Vitamin K intake and status are low in hemodialysis patients

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Vitamin K is essential for the activity of γ-carboxyglutamate (Gla)-proteins including matrix Gla28 protein and osteocalcin; an inhibitor of vascular calcification and a bone matrix protein, respectively. Insufficient vitamin K intake leads to the production of non-carboxylated, inactive proteins and this could contribute to the high risk of vascular calcification in hemodialysis patients. To help resolve this, we measured vitamin K_1 and K_2 intake (4-day food record), and the vitamin K status in 40 hemodialysis patients. The intake was low in these patients (median 140 μg/day), especially on days of dialysis and the weekend as compared to intakes reported in a reference population of healthy adults (mean K_1 and K_2 intake 200 μg/day and 31 μg/day, respectively). Non-carboxylated bone and coagulation proteins were found to be elevated in 33 hemodialysis patients, indicating subclinical hepatic vitamin K deficiency. Additionally, very high non-carboxylated matrix Gla28 protein levels, endemic to all patients, suggest vascular vitamin K deficiency. Thus, compared to healthy individuals, hemodialysis patients have a poor overall vitamin K status due to low intake. A randomized controlled trial is needed to test whether vitamin K supplementation reduces the risk of arterial calcification and mortality in hemodialysis patients.

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Vitamin K is necessary for the function of proteins containing γ-carboxyglutamate (Gla) residues. Well-known vitamin K–dependent proteins (Gla proteins) are vitamin K–dependent coagulation factorsthat are mainly synthesized in the liver. Extrahepatic Gla proteins are osteocalcin (OC), synthesized in bone, and matrix Gla protein (MGP), synthesized in the vasculature and cartilage. Gla residues are formed during a vitamin K–dependent posttranslational carboxylation reaction and are essential for the activity of Gla proteins.[1] Insufficient dietary intake of vitamin K will lead to the production of uncarboxylated (i.e., inactive) proteins.[2]

MGP synthesized by vascular smooth muscle cells is the strongest physiological inhibitor of vascular calcification currently known. Deficiency of carboxylated MGP may contribute substantially to the development and progression of arterial calcification. Vascular calcifications are found in 60–80% of hemodialysis (HD) patients[3,4] and are associated with a high cardiovascular risk, independent of traditional atherogenic risk factors.[5,6] Areas of calcification in vascular tissue are associated with accumulation of uncarboxylated MGP species, which has also been found to precede the development of clinically overt calcification in children on dialysis.[7]

Vitamin K intake may be differentiated for the intake of vitamin K_1 (phylloquinone) and vitamin K_2 (group name for menaquinones). The estimated daily vitamin K_1 intake is 200 ± 98 μg and for vitamin K_2 it is 31 ± 7 μg in the general population.[8–10] Vitamin K_1 and K_2 content of food products has been extensively studied by our laboratory in the past,[11] resulting in a comprehensive dietary vitamin K_1 and K_2 database. Previous studies using this database demonstrated that intake of vitamin K_2 was inversely associated with cardiovascular calcification and mortality.[12–14]

Systemic vitamin K status can be determined by measuring (1) circulating vitamin K_1 and K_2 levels and (2) circulating inactive forms of vitamin K–dependent proteins. Intake of vitamin K–containing food products will readily influence measurements of circulating vitamin K levels. In contrast, measurements of uncarboxylated prothrombin (known as

protein induced by vitamin K absence/antagonism II (PIVKA-II)), uncarboxylated OC (ucOC), and desphospho-uncarboxylated (dp-uc) MGP will reflect utilization of vitamin K in tissues. We recently developed a new MGP assay to measure the dp-ucMGP species. First results show that this inactive, immature MGP species is suited to assess extrahepatic vitamin K status, particularly in the vasculature.[15]

In the present study, we aimed to investigate vitamin K_1 and K_2 intake as well as vitamin K status in HD patients. Vitamin K intake was estimated using a comprehensive dietary vitamin K database.[12,13] Vitamin K status was evaluated with measurements of circulating vitamin K_1 and K_2 levels, as well as of hepatic and extrahepatic vitamin K–dependent proteins, including PIVKA-II, MGP, and OC. With the present study, we expected to obtain new insights in different aspects of vitamin K metabolism in HD patients, and to increase our knowledge on the risk factors and pathogenesis of arterial calcification in these patients. Additionally, the results of this study will give an indication whether HD patients may benefit from an increased dietary vitamin K intake.

RESULTS

Patient population

Vitamin K intake and status were investigated in the entire cohort of 40 HD patients, consisting of 22 male and 18 female patients with a mean age of 62 ± 16 years. The median (range) dialysis vintage was 40 (4–385) months. All baseline characteristics of the patient population are presented in Table 1. As expected, the prevalence of cardiovascular disease (CVD) and hypertension was high. Control of calcium and phosphorus was quite well.

Table 1 | Characteristics of the 40 HD patients

Age (years)	65 (23-86)
Sex (male/female ratio)	22/18
Dialysis vintage (months)	40 (4–385)
Cause of ESRD	
Glomerular disease	9 (23)
Vascular disease, hypertension	10 (25)
Polycystic kidney disease	9 (23)
Diabetic kidney disease	2 (5)
Miscellaneous	6 (15)
Unknown	4 (10)
Hypertension	27 (67.5)
Diabetes	6 (15.0)
Current smoking	6 (15.0)
BMI (kg/m^2)	24.6 ± 3.7
Cardiovascular disease	15 (37.5)
Coronary artery disease	10 (25.0)
Peripheral artery disease	5 (12.5)
Cerebrovascular disease	4 (10)
History of renal transplantation	7 (17.5)
History of parathyroidectomy	5 (12.5)
Hb (mmol/l)	7.2 ± 0.8
Ca (mmol/l)	2.3 ± 0.2
P (mmol/l)	1.6 ± 0.3
Ca × P (nmol^2/l^2)	3.6 ± 0.8
AP (U/l)	72 ± 23
PTH (pmol/l)	15 (1–94)
Cholesterol (mmol/l)	4.0 ± 1.0
Triglycerides (mmol/l)	1.9 ± 0.8
Vitamin D supplementation	37 (92.5%)
Cinacalcet	4 (10)
Oral PB	35 (87.5)
>1 Oral PB	20 (50)
Ca-containing PB	16 (40)
Non-Ca-containing PB	29 (72.5)
Antihypertensive medication	28 (70)
>1 Medication	10 (25)
Iron supplementation	38 (95)
Darbepoetin alpha	37 (92.5)
Dietary intake	
Total energy (kJ/day)	7502 ± 2119
Total protein (g/day)	66 ± 8.5
Total fat (g/day)	62 ± 9
Monounsaturated fat (g/day)	21 ± 4
Polyunsaturated fat (g/day)	11 ± 3
Saturated fat (g/day)	26 ± 5
Fiber (g/day)	20 ± 12
Ca (mg/day)	814 ± 235
Vitamin C (mg/day)	68 ± 31

Abbreviations: AP, alkaline phosphatase; BMI, body mass index; Ca, calcium; ESRD, end-stage renal disease; Hb, hemoglobin; HD, hemodialysis; P, phosphorus; PB, phosphate binders; PTH, parathyroid hormone.
Data are given as mean ± s.d., median (range), or as absolute numbers with percentage of total between parentheses. Cardiovascular disease was defined as a history of coronary artery disease (myocardial infarction, angina pectoris, or evidence of obstructive disease by angiography), cerebrovascular disease (thrombotic stroke or transient ischemic attack), calcific aortic valve disease, or peripheral artery disease (a history of claudication or lower extremity revascularization).

Vitamin K intake

Total vitamin K intake of patients, registered during 4 days, was 140 (30–546) μg/day, consisting of 118 (18–494) μg/day vitamin K_1 and 21 (2–68) μg/day vitamin K_2 (Table 2). As expected, total vitamin K intake was predominantly determined by the intake of vitamin K_1. One-third of the patients ($n = 13$; 33%) had an average total vitamin K intake of < 100 μg/day. Total vitamin K intake was considerably lower on weekend days and dialysis days (Table 2), as compared with week days ($P = 0.003$ for both comparisons). These differences originated from differences in vitamin K_1 intake between these day-types ($P = 0.003$ for both comparisons), without significant differences in vitamin K_2 intake.

There were no differences in total vitamin K, K_1, or K_2 intake between males and females, or between patients with CVD or diabetes mellitus and patients without these conditions (Table 2). In correlation analysis, total vitamin K intake was not associated with age, body mass index, dialysis vintage, or any of the biochemical characteristics.

Vitamin K status

Serum vitamin K_1 concentration showed a broad range (Table 3). Almost half of the patients ($n = 18$; 45%) had circulating vitamin K_1 levels below the lower limit of normal range. Of the menaquinones (vitamin K_2), only MK-4 was measurable in low quantities in the blood. MK-5 through MK-10 were

Table 2 | Vitamin K intake

	Intake (μg/day) of energy-adjusted vitamin K		
	Total	Vitamin K_1	Vitamin K_2
Average all days	140 (30–546)	118 (18–494)	21 (2–68)
Day types			
Week (Monday–Friday)	171 (41–888)	154 (19–882)	16 (2–58)
Weekend (Saturday, Sunday)	53 (13–1075)	21 (6–1064)	19 (0–111)
Hemodialysis	66 (5–452)	44 (5–425)	20 (0–85)
P	<0.001	<0.001	0.624
Patients			
Male (*n*=22)	115 (42–393)	96 (33–360)	19 (2–48)
Female (*n*=18)	178 (30–546)	144 (18–494)	22 (4–68)
P	0.289	0.302	0.471
With CVD (*n*=15)	113 (30–546)	93 (18–494)	18 (3–52)
Without CVD (*n*=25)	162 (42–413)	134 (18–385)	22 (2–68)
P	0.121	0.158	0.451
With DM	139 (66–292)	107 (47–272)	19 (4–48)
Without DM	140 (30–546)	123 (18–494)	22 (2–68)
P	0.791	0.910	0.691

Abbreviations: CVD, cardiovascular disease; DM, diabetes mellitus.
Vitamin K intakes (total, K_1, and K_2) are given for different day types (week, weekend, and dialysis) and for different patient groups (female and male, with and without CVD and DM). Data are given as median (range). *P*-values are based on Kruskal–Wallis tests (for comparison of day types) and Mann–Whitney *U*-tests (for comparison of patient groups).

Table 3 | Vitamin K status

	HD patients	Reference values
Circulating vitamin K (nmol/l)		
Vitamin K_1	0.30 (0.00–5.71)	0.029–2.65
MK-4	0.00 (0.00–1.13)	<Lower detection limit
MK-5 trough MK-10	0.00 (0.00–0.00)	<Lower detection limit
Hepatic vitamin K status		
PIVKA-II (ng/ml)	3.8 (1.4–12.4)	<2
Extrahepatic vitamin K status		
dp-ucMGP (pmol/l)	1868 ± 870	50–700
ucOC (ng/ml)	6.1 ± 28	2–4
cOC (ng/ml)	11.7 ± 2.8	5–9
Ratio ucOC/cOC	0.5 (0.1–0.9)	<1.0

Abbreviations: cOC, carboxylated osteocalcin; dp-ucMGP, desphospho-uncarboxylated MGP; HD, hemodialysis; MGP, matrix Gla protein; PIVKA-II, protein induced by vitamin K absence/antagonism II; ucOC, uncarboxylated OC.
Values of different markers for vitamin K status are given: circulating vitamin K_1 and K_2 levels, PIVKA-II levels for hepatic vitamin K status, and dp-ucMGP and OC levels for extrahepatic vitamin K status. Reference values in healthy subjects are given based on literature (vitamin K_1 and K_2 levels), information provided by manufacturer of the assay (PIVKA-II), and measurements in healthy subjects aged above 40 years (dp-ucMGP and OC). Data are given as mean ± s.d. or median (range).

undetectable in the circulation of the patients (Table 3). Hepatic vitamin K status, as assessed with PIVKA-II measurement, was suboptimal in the patient cohort. Thirty-three patients (82.5%) had elevated PIVKA-II levels. The median level of 3.8 (1.4–12.4) ng/ml was almost twice the upper normal level. Plasma PIVKA-II levels were not associated with any of the baseline characteristics.

Extrahepatic vitamin K status was assessed by dp-ucMGP and OC measurements, reflecting vascular and bone vitamin K status, respectively. Plasma dp-ucMGP levels were elevated in all 40 patients (Table 3), indicating suboptimal vascular vitamin K status in the entire patient cohort. The dp-ucMGP levels were positively correlated with dialysis vintage (Spearman's rho = 0.440, $P = 0.035$). In addition, patients with CVD had significantly higher dp-ucMGP levels (2226 ± 1103 pmol/l) compared with patients without CVD (1653 ± 626 pmol/l; $P = 0.034$). The mean ucOC level was considerably increased compared with average levels found in healthy reference subjects aged >40 years (Table 3). However, as carboxylated OC (cOC) levels were also elevated, the average ucOC/cOC ratio was 0.5, which is within the normal range.

When investigating the associations between the different parameters for vitamin K status, plasma dp-ucMGP levels were positively correlated with PIVKA-II levels (Spearman's rho = 0.384, $P = 0.028$). There were no other significant correlations. Investigation of the associations between vitamin K intake (total, K_1, and K_2) and measurement of vitamin K status, revealed no significant associations.

DISCUSSION

This is the first study fully assessing vitamin K intake and status in HD patients. The main finding was that vitamin K intake in the present HD patient cohort was estimated at 140 μg/day, mainly from vitamin K_1. The second important finding is that both hepatic as well as extrahepatic vitamin K status were strikingly poor, with the most extreme situation for the vascular wall.

Dietary intake of vitamin K in patients was below values previously found in a reference population of healthy adults (mean vitamin K_1 intake 200 μg/day, and vitamin K_2 intake 31 μg/day in the Netherlands).[8–10] Of note, a different method was used in these studies, that is, a food-frequency questionnaire to estimate vitamin K intake. We have recently performed a study among 60 Dutch healthy volunteers using a similar 3-day food record. In this (yet unpublished) study, the intake of vitamin K_1 was 191 μg/day and for vitamin K_2 it was 24.8 μg/day. As these values are consistent with the previous studies in healthy population using a food-frequency questionnaire, it is unlikely that the difference in vitamin K intake between healthy population and HD patients is due to methodological differences. Low vitamin K intake of HD patients may be related to the dietary regimen generally prescribed for HD patients, which includes restriction of sodium and potassium intake.[16] Sodium is present in cheese and potassium is abundantly present in green vegetables, which are the food products containing abundant amounts of vitamin K_1 and K_2, respectively. Nevertheless, vitamin K intake of our patients was still higher than might have been expected as it was previously predicted that a typical renal diet would contain 80 μg or less of vitamin K per day.[17] This may be related to a relatively high green vegetable and cheese intake in the Netherlands. Vitamin K intake was lower on weekend days and dialysis days as compared with week days, reflecting a different dietary pattern on these days. Consistent with the literature, intake of vitamin K_2 only accounts for the minority of vitamin K intake.[8–10] However, intake of vitamin

K_2 is probably highly relevant for cardiovascular health. Vitamin K_2 intake (and not vitamin K_1) was inversely associated with cardiovascular calcification and mortality in the general population.[12–14] Vitamin K_2 is more uniformly distributed over the various tissues, whereas vitamin K_1 is preferentially targeted to the liver.[18] It has been shown for instance that there is a higher accumulation and utilization of MK-4 in the vascular wall compared with vitamin K_1.[19]

Vitamin K status was investigated using measurements of circulating vitamin K, PIVKA-II, dp-ucMGP, and OC. Circulating vitamin K levels were below the normal range in almost half of the patients. The majority of the menaquinones (MK-5 through MK-13) were below the lower detection limit that is usually found in humans.[20] It should be kept in mind that circulating vitamin K concentrations fluctuate, among others influenced by recent dietary intake. Also, circulating vitamin K may not reflect the utilization of vitamin K in tissue. The high PIVKA-II levels found in the vast majority of patients indicate that vitamin K availability in the liver is too low to produce fully carboxylated clotting factors. Although an increased PIVKA-II level may not result in clinical hemostatic problems, it indicates that the hepatic vitamin K status is poor. The high dp-ucMGP levels found in all patients confirm previous findings in HD patients[15,21] and indicate a poor vascular vitamin K status, which may well be a contributing factor in cardiovascular calcification. Plasma dp-ucMGP was the only marker that correlated with PIVKA-II levels, stressing its value as a vascular biomarker for vitamin K status. With respect to OC, both the ucOC and cOC levels were high, resulting in a normal OC ratio. This may be explained by retention of circulating OC (fragments) in uremic serum.[22,23] An alternative explanation may be that end-stage renal disease–associated increased bone turnover resulted in an overall increased OC synthesis. Further investigations are needed to elucidate why both ucOC and cOC levels measured with enzyme-linked immunosorbent assays (ELISAs) are high in HD patients. Overall, the application of OC measurements in HD patients seems to be complicated and OC carboxylation may not be a useful marker for vitamin K status in HD patients.[22]

In general, dietary vitamin K intake in healthy subjects and HD patients is sufficient to maintain normal hemostasis, but may not be sufficient for full carboxylation (activation) of the extrahepatic Gla proteins. Booth and coworkers showed in a cohort of 142 HD patients that vitamin K status in this patient population is suboptimal.[22] Low circulating phylloquinone levels and poor OC carboxylation were found in 29 and 93% of subjects, respectively. A recent study by the same group in 172 subjects with stage 3–5 chronic kidney disease, showed that the criteria for subclinical vitamin K deficiency were met by 6% of the patients based on circulating K_1 measurements, by 60% based on OC carboxylation, and by 97% based on PIVKA-II levels.[24] The present study confirms these findings, with exception of poor OC carboxylation. It should be noted, however, that OC carboxylation was measured with a different technique (a radioimmunoassay based on the different affinities of ucOC and cOC for hydroxyapatite) in these studies from that used in the present study (ELISAs based on monoclonal antibodies against ucOC and cOC). Their outcomes may, therefore, not be comparable with ours. Owing to its lipophilic characteristics and incorporation into lipoproteins, vitamin K is not expected to be removed by HD treatment.[9] It can, therefore, be hypothesized that vitamin K deficiency in HD patients is due to a diminished dietary intake, which may increase the risk of arterial calcification. Our study confirms that the dietary vitamin K intake in HD patients is lower than that in the general population, although we could not demonstrate a correlation between vitamin K intake and markers for vitamin K status. This might be because of the relatively low number of patients.

The recommended daily allowance for vitamin K of 1 μg/day/kg bodyweight is based on intakes of vitamin K_1 needed to maintain adequate synthesis of blood coagulation factors. The (higher) requirements of extrahepatic tissues for maximal carboxylation of Gla proteins were not taken into account in this recommendation, and although vitamin K intake met this intake level for the majority of patients, it is clear that this is too low for maximal carboxylation of extrahepatic Gla proteins. Based on the elevated plasma levels of PIVKA-II, it may even be concluded that the hepatic vitamin K status was not adequate to fully support the carboxylation of the vitamin K–dependent clotting factors. The design of the present study does not allow to investigate whether factors other than low dietary intake may cause insufficient vitamin K status and to evaluate its long-term consequences. Further studies need to be performed to confirm the present results in larger cohorts of chronic kidney disease and dialysis patients, including peritoneal dialysis patients. Also, it may be relevant to evaluate kidney transplant recipients, because these patients may well maintain their previous dietary habits as dialysis patient.

What may be the clinical implications of our findings? Improvement of vitamin K status in HD patients may readily be achieved by food supplements. The bioavailability of vitamin K from supplements is probably better than that from most foods.[20] For OC it is known that, although ucOC is responsive to increased dietary vitamin K, a maximal response can only be achieved with pharmacological vitamin K intakes.[20] The most common vitamin K form in food supplements and multivitamins is K_1. Two commercially available K_2 vitamins are MK-4 and MK-7. The half-life of MK-7 (approximately 3 days), is much longer compared with that of MK-4 and K_1, with a steady-state plasma level reached after 2 weeks of daily supplementation.[25] In a pilot study among 53 HD patients, the administration of MK-7 (dosage 45, 135, or 360 μg/day) resulted in significantly decreased ucOC and dp-ucMGP levels within 6 weeks.[26] In the highest dosage group, the response-to-treatment for dp-ucMGP levels was even 100%. To improve the vitamin K status of HD patients, supplementation may indeed be the best method, as increasing the dietary intake of vitamin K is probably not possible because

of the generally prescribed dietary regimen, as discussed previously. As HD patients are generally prescribed multivitamins, it may be relatively easy to add vitamin K to these preparations. A contraindication for vitamin K supplementation is the use of vitamin K antagonists (oral anticoagulants).

In conclusion, HD patients have a poor overall vitamin K status and vitamin K intake is low compared with healthy Dutch subjects. The high dp-ucMGP levels are strongly suggestive for vascular vitamin K insufficiency to a level that the calcification-inhibitory activity of MGP is impaired, which may contribute to the extremely high risk for arterial calcification in HD patients. Together with preliminary data that vitamin K supplementation may improve the carboxylation status of MGP, these data warrant a well-designed controlled trial to test whether vitamin K supplementation may reduce the risk of arterial calcification and may even reduce mortality in HD patients.

MATERIALS AND METHODS

Patients

The study was performed between 1 October 2009 and 1 February 2010. There was no formal power calculation for this explorative observational study. All patients who had been on regular three times weekly, in-center HD treatment at the Dialysis Center Groningen/University Medical Center Groningen for at least 3 months and who fulfilled the inclusion and exclusion criteria listed below were invited to participate. Patients with a minimum age of 18 years and able to return completed 4-day food record were eligible, irrespective of gender, the underlying primary renal disease, presence of CVD, diabetes mellitus, or traditional atherogenic risk factors. Malignancy in the past 6 months, abnormal liver function, a history of gastrointestinal disease or metabolic disease, or an active infection were the exclusion criteria. Additional exclusion criteria were use of coumarin derivatives, anticonvulsants (such as phenytoin, carbamazepine, and phenobarbital), which may influence vitamin K metabolism, and use of vitamin K supplements. Data were obtained on demographic, clinical, and dialysis-specific characteristics during interview and from review of patient's files. Informed consent was obtained from all participants and the study was approved by the local medical ethics committee. During the inclusion period, there were 163 patients on regular in-center HD. Of these, 40 patients met all inclusion and exclusion criteria and were included in the study.

Vitamin K intake

Energy and nutrient intake were estimated by registration of all food items consumed, including beverages, and the amount consumed during 4 non-consecutive days in food records by the patients, within a time period of 2 weeks. A food record was used, because our aim was to estimate absolute intake of vitamin K rather than relative intake as measured by a food-frequency questionnaire. As this method is completely open ended, it can accommodate any food or food combination reported by the subject. The food record only had pre-specified sections for breakfast, lunch, dinner, and snacks. All participants received verbal and written instructions before receiving the 4-day food record. The food record consisted of 2 week days and 1 weekend day, during which the patient did not undergo HD treatment, and 1 week day during which the patient underwent HD treatment ('dialysis day'). The 4-day food records were collected on other days than the days before and of the blood drawn.

Food records were analyzed using a nutrient software program (part of Evry-Dietist, Ensemble BV, Zoetermeer, The Netherlands), which is based on the Dutch national food compositions (NEVO) table. Energy and nutrient intake were calculated using the 2006 version of the Dutch Food Composition Database (NEVO), which contains data on the nutritional composition of approximately 2780 foods (www.rivm.nl/nevo_en/). Concentrations of vitamin K_1 and K_2 (MK-4 through MK-10) of 260 foods have been added to the NEVO (2006) food database, as described previously.[12] All nutrients were adjusted for total energy intake by using the residual method.[27] Owing to the variance intake on week, weekend, and dialysis days, intake was measured on non-consecutive days on 1 dialysis day, 2 separate week days, and 1 weekend day. These intake measurements were weighted to reflect their frequency in a 2-week period. This resulted in the following weightings: 6/14 for weekdays, 3/14 for weekend days, and 5/14 for dialysis days. In our study population, vegetables contributed 71% of vitamin K_1 intake, cheese contributed 59%, milk products 14%, and meat 18% of vitamin K_2 intake.[27]

Vitamin K status

Vitamin K status was assessed by measurements of circulating vitamin K_1 and K_2 (menaquinones (MK)-4 through MK-10), and the vitamin K–dependent proteins PIVKA-II, dp-ucMGP, ucOC, and cOC. Venous blood samples were taken before the initiation of a HD session after the 2-day dialysis-free interval. Participants were asked to adhere to their dietary habits, but to refrain from consumption of vitamin K-containing food items (e.g., green vegetables and fermented food products) the evening before and the day of blood withdrawal. Before serum preparation, blood was kept for 20 min at room temperature. Plasma and serum were prepared by standard centrifugation and stored at $-80\,^{\circ}C$ until testing.

The circulating concentrations of vitamin K species (vitamin K_1, MK-4 through MK-10) were measured using reversed phase high-performance liquid chromatography as described previously.[11]

PIVKA-II levels were measured with the ASSERACHROM PIVKA-II kit (Diagnostica Stago, Asnières-sur-Mer, France). This ELISA is based on mouse monoclonal F(ab')2 fragments specific for PIVKA-II, without reactivity with native prothrombin.

Circulating dp-ucMGP levels were determined in plasma using a dual-antibody ELISA (VitaK BV, Maastricht, The Netherlands). In this assay, the capture antibody is directed against the non-phosphorylated MGP sequence 3–15 and the detection antibody against the uncarboxylated MGP sequence 35–49. This assay was shown to be particularly suited to assess vascular vitamin K status.[15]

Markers used to evaluate the carboxylation status of circulating OC were ucOC, cOC, and the ratio between ucOC and cOC (ucOC/cOC). Both ucOC and cOC levels were measured with ELISAs based conformation-specific OC antibodies (Takara, Shiga, Japan).

Other biochemical measurements, including calcium, phosphorus, cholesterol, triglycerides, alkaline phosphatase, and parathyroid hormone were performed using standard laboratory techniques.

Statistical analysis

Normally and non-normally distributed continuous variables are presented as mean ± s.d. or as median (range), respectively, and categorical variables as numbers and frequencies. Differences in vitamin K intake between day-types (week, weekend, and dialysis days) were analyzed with Kruskal–Wallis and Mann–Whitney

U-tests. Within-group differences in vitamin K intake and parameters of vitamin K status were analyzed with Kruskal–Wallis and Mann–Withney *U*-tests. Bonferroni correction was applied for multiple comparisons. Bivariate correlation analysis was performed using the Pearson correlation coefficient for normally distributed data and Spearman correlation coefficient for non-normally distributed data. A *P*-value of <0.05 was considered to be statistically significant; reported *P*-values are based on two-tailed tests of statistical significance. All statistical analyses were conducted using SPSS version 15.0 for Windows (SPSS, Chicago, IL).

DISCLOSURE

All the authors declared no competing interests.

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