Phylloquinone and Vitamin D Status: Associations with Incident Chronic Kidney Disease in the Framingham Offspring Cohort

Conall M. O'Seaghdha^{a,b} Shih-Jen Hwang^a Rachel Holden^e Sarah L. Booth^d
Caroline S. Fox^{a,c}

^aNational Heart, Lung and Blood Institute's Framingham Heart Study and the Center for Population Studies, Framingham, Mass., ^bRenal Division and ^cDivision of Endocrinology, Brigham and Women's Hospital and Harvard Medical School, and ^dJean Mayer US Department of Agriculture Human Nutrition Research Center of Aging at Tufts University, Boston, Mass., USA; ^eDivision of Nephrology, Queen's University, Kingston, Ont., Canada

Key Words

Chronic kidney disease · Vitamin K · Vitamin D

Abstract

Background: Cardiovascular risk factors are associated with the development of chronic kidney disease (CKD), and CKD and vascular disease are etiologically linked. Evidence suggests deficiencies of vitamins D and K may adversely affect the cardiovascular system, but data from longitudinal studies are lacking. We hypothesized that deficiencies of vitamins D and K may be associated with incident CKD and/or incident albuminuria amongst members of the general population. **Methods:** We analyzed 1,442 Framingham Heart Study participants (mean age 58 years; 50.5% women), free of CKD (eGFR <60 ml/min/1.73 m²), with a mean follow-up of 7.8 years in 2005–2008. Incident albuminuria was defined using sex-specific cut-offs of urine albumin-to-creatinine ratio (≥ 17 mg/g men and ≥ 25 mg/g women). Baseline log plasma phylloquinone (vitamin K₁) and 25(OH)D levels, analyzed as continuous variables and by quartile, were related to risk of incident CKD (n = 108) and incident albuminuria (n = 106) using logistic regression models adjusted for standard risk factors. **Results:** Participants in the highest phylloquinone quartile (≥ 1.78 nmol/l) had an increased risk of CKD

(multivariable-adjusted OR Q₄ vs. Q₁ 2.39; p = 0.006) and albuminuria at follow-up (multivariable-adjusted OR Q₄ vs. Q₁ 1.95; p = 0.05), whereas no association was observed with continuous phylloquinone levels for either endpoint. Deficiency of 25(OH)D was not associated with incident CKD or albuminuria in either analysis. **Conclusions:** Contrary to our hypothesis, higher plasma phylloquinone levels are associated with an increased risk of incident CKD. Whether plasma phylloquinone is a marker for another unmeasured risk factor requires further study. External validation is necessary given the unexpected nature of these results.

Copyright © 2012 S. Karger AG, Basel

Introduction

Chronic kidney disease (CKD) is associated with cardiovascular disease, such that they commonly coexist and the overlap in their etiologies is considerable [1]. However, vascular calcification is more commonly a feature of cardiovascular disease in CKD patients than is observed in the general population [2]. For example, at the time of initiating renal replacement therapy, the majority of end-stage renal disease patients exhibit extensive arterial calcification, typically involving the coronary ar-

teries, aorta and cardiac valves [3], and the presence and extent of such advanced vascular calcification predicts cardiovascular disease and mortality beyond conventional risk factors [4, 5]. Advances in imaging techniques have permitted the identification of vascular calcification at earlier stages of CKD in humans [6], while data from animal studies indicates the processes that lead to vascular calcification begin with very mild degrees of renal injury, before the altered mineral metabolism of secondary hyperparathyroidism develops [7].

Deficiencies of vitamins K and D are commonly observed across the spectrum of kidney disease stages [8]. Both are fat-soluble nutrients essential to bone health [9, 10], and deficiencies have been linked to vascular calcification in animals [11–13], members of the general population [14, 15] and people with kidney disease [6, 12]. Vitamin K administration causes regression of warfarin-induced medial elastocalcinosis in rats [16]. In the case of vitamin D, supplementation has been shown to prevent the development of albuminuria in an animal model of diabetic nephropathy [17, 18]. Furthermore, evidence from a recent randomized controlled trial indicates that treatment with the vitamin D agonist, paricalcitol, reduces proteinuria in established diabetic nephropathy [19].

In light of these observations, we hypothesized that deficiencies of vitamins D and K may be associated with incident kidney disease. Using data from the Framingham Offspring cohort, we tested this hypothesis by relating measures of vitamin K status (as measured by plasma phyloquinone level (vitamin K₁)) and vitamin D status (plasma 25(OH)D) to risk of incident CKD and incident albuminuria at 8 years' follow-up in a community-based sample of men and women.

Methods

Study Sample

Participants were drawn from the Framingham Offspring Study cohort [20]. Offspring participants underwent assessment in 4- to 8-year cycles, which included physical examination, blood biochemistries, assessment of cardiovascular risk factors and physician interview. Samples for vitamins K and D were collected between 1997 and 1999 [the end of examination cycle 6 (1995–1998) and the beginning of cycle 7 (1998–2001)]. In total, 1,599 participants had baseline phyloquinone levels drawn and follow-up creatinine measures available. Of these, 147 were excluded due to baseline CKD, and 10 due to missing covariates, resulting in a final study sample of 1,442. For the 25(OH)D analysis, the total sample size was 1,438. All participants provided written informed consent, and the institutional review boards of the Boston University Medical Center approved the study.

Exposure Measurement: Vitamins K and D

Fasting morning blood samples were drawn and plasma was stored at -80°C until processing. Vitamin K status was assessed by plasma phyloquinone level, as measured by high-pressure liquid chromatography [21]. Low and high control specimens had average values of 0.56 and 3.15 nmol/l, with coefficients of variation (total CVs) of 15.2 and 10.9%, respectively. 25(OH)D status was estimated by measuring plasma 25(OH)D level using radioimmunoassay (<http://www.diasorin.com>).

Primary Outcome Assessment: eGFR and Incident CKD

The primary outcome was development of incident CKD, defined as $\text{eGFR} < 60 \text{ ml/min/1.73 m}^2$ using the Modification of Diet in Renal Disease (MDRD) equation [22], by the eighth examination cycle (2005–2008). Serum creatinine levels were measured using the modified Jaffé method. Calibration of serum creatinine values to the Cleveland Clinic Laboratory standard was performed using the correction factor of 0.23 mg/dl (20.33 $\mu\text{mol/l}$) [23]. We utilized an alternate definition of incident CKD as a secondary outcome, CKDi25: $\text{eGFR} < 60 \text{ ml/min}$ at follow-up and at least 25% decline in eGFR from baseline.

Secondary Outcome Assessment: Incident Albuminuria

Incident albuminuria was defined using the sex-specific cut-offs of urine albumin-to-creatinine ratio (UACR) $\geq 17 \text{ mg/g}$ in men and $\geq 25 \text{ mg/g}$ in women [24]. UACR was measured on spot morning urine samples collected between 1995 and 1998. After collection, urine samples were stored at -20°C and then transitioned to -80°C until quantification in October 1998 in Children's Hospital, Boston, Mass., USA. Urinary albumin concentration was measured using immunoturbidimetry (Tina-quant Albumin assay; Roche Diagnostics; <http://www.roche-diagnostics.us/>) and urinary creatinine levels were measured using the Jaffé method; [25] the intra-assay coefficient of variation varied from 1.7 to 3.8%.

Covariate Assessment

Participants underwent blood testing and were assessed for CKD risk factors. High-density lipoprotein cholesterol (HDL-C) and blood glucose were measured on fasting morning blood samples. Diabetes was defined as fasting blood glucose of 126 mg/dl (7 mmol/l) or greater or use of diabetic medication. Systolic and diastolic blood pressure measurements were taken as the mean of two physician readings using a mercury sphygmomanometer. Hypertension was defined as a systolic BP $\geq 140 \text{ mm Hg}$ or a diastolic BP $\geq 90 \text{ mm Hg}$ or self-reported use of antihypertensive medications. Body mass index (BMI) was defined as an individual's weight in kilograms divided by height in meters squared. Current smoking status was defined by self-report. Season was also included as a covariate due to seasonal influences on vitamins D status [26]. Season was defined as: June–August, summer; September–November, fall; December–February, winter; March–May, spring – with fall, winter and spring being entered as dichotomous variables and summer as the reference.

Statistical Analyses

Phyloquinone was log-transformed to approximate normality due to a skewed distribution (skewness 7.4). Following log transformation the distribution skewness improved to -0.3 and kurtosis was 1.06. Baseline characteristics of study participants

were calculated by quartile (Q) of phyloquinone level and the statistical significance of differences was compared using χ^2 tests for categorical variables and one-way ANOVA for continuous variables. Pearson's correlation coefficients were used to assess associations between plasma phyloquinone level with age, BMI, systolic blood pressure, HDL-C, log triglycerides, eGFR, UACR and 25(OH)D.

Baseline phyloquinone level was considered both in quartiles and as a continuous variable (per 1 standard deviation (SD) increase). The association between phyloquinone quartile and risk of incident CKD was tested using logistic regression models. Three sets of regression models were constructed: (1) adjusting for age and sex, (2) a multivariable model adjusting for age, sex, diabetes, systolic blood pressure, hypertension treatment, HDL-C, BMI, current smoking and eGFR, and (3) additional adjustment to model 2 for the proportion of circulating undercarboxylated osteocalcin (%ucOC), a sensitive measure of vitamin K status. In these regression models, the reference category was Q₁ (lowest phyloquinone level).

25(OH)D was also analyzed as both a continuous variable and by quartiles, and related to risk of incident CKD and albuminuria using logistic regression models. Participants in the lowest quartile plasma 25(OH)D levels were used as the reference group. Identical regression models were used as for the phyloquinone analysis. All analyses were performed using SAS, version 9.1 (SAS Institute, Cary, N.C., USA).

Secondary Analyses

We examined incident albuminuria as a secondary outcome, defined using the sex-specific cut-points of UACR ≥ 17 mg/g (men) or ≥ 25 mg/g (women) [27]. As for the CKD analysis, the association between vitamin quartile and risk of albuminuria was tested using two sets of logistic regression models: (1) age- and sex-adjusted, and (2) a multivariable model adjusted for age, sex, diabetes, HDL-C, log triglycerides, current smoking, eGFR and baseline log UACR.

As an additional secondary analysis, we also adjusted for dietary phyloquinone intake, assessed by food frequency questionnaire, in the multivariable model for incident CKD.

Results

Baseline Characteristics

Baseline characteristics of the cohort, by quartile of baseline plasma phyloquinone level, are presented in table 1a. Participants with higher phyloquinone levels at baseline were more likely to be men and have hypertension but less likely to smoke tobacco. There were no significant differences in baseline eGFR or albuminuria across quartiles. Data by quartile of baseline plasma 25(OH)D are shown in table 1b.

Age- and sex-adjusted cross-sectional correlations of log plasma phyloquinone and plasma 25(OH)D with established CKD risk factors are presented in table 2. log plasma phyloquinone was correlated with log plasma tri-

glyceride level ($r = 0.20$; $p < 0.0001$), and inversely correlated with plasma HDL-C ($r = -0.11$; $p < 0.0001$). Plasma 25(OH)D was inversely correlated with BMI ($r = -0.22$; $p < 0.0001$). Weaker correlations were observed between plasma 25(OH)D and log plasma triglyceride level ($r = -0.09$; $p = 0.0002$), plasma HDL-C ($r = 0.13$; $p < 0.0001$) and systolic blood pressure ($r = -0.06$; $p = 0.02$).

Incident CKD by Vitamin K Status

Of 1,442 study participants, 108 (7.5%) developed incident CKD over a mean of 7.8 years' follow-up. In an analysis by baseline phyloquinone quartile, participants in Q₄ (≥ 1.78 nmol/l; highest plasma phyloquinone level) demonstrated an increased risk of incident CKD when compared with Q₁ (≤ 0.55 nmol/l; lowest) in age- and sex-adjusted (OR 2.20; 95% confidence interval (CI) 1.20–4.00; $p = 0.01$) and multivariable-adjusted models (OR 2.39 (1.28–4.46); $p = 0.006$; table 3). The inclusion of %ucOC in the multivariable incident CKD logistic regression model did not materially alter the results, and %ucOC was not associated with incident CKD ($p = 0.2$).

When analyzed as a continuous variable, plasma phyloquinone was not associated with incident CKD (multivariable-adjusted OR per SD increase in plasma phyloquinone: 0.99 (0.83–1.19); $p = 1.0$; table 3). There was no evidence of effect modification by sex (p value range for sex-interaction terms: 0.5–1.0).

Incident CKD by 25(OH)D Status

Of 1,438 study participants for the 25(OH)D analysis, 108 (7.5%) developed incident CKD over the study period. When analyzed by baseline quartile of 25(OH)D level, no association with risk of CKD was observed for any quartile compared to the referent in age- and sex-adjusted (for example, OR Q₄ vs. Q₁: 1.30 (0.74–2.27); $p = 0.4$) and multivariable-adjusted models (OR 1.44 (0.79–2.61); $p = 0.2$; table 4). When analyzed as a continuous variable, plasma 25(OH)D was not associated with incident CKD in either age- and sex-adjusted or multivariable-adjusted models (multivariable-adjusted OR per SD increase in plasma 25(OH)D level: 0.85 (0.69–1.04); $p = 0.1$, table 4). There was no evidence of effect modification by sex (p value for sex-interaction terms ranges from 0.4 to 0.9).

Secondary Analyses

Alternate Definition of CKD

Applying a more stringent alternate definition of CKD (follow-up eGFR < 60 ml/min and at least 25% decline in eGFR from baseline) yielded fewer cases than the primary analysis ($n = 62$; 4.3%). However, results were similar

Table 1. Baseline characteristics of study participants by quartile of **a** plasma phylloquinone (vitamin K₁) level, nmol/l, and **b** plasma 25(OH)D level, ng/ml (data presented as mean with SD in parentheses for continuous variables or percent with number in parentheses for categorical data)

a

Characteristic	Q ₁	Q ₂	Q ₃	Q ₄	p*
Phylloquinone level, nmol/l (range)	0.35 (0.05–0.55)	0.76 (0.56–0.98)	1.33 (0.99–1.77)	3.37 (1.78–35.02)	<0.0001
Participants, n	361	357	361	363	–
Age, years	58 (9)	58 (9)	58 (8)	58 (8)	0.4
Female sex, % (n)	54 (194)	55 (195)	49 (175)	46 (165)	0.007
Body mass index	27.8 (5.8)	27.6 (5.2)	28.2 (5.1)	28.7 (4.7)	0.02
Systolic blood pressure, mm Hg	124 (17)	125 (17)	127 (18)	127 (16)	0.01
Hypertension, % (n)	33 (120)	33 (119)	37 (133)	46 (167)	0.0009
Hypertension treatment, % (n)	23 (84)	22 (80)	25 (90)	34 (123)	0.002
Smoking, % (n)	18 (64)	13 (46)	13 (46)	11 (40)	0.05
Diabetes mellitus, % (n)	8 (28)	8 (27)	6 (23)	9 (31)	0.95
Total cholesterol, mg/dl	190	200	203	208	<0.0001
LDL-C, mg/dl	117	123	125	125	0.002
HDL-C, mg/dl	53 (15)	53 (16)	52 (16)	48 (17)	0.004
Triglycerides, mg/dl (25th, 75th)	85 (66, 117)	92 (72, 124)	105 (77, 157)	121 (89, 170)	<0.0001
eGFR, ml/min/1.73 m ²	86 (17)	86 (20)	87 (17)	89 (21)	0.8
Chronic kidney disease, %	5.26 (19/361)	7.84 (28/329)	6.93 (25/361)	9.92 (36/363)	0.2
UACR, mg/g (25th, 75th)	5.9 (2.8, 13.6)	6.4 (3.1, 14.1)	5.6 (2.3, 11.9)	5.0 (1.9, 11.7)	0.3
%ucOC	20.6	17.6	17.1	14.3	<0.0001
Dietary phylloquinone intake, µg/day	150 (137)	156 (86)	169 (101)	164 (94)	0.02

b

Characteristic	Q ₁	Q ₂	Q ₃	Q ₄	p**
Plasma 25(OH) D, ng/ml	2.2–14.6	14.7–19.1	19.2–24.0	24.1–58.5	–
Participants, n	358	357	365	358	–
Age, years	58 (9)	58 (9)	58 (9)	58 (8)	0.4
Female sex, % (n)	49 (176)	47 (166)	51 (186)	56 (200)	0.03
Season of blood draw	–	–	–	–	–
Spring, % (n)	39 (138)	28 (99)	27 (100)	20 (72)	<0.0001
Summer, % (n)	3 (10)	5 (17)	10 (38)	23 (83)	<0.0001
Fall, % (n)	16 (58)	32 (113)	37 (136)	37 (135)	<0.0001
Winter, % (n)	43 (152)	36 (127)	25 (91)	19 (68)	<0.0001
Body mass index	29.6 (6.1)	28.8 (5.5)	27.3 (4.4)	26.6 (4.1)	<0.0001
Systolic blood pressure, mm Hg	128 (17)	126 (16)	124 (17)	125 (18)	0.008
Hypertension, % (n)	54 (193)	53 (187)	46 (169)	50 (178)	0.07
Hypertension treatment, % (n)	28 (100)	26 (92)	27 (98)	24 (87)	0.4
Smoking, % (n)	17 (60)	13 (48)	12 (45)	12 (43)	0.06
Diabetes mellitus, % (n)	10 (37)	7 (26)	7 (26)	6 (20)	0.04
HDL-C, mg/dl	49 (16)	49 (15)	53 (17)	55 (16)	<0.0001
Triglycerides, mg/dl (25th, 75th)	104 (79, 148)	109 (79, 164)	93 (68, 134)	94 (69, 131)	0.008
eGFR, ml/min/1.73 m ²	89 (18)	89 (20)	87 (19)	84 (17)	0.3
UACR mg/g (25th, 75th)	4.6 (2.0, 9.3)	4.7 (1.9, 7.8)	4.8 (1.8, 8.6)	5.0 (2.6, 9.8)	0.9

HDL = High-density lipoprotein; LDL = low-density lipoprotein; eGFR = estimated glomerular filtration rate; UACR = urine albumin:creatinine ratio; %ucOC = proportion of circulating undercarboxylated osteocalcin.

* p values are for significance of trend across quartiles, adjusted for age and sex (except age, which is sex-adjusted and sex, which is age-adjusted). Mean phylloquinone level: 1.46 nmol/l.

** p values are for significance of trend across groups, adjusted for age and sex (except age, which is sex-adjusted and sex, which is age-adjusted). Mean 25(OH)D level: 19.7 ng/ml.

Table 2. Age- and sex-adjusted cross-sectional partial Pearson correlation coefficients of log plasma phyloquinone (vitamin K₁) and 25(OH)D levels with kidney disease covariates

	Phylloquinone (vitamin K ₁)		25(OH)D	
	r	p value	r	p value
Body mass index	0.05	0.4	-0.22	<0.0001
Systolic blood pressure	0.04	0.17	-0.06	0.02
High-density lipoprotein cholesterol	-0.11	<0.0001	0.13	<0.0001
Estimated glomerular filtration rate	-0.002	0.93	-0.03	0.29
Urine albumin:creatinine ratio*	-0.022	0.46	0.006	0.83
log triglycerides	0.20	<0.0001	-0.09	0.0002
25(OH)D	-0.03	0.24	–	–

Table 3. Results of logistic regression for quartile and continuous analyses of plasma phyloquinone level and incident kidney disease¹

	log plasma phyloquinone (vitamin K ₁), nmol/l							
	Q ₂ vs. Q ₁		Q ₃ vs. Q ₁		Q ₄ vs. Q ₁		per 1 SD increase	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Incident CKD, 108/1,442 events (7.5%)								
Age- and sex-adjusted	1.64 (0.88–3.07)	0.1	1.44 (0.76–2.72)	0.3	2.20 (1.20–4.00)	0.01	0.97 (0.81–1.18)	0.8
Multivariable-adjusted ²	1.66 (0.87–3.17)	0.1	1.58 (0.82–3.05)	0.2	2.39 (1.28–4.46)	0.006	0.99 (0.83–1.19)	1.0
Incident albuminuria ³ , 106/1,151 events (9.2%)								
Age- and sex-adjusted	2.21 (1.14–4.25)	0.02	2.21 (1.16–4.22)	0.02	1.89 (0.98–3.67)	0.06	0.92 (0.76–1.11)	0.4
Multivariable-adjusted ⁴	2.17 (1.11–4.22)	0.02	2.21 (1.15–4.25)	0.02	1.95 (0.99–3.82)	0.05	0.93 (0.78–1.12)	0.5

CKD = Chronic kidney disease; eGFR = estimated glomerular filtration rate; SD = standard deviation. CKD defined as eGFR of <60 ml/min/1.73 m². 1 SD of plasma phyloquinone: nmol/l.

¹ Quartile analysis presents odds of incident CKD by baseline quartile of log plasma phyloquinone (vitamin K₁), with lowest quartile as reference. Continuous analysis presents odds of incident CKD per SD increase in log plasma phyloquinone.

² Multivariable model adjusted for age, sex, diabetes, systolic blood pressure, hypertension treatment, HDL-C, log triglycerides, BMI, current smoking and eGFR.

³ Sex-specific cut-offs for quantitative albuminuria: urine albumin:creatinine ratio ≥17 mg/g in men and ≥25 mg/g in women.

⁴ Multivariable model adjusted for age, sex, diabetes, HDL-C, current smoking, eGFR and baseline log urine albumin-to-creatinine ratio.

with participants in the highest phyloquinone quartile demonstrating increased risk of CKD at follow-up when compared with the lowest in both models (age- and sex-adjusted OR 2.83 (1.32–6.08); *p* = 0.008; multivariable-adjusted OR 2.82 (1.28–6.22); *p* = 0.01; data not shown), whereas no association with CKD risk seen in continuous analysis (multivariable-adjusted OR per SD decrease in phyloquinone level 1.01 (0.83–1.23); *p* = 0.9; data not shown).

For 25(OH)D, results were also similar to the primary analysis, with no evidence of association between plasma 25(OH)D level and CKD risk observed in quartile-based (multivariable-adjusted OR 1.43 (0.69–2.96); *p* = 0.3; data

not shown) or continuous analyses (multivariable-adjusted OR per SD decrease in 25(OH)D level 1.10 (0.85–1.41); *p* = 0.5; data not shown).

Incident Albuminuria

Of 1,151 participants with urinary data available, 106 developed new-onset albuminuria at follow-up (9.2%). In quartile-based analyses, risk of incident albuminuria was increased in all upper phyloquinone quartiles when compared to lowest (multivariable-adjusted OR for Q₂ vs. Q₁: 2.17 (1.11–4.22); *p* = 0.02; Q₃ vs. Q₁: 2.21 (1.15–4.25); *p* = 0.02; Q₄ vs. Q₁: 1.95 (0.99–3.82); *p* = 0.05; table 3). There was no association observed between plasma phyl-

Table 4. Results of logistic regression for quartile and continuous analyses of plasma 25(OH)D level and incident kidney disease¹

	Plasma 25(OH)D level, ng/ml							
	Q ₂ vs. Q ₁		Q ₃ vs. Q ₁		Q ₄ vs. Q ₁		per 1 SD increase	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Incident CKD, 108/1,438 events (7.5%)								
Age- and sex-adjusted	0.73 (0.39–1.37)	0.3	1.12 (0.63–1.99)	0.7	1.30 (0.74–2.27)	0.4	0.87 (0.71–1.06)	0.2
Multivariate-adjusted ²	0.78 (0.41–1.50)	0.5	1.16 (0.63–2.12)	0.6	1.44 (0.79–2.61)	0.2	0.85 (0.69–1.04)	0.1
Incident albuminuria ³ , 106/1,345 events (7.8%)								
Age- and sex-adjusted	0.83 (0.47–1.48)	0.5	0.84 (0.47–1.49)	0.5	0.80 (0.45–1.43)	0.5	1.06 (0.87–1.32)	0.5
Multivariate-adjusted ⁴	0.87 (0.48–1.57)	0.6	0.91 (0.51–1.65)	0.8	0.91 (0.50–1.66)	0.8	1.01 (0.82–1.25)	0.9

CKD = Chronic kidney disease; eGFR = estimated glomerular filtration rate; SD = standard deviation. CKD defined as eGFR of <60 ml/min/1.73 m². 1 SD of plasma 25(OH)D: ng/ml.

¹ Quartile analysis presents odds of incident CKD by baseline quartile of plasma 25(OH)D, with lowest quartile as reference. Continuous analysis presents odds of incident CKD per SD increase in plasma 25(OH)D.

² Multivariable model adjusted for age, sex, diabetes, systolic blood pressure, hypertension treatment, HDL-C, log triglycerides, BMI, current smoking and eGFR.

³ Sex-specific cut-offs for quantitative albuminuria: urine albumin:creatinine ratio ≥17 mg/g in men and ≥25 mg/g in women.

⁴ Multivariable model adjusted for age, sex, diabetes, HDL-C, current smoking, eGFR and baseline log urine albumin:creatinine ratio.

loquinone level analyzed as a continuous variable and incident albuminuria ($p = 0.5$; table 3). Finally, no association with incident albuminuria was observed in any analysis of 25(OH)D (multivariable-adjusted OR 0.91 (0.50–1.66); $p = 0.8$ for Q₄ vs. reference; table 4).

Dietary Phylloquinone Intake

Of 1,442 participants in the primary analysis, 1,293 had dietary information available. At baseline, there was a trend for greater dietary phylloquinone intake by plasma quartile (p for trend 0.02; table 1). However, additional adjustment for dietary phylloquinone in the multivariable model of incident CKD did not materially affect the results (multivariable-adjusted OR for Q₂ vs. Q₁: OR 1.81 (95% CI 0.91–3.61, $p = 0.09$); Q₃ vs. Q₁: OR 1.87 (95% CI 0.94–3.71, $p = 0.07$); Q₄ vs. Q₁: OR 2.65 (95% CI 1.37–5.13, $p = 0.004$).

Discussion

The findings of our study are twofold. First, contrary to our initial hypothesis, we observed no association between lower circulating phylloquinone levels and any of the three study endpoints, namely incident CKD, incident CKD with evidence of progression, and incident albuminuria. In fact, an unexpected excess risk for these

endpoints was detected in the highest phylloquinone quartile. Second, 25(OH)D deficiency was not associated with incident CKD or incident albuminuria.

Vitamin K is a co-factor in the post-translational γ -carboxylation of glutamate residues of several vitamin K-dependent proteins, including matrix Gla protein, an inhibitory regulator of tissue mineralization in the arterial wall. Scientific interest in a potential role for vitamin K in vascular biology and cardiovascular health was stimulated by the demonstration of a lethal phenotype of vascular calcification in the matrix Gla protein knockout mouse [28]. Results from subsequent human studies supported this idea. For example, in a 3-year follow-up study of 388 healthy men and postmenopausal women, phylloquinone 500 μ g/day conferred a protective effect against progression of vascular calcification, assessed by coronary calcium score, when compared to placebo [29]. Furthermore, a placebo-controlled trial of phylloquinone supplementation in 108 postmenopausal women demonstrated improved vascular compliance, distensibility, and intima media thickness in the treatment arm [30]. Also, plasma phylloquinone levels were inversely associated with circulating inflammatory markers in the Framingham Offspring cohort [31]. In the setting of kidney disease, deficiencies of vitamins K and D are prevalent in patients with advanced CKD (stages 3–5) [8]. However it should be noted that these deficiencies appear to be a

function of an overall decline in nutritional status observed in CKD patients, as sufficiency of both vitamins was predicted by measures of improved nutritional status. In the present study, participants were free of kidney disease at the time of vitamin assay and were a far less sick cohort in general. As such, poor nutrition would not be expected to be a factor.

It is thus unexpected that we observed an increased risk of incident CKD in individuals with higher plasma phylloquinone levels in the present study. There is no tolerable upper intake limit set as there are no known cases of toxicity due to vitamin K [32]. Unlike other fat-soluble vitamins, vitamin K is not stored in any significant quantity in the liver, therefore toxic levels are rarely achieved. On the contrary, plasma phylloquinone levels are linearly associated with a 'healthy' dietary intake of green vegetables [33]. Consistent with these observations, adjusting for dietary phylloquinone intake or %ucOC in the multivariable model did not materially alter the results, and neither variable was associated with incident CKD. These observations suggest the excess risk of kidney disease seen in the highest plasma phylloquinone quartile is not directly mediated by dietary phylloquinone intake or phylloquinone γ -carboxylase bioactivity.

With no data to support a direct toxic effect of phylloquinone, how might these results be explained? First, this may be an epiphenomenon, and plasma phylloquinone may be a marker of an unmeasured biochemical, genetic or environmental CKD risk factor in our dataset. For example, although variability in biomarkers of vitamin K status is mostly attributed to non-genetic factors [34], polymorphisms in vitamin K epoxide reductase C1 (*VKORC1*), for which phylloquinone is a substrate, have been shown to be associated with cross-sectional measures of plasma phylloquinone [35, 36]. *VKORC1* haplotypes have also been shown to be associated with vascular calcification in rats [37], accelerated renal allograft loss in humans [38] and aortic calcification in the Rotterdam Study [36]. As such, it is possible that similar polymorphisms in genes involved in phylloquinone metabolism may result in higher plasma phylloquinone levels due to reduced phylloquinone recycling or metabolism, i.e. a functional phylloquinone deficiency state. A similar functional deficiency could result from a variety of factors, such as altered phylloquinone transport, disrupted cellular uptake of phylloquinone or altered conversion of phylloquinone to vitamin K₂, and these potential mechanisms are interesting avenues for further study. A second possible explanation for these findings may be that individuals at risk for CKD have a different response to di-

etary phylloquinone, which may account for higher circulating levels. Clinical trials of phylloquinone to date have primarily focused on bone disease and have tended to screen out individuals with renal abnormalities. Finally, it is possible that this finding may be a false positive, and external replication of these results is required. However, we observed decreasing %ucOC with increasing phylloquinone quartiles (*p* for trend <0.0001), indicative of increasing phylloquinone bioactivity occurring in line with plasma levels, which argues against artifactual phylloquinone elevation in the highest quartile.

The fact that phylloquinone was only associated with CKD risk in the quartile analysis, and not when analyzed as continuous variable, may perhaps be explained by the presence of a 'threshold effect'. Similar threshold relationships have been noted for other biomarkers of incident CKD in the general population, such as plasma phosphorus level [39].

Evidence that 25(OH)D deficiency plays a role in kidney disease initiation is lacking, although limited animal data suggests it may influence progression of kidney disease. For example, activated vitamin D negatively regulates both the renin-angiotensin system [40] and production of TGF- β_1 [41], a key promoter of renal fibrosis in mice. Furthermore, 25(OH)D may be necessary for maintenance of podocyte structure and prevention of pathologic mesangial cell proliferation in response to renal injury [42]. Studies in humans of 25(OH)D and progression of kidney disease are scarce, although two small studies suggest a potential benefit of active vitamin D use in slowing the progression of kidney disease [43, 44]. Furthermore, a recent NHANES III analysis found that participants with 25(OH)D levels <15 ng/ml were more likely to progress to end-stage renal disease compared to those without deficiency [45]. Importantly, the excess risk for developing ESRD was primarily seen in non-Hispanic black individuals in that study. The fact that Framingham participants are white and generally of northern European descent is a critical difference, and may explain the lack of association in the present analysis.

Contrasting with these earlier studies, we did not observe any association with 25(OH)D deficiency and incident CKD or albuminuria. It should be emphasized that half of the cohort had 25(OH)D levels below current recommended guidelines, hence there was a sufficient distribution with which to identify an effect if it truly existed. Consistent with our null findings, two recent systemic reviews found that the available evidence that vitamin D influences cardiovascular outcomes is incon-

sistent and contradictory [46, 47]. Furthermore, a recent Institute of Medicine Committee report concluded that the evidence that vitamin D supplementation reduces the risk of non-skeletal chronic diseases is inconclusive, fails to establish a cause-and-effect relationship and is insufficient to inform nutritional recommendations [48]. While hypothesis-generating observational and pharmaco-epidemiological studies have stimulated much interest in the potential beneficial effects of vitamin D therapy in CKD amongst other diseases, large randomized controlled trials are now required to test the hypothesis that vitamin D therapy improves clinical outcomes. The planned NIH-sponsored VITAL study (ClinicalTrials.gov Identifier: NCT01169259) will attempt to clarify the role of vitamin D supplementation for these indications.

Our study has important implications. Existing observational studies that suggest a beneficial effect of phylloquinone on cardiovascular risk generally utilize dietary intake estimates rather than plasma levels [49–51]. These studies may be confounded by phylloquinone intake being primarily a marker of a healthy lifestyle. As the present study indicates potential harm associated with higher plasma phylloquinone, it is essential to validate these findings in independent samples and determine the mechanism of excess CKD risk, which may be independent of dietary intake.

The richness of the dataset with well-defined cardiovascular disease risk factors and long duration of follow-up considerably strengthens our analysis. However, several limitations must also be acknowledged. First, higher cross-sectional rates of hypertension, use of antihypertensive medications and obesity were present in the highest phylloquinone group. While every effort was made to adjust for these CKD risk factors, the possibility of residual confounding cannot be completely ruled out. Second, CKD was defined using a single creatinine measure, which may have resulted in some misclassification. However, if misclassification occurred, it would be expected to bias our results towards the null and would not account for the positive association with CKD risk seen in the highest phylloquinone quartile. Third, there is no agreed global biomarker of vitamin K status, and each of the available markers address a different component of absorption, transport and function. For that reason, we chose plasma phylloquinone as a validated biomarker of exposure to vitamin K. Plasma phylloquinone reflects recent dietary intake and supplement use and responds to manipulation of phylloquinone, as validated in controlled human feeding studies [33]. The major limitation

of this marker is its fluctuations in response to short-term changes in dietary phylloquinone intake. However, given our large sample size, this variability is likely modest and would be expected to attenuate our findings. Fourth, although the use of a single plasma phylloquinone measure as an indicator of long-term vitamin K status is imperfect, it is an acceptable measure for ranking participants over a range of levels [33]. Fifth, unlike incident cardiovascular events, the development of CKD is identified through the scheduled examination cycle. Consequently, no exact incident time for CKD can be ascertained and a survival-type analysis is not possible. Finally, the Framingham Offspring cohort participants are generally older, of northern European descent, and reside in the northeastern United States. As such, our findings should not be generalized to other ethnic/racial groups, younger individuals, or those residing in sunnier climates and have limited use of sunscreen.

Despite these limitations, we have identified that deficiency of vitamins K or D is not associated with the development of CKD. Further, an unexplained excess risk of CKD was observed in individuals with the highest plasma phylloquinone levels. Future research should be directed toward replicating these findings in independent samples.

Disclosure Statement

This material is based upon work supported by the US Department of Agriculture, under agreement No. 58-1950-4-401, the National Institute of Health (AG14759). The Framingham Heart Study is supported by the National Heart, Lung, and Blood Institute (N01-HC-25195). The funding source had no role in the design, conduct, or reporting of the study or in the decision to submit the report for publication. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the US Department of Agriculture.

References

- 1 Ronco C, Haapio M, House AA, Anavekar N, Bellomo R: Cardiorenal syndrome. *J Am Coll Cardiol* 2008;52:1527–1539.
- 2 Russo D, Palmiero G, De Blasio AP, Balletta MM, Andreucci VE: Coronary artery calcification in patients with CRF not undergoing dialysis. *Am J Kidney Dis* 2004;44:1024–1030.
- 3 Hujairi NM, Afzali B, Goldsmith DJ: Cardiac calcification in renal patients: what we do and don't know. *Am J Kidney Dis* 2004;43: 234–243.

- 4 Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM: Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension* 2001;38:938–942.
- 5 Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM: Impact of aortic stiffness on survival in end-stage renal disease. *Circulation* 1999;99:2434–2439.
- 6 Garcia-Canton C, Bosch E, Ramirez A, Gonzalez Y, Auyanet I, Guerra R, et al: Vascular calcification and 25-hydroxyvitamin D levels in non-dialysis patients with chronic kidney disease stages 4 and 5. *Nephrol Dial Transplant* 2011;26:2250–2256.
- 7 Hruska KA, Mathew S, Davies MR, Lund RJ: Connections between vascular calcification and progression of chronic kidney disease: therapeutic alternatives. *Kidney Int Suppl* 2005;99:142–151.
- 8 Holden RM, Morton AR, Garland JS, Pavlov A, Day AG, Booth SL: Vitamins K and D status in stages 3–5 chronic kidney disease. *Clin J Am Soc Nephrol* 2010;5:590–597.
- 9 Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, Torgerson DJ: Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med* 2006;166:1256–1261.
- 10 Wolff AE, Jones AN, Hansen KE: Vitamin D and musculoskeletal health. *Nat Clin Pract Rheumatol* 2008;4:580–588.
- 11 Shobeiri N, Adams MA, Holden RM: Vascular calcification in animal models of CKD: a review. *Am J Nephrol* 2010;31:471–481.
- 12 Krueger T, Westenfeld R, Ketteler M, Schurgers LJ, Floege J: Vitamin K deficiency in CKD patients: a modifiable risk factor for vascular calcification? *Kidney Int* 2009;76:18–22.
- 13 Zittermann A, Schleithoff SS, Koerfer R: Vitamin D and vascular calcification. *Curr Opin Lipidol* 2007;18:41–46.
- 14 Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, et al: Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. *J Nutr* 2004;134:3100–3105.
- 15 Young KA, Snell-Bergeon JK, Naik RG, Hokanson JE, Tarullo D, Gottlieb PA, et al: Vitamin D deficiency and coronary artery calcification in subjects with type 1 diabetes. *Diabetes Care* 2011;34:454–458.
- 16 Schurgers LJ, Spronk HM, Soute BA, Schiffrin PM, DeMey JG, Vermeer C: Regression of warfarin-induced medial elastocalciosis by high intake of vitamin K in rats. *Blood* 2007;109:2823–2831.
- 17 Deb DK, Sun T, Wong KE, Zhang Z, Ning G, Zhang Y, et al: Combined vitamin D analog and AT1 receptor antagonist synergistically block the development of kidney disease in a model of type 2 diabetes. *Kidney Int* 2010;77:1000–1009.
- 18 Zhang Y, Deb DK, Kong J, Ning G, Wang Y, Li G, et al: Long-term therapeutic effect of vitamin D analog doxercalciferol on diabetic nephropathy: strong synergism with AT1 receptor antagonist. *Am J Physiol Renal Physiol* 2009;297:F791–F801.
- 19 De Zeeuw D, Agarwal R, Amdahl M, Audhya P, Coyne D, Garimella T, et al: Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. *Lancet* 2010;376:1543–1551.
- 20 Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP: An investigation of coronary heart disease in families. The Framingham Offspring Study. *Am J Epidemiol* 1979;110:281–290.
- 21 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;282:408–421.
- 22 Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;130:461–470.
- 23 Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, et al: Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 2002;39:920–929.
- 24 Eknoyan G, Hostetter T, Bakris GL, Hebert L, Levey AS, Parving HH, et al: Proteinuria and other markers of chronic kidney disease: a position statement of the National Kidney Foundation (NKF) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). *Am J Kidney Dis* 2003;42:617–622.
- 25 Hsu CC, Brancati FL, Astor BC, Kao WH, Steffes MW, Folsom AR, et al: Blood pressure, atherosclerosis, and albuminuria in 10,113 participants in the atherosclerosis risk in communities study. *J Hypertens* 2009;27:397–409.
- 26 Dawson-Hughes B, Harris SS, Dallal GE: Plasma calcidiol, season, and serum parathyroid hormone concentrations in healthy elderly men and women. *Am J Clin Nutr* 1997;65:67–71.
- 27 Mattix HJ, Hsu CY, Shaykevich S, Curhan G: Use of the albumin/creatinine ratio to detect microalbuminuria: implications of sex and race. *J Am Soc Nephrol* 2002;13:1034–1039.
- 28 Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, et al: Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 1997;386:78–81.
- 29 Shea MK, O'Donnell CJ, Hoffmann U, Dallal GE, Dawson-Hughes B, Ordovas JM, et al: Vitamin K supplementation and progression of coronary artery calcium in older men and women. *Am J Clin Nutr* 2009;89:1799–1807.
- 30 Braam LA, Hoeks AP, Brouns F, Hamulyak K, Gerichhausen MJ, Vermeer C: Beneficial effects of vitamins D and K on the elastic properties of the vessel wall in postmenopausal women: a follow-up study. *Thromb Haemost* 2004;91:373–380.
- 31 Shea MK, Booth SL, Massaro JM, Jacques PF, D'Agostino RB Sr, Dawson-Hughes B, et al: Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. *Am J Epidemiol* 2008;167:313–320.
- 32 Institute of Medicine 2001: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, National Academy Press, 2001.
- 33 McKeown NM, Jacques PF, Gundberg CM, Peterson JW, Tucker KL, Kiel DP, et al: Dietary and nondietary determinants of vitamin K biochemical measures in men and women. *J Nutr* 2002;132:1329–1334.
- 34 Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino RB Sr, et al: Genetic and non-genetic correlates of vitamins K and D. *Eur J Clin Nutr* 2009;63:458–464.
- 35 Crosier MD, Peter I, Booth SL, Bennett G, Dawson-Hughes B, Ordovas JM: Association of sequence variations in vitamin K epoxide reductase and γ -glutamyl carboxylase genes with biochemical measures of vitamin K status. *J Nutr Sci Vitaminol (Tokyo)* 2009;55:112–119.
- 36 Teichert M, Visser LE, van Schaik RH, Hofman A, Uitterlinden AG, De Smet PA, et al: Vitamin K epoxide reductase complex subunit 1 (VKORC1) polymorphism and aortic calcification: the Rotterdam Study. *Arterioscler Thromb Vasc Biol* 2008;28:771–776.
- 37 Kohn MH, Price RE, Pelz HJ: A cardiovascular phenotype in warfarin-resistant VKORC1 mutant rats. *Artery Res* 2008;2:138–147.
- 38 Quteineh L, Verstuyft C, Durrbach A, Lettierie A, Ferlicot S, Charpentier B, et al: Impact of VKORC1 haplotypes on long-term graft function in kidney transplantation. *Transplantation* 2008;86:779–783.
- 39 O'Seaghdha CM, Hwang SJ, Muntner P, Melamed ML, Fox CS: Serum phosphorus predicts incident chronic kidney disease and end-stage renal disease. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association 2011; 26:2885–2890.
- 40 Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP: 1,25-Dihydroxyvitamin D₃ is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 2002;110:229–238.
- 41 Makibayashi K, Tatematsu M, Hirata M, Fukushima N, Kusano K, Ohashi S, et al: A vitamin D analog ameliorates glomerular injury on rat glomerulonephritis. *Am J Pathol* 2001;158:1733–1741.

- 42 Migliori M, Giovannini L, Panichi V, Filippi C, Taccola D, Origlia N, et al: Treatment with 1,25-dihydroxyvitamin D₃ preserves glomerular slit diaphragm-associated protein expression in experimental glomerulonephritis. *Int J Immunopathol Pharmacol* 2005;18:779–790.
- 43 Chan JC, Kodroff MB, Landwehr DM: Effects of 1,25-dihydroxyvitamin-D₃ on renal function, mineral balance, and growth in children with severe chronic renal failure. *Pediatrics* 1981;68:559–571.
- 44 Coen G, Mazzaferro S, Manni M, Fondi G, Perruzza I, Pasquali M, et al: No acceleration and possibly slower progression of renal failure during calcitriol treatment in predialysis chronic renal failure. *Nephrol Dial Transplant* 1994;9:1520.
- 45 Melamed ML, Astor B, Michos ED, Hostetter TH, Powe NR, Muntner P: 25-Hydroxyvitamin D levels, race, and the progression of kidney disease. *J Am Soc Nephrol* 2009;20:2631–2639.
- 46 Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, et al: Systematic review: vitamin D and cardiometabolic outcomes. *Ann Intern Med* 2010;152:307–314.
- 47 Wang L, Manson JE, Song Y, Sesso HD: Systematic review: vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med* 2010;152:315–323.
- 48 Institute of Medicine 2011: Dietary Reference Intakes for Calcium and Vitamin D. Washington, National Academy Press, 2010.
- 49 Erkkila AT, Booth SL, Hu FB, Jacques PF, Lichtenstein AH: Phylloquinone intake and risk of cardiovascular diseases in men. *Nutr Metab Cardiovasc Dis* 2007;17:58–62.
- 50 Erkkila AT, Booth SL, Hu FB, Jacques PF, Manson JE, Rexrode KM, et al: Phylloquinone intake as a marker for coronary heart disease risk but not stroke in women. *Eur J Clinical Nutr* 2005;59:196–204.
- 51 Braam L, McKeown N, Jacques P, Lichtenstein A, Vermeer C, Wilson 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;104:1410–1414.