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Original Contribution

Vitamin K and Vitamin D Status: Associations with Inflammatory Markers in the Framingham Offspring Study

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In vitro data suggest protective roles for vitamins K and D in inflammation. To examine associations between vitamins K and D and inflammation in vivo, the authors used multiple linear regression analyses, adjusted for age, sex, body mass index, triglyceride concentrations, use of aspirin, use of lipid-lowering medication, season, menopausal status, and hormone replacement therapy. Participants were from the Framingham Offspring Study (1997–2001; $n = 1{,}381$; mean age = 59 years; 52% women). Vitamin K status, measured by plasma phylloquinone concentration and phylloquinone intake, was inversely associated with circulating inflammatory markers as a group and with several individual inflammatory biomarkers ($p < 0.01$). Percentage of undercarboxylated osteocalcin, a functional measure of vitamin K status, was not associated with overall inflammation but was associated with C-reactive protein ($p < 0.01$). Although plasma 25-hydroxyvitamin D was inversely associated with urinary isoprostane concentration, an indicator of oxidative stress ($p < 0.01$), overall associations between vitamin D status and inflammation were inconsistent. The observation that high vitamin K status was associated with lower concentrations of inflammatory markers suggests that a possible protective role for vitamin K in inflammation merits further investigation.

inflammation; vitamin D; vitamin K

Abbreviations: CV, coefficient of variation; SD, standard deviation.

Cardiovascular disease and osteoporosis are major age-related health concerns that contribute to morbidity and mortality in the elderly (1). Inflammation is characteristic of these two chronic diseases (2, 3). Several proinflammatory cytokines, such as interleukin-6, osteoprotegerin, and tumor necrosis factor-α, are implicated in the process of vascular calcification and the regulation of bone remodeling (4, 5). The reciprocal effect of inflammatory cytokines on vascular and bone tissue may partially explain the simultaneous manifestation of bone loss and vascular calcification (6, 7).

Vitamins K and D are fat-soluble vitamins that have been implicated in both cardiovascular and bone health, and more recently in the activity of proinflammatory cytokines.

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Vitamin K is an established cofactor in the γ-carboxylation of vitamin K-dependent proteins. Two vitamin K-dependent proteins, osteocalcin and matrix γ-carboxyglutamate (matrix-Gla) protein, are present in skeletal and vascular tissue, respectively (8), and a role for vitamin K in cardiovascular and skeletal health has been reported (9, 10). Vitamin K also is associated with decreased production of proinflammatory cytokines in studies carried out in vitro (11–13). With the exception of one small study conducted in patients with chronic kidney disease (14), studies of the relations between vitamin K and inflammatory cytokines to date have primarily been carried out in vitro (11, 13).

The importance of vitamin D for optimal calcium homeostasis and bone metabolism is well-recognized (15), and there is some suggestion of a role for vitamin D in reducing cardiovascular disease risk (16, 17). Furthermore, in vitro data suggest that the biologically active form of vitamin D (1,25-dihydroxyvitamin D or calcitriol) has several immunomodulatory functions, including suppression of proinflammatory cytokine expression and regulation of immune cell activity (18). In vivo, vitamin D supplementation has been associated with a reduction in proinflammatory cytokines in patients with osteoporosis (19) and heart failure (20) but not in healthy persons (21).

We hypothesized that vitamin K and vitamin D status are inversely associated with measures of inflammation in older adults. To test this hypothesis, we examined cross-sectional associations between dietary and biochemical measures of vitamin K status (plasma phylloquinone, serum percentage of undercarboxylated osteocalcin, and phylloquinone intake) and vitamin D status (plasma 25-hydroxyvitamin D and vitamin D intake) and a panel of circulating proinflammatory biomarkers (C-reactive protein, CD40 ligand, P-selectin, osteoprotegerin, tumor necrosis factor-α, tumor necrosis factor receptor-2, intercellular adhesion molecule-1, interleukin-6, monocyte chemoattractant protein, myeloperoxidase, urinary isoprostanes, fibrinogen, and lipoprotein phospholipase A_2 mass and activity) in the Framingham Offspring Study cohort, a community-based sample of men and women.

MATERIALS AND METHODS

The design and selection criteria of the Framingham Offspring Study have been described elsewhere (22). Every 4–8 years, Offspring participants undergo extensive evaluations that include medical history, medication use, physical examinations, blood biochemical analyses, and assessment of cardiovascular disease risk factors. Participants were excluded from the present investigation, which took place between 1997 and 2001, if they were currently taking steroidal antiinflammatory medication ($n = 219$) or anticoagulant medication ($n = 41$), did not have a valid food frequency questionnaire ($n = 59$), or did not have data on all of the measures of inflammatory markers ($n = 271$), excluding tumor necrosis factor-α or urinary isoprostanes. Of the 1,850 eligible participants, data on 1,381 persons (669 male, 712 female) were available for analysis.

Self-reported information on alcohol use, dietary intake, and, for women, menopausal status and hormone replacement therapy was routinely collected. Nutrient intakes from foods and supplements, including phylloquinone (vitamin K_1) and vitamin D, were assessed using the Willett food frequency questionnaire (23). Questionnaires were considered invalid and data were excluded from analysis if the participant reported an energy intake of less than 2.51 MJ/day or more than 16.74 MJ/day (600 kcal/day and 4,000 kcal/day, respectively) or if the participant had left more than 12 items blank on the food frequency questionnaire ($n = 59$). This study was approved by the institutional review boards at Tufts University and Boston University Medical Center. All participants gave written informed consent.

Fasting (>10 hours) blood samples were collected between 1997 and 1999, and plasma/serum was stored at −80°C until analysis. The measures used for assessment of vitamin status straddled the end of cycle 6 (1995–1998) ($n = 694$) and the beginning of cycle 7 (1998–2001) ($n = 687$), because of the award date of the vitamin assay research grant. Vitamin K status was assessed through measures of plasma phylloquinone and serum percentage of undercarboxylated osteocalcin. A high percentage of undercarboxylated osteocalcin is indicative of low vitamin K status in bone. Plasma phylloquinone concentrations were determined using reverse-phase high performance liquid chromatography, as described elsewhere (24). Low and high control specimens had average values of 0.56 nmol/liter and 3.15 nmol/liter, with coefficients of variation (CVs) of 15.2 percent and 10.9 percent, respectively. Serum total osteocalcin and undercarboxylated osteocalcin were measured by radioimmunoassay, using the method of Gundberg et al. (25). Percentage of undercarboxylated osteocalcin, a functional marker of vitamin K status, was determined by the amount of osteocalcin that did not bind to hydroxyapatite in vitro. Since this binding varied with the amount of total osteocalcin in the sample, undercarboxylated osteocalcin was expressed as a percentage of total osteocalcin (26). The CVs for the three control serum samples, which had average total osteocalcin results of 3.4 μg/liter, 7.1 μg/liter, and 11.9 μg/liter, were 22.3 percent, 12.8 percent, and 7.8 percent, respectively. Vitamin D status was estimated by measuring plasma 25-hydroxyvitamin D concentration, the standard measure of vitamin D status (which reflects both sun-induced synthesis in the skin and dietary intake), using a radioimmunoassay (DiaSorin Inc., Stillwater, Minnesota). The CVs for the control values of 36 nmol/liter and 137 nmol/liter were 8.5 percent and 13.2 percent, respectively.

The following inflammatory biomarkers were measured in duplicate from samples taken during the seventh examination cycle (1998–2001), using commercially available enzyme-linked immunoassay kits: plasma CD40 ligand (Bender MedSystems, Inc., Burlingame, California; intraassay CV = 5.2 percent (standard deviation (SD), 6.4)), plasma P-selectin (R&D Systems, Inc., Minneapolis, Minnesota; intraassay CV = 3.2 percent (SD, 2.4)), plasma osteoprotegerin (BioMedica Gesellschaft mbH, Vienna, Austria; distributed by ALPCO Diagnostics, Salem, New Hampshire; intraassay CV = 3.7 percent (SD, 2.9)), plasma tumor necrosis factor-α (R&D Systems; intraassay CV = 8.8 percent), plasma tumor necrosis factor receptor-2 (R&D systems; intraassay CV = 2.3 percent (SD, 1.6)), serum soluble intercellular adhesion molecule-1 (R&D systems;

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intraassay CV = 3.9 percent (SD, 2.9)), serum interleukin-6 (R&D Systems; intraassay CV = 3.1 percent (SD, 2.2)), serum monocyte chemoattractant protein-1 (R&D systems; intraassay CV = 3.8 percent (SD, 3.3)), serum myeloperoxidase (Oxis International, Inc., Foster City, California; intraassay CV = 3.2 percent (SD, 2.7)), and urinary isoprostanes indexed to urinary creatinine (27) (Cayman Chemical, Inc., Ann Arbor, Michigan; intraassay CV = 9.6 percent (SD, 6.8)). Single determinants of serum C-reactive protein were made using a high-sensitivity assay (Dade Behring Inc., Deerfield, Illinois; intraassay CV = 3.2 percent) (28). Fibrinogen was measured in duplicate using the clot-time method of Claus (29) with Diagnostica Stago reagents (Diagnostica Stago, Inc., Parsippany, New Jersey; intraassay CV = 1.1 percent (SD, 1.1)). Lipoprotein phospholipase A_2 mass and activity were measured by diaDexus, Inc. (San Francisco, California) and GlaxoSmithKline (Philadelphia, Pennsylvania), respectively (intraassay CVs of 4.3 percent (SD, 7.8) and 4.3 percent (SD, 7.8), respectively).

Statistical analyses

To improve the symmetry of skewed distributions, data on all of the inflammatory markers and markers of vitamin K status, including intake, were logarithmically transformed for analysis. We generated an inflammation index to create an indicator of overall inflammation by summing the normalized deviates of the individual markers of inflammation (30). The inflammation index was correlated with the log of each individual marker (Pearson $r = 0.12$–0.62; all p's < 0.01). In multivariable-adjusted linear regression models, the measures of vitamin K status and vitamin D status were used as continuous regressor variables (each in separate models) and the index or biomarkers of inflammation were used as the dependent variables (one at a time). Although we assessed associations of measures of vitamin K and D status with plasma tumor necrosis factor-α and urinary isoprostanes, these two inflammatory markers were not included in the inflammation index because the numbers of persons with these measures ($n = 992$ and $n = 1{,}087$, respectively) were lower than the numbers with the other measures (specimen collection for their assays occurred later in the examination cycle); including them in the inflammation index would have reduced the sample size for the inflammation index models.

Additional covariates, which were taken from the same examination cycle (cycle 6 or 7) as the measures of vitamin K and D status, included triglycerides, body mass index (weight (kg)/height $(m)^2$), use of aspirin, use of lipid-lowering medication, menopausal status, and hormone replacement therapy. The covariates selected were those that were determined to be statistically significantly correlated with the corresponding biochemical measures of vitamin status (M. K. S., unpublished data). Since seasonal differences in vitamin D status and percentage of undercarboxylated osteocalcin have been reported (31, 32), we included season as a covariate in the models to assess associations between vitamin D status and percentage of undercarboxylated osteocalcin and the markers of inflammation. We chose to report changes in inflammation associated with a twofold increase in plasma phylloquinone level, phylloquinone intake, serum percentage of undercarboxylated osteocalcin, and plasma 25-hydroxyvitamin D level, since these increments of change were deemed plausible on the basis of mean values and ranges for the vitamin status measures. Our primary analyses focused on the associations between measures of vitamin status and the inflammation index. Associations between measures of vitamin status and individual markers of inflammation were considered in secondary analyses. In subsequent analyses, we excluded persons with prevalent cardiovascular disease at examination 7. A p value of 0.01 or less was considered statistically significant. All analyses were performed using SAS 9.1 (SAS Institute, Inc., Cary, North Carolina).

We tested for effect modification by sex and age for each inflammatory marker by entering product terms (age × vitamin status or sex × vitamin status) into the multiple linear regression models. We also checked effect modification by examination cycle, because the measures of vitamin K status and vitamin D status were not consistently taken from the same examination as the measures of inflammation. To reduce the likelihood of type 1 error, we used a Bonferroni adjustment and considered interactions to be significant if the p value was less than 0.003. None of the interaction terms we tested were significant at this level, and therefore they were not included in the final statistical models.

RESULTS

Study participants' characteristics were typical of those of a community-based cohort (table 1). Participants were middle-aged to elderly (mean age = 59 years; range, 35–89 years), and 51.6 percent were female. The prevalence of reported use of osteoporosis medication was 6.2 percent, and that of use of lipid-lowering medication was 20.5 percent. Mean concentrations of plasma phylloquinone, plasma 25-hydroxyvitamin D, and serum percentage of undercarboxylated osteocalcin and their standard deviations were within previously reported reference ranges for these assays (26, 33, 34).

Vitamin K status, as measured by plasma phylloquinone concentration and phylloquinone intake, was significantly inversely associated with the overall inflammation index, which represented the sum of the normalized deviates of the individual markers (table 2). When we reran the statistical analyses with inclusion of tumor necrosis factor-α and urinary isoprostanes in the summary statistic, the associations were similar in direction and significance, although the association between vitamin K intake and the summary statistic was attenuated slightly ($p = 0.002$) because of the smaller sample size (data not shown).

Secondary analyses of the individual markers demonstrated significant ($p < 0.01$) associations with five of the 14 markers (table 2). In multivariable-adjusted analyses, a twofold higher plasma phylloquinone concentration was associated with a 15 percent lower CD40 ligand concentration, a 3 percent lower intracellular adhesion molecule-1 concentration, an 8 percent lower interleukin-6 concentration, a 4 percent lower serum osteoprotegerin concentration, and a 4 percent lower tumor necrosis factor receptor-2 concentration. Usual dietary phylloquinone intake was also

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TABLE 1. Characteristics of participants ($n = 1,381$), Framingham Offspring Study, 1997–2001

	No.	%	Mean (SD*)	Range
Clinical characteristics				
Age (years)			59 (8)	
Body mass index†			28.1 (5.2)	
Triglyceride concentrations (mg/dl)			135 (86)	
Waist circumference (cm)			39.1 (5.5)	
Alcohol consumption (ounces‡/month)			139 (289)	
Female sex	712	51.6		
Smoking	173	12.5		
Diabetes	159	11.5		
Hypertension	584	42.3		
Postmenopausal	593	83.4		
Hormone replacement therapy (if postmenopausal)	239	33.6		
Lipid-lowering treatment	283	20.5		
Osteoporosis treatment	86	6.2		
Prevalent cardiovascular disease	161	11.7		
Vitamin K status				
Plasma phylloquinone level (nmol/liter)			1.5 (1.9)	0.1–25.6
Percentage of undercarboxylated osteocalcin			17.4 (16.8)	0–79.7
Phylloquinone intake (μg/day)			156 (118)	17–2,059
Vitamin D status				
Plasma 25-hydroxyvitamin D level (nmol/liter)			49.4 (18.6)	5.5–146.3
Vitamin D intake (IU/day)			426 (317)	23–2,589
Measures of inflammation				
Inflammation summary statistic			−0.3 (4.9)	−12.1 to 28.4
CD40 ligand (ng/ml)			3.4 (4.8)	0.1–29.5
C-reactive protein (mg/liter)			3.8 (5.3)	0.2–66.2
Fibrinogen (mg/dl)			375 (71)	181–676
Intercellular adhesion molecule-1 (mg/ml)			259 (83)	130–1,328
Interleukin-6 (pg/ml)			3.6 (3.8)	0.4–51.2
Lipoprotein phospholipase A_2 activity (nmol/minute/ml)			144 (36)	41–364
Lipoprotein phospholipase A_2 mass (ng/ml)			302 (95)	78–886
Monocyte chemoattractant protein-1 (pg/ml)			322 (121)	31–2,140
Myeloperoxidase (mg/ml)			47.9 (32.5)	4.9–377.0
Osteoprotegerin (pmol/liter)			5.5 (1.8)	0.6–26.9
P-selectin (ng/ml)			36.1 (14.1)	2.5–175.8
Tumor necrosis factor-α (pg/ml)			1.4 (1.3)	0.3–21.1
Tumor necrosis factor receptor-2 (pg/ml)			2,158 (769)	892–8,215
Urinary isoprostane (pg/ml)			1,559 (1,369)	31–11,125

* SD, standard deviation.
† Weight (kg)/height $(m)^2$.
‡ 1 ounce = 29.6 ml.

significantly ($p \leq 0.01$) inversely associated with concentrations of C-reactive protein, fibrinogen, interleukin-6, myeloperoxidase, osteoprotegerin, and urinary isoprostane and with lipoprotein phospholipase A_2 mass (table 2). Percentage of undercarboxylated osteocalcin and plasma 25-hydroxyvitamin D were not significantly associated with overall inflammation, as indicated by the inflammation index (tables 2 and 3). However, plasma 25-hydroxyvitamin D was significantly inversely associated with urinary isoprostane concentration ($p < 0.01$), a measure of oxidative stress that was not included in the inflammation index.

Exclusion of persons with prevalent cardiovascular disease did not change associations between plasma phylloquinone and markers of inflammation. The association between

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TABLE 2. Cross-sectional association between log measures of vitamin K status and markers of inflammation in men and women, Framingham Offspring Study, 1997–2001

	Estimated change in inflammation marker* per twofold change in vitamin K status					
	Increase in plasma phylloquinone (nmol/liter)		Increase in phylloquinone intake (μg/day)		Increase in serum percentage of undercarboxylated osteocalcin	
	Estimated change†	*p* value	Estimated change†	*p* value	Estimated change‡	*p* value
Inflammation summary statistic	0.62	<0.001	0.78	<0.001	1.06	0.53
CD40 ligand	0.85	0.007	0.96	0.25	1.01	0.90
C-reactive protein	0.90	0.03	0.92	0.014	0.85	0.007
Fibrinogen	0.98	0.08	0.99	0.03	1.01	0.89
Intercellular adhesion molecule-1	0.97	0.006	1.00	0.51	0.98	0.16
Interleukin-6	0.92	0.009	0.94	0.008	0.96	0.18
Lipoprotein phospholipase A_2 activity	1.01	0.47	0.99	0.14	1.01	0.41
Lipoprotein phospholipase A_2 mass	1.01	0.48	0.98	0.01	1.04	0.02
Monocyte chemoattractant protein-1	0.97	0.13	0.98	0.07	1.04	0.03
Myeloperoxidase	0.97	0.26	0.94	0.001	0.99	0.65
Osteoprotegerin	0.96	0.008	0.97	0.004	1.00	0.75
P-selectin	0.99	0.75	0.99	0.24	1.03	0.12
Tumor necrosis factor-α	1.00	0.99	0.99	0.65	1.04	0.13
Tumor necrosis factor receptor-2	0.96	0.004	0.99	0.10	0.98	0.10
Urinary isoprostanes	0.88	0.02	0.89	<0.001	0.98	0.68

* Serum measures, unless otherwise indicated.

† Covariates: sex, age, body mass index, triglyceride concentrations, use of aspirin, use of lipid-lowering medication, menopausal status, and hormone replacement therapy.

‡ Adjusted for season as well as the covariates listed above.

percentage of undercarboxylated osteocalcin and C-reactive protein was attenuated ($p = 0.02$). However, in these same persons, the inverse association between phylloquinone intake and C-reactive protein reached statistical significance ($p = 0.009$), and plasma 25-hydroxyvitamin D was significantly inversely associated with interleukin 6 ($p = 0.009$) (data not shown).

DISCUSSION

In our community-based sample, there was an inverse association between vitamin K status, as measured by plasma phylloquinone level and phylloquinone intake, and overall circulating markers of inflammation. In the same cohort, vitamin D status, as measured by plasma 25-hydroxyvitamin D level, was not consistently associated with systemic inflammatory markers.

Our findings are in general agreement with those from in vitro studies that found a decrease in the production of inflammatory markers, including interleukin-6, by human macrophage and fibroblast cells with vitamin K treatment (11, 13). The current study expanded our knowledge of this putative role of vitamin K because our panel consisted of 14 biomarkers of inflammation, many of which have not been previously studied with respect to vitamin K. In contrast, our findings in this community sample did not support in vitro data which suggest that treatment with different forms of vitamin D may reduce production of inflammatory cytokines (35, 36). However, we acknowledge that in in vitro studies, the active vitamin D metabolite (calcitriol) was more effective in influencing cytokine production, while in vivo 25-hydroxyvitamin D was used as the estimate of vitamin D status, since circulating concentrations of calcitriol are tightly controlled. Most studies in which a beneficial effect of vitamin D on inflammatory cytokines was reported were based on persons diagnosed with chronic diseases (19, 20, 37). The Framingham Offspring Study is a study of a generally healthier, older cohort. Our results are similar to those of a single study by Gannage-Yared et al. (21), who reported that supplementation with 25-hydroxyvitamin D did not influence concentrations of circulating cytokines in a small sample ($n = 47$) of healthy postmenopausal women.

The mechanisms by which vitamin K influences biomarkers of inflammation are not known, although there is some suggestion that vitamin K suppresses inflammation by decreasing the expression of genes for individual cytokines, such as interleukin-6 and osteoprotegerin (13, 38). Interleukin-6 and osteoprotegerin were the two markers that were inversely associated with both plasma phylloquinone concentration and phylloquinone intake in our study. Vitamin K, interleukin-6, and osteoprotegerin are all implicated in bone resorption and the regulation of vascular calcification (39–41). Lower osteoprotegerin concentrations in skeletal and vascular tissue are associated with an increase in bone resorption and vascular calcification (42), whereas patients with osteoporosis and cardiovascular disease are

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TABLE 3. Cross-sectional association between measures of vitamin D status and markers of inflammation in men and women, Framingham Offspring Study, 1997–2001

	Estimated change in inflammation marker* per twofold increase in vitamin D status			
	Plasma 25-hydroxyvitamin D (nmol/liter)		Vitamin D intake (IU/day)	
	Estimated change†	*p* value	Estimated change†	*p* value
Inflammation summary statistic	0.98	0.06	0.98	0.73
CD40 ligand	1.00	0.83	1.09	0.002
C-reactive protein	1.01	0.23	0.98	0.63
Fibrinogen	1.00	0.19	1.00	0.16
Intercellular adhesion molecule-1	1.00	0.25	1.00	0.96
Interleukin-6	1.00	0.02	1.00	0.90
Lipoprotein phospholipase A_2 activity	1.00	0.45	0.99	0.45
Lipoprotein phospholipase A_2 mass	1.00	0.14	0.99	0.35
Monocyte chemoattractant protein-1	1.00	0.07	0.99	0.69
Myeloperoxidase	1.00	0.10	0.98	0.19
Osteoprotegerin	1.00	0.74	1.00	0.93
Plasma tumor necrosis factor receptor-2	1.00	0.79	1.00	0.83
P-selectin	1.00	0.97	0.97	0.30
Tumor necrosis factor-α	1.00	0.39	0.98	0.29
Urinary isoprostanes	0.997	0.007	0.79	0.01

* Serum measures, unless otherwise indicated.

† Covariates: sex, age, body mass index, triglyceride concentrations, use of aspirin, use of lipid-lowering medication, season, menopausal status, and hormone replacement therapy.

reported to have increased concentrations of circulating osteoprotegerin (43, 44). Schoppet et al. (45) have proposed that these observed increases in circulating osteoprotegerin concentrations in patients with skeletal and/or vascular pathology are an incomplete compensatory response to factors leading to increased bone resorption and/or atherosclerosis.

It has been assumed that any role of vitamin K in bone or cardiovascular health is mediated through its action as a cofactor in the carboxylation of vitamin K-dependent proteins, including osteocalcin. Whereas we observed an association between phylloquinone intake and plasma concentrations and overall inflammation, there was no association between percentage of undercarboxylated osteocalcin and overall inflammation. Percentage of undercarboxylated osteocalcin is used as a measure of the amount of vitamin K available for carboxylation in extrahepatic tissues. It is possible that vitamin K modulates inflammation by a mechanism that does not involve its role in γ-carboxylation. The in vitro studies suggest a direct effect of vitamin K on gene expression that is not related to carboxylation of vitamin K-dependent proteins. Further, it has been shown that vitamin K has a protective effect against oxidative stress that is independent of carboxylation, which may be an alternative antiinflammatory mechanism associated with vitamin K (46). The variation in biochemical measures of vitamin K status is determined by both dietary and nondietary factors (M. K. S., unpublished data), which may explain why the significance of associations of biochemical measures of vitamin K status and phylloquinone intake with individual markers of inflammation was not always consistent.

Calcitriol has been postulated to regulate immune function through the nuclear vitamin D receptor, which is expressed by most cells of the immune system. In vivo, the reported immunoregulatory effects of active vitamin D occur at very high concentrations (18). Since the conversion of 25-hydroxyvitamin D to calcitriol is tightly regulated and the range of circulating calcitriol concentrations is narrow in the absence of chronic disease (47), the reported effects of vitamin D on inflammation may not have been detected by our measures of vitamin D status in this community sample of healthy persons, similarly to the findings of Gannage-Yared et al. (21). Vitamin D status, as measured by plasma 25-hydroxyvitamin D, was inversely associated with urinary isoprostane concentration, a measure of oxidative stress. This inverse association is in general agreement with the findings of Lin et al. (48), who reported a protective role of vitamin D in reducing oxidative stress by acting to terminate the lipid peroxidation chain reaction.

Strengths and limitations

There are several limitations to this study. Importantly, we tested associations between multiple biomarkers of inflammation and measures of vitamin K and D status, and we cannot discount the possibility that significant associations may have been due to chance because of multiple testing.

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By using $p \leq 0.01$ as the level of significance, we hoped to decrease this possibility. Certain health conditions, such as prevalent cardiovascular disease, are associated with increases in inflammatory biomarkers and with unhealthy lifestyle patterns that may influence vitamin K and D status. However, results were not substantively changed in analyses excluding persons with diagnosed cardiovascular disease. Conversely, phylloquinone is present in foods that are generally consumed as part of a healthy diet (49), which may partially account for the reduction in inflammatory markers associated with higher intakes of phylloquinone. Furthermore, although in vitro data suggest that calcitriol, the biologically active form of vitamin D, is involved in immunoregulation, we did not measure this form of vitamin D because circulating levels are tightly regulated. The Offspring cohort participants are primarily older, primarily of northern European descent, and predominantly reside in the northeastern United States; thus, our findings cannot be generalized to other ethnic/racial groups, younger persons, or persons residing in sunnier climates.

We acknowledge that the measurement of some of the vitamin concentrations at examination 6 (49 percent), approximately 3 years prior to the measurement of inflammatory markers at examination 7, may have led to some misclassification; however, we did not observe significant effect modification by examination. The cross-sectional study design precluded causal inferences in interpreting our results. Although we modeled the relation of vitamins to inflammatory markers (dependent variables), we acknowledge that inflammatory markers may influence vitamin concentrations, or both may be related via other unmeasured intermediate factors. Finally, although the associations were statistically significant, the clinical significance of the very modest changes we observed is uncertain. Many of the statistically significant associations would not have survived a strict Bonferroni correction, and our findings need to be replicated in other cohorts.

Balanced against these limitations are the novelty of the analyses and the routine ascertainment of vitamins, inflammatory biomarkers, and covariates in a community-based sample of men and women.

Clinical and research implications

Our findings provide one potential alternative mechanism for a putative protective effect of vitamin K in the progression of cardiovascular disease and osteoporosis, since both diseases are characterized by inflammation. Limited in vitro data support the inverse association between vitamin K and interleukin-6, and this may influence the association between vitamin K and other cytokines, such as osteoprotegerin. Further research to better elucidate mechanisms underlying the associations between vitamin K and inflammatory cytokines is warranted.

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Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the US Department of Agriculture.

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REFERENCES

1. Baldini V, Mastropasqua M, Francucci CM, et al. Cardiovascular disease and osteoporosis. J Endocrinol Invest 2005;28: 69–72.
2. Clowes JA, Riggs BL, Khosla S. The role of the immune system in the pathophysiology of osteoporosis. Immunol Rev 2005;208:207–27.
3. Libby P. Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr 2006;83(suppl):456S–60S.
4. Doherty TM, Asotra K, Fitzpatrick LA, et al. Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. Proc Natl Acad Sci U S A 2003;100: 11201–6.
5. Collin-Osdoby P, Rothe L, Anderson F, et al. Receptor activator of NF-kappa B and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. J Biol Chem 2001;276:20659–72.
6. Bagger YZ, Tanko LB, Alexandersen P, et al. Radiographic measure of aorta calcification is a site-specific predictor of bone loss and fracture risk at the hip. J Intern Med 2006;259: 598–605.
7. Kiel DP, Kauppila LI, Cupples LA, et al. Bone loss and the progression of abdominal aortic calcification over a 25 year period: The Framingham Heart Study. Calcif Tissue Int 2001; 68:271–6.
8. Shea MK, Booth SL. Role of vitamin K in the regulation of calcification. In: Burckhardt P, Dawson-Hughes B, Heaney RP, eds. Nutritional aspects of osteoporosis 2006. Amsterdam, the Netherlands: Elsevier Publishers BV, 2007:273–81.
9. Erkkila AT, Booth SL, Hu FB, et al. Phylloquinone intake and risk of cardiovascular diseases in men. Nutr Metab Cardiovasc Dis 2007;17:58–62.
10. Booth SL, Broe KE, Peterson JW, et al. Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab 2004;89:4904–9.
11. Reddi K, Henderson B, Meghji S, et al. Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds. Cytokine 1995;7:287–90.
12. Koshihara Y, Hoshi K, Shiraki M. Vitamin K2 (menatetrenone) inhibits prostaglandin synthesis in cultured human osteoblast-like periosteal cells by inhibiting prostaglandin H synthase activity. Biochem Pharmacol 1993;46:1355–62.
13. Ohsaki Y, Shirakawa H, Hiwatashi K, et al. Vitamin K suppresses lipopolysaccharide-induced inflammation in the rat. Biosci Biotechnol Biochem 2006;70:926–32.
14. Sasaki N, Kusano E, Takahashi H, et al. Vitamin K2 inhibits glucocorticoid-induced bone loss partly by preventing the

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reduction of osteoprotegerin (OPG). J Bone Miner Metab 2005;23:41–7.
15. Bischoff-Ferrari HA, Dietrich T, Orav EJ, et al. Positive association between 25-hydroxy vitamin D levels and bone mineral density: a population-based study of younger and older adults. Am J Med 2004;116:634–9.
16. Zittermann A, Schleithoff SS, Koerfer R. Putting cardiovascular disease and vitamin D insufficiency into perspective. Br J Nutr 2005;94:483–92.
17. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004;80(suppl):1678S–88S.
18. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. J Steroid Biochem Mol Biol 2005;97:93–101.
19. Inanir A, Ozoran K, Tutkak H, et al. The effects of calcitriol therapy on serum interleukin-1, interleukin-6 and tumour necrosis factor-alpha concentrations in post-menopausal patients with osteoporosis. J Int Med Res 2004;32:570–82.
20. Schleithoff SS, Zittermann A, Tenderich G, et al. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 2006;83:754–9.
21. Gannage-Yared MH, Azoury M, Mansour I, et al. Effects of a short-term calcium and vitamin D treatment on serum cytokines, bone markers, insulin and lipid concentrations in healthy post-menopausal women. J Endocrinol Invest 2003; 26:748–53.
22. Kannel WB, Feinleib M, McNamara PM, et al. An investigation of coronary heart disease in families. The Framingham Offspring Study. Am J Epidemiol 1979;110:281–90.
23. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
24. 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.
25. Gundberg CM, Hauschka PV, Lian JB, et al. Osteocalcin: isolation, characterization, and detection. Methods Enzymol 1984;107:516–44.
26. Gundberg CM, Nieman SD, Abrams S, et al. Vitamin K status and bone health: an analysis of methods for determination of undercarboxylated osteocalcin. J Clin Endocrinol Metab 1998;83:3258–66.
27. Keaney JF Jr, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol 2003; 23:434–9.
28. Loucks EB, Sullivan LM, Hayes LJ, et al. Association of educational level with inflammatory markers in the Framingham Offspring Study. Am J Epidemiol 2006;163:622–8.
29. Clauss A. Rapid physiological coagulation method in determination of fibrinogen. (In German). Acta Haematol 1957;17: 237–46.
30. Fox CS, Guo CY, Larson MG, et al. Relations of inflammation and novel risk factors to valvular calcification. Am J Cardiol 2006;97:1502–5.
31. Jacques PF, Felson DT, Tucker KL, et al. Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. Am J Clin Nutr 1997;66:929–36.
32. McKeown NM, Jacques PF, Gundberg CM, et al. Dietary and nondietary determinants of vitamin K biochemical measures in men and women. J Nutr 2002;132:1329–34.
33. Sadowski JA, Hood SJ, Dallal GE, et al. Phylloquinone in plasma from elderly and young adults: factors influencing its concentration. Am J Clin Nutr 1989;50:100–8.
34. Looker AC, Dawson-Hughes B, Calvo MS, et al. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. Bone 2002;30: 771–7.
35. Muller K, Haahr PM, Diamant M, et al. 1,25-Dihydroxyvitamin D3 inhibits cytokine production by human blood monocytes at the post-transcriptional level. Cytokine 1992;4:506–12.
36. Panichi V, De Pietro S, Andreini B, et al. Calcitriol modulates in vivo and in vitro cytokine production: a role for intracellular calcium. Kidney Int 1998;54:1463–9.
37. Mahon BD, Gordon SA, Cruz J, et al. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol 2003;134:128–32.
38. Katsuyama H, Otsuki T, Tomita M, et al. Menaquinone-7 regulates the expressions of osteocalcin, OPG, RANKL and RANK in osteoblastic MC3T3E1 cells. Int J Mol Med 2005; 15:231–6.
39. Kwan Tat S, Padrines M, Theoleyre S, et al. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev 2004;15:49–60.
40. Vik A, Mathiesen EB, Notø AT, et al. Serum osteoprotegerin is inversely associated with carotid plaque echogenicity in humans. Atherosclerosis 2007;191:128–34.
41. Mysliwska J, Wieckiewicz J, Hak L, et al. Interleukin 6 polymorphism corresponds to the number of severely stenosed coronary arteries. Eur Cytokine Netw 2006;17:181–8.
42. Yasuda H, Shima N, Nakagawa N, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci U S A 1998;95:3597–602.
43. Schoppet M, Sattler AM, Schaefer JR, et al. Increased osteoprotegerin serum levels in men with coronary artery disease. J Clin Endocrinol Metab 2003;88:1024–8.
44. Yano K, Tsuda E, Washida N, et al. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 1999;14:518–27.
45. Schoppet M, Preissner K, Hofbauer LC. RANK ligand and osteoprotegerin: paracrine regulators of bone metabolism and vascular function. Arterioscler Thromb Vasc Biol 2002; 22:549–53.
46. Li J, Lin JC, Wang H, et al. Novel role of vitamin K in preventing oxidative injury to developing oligodendrocytes and neurons. J Neurosci 2003;23:5816–26.
47. Barger-Lux MJ, Heaney RP, Dowell S, et al. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporos Int 1998;8:222–30.
48. Lin AM, Chen KB, Chao PL. Antioxidative effect of vitamin D3 on zinc-induced oxidative stress in CNS. Ann N Y Acad Sci 2005;1053:319–29.
49. Braam L, McKeown N, Jacques P, et al. Dietary phylloquinone intake as a potential marker for a heart-healthy dietary pattern in the Framingham Offspring cohort. J Am Diet Assoc 2004; 104:1410–14.

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