

Statistical analysis. Differences in baseline characteristics were compared by using the Mann-Whitney *U* test (continuous outcomes) or the chi-square test (categorical outcomes). Cox models were used to calculate the unadjusted and adjusted HRs and 95% CIs for incident CVD among participants with low plasma phylloquinone compared with those with adequate plasma phylloquinone and in those with high (dp)ucMGP compared with those with lower plasma (dp)ucMGP. Effect modification by hypertension treatment, sex, and race was assessed by using interaction terms (i.e., treated hypertension × low plasma phylloquinone). Models were first adjusted for demographic characteristics (age, sex, race, site, and education), TGs, HDL and LDL cholesterol, lipid-lowering medication use, glucose, insulin, diabetes medication use, BMI, SBP, DBP, hypertension medication, IL-6, anti-inflammatory medication, and estimated glomerular filtration rate. Fully adjusted models accounted for healthy lifestyle characteristics (Healthy Eating Index, physical activity, smoking history, alcohol use, 25-hydroxyvitamin D, and season). To account for the selection of the

TABLE 1 Participant characteristics at baseline according to plasma phylloquinone concentration[1]

	<0.2 nmol/L (*n* = 52)	≥0.2 nmol/L (*n* = 1009)	*P*[2]
Age, y	74 ± 4	74 ± 5	0.91
Female sex, *n* (%)	33 (64)	582 (58)	0.41
Black race, *n* (%)	23 (44)	389 (39)	0.41
Site: Pittsburgh, *n* (%)	33 (64)	505 (50)	0.06
BMI, kg/m^2	27.2 ± 9.0	27.3 ± 6.0	0.55
Systolic blood pressure, mm Hg	139 ± 24	132 ± 24	0.37
Diastolic blood pressure, mm Hg	75 ± 17	71 ± 15	0.20
TGs,[3] mg/dL	108 ± 44	122 ± 67	0.10
LDL cholesterol,[3] mg/dL	128 ± 39	121 ± 46	0.18
HDL cholesterol,[3] mg/dL	63 ± 27	54 ± 20	0.04
IL-6,[4] pg/mL	2.9 ± 3.0	2.4 ± 2.0	0.01
Glucose,[4] mg/dL	92 ± 18	92 ± 16	0.26
Insulin,[4] μIU/mL	6.5 ± 5.0	7.0 ± 5.0	0.56
25-Hydroxyvitamin D,[3] nmol/L	69 ± 32	64 ± 37	0.62
eGFR, mL · min^{-1} · 1.73 m^{-2}	68 ± 16	69 ± 15	0.61
Knee pain, *n* (%)	35 (67)	435 (43)	<0.01
Short Physical Performance Battery score (range: 0–4) (13)	2.2 ± 0.7	2.3 ± 0.6	0.73
Lipid-lowering medication, *n* (%)	6 (12)	125 (12)	0.86
Anti-inflammatory medication, *n* (%)	23 (44)	487 (48)	0.57
Diabetes medication, *n* (%)	1 (2)	49 (5)	0.33
Hypertension medication, *n* (%)	18 (35)	472 (47)	0.09
Untreated hypertension, *n* (%)	13 (25)	140 (14)	0.08
Education, *n* (%)			
Less than high school	17 (33)	227 (23)	0.19
High school graduate	13 (25)	337 (33)	
College graduate	22 (42)	445 (44)	
Smoking, pack-years	3 ± 43	1 ± 21	0.52
Alcohol consumption			
None	16 (31)	487 (48)	0.08
<1 drink/wk[5]	17 (33)	216 (21)	
1–7 drinks/wk[5]	14 (27)	230 (23)	
>1 drink/d[5]	5 (10)	76 (8)	
Healthy Eating Index (range: 0–100)	70 ± 15	71 ± 18	0.77
Walking, min/wk	40 ± 95	30 ± 180	0.30
Season, *n* (%)			
December–February	6 (12)	273 (27)	<0.01
March–May	5 (10)	325 (32)	
June–August	14 (27)	165 (16)	
September–November	27 (52)	246 (24)	
(dp)ucMGP[4] ≥574 pmol/L (highest tertile, of a total *n* = 635), *n* (%)	17 (36)	187 (32)	0.54

[1] Values are medians ± IQRs unless indicated otherwise. (dp)ucMGP, (desphospho)uncarboxylated matrix Gla protein; eGFR, estimated glomerular filtration rate.
[2] Based on Mann-Whitney *U* test (continuous) or chi-square test (categorical).
[3] Measured in serum.
[4] Measured in plasma.
[5] One drink = 14 g alcohol.

knee OA subcohort, we also included knee pain and lower extremity function as covariates (13). The proportional hazards assumption was satisfied throughout. Given the differences between the 731 randomly chosen participants who were not in the Knee OA substudy with plasma phylloquinone measures and those in the Knee OA substudy with both plasma phylloquinone and (dp)ucMGP measures, we conducted a sensitivity analysis of plasma phylloquinone by using only participants in the Knee OA substudy [the same participants used in the analysis of (dp)ucMGP] following this same statistical approach. Analyses were carried out by using SPSS (version 22; IBM Corporation), and significance was set at $P < 0.05$.

Results

Baseline characteristics according to plasma phylloquinone status are shown in **Table 1**. Those with plasma phylloquinone <0.2 nmol/L had higher circulating IL-6, higher HDL cholesterol, and knee pain. We also detected seasonal differences in plasma phylloquinone.

Over the median 12.1 y of follow-up, 275 of the 1061 included participants developed clinical CVD. Low plasma phylloquinone was not associated with incident CVD overall (**Table 2**). However, the association between low plasma phylloquinone and incident CVD differed by hypertension treatment status (interaction term $P = 0.03$). Among participants treated for hypertension ($n = 490$; 135 developed CVD), those with low plasma phylloquinone were more likely to develop CVD than were those who did not have low phylloquinone (fully adjusted HR: 2.94; 95% CI: 1.41, 6.13; $P < 0.01$). In contrast, there was no association between low plasma phylloquinone and incident CVD among those with untreated hypertension ($n = 153$; 48 developed CVD) or those free of hypertension ($n = 418$; 92 events) (**Figure 2**, **Table 3**). The results were similar when analyses were restricted to participants in the Knee OA substudy (**Supplemental Tables 1** and **2**).

In the subgroup of participants available for the (dp)ucMGP analysis ($n = 635$), 169 developed clinical CVD. There was no association between elevated (dp)ucMGP (≥574 pmol/L; the highest tertile) and incident CVD (Table 3). The association between high (dp)ucMGP and incident CVD did not differ by hypertension treatment status (interaction term $P = 0.72$). High (dp)ucMGP was not significantly associated with CVD when defined as the highest 5% of the distribution (≥1012 pmol/L) [fully adjusted HR for CVD among those with (dp)ucMGP ≥1012 pmol/L compared with those with <1012 pmol/L: 1.45; 95% CI: 0.72, 2.92; $P = 0.30$]. No significant effect modification by sex or race with plasma phylloquinone or (dp)ucMGP was detected.

Discussion

In community-dwelling older adults, overall, neither low plasma phylloquinone nor high (dp)ucMGP was significantly associated with incident CVD over 12 y of follow-up. Low plasma phylloquinone however, was associated with an increased risk of CVD among individuals treated for hypertension. No such association was observed in those not treated for hypertension. These results are consistent with previous analyses of vitamin K and CAC progression (indicative of subclinical CVD) (8) and potentially extend the relevance of vitamin K to clinical CVD in persons treated for hypertension, in a third and independent cohort. In MESA, those taking hypertension medication who also had low serum phylloquinone were significantly more likely to have CAC progression over 2.5 y, although low serum phylloquinone was not significantly associated with CAC progression when all participants were analyzed together (8). In a replicative post hoc analysis of a randomized trial (7), 3 y of phylloquinone supplementation reduced CAC progression in older adults taking hypertension medication but not in those not taking hypertension medication (8). The magnitude of the effect of low plasma phylloquinone we detected in those taking hypertension medication here (HR: 2.94; 95% CI: 1.41, 6.13) is consistent with the effect detected with respect to subclinical CVD progression in MESA (OR; 2.37; 95% CI: 1.38, 4.09) (8). However, the 95% CI suggests that the precision of the estimated effect of low phylloquinone on CVD merits improvement in larger hypertension studies.

Phylloquinone is the primary circulating form of vitamin K and is also the predominant form in Western diets. The main dietary sources of phylloquinone (green leafy vegetables and vegetable oils) are characteristic of healthy diets, and circulating phylloquinone (the form we measured) can reflect healthy diets and lifestyles (30). We adjusted our models for the Healthy Eating Index, physical activity, and other potential lifestyle confounders. Additional adjustment for fruit and vegetable intake did not change our results (data not shown). Nonetheless, residual confounding cannot be discounted. Several observational studies found that higher dietary menaquinone (vitamin K-2) intake, but not dietary phylloquinone intake, was associated with lower CVD risk (31–33). Menaquinones, found mostly in meat, some dairy products, and fermented soybean

TABLE 2 Association between low vitamin K status and CVD risk in Health ABC participants over 12 y of follow-up[1]

	Plasma phylloquinone <0.2 nmol/L compared with ≥0.2 nmol/L ($n = 1061$; 275 events)		Plasma (dp)ucMGP ≥574 pmol/L compared with <574 pmol/L ($n = 635$; 169 events)	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Unadjusted	1.20 (0.74, 1.97)	0.46	1.11 (0.81, 1.53)	0.53
Model 2[2]	1.42 (0.86, 2.34)	0.17	1.03 (0.73, 1.44)	0.88
Model 3[3]	1.27 (0.75, 2.13)	0.38	1.02 (0.72, 1.45)	0.90

[1] HRs (95% CIs) were based on Cox regression. CVD, cardiovascular disease; (dp)ucMGP, (desphospho)uncarboxylated matrix Gla protein; Health ABC, Health, Aging, and Body Composition Study.
[2] Adjusted for age, sex, race, site, education, TGs, LDL cholesterol, HDL cholesterol, fasting glucose, insulin, BMI, systolic blood pressure, diastolic blood pressure, IL-6, estimated glomerular filtration rate, lipid-lowering medication use, hypertension medication use, diabetes medication use, and anti-inflammatory medication use.
[3] Adjusted for covariates in model 2 plus for Healthy Eating Index, walking time per week, smoking (pack-years), season, serum 25-hydroxyvitamin D, alcohol use, knee pain, and lower extremity function.

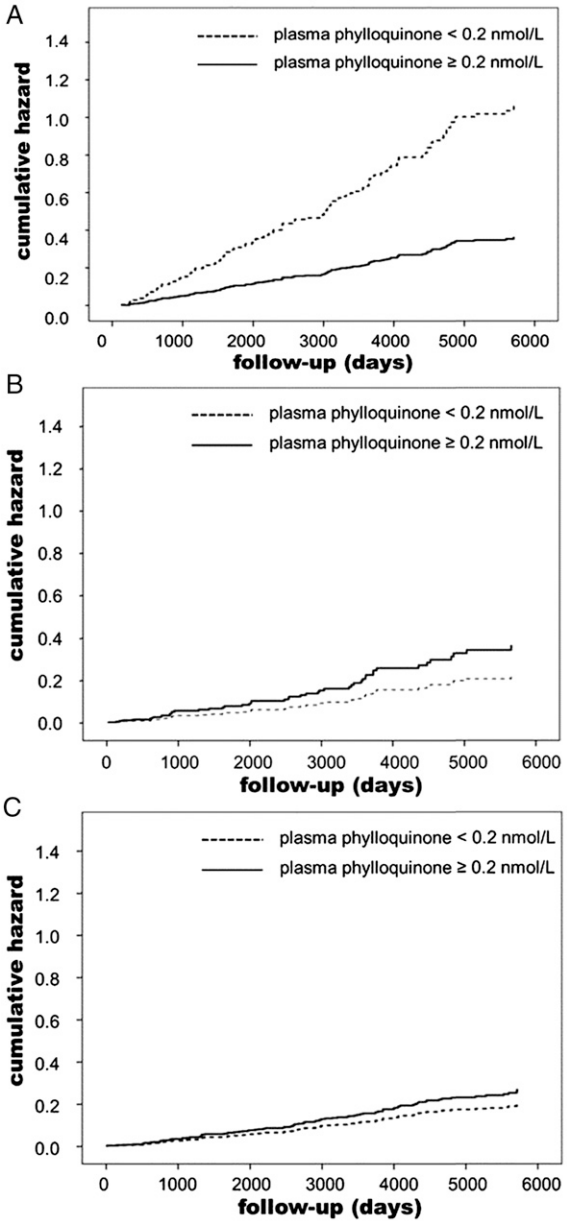

FIGURE 2 Fully adjusted cumulative hazards for incident CVD in Health ABC participants with plasma vitamin K <0.2 and ≥0.2 nmol/L, according to hypertension status, in participants treated for hypertension (n = 490; 135 events) (A), participants with untreated hypertension (n = 153; 48 events) (B), and participants free of hypertension (n = 414; 82 events) (C). Values were adjusted for age, sex, race, site, education, TGs, LDL cholesterol, HDL cholesterol, fasting glucose, insulin, BMI, systolic blood pressure, diastolic blood pressure, IL-6, estimated glomerular filtration rate, lipid-lowering medication use, diabetes medication use, anti-inflammatory medication use, Healthy Eating Index, walking time per week, smoking (pack-years), season, serum 25-hydroxyvitamin D, alcohol use, knee pain, and lower extremity function. CVD, cardiovascular disease; Health ABC, Health, Aging, and Body Composition Study.

products, are not thought to be important contributors to dietary vitamin K intake in the United States. The studies that found that menaquinone intake was inversely associated with CVD were conducted in countries where fermented cheeses are heavily consumed (31–33). It is plausible that menaquinones are more relevant to CVD and equally plausible that other compounds in fermented cheeses were associated with CVD, for which menaquinone intake served as a marker (34). Menaquinones are not generally detectable in circulation unless taken in high doses (either in food or supplement form) (35) and were not detected in the Health ABC.

The finding that plasma (dp)ucMGP was not associated with incident CVD in the Health ABC is consistent with a secondary analysis of the randomized phylloquinone supplementation trial that found that (dp)ucMGP was not associated with CAC progression, even though it decreased in response to supplementation (7, 23). It is also consistent with a case-cohort analysis of the Dutch EPIC (European Prospective Investigation into Cancer and Nutrition) cohort that found no association between (dp)ucMGP and incident myocardial infarction or other ischemic heart disease, stroke, or sudden death over 11.5 y (11). Others have reported that higher (dp)ucMGP was associated with more CVD (9, 10). The race composition of the Health ABC may explain why our results are not consistent with these studies in whites. To the best of our knowledge, this is the first population-based study to analyze the association of (dp)ucMGP with CVD in a cohort that included blacks. In this cohort, plasma phylloquinone and (dp)ucMGP were positively correlated in whites but not blacks (12), suggesting that the relation between (dp)ucMGP and vitamin K status could differ. Although we did not detect a significant interaction between high (dp)ucMGP and race with respect to CVD, we may have been underpowered to do so. Future studies in racially and ethnically diverse groups are needed to clarify if the association between (dp)ucMGP and CVD differs according to race-ethnicity. It is also plausible that the (dp)ucMGP in circulation does not entirely reflect all of the vitamin K–dependent mechanisms in the vascular wall, as has been shown by genetically modified animal models (36).

This study builds on previous work, because we evaluated the longitudinal association between vitamin K status and incident clinical CVD in older adults over a median follow-up of >12 y. Our primary outcome combined coronary artery disease, ischemic heart disease, and stroke because vitamin K's potential role may be relevant to both and hypertension is a common risk factor. Participants who were not treated for hypertension were classified as "untreated" or "free of hypertension" on the basis of blood pressure readings obtained on a single day, which may have resulted in some misclassification (37). Plasma phylloquinone and (dp)ucMGP were measured from a single blood draw. Repeated measures over time would be more reflective of long-term status and reduce misclassification bias. When examining cohort data, it is likely that the use of hypertension medications identifies individuals with a longer history of and/or more severe hypertension, particularly when evaluating older adults that have access to health care. The interaction we detected may be due to more severe hypertension overall. Different classes of hypertension medications appear to be associated with residual risk for different cardiovascular endpoints (38), and it is also plausible that vitamin K is more relevant to some classes of medications than others (39, 40). We were not sufficiently powered to identify differences in subgroups of participants treated with specific classes of medications. Larger studies are needed to clarify the relevance of vitamin K status to CVD in subgroups of hypertensive individuals: for example, on the basis of blood pressure control, medication class(es), and/or treatment duration.

Vitamin K status was initially measured only in the Health ABC Knee OA substudy participants, who were selected on the basis of knee pain symptoms. Plasma phylloquinone was additionally measured in a subgroup of randomly chosen

TABLE 3 Risk of incident CVD in Health ABC participants according to hypertension treatment status, comparing those with plasma phylloquinone <0.2 nmol/L with those with ≥0.2 nmol/L over 12 y of follow-up[1]

	Treated hypertension (n = 490; 135 events)		Untreated hypertension (n = 153; 48 events)		No hypertension (n = 414; 82 events)	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Unadjusted	1.74 (0.88, 3.42)	0.11	0.61 (0.19, 1.96)	0.40	1.11 (0.45, 2.73)	0.82
Model 2[2]	2.47 (1.22, 5.00)	0.01	0.74 (0.20, 2.67)	0.64	1.14 (0.46, 2.87)	0.78
Model 3[3]	2.94 (1.41, 6.13)	<0.01	0.61 (0.17, 2.77)	0.59	0.75 (0.27, 2.05)	0.57

[1] HRs (95% CIs) were based on Cox regression. CVD, cardiovascular disease; Health ABC, Health, Aging, and Body Composition Study.
[2] Adjusted for age, sex, race, site, education, TGs, LDL cholesterol, HDL cholesterol, fasting glucose, insulin, BMI, systolic blood pressure, diastolic blood pressure, IL-6, estimated glomerular filtration rate, lipid-lowering medication use, diabetes medication use, and anti-inflammatory medication use.
[3] Adjusted for covariates in model 2 plus for Healthy Eating Index, walking time per week, smoking (pack-years), season, serum 25-hydroxyvitamin D, alcohol use, knee pain, and lower extremity function.

participants who were not included in the Knee OA substudy. This sampling did not appear to influence our findings, because the inclusion of an indicator variable reflecting this sampling did not change the results of our models. The fact that Health ABC participants were selected to include well-functioning adults between 70 and 79 y old and most participants analyzed here were in the knee OA substudy may limit the generalizability. We defined low plasma phylloquinone as <0.2 nmol/L on the basis of previous work in this cohort (14), which was present in 5% of Health ABC participants included here. The prevalence of circulating vitamin K below this level is reported to be ≤25% in other cohorts (8). The lower prevalence in Health ABC may reflect the cohort's overall health status at the time phylloquinone was measured. The benefits of remediating low phylloquinone may affect a larger segment of the population than suggested here. In MESA, we detected differences in CAC progression on the basis of a threshold of 1.0 nmol/L, the concentration achieved when recommended intakes are met (8), but we did not see differences in incident CVD risk at this threshold in Health ABC (data not shown). It is plausible that a lower threshold may apply to clinical CVD (evaluated here) instead of subclinical CVD [evaluated previously (8)]. There is no established threshold for low circulating phylloquinone. Further studies are needed to clarify the concentration sufficient to meet all physiologic needs. Our results are not applicable to patients taking warfarin because warfarin users were excluded. Although our study is strengthened by the longitudinal design, causality cannot be ascertained.

In conclusion, consistent with previous analyses (8), we found that low plasma phylloquinone was a risk factor for incident CVD in older men and women treated for hypertension but was not associated with CVD in those not treated for hypertension. Additional evidence from larger clinical studies is needed to clarify the importance of vitamin K to CVD in this segment of the population.

Acknowledgments

MKS, SLB, and SBK conceived of and designed the study and drafted the manuscript; MKS, SLB, DEW, CV, and SBK acquired, analyzed, or interpreted the data; MKS, SLB, DEW, TEB, AMK, RAM, EMS, CLW, CV, and SBK critically revised the manuscript for important intellectual content; and MKS performed the statistical analysis and had primary responsibility for the final content. All of the authors read and approved the final manuscript.

References

1. Miniño AM. Death in the United States, 2011. NCHS Data Brief 2013;115:1–8.
2. Satariano WA, Guralnik JM, Jackson RJ, Marottoli RA, Phelan EA, Prohaska TR. Mobility and aging: new directions for public health action. Am J Public Health 2012;102:1508–15.
3. Berkner KL, Runge KW. The physiology of vitamin K nutriture and vitamin K-dependent protein function in atherosclerosis. J Thromb Haemost 2004;2:2118–32.
4. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. Arterioscler Thromb Vasc Biol 1998;18:1400–7.
5. Schurgers LJ, Spronk HM, Skepper JN, Hackeng TM, Shanahan CM, Vermeer C, Weissberg PL, Proudfoot D. Post-translational modifications regulate matrix Gla protein function: importance for inhibition of vascular smooth muscle cell calcification. J Thromb Haemost 2007;5:2503–11.
6. Schurgers LJ, Spronk HM, Soute BA, Schiffers PM, DeMey JG, Vermeer C. Regression of warfarin-induced medial elastocalcinosis by high intake of vitamin K in rats. Blood 2007;109:2823–31.
7. Shea MK, O'Donnell CJ, Hoffmann U, Dallal GE, Dawson-Hughes B, Ordovas JM, Price PA, Williamson MK, Booth SL. Vitamin K supplementation and progression of coronary artery calcium in older men and women. Am J Clin Nutr 2009;89:1799–807.
8. Shea MK, Booth SL, Miller ME, Burke GL, Chen H, Cushman M, Tracy RP, Kritchevsky SB. Association between circulating vitamin K1 and coronary calcium progression in community-dwelling adults: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr 2013;98:197–208.
9. Dalmeijer GW, van der Schouw YT, Magdeleyns EJ, Vermeer C, Verschuren WM, Boer JM, Beulens JW. Matrix Gla protein species and risk of cardiovascular events in type 2 diabetic patients. Diabetes Care 2013;36:3766–71.
10. van den Heuvel EG, van Schoor NM, Lips P, Magdeleyns EJ, Deeg DJ, Vermeer C, den Heijer M. Circulating uncarboxylated matrix Gla protein, a marker of vitamin K status, as a risk factor of cardiovascular disease. Maturitas 2014;77:137–41.
11. Dalmeijer GW, van der Schouw YT, Magdeleyns EJ, Vermeer C, Verschuren WM, Boer JM, Beulens JW. Circulating desphospho-uncarboxylated matrix gamma-carboxyglutamate protein and the risk of coronary heart disease and stroke. J Thromb Haemost 2014;12:1028–34.
12. Shea MK, Booth SL. Concepts and controversies in evaluating vitamin K status in population-based studies. Nutrients 2016;8: doi 10.3390/nu801008.
13. Houston DK, Tooze JA, Neiberg RH, Hausman DB, Johnson MA, Cauley JA, Bauer DC, Cawthon PM, Shea MK, Schwartz GG, et al. 25-Hydroxyvitamin D status and change in physical performance and strength in older adults: the Health, Aging, and Body Composition Study. Am J Epidemiol 2012;176:1025–34.
14. Shea MK, Kritchevsky SB, Hsu FC, Nevitt M, Booth SL, Kwoh CK, McAlindon TE, Vermeer C, Drummen N, Harris TB, et al. The association between vitamin K status and knee osteoarthritis features in older adults: the Health, Aging and Body Composition Study. Osteoarthritis Cartilage 2015;23:370–8.

15. Shea MK, Loeser RF, Hsu FC, Booth SL, Nevitt M, Simonsick EM, Strotmeyer ES, Vermeer C, Kritchevsky SB. Vitamin K status and lower extremity function in older adults: the Health Aging and Body Composition Study. J Gerontol A Biol Sci Med Sci 2016;71:1348–55.
16. Simonsick EM, Newman AB, Nevitt MC, Kritchevsky SB, Ferrucci L, Guralnik JM, Harris T. Measuring higher level physical function in well-functioning older adults: expanding familiar approaches in the Health ABC Study. J Gerontol A Biol Sci Med Sci 2001;56:M644–9.
17. Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, et al. Inflammatory markers and onset of cardiovascular events: results from the Health ABC Study. Circulation 2003;108:2317–22.
18. Davidson KW, Sadowski JA. Determination of vitamin K compounds in plasma or serum by high-performance liquid chromatography using postcolumn chemical reduction and fluorimetric detection. Methods Enzymol 1997;282:408–21.
19. Card DJ, Shearer MJ, Schurgers LJ, Harrington DJ. The external quality assurance of phylloquinone (vitamin K1) analysis in human serum. Biomed Chromatogr 2009;23:1276–82.
20. Booth SL, Martini L, Peterson JW, Saltzman E, Dallal GE, Wood RJ. Dietary phylloquinone depletion and repletion in older women. J Nutr 2003;133:2565–9.
21. Wallin R, Schurgers LJ, Loeser RF. Biosynthesis of the vitamin K-dependent matrix Gla protein (MGP) in chondrocytes: a fetuin-MGP protein complex is assembled in vesicles shed from normal but not from osteoarthritic chondrocytes. Osteoarthritis Cartilage 2010;18:1096–103.
22. Hawker GA, Croxford R, Bierman AS, Harvey PJ, Ravi B, Stanaitis I, Lipscombe LL. All-cause mortality and serious cardiovascular events in people with hip and knee osteoarthritis: a population based cohort study. PLoS One 2014;9:e91286.
23. Shea MK, O'Donnell CJ, Vermeer C, Magdeleyns EJ, Crosier MD, Gundberg CM, Ordovas JM, Kritchevsky SB, Booth SL. Circulating uncarboxylated matrix Gla protein is associated with vitamin K nutritional status, but not coronary artery calcium, in older adults. J Nutr 2011;141:1529–34.
24. Cranenburg EC, Koos R, Schurgers LJ, Magdeleyns EJ, Schoonbrood TH, Landewe RB, Brandenburg VM, Bekers O, Vermeer C. Characterisation and potential diagnostic value of circulating matrix Gla protein (MGP) species. Thromb Haemost 2010;104:811–22.
25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
26. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461–70.
27. Anderson AL, Harris TB, Tylavsky FA, Perry SE, Houston DK, Lee JS, Kanaya AM, Sahyoun NR. Dietary patterns, insulin sensitivity and inflammation in older adults. Eur J Clin Nutr 2012;66:18–24.
28. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, Lee JS, Sahyoun NR, Visser M, Kritchevsky SB. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr 2008;87:150–5.
29. Shahar DR, Yu B, Houston DK, Kritchevsky SB, Lee JS, Rubin SM, Sellmeyer DE, Tylavsky FA, Harris TB. Dietary factors in relation to daily activity energy expenditure and mortality among older adults. J Nutr Health Aging 2009;13:414–20.
30. Braam L, McKeown N, Jacques P, Lichtenstein A, Vermeer C, Wilson P, Booth S. Dietary phylloquinone intake as a potential marker for a heart-healthy dietary pattern in the Framingham offspring cohort. J Am Diet Assoc 2004;104:1410–4.
31. Beulens JW, Bots ML, Atsma F, Bartelink ML, Prokop M, Geleijnse JM, Witteman JC, Grobbee DE, van der Schouw YT. High dietary menaquinone intake is associated with reduced coronary calcification. Atherosclerosis 2009;203:489–93.
32. Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, Hofman A, Witteman JC. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam study. J Nutr 2004;134:3100–5.
33. Gast GC, de Roos NM, Sluijs I, Bots ML, Beulens JW, Geleijnse JM, Witteman JC, Grobbee DE, Peeters PH, van der Schouw YT. A high menaquinone intake reduces the incidence of coronary heart disease. Nutr Metab Cardiovasc Dis 2009;19:504–10.
34. Praagman J, Dalmeijer GW, van der Schouw YT, Soedamah-Muthu SS, Monique Verschuren WM, Bas Bueno-de-Mesquita H, Geleijnse JM, Beulens JW. The relationship between fermented food intake and mortality risk in the European Prospective Investigation into Cancer and Nutrition-Netherlands cohort. Br J Nutr 2015;113:498–506.
35. Karl JP, Fu X, Dolnikowski GG, Saltzman E, Booth SL. Quantification of phylloquinone and menaquinones in feces, serum, and food by high-performance liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2014;963:128–33.
36. Murshed M, Schinke T, McKee MD, Karsenty G. Extracellular matrix mineralization is regulated locally: different roles of two gla-containing proteins. J Cell Biol 2004;165:625–30.
37. Perry HM Jr., Miller JP. Difficulties in diagnosing hypertension: implications and alternatives. J Hypertens 1992;10:887–96.
38. Blacher J, Evans A, Arveiler D, Amouyel P, Ferrières J, Bingham A, Yarnell J, Haas B, Montaye M, Ruidavets JB, et al. Residual cardiovascular risk in treated hypertension and hyperlipidaemia: the PRIME study. J Hum Hypertens 2010;24:19–26.
39. Jia G, Stormont RM, Gangahar DM, Agrawal DK. Role of matrix Gla protein in angiotensin II–induced exacerbation of vascular stiffness. Am J Physiol Heart Circ Physiol 2012;303:H523–32.
40. Mendoza FJ, Martinez-Moreno J, Almaden Y, Rodriguez-Ortiz ME, Lopez I, Estepa JC, Henley C, Rodriguez M, Aguilera-Tejero E. Effect of calcium and the calcimimetic AMG 641 on matrix-Gla protein in vascular smooth muscle cells. Calcif Tissue Int 2011;88:169–78.