

Vitamins K and D Status in Stages 3–5 Chronic Kidney Disease

Rachel M. Holden,* A. Ross Morton,* Jocelyn S. Garland,* Andrey Pavlov,[†]
Andrew G. Day,[‡] and Sarah L. Booth[‡]

*Division of Nephrology, Queen's University, Kingston, Ontario, Canada; [†]Clinical Research Centre, Kingston General Hospital, Kingston, Ontario, Canada; and [‡]Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center of Aging at Tufts University, Boston, Massachusetts

Background and objectives: Vitamin K, vitamin K-dependent proteins, and vitamin D may be involved in the regulation of calcification in chronic kidney disease (CKD).

Design, setting, participants, & measurements: Vitamin K and D status was measured as dietary intake, plasma phylloquinone, serum percent uncarboxylated osteocalcin (%ucOC), proteins induced by vitamin K absence (PIVKA-II), Vitamin K Epoxide Reductase single-nucleotide polymorphism, apolipoprotein E genotype, and plasma 25-hydroxyvitamin D (25(OH)D) in 172 subjects with stage 3 to 5 CKD. Nutritional status was determined by subjective global assessment.

Results: Subclinical vitamin K deficiency criteria was met by 6% (phylloquinone), 60% (%ucOC), and 97% (PIVKA-II) of subjects, whereas 58.3% and 8.6% had 25(OH)D insufficiency and deficiency, respectively. Dietary vitamin K intake was associated with higher phylloquinone and lower PIVKA-II. There were positive correlations between phylloquinone and the presence of stable weight, and the absence of subcutaneous fat loss or muscle wasting. 25(OH)D levels were positively associated with stable weight and albumin ($P < 0.001$). PIVKA-II levels were associated with apolipoprotein E genotype. Higher %ucOC and lower 25(OH)D were similarly associated with CKD stage, parameters of mineral metabolism, and urine albumin to creatinine ratio.

Conclusions: These data indicate that a suboptimal vitamin K and D status is prevalent in patients with CKD. Sufficiency of both vitamins K and D was similarly predicted by measures of overall improved nutritional status. Proteinuria was associated with both a suboptimal vitamin D status as well as worse peripheral vitamin K status.

Clin J Am Soc Nephrol 5: 590–597, 2010. doi: 10.2215/CJN.06420909

The majority of patients with stage 3 to 5 chronic kidney disease (CKD) will die of a cardiovascular event before they require renal replacement therapy (RRT). CKD-specific risk factors contribute substantially to vascular calcification and the burden of cardiovascular disease in this patient population, which is not true for the general population (1). Abnormal mineral metabolism is, in part, responsible for the severity of vascular calcification observed (1). *In vitro* studies indicate that in the presence of hyperphosphatemia, vascular smooth muscle cells transform into osteoblast-like cells that can express proteins that regulate mineralization (2). Two of these proteins, matrix Gla protein (MGP) and osteocalcin (OC), are regulators of tissue mineralization in the arterial wall and bone, respectively (3). OC and matrix Gla protein expressions are up-regulated by vitamin D and dependent on vitamin K for their calcium-binding capacity (4). We and others have demonstrated that subclinical vitamin K deficiency is prevalent in patients requiring RRT (5–8), whereas a suboptimal vitamin D

status at the point of dialysis initiation has been linked to mortality in the CKD population (9). However, to the best of our knowledge, the dietary and nondietary correlates of vitamin K and vitamin D biochemical markers have not been studied concurrently in patients who are in earlier stages of CKD (stages 3 to 5).

Phylloquinone (vitamin K₁) is the primary form of vitamin K in circulation and reflects recent dietary intake (10). OC is the major noncollagenous protein product of the mature osteoblast. The proportion of circulating OC that is not γ -carboxylated (%ucOC) is considered a sensitive measure of vitamin K status in bone in the non-CKD population. Elevated protein induced in the vitamin K absence or antagonism-factor II (PIVKA-II) reflects abnormalities in vitamin K-dependent prothrombin before prolongation of coagulation occurs and increases with dietary vitamin K restriction. PIVKA-II has the added advantage in this population of not being affected by kidney function. Vitamin K status may also be determined by variations in the genes involved in the transport or uptake of vitamin K into liver and bone (apolipoprotein E (apoE)) or those involved in the tissue-specific recycling of vitamin K (Vitamin K Epoxide Reductase (VKOR)). There are few studies evaluating the effect of variations within these two genes and vitamin K status in either the general population or in those with CKD (6,11–13).

Received September 11, 2009. Accepted January 25, 2010.

Published online ahead of print. Publication date available at www.cjasn.org.

Correspondence: Dr. Rachel M. Holden, 3048C Etherington Hall, Queen's University, Kingston, ON, Canada K7L 3V6. Phone: 613-533-3134; Fax: 613-533-3292; E-mail: holdenr@kgh.kari.net

Circulating concentrations of 25-hydroxyvitamin D (25(OH)D) are a sensitive measure of vitamin D status that reflect both intake and endogenous production in response to ultraviolet B exposure (14,15). Several epidemiologic studies have reported that patients with stage 3 to 5 CKD have lower 25(OH)D concentrations than the general population (7,8), but this appears to be independent of the actual estimated GFR (eGFR). The identification of extrarenal 1 α -hydroxylase suggests that 25(OH)D status is an important consideration in patients with CKD (16). The objective of this study was to determine both the dietary and nondietary determinants of vitamin K and vitamin D status in a cohort of patients with stage 3 to 5 CKD not requiring RRT.

Materials and Methods

Study Population

This cross-sectional study included 174 patients with stage 3 to 5 CKD receiving nephrology care at Kingston General Hospital, Ontario, Canada. Consecutive patients were approached at their CKD clinic visit. All patients provided informed consent according to the Declaration of Helsinki. Procedures were in accordance with the ethics standards of Queen's University and approved by the Tufts Medical Center Institutional Review Board (Boston, MA). Of 791 patients considered for this study, 210 consented for enrollment and 23 subsequently withdrew. Patients were not enrolled for the following reasons: unwilling to participate ($n = 217$); warfarin or bisphosphonate exposure ($n = 104$); and not CKD stages 3 to 5 ($n = 275$). Of the 172 patients completing the study, blood samples were available for analysis as follows: phylloquinone ($n = 162$), %ucOC ($n = 163$), PIVKA-II ($n = 108$), 25(OH)D ($n = 163$), apoE genotype ($n = 161$), and VKOR polymorphism ($n = 161$). Fewer PIVKA-II samples were available because of an error in sample storage that occurred in the first 70 patients enrolled in the study. All 172 patients underwent clinical evaluation of nutritional status (subjective global assessment (SGA)) by a registered renal dietician, and 168 successfully completed their food frequency questionnaire (FFQ).

Analytical Measures

Clinical data were abstracted by patient interview and chart review and included age, gender, eGFR, history of coronary artery disease, history of peripheral vascular disease (PVD), history of diabetes mellitus, etiology of CKD, and smoking status. The eGFR was determined by the four-variable Modification of Diet in Renal Disease (MDRD) equation. Cardiovascular disease was determined by both current symptoms (patient interview) using Canadian Cardiovascular Society criteria for heart failure and angina and history of cardiovascular events, as well as detailed chart review. PVD included transient ischemic attack, cerebral vascular accident, and claudication. Weight and height were collected to calculate body mass index (BMI). Current smokers were defined as patients smoking at least one cigarette per day during the previous 6 months.

Laboratory Measures

Laboratory measures included ionized calcium (mmol/L), phosphate (mmol/L), intact parathyroid hormone (PTH) (pmol/L), alkaline phosphatase (IU/L), serum albumin (g/L), serum C-reactive protein (hsCRP) (mg/L), total cholesterol (mmol/L), LDL cholesterol (mmol/L), HDL cholesterol (mmol/L), and triglycerides (mmol/L). Intact PTH was assessed by means of electrochemiluminescence (Roche, Basel, Switzerland) modular analytics E170 immunoassay. Serum albumin was measured by the bromocresol green method (Roche, Basel, Swit-

zerland). hsCRP was assessed using a Roche modular immunoturbidimetric assay. Serum creatinine (Roche Creatinine Plus Modular) was used in the MDRD Study equation to determine eGFR. All of the aforementioned analyses were performed in the laboratory at Kingston General Hospital to minimize interlaboratory variability. Blood samples were stored at -80°C before the analysis of phylloquinone, %ucOC, and PIVKA-II. Fasting phylloquinone concentrations were measured using reversed-phase HPLC (17). OC was measured in serum by an RIA that uses human OC for standard and tracer and a polyclonal antibody that recognizes intact OC and the large N-terminal midmolecule fragment. ucOC was determined in this assay as OC that does not bind *in vitro* to hydroxyapatite (18). The ucOC is expressed as a percentage of the total OC that is not carboxylated (%ucOC), and a value $>20\%$ is consistent with subclinical vitamin K deficiency. This cutoff point is specific to this assay and should not be considered generalizable to other assays used for determination of %ucOC. PIVKA-II concentrations were determined by ELISA (Diagnostic Stago, Parsippany, NJ). Genotyping of both apoE and the Vitamin K Epoxide Reductase Complex Subunit (VKORC1) G1542C (rs8050894) single-nucleotide polymorphism was performed by PCR/restriction fragment length polymorphism analysis using real-time PCR using TaqMan-based assays implemented on an Applied Biosystems ABI Prism 7000 instrument (AMJ Laboratory Services Ltd., Millgrove, Canada). The G1542C VKORC1 polymorphism is associated with decreased warfarin doses in heterozygous and homozygous mutant patients (19). Plasma 25(OH)D was measured by RIA (DiaSorin; MDS Laboratory Services, Toronto, Canada).

Dietary Vitamin K Intake

Dietary intake of vitamin K and vitamin D was assessed by a semi-quantitative 126-item FFQ validated for vitamin K intake in North America (20). Successfully completed FFQs ($n = 168$) were sent to the Harvard School of Public Health for analysis of habitual intakes of vitamin K, vitamin D, and total energy over the previous 12 months. The FFQ results of four patients were excluded from the analysis because of implausible dietary caloric intake (≥ 3500 kcal/d).

Clinical Assessment of Nutrition

The SGA combines data from subjective and objective aspects of medical history (weight change, dietary intake change, gastrointestinal symptoms, and changes in functional capacity) and physical examination (loss of subcutaneous fat, muscle wasting, ankle or sacral edema, and ascites). Patients are categorized into three distinct classes: well nourished, moderately malnourished, and severely malnourished. The SGA has been validated in many diverse populations, including individuals with CKD (21,22).

Statistical Analyses

All data were analyzed using the SAS software package (version 9.1; SAS Institute, Cary, NC). Descriptive statistics (mean \pm SD for continuous data and frequency for categorical values) were generated for all variables. Spearman correlation coefficient was used to evaluate bivariate relationships between continuous and binary variables. An Eta statistic was used to evaluate the bivariate relationship between categorical variables with more than two levels (*i.e.*, VKOR and stage of CKD). Multivariable linear regression models were applied to determine independent predictors of vitamin K and D status using the backward-selection method. Variables with a strong positive skew were log transformed before regression modeling. All statistical tests were two sided, and an unadjusted P value of 0.05 or less was considered statistically significant.

Results

The demographics of the study population are listed in Table 1. There were 172 patients with stage 3 to 5 CKD enrolled, of

whom 36 (20.7%) were moderately malnourished. No cases of severe malnutrition were identified.

There was no association between dietary intake of vitamin K

Table 1. Demographic and clinical features of study population

Variables	<i>n</i> (%)	Mean ± SD	Range
Clinical characteristics (<i>n</i> = 172)			
age, yrs		61 ± 14	22 to 85
gender (male)	106 (60.9)		
eGFR, ml/min per 1.73 m ²		26 ± 12	5.3 to 58
CKD stage 3	52 (29.9)		
CKD stage 4	89 (51.1)		
CKD stage 5	33 (19.0)		
etiology of CKD			
diabetes mellitus	78 (44.8)		
renovascular disease	35 (20.1)		
glomerular disease	20 (11.5)		
other	41 (23.6)		
cardiovascular disease	32 (18.4)		
peripheral vascular disease	21 (12.1)		
smoking	28 (16.1)		
Nutritional parameters (<i>n</i> = 172)			
BMI, kg/m ²		31.4 ± 7.6	15.0 to 64.9
SGA, score/7		6.2 ± 0.9	3.0 to 7.0
well nourished	138 (79.3)		
moderately malnourished	36 (20.7)		
total energy, kcal/d		1839 ± 633	548 to 3779
Vitamin K status			
dietary vitamin K intake, μg/d (<i>n</i> = 168)		130 ± 103	17.3 to 740
phylloquinone concentration, nmol/L (<i>n</i> = 162)		2.1 ± 2.4	0 to 19.3
%ucOC (<i>n</i> = 163)		26.8 ± 22.3	0 to 89.0
PIVKA-II (<i>n</i> = 108)		3.62 ± 1.4	1.7 to 9.0
VKOR polymorphism (<i>n</i> = 161)			
G/G	18 (10.3)		
G/C	82 (47.1)		
C/C	63 (36.2)		
apolipoprotein E genotype (<i>n</i> = 161)			
E4	55 (34.2)		
no E4	106 (65.8)		
Vitamin D status (<i>n</i> = 163)			
vitamin D intake (diet + supplements), IU		473 ± 379	28.1 to 2091
25(OH)D, nmol/L		67.2 ± 26.0	24 to 151
Laboratory measures (<i>n</i> = 172)			
phosphate, mmol/L		1.3 ± 0.3	0.5 to 2.4
alkaline phosphatase, mmol/L		85.2 ± 30.8	28.0 to 217
parathyroid hormone, pmol/L		13.8 ± 13.3	0.8 to 86.7
albumin, g/L		40.6 ± 4.7	22.2 to 51.8
total cholesterol, mmol/L		4.7 ± 1.4	0.7 to 12.8
LDL cholesterol, mmol/L		2.3 ± 1.0	0.4 to 5.7
HDL cholesterol, mmol/L		1.3 ± 0.4	0.5 to 3.5
triglycerides, mmol/L		2.5 ± 2.3	0.6 to 25.6
CRP, mg/L		6.3 ± 7.3	0.5 to 38.8
urinary ACR		103 ± 147	0.3 to 724

ACR, albumin to creatinine ratio.

and stage of CKD. Patients were more likely to have higher vitamin K intake if they consumed more calories ($P < 0.001$), were female ($P = 0.004$), were older ($P = 0.04$), and if they did not report symptoms of anorexia at their SGA ($P = 0.01$).

Subclinical vitamin K deficiency (normal range: 0.29 to 2.65 nmol/L) was identified in 10 patients (6.0%). As shown in Tables 2 and 3, higher phylloquinone concentrations were associated with dietary vitamin K intake, higher total cholesterol, triglycerides, and LDL cholesterol, but not HDL cholesterol,

whereas lower concentrations of phylloquinone were associated with a previous history of PVD. Nutritional variables associated with higher phylloquinone concentrations included stable weight, presence of subcutaneous fat, and the absence of muscle wasting. The single independent predictor of phylloquinone concentration was triglyceride concentration (Table 4). There was no association between phylloquinone concentration and stage of CKD. Subclinical vitamin K deficiency criteria defined as $>20\%$ ucOC was met by 98 patients (60%). Higher

Table 2. Cross-sectional correlations among measures of vitamin K and D status and demographic, nutritional, and laboratory variables^a

Variables	Phylloquinone		%ucOC		PIVKA-II		25(OH)D	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Demographic variables								
age, yrs	−0.03	0.72	−0.05	0.55	−0.05	0.59	−0.01	0.95
gender (male)	−0.01	0.92	0.12	0.14	0.08	0.42	−0.01	0.86
eGFR, ml/min per 1.73 m ²	−0.04	0.62	−0.26	0.01	−0.12	0.23	0.20	0.009
CKD stage ^b	0.12	0.32	0.32	<0.01	0.15	0.32	0.21	0.023
etiology of CKD ^c	0.06	0.89	0.26	0.01	0.12	0.67	−0.29	0.003
diabetes mellitus	−0.15	0.07	−0.20	0.01	0.00	0.98	0.16	0.05
renovascular disease	0.06	0.48	0.05	0.52	−0.11	0.26	0.10	0.22
glomerular	0.10	0.20	−0.12	0.14	0.01	0.93	0.17	0.03
other	−0.01	0.93	0.23	<0.01	0.05	0.60	−0.33	<0.01
CVD	−0.02	0.81	0.14	0.08	0.16	0.12	−0.22	0.52
PVD	−0.16	0.03	0.04	0.64	−0.03	0.80	−0.05	0.03
Smoking status	0.10	0.44	0.09	0.52	0.20	0.14	0.28	0.01
Nutritional variables								
BMI, kg/m ²	0.09	0.23	0.07	0.40	−0.01	0.89	−0.28	<0.001
SGA, score/7	0.11	0.17	0.02	0.79	0.08	0.41	−0.08	0.31
weight change	0.19	0.02	−0.06	0.43	−0.08	0.44	0.17	0.03
food intake	0.06	0.47	−0.05	0.56	−0.08	0.41	0.07	0.37
anorexia	0.05	0.50	0.02	0.77	0.03	0.78	0.05	0.54
subcutaneous fat loss	0.18	0.02	0.09	0.28	−0.04	0.68	0.00	0.98
muscle wasting	0.21	0.01	−0.03	0.67	0.06	0.53	0.01	0.85
total energy, kcal/d	0.05	0.54	−0.09	0.24	−0.10	0.33	0.03	0.68
Laboratory variables								
phosphate, mmol/L	−0.01	0.92	0.32	<0.001	0.14	0.15	−0.18	0.02
calcium, mmol/L	0.11	0.17	−0.06	0.45	0.00	0.99	0.25	0.001
ALP, mmol/L	−0.11	0.18	0.17	0.03	0.00	0.98	−0.17	0.03
PTH, pmol/L	−0.12	0.13	0.38	<0.001	−0.14	0.17	−0.21	0.01
albumin, g/L	−0.08	0.71	−0.14	0.07	−0.06	0.53	0.41	<0.001
total cholesterol, mmol/L	0.38	<0.001	−0.08	0.34	0.12	0.24	0.13	0.09
LDL cholesterol, mmol/L	0.25	0.01	−0.09	0.26	0.03	0.76	0.19	0.02
HDL cholesterol, mmol/L	−0.03	0.74	−0.04	0.63	−0.12	0.25	0.21	0.01
triglycerides, mmol/L	0.56	<0.001	−0.08	0.34	0.22	0.03	−0.05	0.53
CRP, mg/L	−0.08	0.34	0.21	0.01	0.15	0.16	−0.23	0.01
urinary ACR	0.15	0.06	0.32	<0.001	0.12	0.25	−0.38	<0.001

^aAll *r* values are Spearman correlation coefficient unless noted otherwise. ALP, alkaline phosphatase; CVD, cardiovascular disease; ACR, albumin to creatinine ratio.

^b*r* values are not Spearman correlation coefficient; the reported statistic is Eta (square root of the ANOVA R^2), and the *P* value is for the ANOVA F-test.

^cSpearman ρ for the dummy variable, supplied with the raw *P* value, and the *P* value-adjusted multiple comparison by the stepdown Bonferroni method.

Table 3. Correlations between vitamin K biomarkers and vitamin D (25(OH)D) and predictors of vitamin K status (dietary vitamin K intake, apolipoprotein E genotype, VKOR polymorphism)

Variables	Phylloquinone	P	%ucOC	P	PIVKA-II	P
Vitamin K						
phylloquinone			−0.15	0.06	−0.05	0.60
%ucOC	−0.15	0.06			0.01	0.99
PIVKA-II	−0.05	0.60	0.01	0.99		
Dietary vitamin K intake	0.15	0.05	−0.08	0.29	−0.20	0.05
VKOR polymorphism ^a	0.13	0.28	0.09	0.53	0.05	0.897
apoE4 allele <i>versus</i> all others	0.00	0.96	0.00	0.99	0.23	0.021
Vitamin D						
25(OH)D	−0.15	0.06	−0.01	0.94	−0.16	0.12

^aEta statistic.

Table 4. Multivariable linear regression models and significant predictors of phylloquinone, %ucOC, PIVKA-II, and 25(OH)D

	β Coefficient	95% Confidence Interval	P
Log phylloquinone; $R^2 = 0.31$ ($n = 156$)			
log triglycerides	0.95	0.72 to 1.19	<0.001
log dietary vitamin K	0.19	−0.01 to 0.39	0.06
%ucOC (positive); $R^2 = 0.04$ ($n = 105$)			
log CRP	3.15	0.26 to 6.05	0.03
Log PIVKA-II; $R^2 = 0.20$ ($n = 93$)			
log phylloquinone	−0.08	−0.16 to −0.01	0.03
log triglycerides	0.19	0.05 to 0.32	0.007
log dietary vitamin K	−0.12	−0.21 to −0.03	0.011
ApoE4 allele	0.16	0.03 to 0.29	0.016
Log 25(OH)D; $R^2 = 0.28$ ($n = 156$)			
log phosphate	−0.19	−0.38 to −0.01	0.039
log calcium	0.21	0.10 to 0.33	<0.001
CKD stage 3	0.23	0.07 to 0.39	0.006
CKD stage 4	0.13	−0.01 to −0.27	0.075
log BMI	−0.28	−0.51 to −0.06	0.015
albumin	0.03	0.02 to 0.04	<0.0001

%ucOC (and therefore worse vitamin K status) was associated with stage of CKD, diabetes mellitus, higher alkaline phosphatase, PTH, CRP, and urinary albumin loss (Table 2). Higher values of %ucOC were independently associated with CRP.

Elevated PIVKA-II concentrations (normal range <2 nM/L) (and therefore suboptimal vitamin K status) were identified in 97% of patients. Higher PIVKA-II concentrations were associated with triglycerides and the apoE4 allele. Independent predictors of PIVKA-II included dietary intake of vitamin K, phylloquinone, and triglyceride concentrations, as well as the apoE4 allele (presence of apoE4 increases PIVKA-II by an average of 16%).

There was no association between the polymorphisms of VKOR and any of the biomarkers of vitamin K status.

A total of 58.3% of patients had 25(OH)D insufficiency (defined as <75 nmol/L), and 8.6% had 25(OH)D deficiency (defined as <37.5 nmol/L) (Table 1). Higher total intake of vitamin D was

associated with higher serum 25(OH)D concentrations, the absence of anorexia, and lower PTH concentrations (Table 2).

Lower 25(OH)D concentrations were associated with poor kidney function and with cardiac risk factors, such as high BMI, high CRP, and higher urinary albumin to creatinine ratio, as well as with a prior history of PVD. Patients with diabetes mellitus had significantly lower 25(OH)D concentrations than all other groups. Lower 25(OH)D concentrations were, as expected, associated with lower total calcium and higher concentrations of phosphate, PTH, and alkaline phosphatase. Nutritional factors associated with higher 25(OH)D concentrations were stable weight, higher albumin, HDL cholesterol, and LDL cholesterol concentrations (Table 2).

We did not find any association between 25(OH)D and any measure of vitamin K status. Independent predictors of lower 25(OH)D concentrations included higher CRP and a more advanced stage of CKD as well as phosphate, calcium, and BMI.

Discussion

This study confirms that a substantial proportion of patients with stages 3 to 5 CKD have a suboptimal status of both vitamin K and D. Whereas poor vitamin D status was associated with poor renal function, the associations between individual measures of vitamin K status and renal function were less consistent.

Higher dietary intakes of vitamin K were associated with overall healthier dietary patterns, consistent with findings from studies of community-dwelling adults (23,24). Higher intake of vitamin K was weakly associated with higher circulating concentrations of phylloquinone and independently associated with lower concentrations of PIVKA-II (indicative of improved peripheral vitamin K status).

Individuals with stage 3 to 5 CKD have substantially higher phylloquinone concentrations (mean: 2.1 ± 2.4 nmol/L) than we have previously reported among individuals with CKD who require hemodialysis (mean: 0.99 ± 1.12 nmol/L) or peritoneal dialysis (median: 0.7 nmol/L; range: 0 to 2.2 nmol/L) (5,6). Consistent with the literature from healthy community-dwelling populations, we found very strong correlations between phylloquinone concentrations and total cholesterol, LDL cholesterol, and triglycerides. The lipid abnormalities that accompany CKD, primarily characterized by hypertriglyceridemia and low HDL cholesterol, may be implicated. However, when we expressed vitamin K as a ratio of phylloquinone concentrations to triglyceride concentrations, our overall findings did not change (data not shown). This led us to conclude that patients who were clinically better nourished (stable weight, absence of muscle wasting, or loss of subcutaneous fat) have better vitamin K status, which was not unexpected.

Measurement of the circulating carboxylation fraction of vitamin K-dependent proteins (VKDPs) is a sensitive functional indicator of subclinical vitamin K deficiency. Considering %uOC, 60% of patients in this study met criteria for subclinical vitamin K deficiency. The mean %uOC was lower than we have reported in individuals requiring RRT (5,6). However, %uOC may not be an ideal marker of vitamin K status in advanced CKD because of the combination of bone resorption that occurs with underlying secondary hyperparathyroidism and the potential for retention of OC fragments in the setting of reduced kidney function. Indeed, we observed strong correlations in this cohort between %uOC and stage of CKD, parameters of mineral metabolism and urinary loss of protein. To the best of our knowledge, the relationship between urinary albumin loss and worse vitamin K status in bone has not been reported.

Given the concerns regarding the utility of %uOC as a biomarker in patients with CKD, we also evaluated the carboxylation status of prothrombin as a potentially superior marker of vitamin K deficiency in this population. In this study, more than 90% of patients had elevated PIVKA-II concentrations, confirming that a peripheral subclinical vitamin K status is highly prevalent. PIVKA-II concentrations were independently associated with dietary intake, phylloquinone concentrations, and triglycerides.

There is growing interest in the role of the genetic contribution to the large interindividual variation in vitamin K biomar-

kers. In this small sample, we found no effect of VKORC1 haplotypes on any vitamin K biomarkers. ApoE4 carriers may be at risk for undercarboxylated VKDPs due to rapid clearance of phylloquinone in the liver. In our study, the only significant independent association we found was between the apoE4 allele and higher PIVKA-II concentrations. At present, the direction and magnitude of the effect of apoE genotype on γ -carboxylation status of peripheral VKDPs remain controversial (11,12). However, this study and our earlier study suggest that the apoE4 allele may be associated with poorer γ -carboxylation of VKDPs in individuals with CKD. Therefore, the apoE4 allele, carried by 34% of this CKD population, may potentially represent a nonmodifiable risk factor influencing vitamin K status (6).

Our data are concordant with those of the large Third National Health and Nutrition Survey population study, which showed significant 25(OH)D deficiency in persons with stage 4 CKD (25). Deficiency of 25(OH)D has been associated with all-cause mortality in the CKD population by mechanisms which may be independent of its role in mineral metabolism (26). Similar to others, we have observed lower 25(OH)D concentrations in association with higher urinary albumin to creatinine ratio, which likely reflect losses of 25(OH)D bound to its carrier protein (27). Although the majority of the data are from the pediatric literature, this may have particular relevance in patients with the nephrotic syndrome where cholecalciferol and calcium supplementation help maintain bone mineral density in a group of patients where the role of bisphosphonates is in doubt.

We found a significant inverse association between 25(OH)D levels and hsCRP, a relationship that has not been found in studies of patients with normal renal function. However, no change has been found in CRP concentrations in hemodialysis patients with elevated CRP who have received cholecalciferol supplementation (28). The administration of 25(OH)D to CKD patients has been the subject of a number of small studies with primarily biochemical endpoints. Ergocalciferol (vitamin D₂) has been shown to have a modest effect on PTH levels (29,30), whereas cholecalciferol had no effect in a small, double-blind, randomized trial of 20 patients (31). Given the paucity of good clinical data, Kalantar-Zadeh and Kovesdy (32) have called for randomized, controlled trials of both nutritional and pharmacologic supplements of vitamin D.

Although both vitamin K and vitamin D insufficiency coexisted in our study population, we found no association between 25(OH)D and markers of vitamin K deficiency, such as %uOC and PIVKA-II. This may simply reflect that both were equally sufficient or insufficient in individual patients. However, the results of this study do confirm that a significant proportion of patients with stage 3 CKD have subclinical vitamin K deficiency and are either insufficient or deficient in vitamin D. The major limitation of this study is its cross-sectional nature; thus, causal relationships cannot be defined. No longitudinal data have been reported here; thus, relationships between variables may change over time in different individuals. Finally, the stage of kidney disease was defined by a single creatinine measure. Balanced against these limitations, we have identified

that sufficiency of both vitamins K and D was similarly predicted by measures of overall improved nutritional status and, as such, malnourished patients should be identified as high risk. Urinary loss of protein predicted both a suboptimal vitamin D status as well as worse peripheral vitamin K status, suggesting that the proteinuric subgroup of patients with CKD may also need special consideration. Future research in the CKD population should be directed toward describing the relationship between vitamin K and D status and important clinical outcomes, such as vascular calcification (33) or loss of bone mineral density, and should also consider the effect of intervention with supplementation.

Acknowledgments

This research was conducted on the basis of work supported by the Kidney Foundation of Canada and by the Agricultural Research Service, U.S. Department of Agriculture (cooperative agreement 58-1950-7 to 707). The authors gratefully acknowledge the assistance of Francis MacLeod, James Peterson, Michelle Ival, Lisa Bartsch, Darlene Broad, and Sarah Mahdavi. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the U.S. Department of Agriculture.

Disclosures

None.

References

- Garland JS, Holden RM, Groome PA, Lam M, Nolan RL, Morton AR, Pickett W: Prevalence and associations of coronary artery calcification in patients with stages 3 to 5 CKD without cardiovascular disease. *Am J Kidney Dis* 52: 849–858, 2008
- Shanahan CM: Vascular calcification. *Curr Opin Nephrol Hypertens* 14: 361–367, 2005
- Spronk HM, Soute BA, Schurgers LJ, Cleutjens JP, Thijssen HH, De Mey JG, Vermeer C: Matrix Gla protein accumulates at the border of regions of calcification and normal tissue in the media of the arterial vessel wall. *Biochem Biophys Res Commun* 289: 485–490, 2001
- Proudfoot D, Shanahan CM: Molecular mechanisms mediating vascular calcification: Role of matrix Gla protein. *Nephrology* 11: 455–461, 2006
- Holden RM, Iliescu E, Morton AR, Booth SL: Vitamin K status of Canadian peritoneal dialysis patients. *Perit Dial Int* 28: 415–418, 2008
- Pilkey RM, Morton AR, Boffa MB, Noordhof C, Day AG, Su Y, Miller LM, Koschinsky ML, Booth SL: Subclinical vitamin K deficiency in hemodialysis patients. *Am J Kidney Dis* 49: 432–439, 2007
- Gonzalez EA, Sachdeva A, Oliver DA, Martin KJ: Vitamin D insufficiency and deficiency in chronic kidney disease. A single center observational study. *Am J Nephrol* 24: 503–510, 2004
- Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL: Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the study to evaluate early kidney disease. *Kidney Int* 71: 31–38, 2007
- Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA Jr., Tonelli M, Thadhani R: Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int* 72: 1004–1013, 2007
- Sadowski JA, Hood SJ, Dallal GE, Garry PJ: Phylloquinone in plasma from elderly and young adults: Factors influencing its concentration. *Am J Clin Nutr* 50: 100–108, 1989
- Beavan SR, Prentice A, Stirling DM, Dibba B, Yan L, Harrington DJ, Shearer MJ: Ethnic differences in osteocalcin gamma-carboxylation, plasma phylloquinone (vitamin K1) and apolipoprotein E genotype. *Eur J Clin Nutr* 59: 72–81, 2005
- Yan L, Zhou B, Nigdikar S, Wang X, Bennett J, Prentice A: Effect of apolipoprotein E genotype on vitamin K status in healthy older adults from China and the UK. *Br J Nutr* 94: 956–961, 2005
- Kohlmeier M, Saupé J, Shearer MJ, Schaefer K, Asmus G: Bone health of adult hemodialysis patients is related to vitamin K status. *Kidney Int* 51: 1218–1221, 1997
- Holick MF: Vitamin D deficiency. *N Engl J Med* 357: 266–281, 2007
- Jones G, Horst R, Carter G, Makin HL: Contemporary diagnosis and treatment of vitamin D-related disorders. *J Bone Miner Res* 22[Suppl 2]: V11–V15, 2007
- Gal-Moscovici A, Sprague SM: Role of vitamin D deficiency in chronic kidney disease. *J Bone Miner Res* 22 Suppl 2: V91–V94, 2007
- 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* 282: 408–421, 1997
- Gundberg CM, Nieman SD, Abrams S, Rosen H: Vitamin K status and bone health: An analysis of methods for determination of undercarboxylated osteocalcin. *J Clin Endocrinol Metab* 83: 3258–3266, 1998
- Vecsler M, Loebstein R, Almog S, Kurnik D, Goldman B, Halkin H, Gak E: Combined genetic profiles of components and regulators of the vitamin K-dependent γ -carboxylation system affect individual sensitivity to warfarin. *Thromb Haemost* 95: 205–211, 2006
- McKeown NM, Jacques PF, Gundberg CM, Peterson JW, Tucker KL, Kiel DP, Wilson PW, Booth SL: Dietary and nondietary determinants of vitamin K biochemical measures in men and women. *J Nutr* 132: 1329–1334, 2002
- Steiber A, Leon JB, Secker D, McCarthy M, McCann L, Serra M, Sehgal AR, Kalantar-Zadeh K: Multicenter study of the validity and reliability of subjective global assessment in the hemodialysis population. *J Ren Nutr* 17: 336–342, 2007
- Tapiawala S, Vora H, Patel Z, Badve S, Shah B: Subjective global assessment of nutritional status of patients with chronic renal insufficiency and end stage renal disease on dialysis. *J Assoc Physicians India* 54: 923–926, 2006
- Erkkila AT, Booth SL, Hu FB, Jacques PF, Manson JE, Rexrode KM, Stampfer MJ, Lichtenstein AH: Phylloquinone intake as a marker for coronary heart disease risk but not stroke in women. *Eur J Clin Nutr* 59: 196–204, 2005
- Erkkila AT, Booth SL, Hu FB, Jacques PF, Lichtenstein AH: Phylloquinone intake and risk of cardiovascular diseases in men. *Nutr Metab Cardiovasc Dis* 17: 58–62, 2007

25. Chonchol M, Scragg R: 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutritional Examination Survey. *Kidney Int* 71: 134–139, 2007
26. Barreto DV, Barreto FC, Liabeurf S, Temmar, M Boitte F, Choukroun G, Fournier A, Massy ZA: Vitamin D affects survival independently of vascular calcification in chronic kidney disease. *Clin J Am Soc Nephrol* 4: 1128–1135, 2009
27. Sato KA, Gray RW, Lemann J Jr: Urinary excretion of 25-hydroxyvitamin D in health and the nephrotic syndrome. *J Lab Clin Med* 99: 325–330, 1982
28. Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C: Daily oral 25-hydroxycholecalciferol supplementation for vitamin D deficiency in haemodialysis patients: Effects on mineral metabolism and bone markers. *Nephrol Dial Transplant* 23: 3670–3676, 2008
29. Deville J, Thorp ML, Tobin L, Gray E, Johnson ES, Smith DH: Effect of ergocalciferol supplementation on serum parathyroid hormone and serum 25-hydroxyvitamin D in chronic kidney disease. *Nephrology* 11: 555–559
30. Al-Aly Z, Qazi RA, Gonzalez EA, Zeringue A, Martin KJ: Changes in serum 25-hydroxyvitamin D and plasma intact PTH levels following treatment with ergocalciferol in patients with CKD. *Am J Kidney Dis* 50: 59–68
31. Zisman AL, Hristova M, Ho LT, Sprague SM: Impact of ergocalciferol treatment on vitamin D deficiency on serum parathyroid hormone concentrations in chronic kidney disease. *Am J Nephrol* 27: 36–43, 2007
32. Kalantar-Zadeh K, Kovesdy CP: Is it worth correcting hyperparathyroidism if hyperphosphatemia and hypocalcemia worsen? A cinacalcet story. *Am J Kidney Dis* 53: 183–188, 2009
33. 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* 76: 18–22, 2009