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Overweight-obesity is associated with decreased vitamin K2 levels in hemodialysis patients

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Abstract

Objectives: Obesity is an important risk factor for morbidity and mortality. Vitamin K2 is involved in the production of bone and matrix amino acid g-carboxy-glutamic acid (Gla) proteins (vitamin K-dependent proteins [VKDPs]), regulating bone and vascular calcification (VC). Bone Gla protein (BGP) is involved both in bone mineralization and VCs. We assessed the relationships between vitamin K levels and body mass index (BMI) according to the hypothesis that the impact of BMI on mortality is partly driven by low vitamin K levels.

Methods: The Vitamin K Italian (VIKI) study included 387 hemodialysis patients from 18 dialysis centers in Italy. We determined plasma levels of bone markers: vitamin K levels, VKDPs, vitamin 25(OH)D, alkaline phosphatase (ALP), parathyroid hormone (PTH), calcium (Ca), phosphorus (P) and routine biochemistry. BMI was classified into the following categories: underweight (BMI < 18.5 kg/m^2), normal weight (18.5 ≤ BMI < 25 kg/m^2), overweight (25 ≤ BMI < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2).

Results: 45.2% of patients were overweight or obese. Stratification by BMI demonstrated lower median menaquinone-7 (MK7)/triglycerides levels in obese patients (0.42 ng/mg [0.19, 0.87], p = 0.005). BGP levels were lower in overweight and obese patients (152 mcg/L [83.2, 251] and 104 mcg/L [62.7, 230], p = <0.001). Furthermore, there was an inverse correlation between MK7/triglycerides levels and BMI (regression coefficient β = −0.159; p = 0.003). In multiple linear regression, there was an inverse relationship between BGP levels and BMI (β = −0.119; p = 0.012).

Conclusions: These data are the first to report an inverse relationship between Vitamin K2 levels and BMI in hemodialysis patients. Further studies are needed to confirm these findings and to determine if lower levels of Vitamin K are related to greater morbidity and mortality in this at-risk population.

Keywords: BMI; hemodialysis; obesity; vitamin K.

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Introduction

Obesity is a global epidemic, and its prevalence has nearly tripled between 1975 and 2016. Obesity is also associated with higher risk of type 2 diabetes mellitus, dyslipidemia, arterial hypertension and CKD [1–3]. In end-stage renal disease (ESRD), large epidemiologic studies reported a U-shaped association between body mass index (BMI) and death [4, 5]. Although proposed mechanisms for this "obesity paradox" [5] in hemodialysis patients include comorbidities and malnutrition, identification of biochemical mediators, possibly associated with adverse outcomes, is needed in dialysis patients.

Vitamin K is a fat-soluble vitamin existing in two biologically active forms: vitamin K1 or phylloquinone and vitamin K2 or menaquinone, which includes 12 different menaquinones (from MK2 to MK11) [6], the most studied of which are MK4 and menaquinone-7 (MK7) [7, 8]. Vitamin K acts as the coenzyme of a carboxylase that determines carboxylation of glutamic acid residues, resulting in the formation of the amino acid g-carboxy-glutamic acid (Gla). In the liver, this reaction controls the production of vitamin K-dependent proteins (VKDPs), such as coagulation factors, and in extrahepatic tissues the VKDPs, bone and matrix Gla proteins (BGP and MGP, respectively) [7, 8]. BGP is a small protein produced by osteoblasts under the control of vitamin D. It contains three GLA residues that enable its binding to hydroxyapatite in bone [9]. BGP knockout mice develop hyperostosis, showing that it has a role in promoting normal bone mineralization [10]. Vitamin K deficiency in bone can be measured indirectly by measuring undercarboxylated BGP (ucBGP). MGP is a potent inhibitor of vascular calcification (VC), and it is produced by osteoclasts, chondrocytes and vascular smooth muscle cells (VSMCs) [11]. MGP knockout mice experience pathological fractures due to severe osteoporosis and widespread VC [12].

In a secondary analysis of the VIKI study, we evaluated the relationship between vitamin K levels and BMI value in hemodialysis patients according to the hypothesis that the impact of BMI on mortality is in part driven by low vitamin K levels (Figure 1).

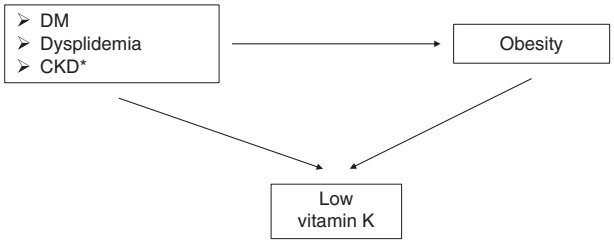

Figure 1: Interconnection between obesity and vitamin K levels (*Schurgers et al. [11]).

Materials and methods

This study is a secondary analysis of the VIKI study, involving 18 dialysis centers in Italy [13]. Ethics committees were approved for the study (approval dates ranged from July 14, 2008 to October 26, 2009), in accordance with the regulations in place related to observational studies. Patient enrollment took place between November 2008 and November 2009, and follow-up to assess vital status was performed in December 2011. We included adult patients of both genders who had been on hemodialysis for >1 year, provided that they gave their informed consent, in writing, for the use of their medical records for the study. We excluded patients who had a life expectancy <6 months, cancer (with the exception of basal cell carcinoma), coagulation disorders or conditions that, according to the investigator, could interfere with the study outcome. We collected the following information: demographic data (initials or ID number, gender, age); renal failure history (cause, type of hemodialysis, duration of hemodialysis in months, transplantation history); lifestyle (smoking status, alcohol consumption) and medical history. BMI was classified into the following categories: underweight (BMI < 18.5 kg/m^2), normal weight (18.5 ≤ BMI < 25 kg/m^2), overweight (25 ≤ BMI < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2).

Laboratory determination

Parathyroid hormone (PTH)

The method for quantitative determination of PTH in serum was the automated LIAISON® N-Tact® PTH Assay 310910 (DiaSorin Inc., Stillwater, MN, USA), a direct, two-site, sandwich-type chemiluminescence immunoassay (CLIA) carried out on the LIAISON® (DiaSorin Inc., Stillwater, MN, USA) instrument. The analytical sensitivity was 1 pg/mL and the intra-assay and inter-assay coefficients of variation (CVs) were 3.7–6.3 and 3.5%–5.3%, respectively.

25-OH vitamin D

For quantitative determination of total 25-OH vitamin D (both D2 and D3 form) in serum, we used the automated LIAISON® 25 OH Vitamin D TOTAL Assay 310600, a direct competitive CLIA executed on the LIAISON (DiaSorin Inc., Stillwater, MN, USA) instrument. The analytical sensitivity was <10 nmol/L, and the intra-assay CV was between 2.9% and 5.5%, while the inter-assay CV was 6.3%–12.9%.

Total BGP

The method for the quantitative determination of total BGP in serum was the automated LIAISON® Osteocalcin Assay 310950 (DiaSorin

Inc., Stillwater, MN, USA), a direct, two-site, sandwich-type CLIA executed on the LIAISON® (DiaSorin Inc., Stillwater, MN, USA) instrument. The analytical sensitivity was <0.3 ng/mL and the intra-assay CV was 3%–8%, while the inter-assay CV was 4%–9%.

Undercarboxylated BGP (ucBGP)

For quantitative determination of the *ucBGP*, we used the Glu-osteocalcin Enzyme Immuno Assay (EIA) Kit MK118 (Takara Bio Inc., Otsu, Shiga, Japan), a manual solid-phase EIA based on a sandwich method that utilizes two mouse monoclonal anti-ucBGP antibodies to detect ucBGP by a two-step procedure. One of the mouse monoclonal anti-ucBGPs is immobilized onto the micro-titer plate and blocked against non-specific binding. Samples are added to each well and incubated. The second step is to wash the plate and to add the second anti-BGP labeled with per-oxidase (POD). The reaction between POD and substrate (H_2O_2 and 3,3′, 5,5′ tetramethyl-benzidine) results in color development with intensities proportional to the amount of ucBGP present. The analytical sensitivity was 0.25 ng/mL and the intra-assay and inter-assay CVs were 4.4–6.7 and 5.7%–9.9%, respectively.

Total matrix GLA protein (MGP)

The quantitative determination of MGP was performed using the Human MGP-Matrix Gla Protein Kit (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria). It is a manual competitive ELISA method designed to detect MGP in serum. The analytical sensitivity was 0.3 nmol/L, and the intra-assay and inter-assay CVs were 5%–6% and 7%–9%, respectively.

Undercarboxylated MGP (ucMGP)

The measurement of the total ucMGP was performed by VitaK using a competitive ELISA, as described previously [14]. The analytical sensitivity was 21 nmol/L, and the intra-assay and inter-assay CVs have been found to be 8.9% and 11.4%, respectively.

We also measured vitamin K components (see Appendix).

Statistics

Data are summarized as mean ± standard deviation (SD) for normally distributed variables or as median and interquartile range (IQ) for non-normally distributed variables, and percentages for all categorical variables. The normal distribution of continuous variables was tested by the Shapiro-Wilk test. Categorical variables across BMI groups were analyzed by the chi-squared (χ^2) test or Fisher's exact method. Continuous variables among more than two groups were compared by the one-way ANOVA or the Kruskall-Wallis tests, as appropriate. To assess the independent correlates of BGP and MK7/triglycerides (dependent variables), multiple linear regression models were built up by including all variables which resulted to be associated with the outcome variables with a $p < 0.10$ at univariate analysis. All statistical analyses were performed using statistical SPSS 15.0 package. A value of $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of patients classified on the basis of BMI categories are presented in Table 1. 45.72% were overweight or obese, 4.39% were underweight and 50.39% had normal weight. A high prevalence of underweight women (82% in the group with BMI less than 18.5 kg/m^2) was observed. Obese and overweight patients had higher prevalence of diabetes and shorter dialysis vintage than patients who were underweight or with normal weight. Higher triglycerides and lower HDL levels were observed in obese and overweight patients; these patients received statins (41.7%), oral antidiabetic drugs (4%) and insulin (21.7%). There were no between-group differences in medications aimed at correcting MBD-CKD, namely calcium carbonate, sevelamer, lanthanum, calcitriol, vitamin D analogs and calciomimetics (Table 2).

On univariate analyses, total and ucBGP levels were inversely related to BMI (Figure 2). By contrast, no association was found between this metric and total MGP and ucMGP (Table 1). Obese patients had higher K1 and K1/triglycerides levels as compared to underweight patients. Furthermore, obese patients had lower MK7/triglycerides levels than normal weight patients (Figure 3 and Table 3). Multiple regression analysis adjusted for a series of potential confounders showed that BMI was independently related to MK7/triglycerides levels ($\beta = -0.159$; $p = 0.003$) (Table 4) and this was also when the same analysis was carried out according to BGP ($\beta = -0.119$; $p = 0.012$) (Table 5).

Discussion

We found that obese hemodialysis patients had lower MK7 levels as compared to non-obese patients. These are the first data to demonstrate an inverse relationship between MK7 (Vitamin K2) and BMI in hemodialysis patients. Furthermore, in our study, total BGP concentrations have been shown to be reduced in patients with greater BMI.

Vitamin K deficiency and obesity are both risk factors for cardiovascular diseases in CKD patients. MK7 is the most widely known menaquinone. It is present in fermented and some animal-derived foods, and it has greater

Table 1: Main characteristics of the patients.

Variable	Patients underweight BMI < 18.5 (n = 17, 4.39%)	Patients normal weight 18.5 ≤ BMI < 25 (n = 195, 50.39%)	Patients overweight 25 ≤ BMI < 30 (n = 118, 30.49%)	Patients obese BMI ≥ 30 (n = 57, 14.73%)	p-Value
Gender, female, n (%)	14 (82.35%)	77 (39.49%)	36 (30.51%)	18 (31.58%)	**<0.001**
Age, years (median)	71 (54, 72)	68 (53, 74)	68 (55, 74)	65 (58, 70)	0.717
Weight, kg, mean ± SD (not normal distributed by all group)	48.59 ± 6.46	62.27 ± 8.71	76.13 ± 9.09	91.38 ± 11.29	**<0.001**
Weight, kg (median)	46.5 (43.5, 53)	61.5 (57, 68)	75 (70.3, 81)	89.5 (85, 99)	**<0.001**
Height, mean ± SD	1.67 ± 0.10	1.67 ± 0.09	1.67 ± 0.09	1.67 ± 0.09	0.987
BMI, kg/cm^2 (median)	17.3 (16.99, 17.67)	22.4 (20.96, 23.83)	27.02 (25.67, 28.39)	31.87 (31.22, 33.75)	**<0.001**
Smoker, n (%) (n = 370)					0.298
Yes	14 (93.33%)	116 (62.37%)	72 (62.61%)	32 (59.26%)	
No	0 (0%)	42 (22.58%)	29 (25.22%)	14 (25.93%)	
Ex	1 (6.67%)	28 (15.05%)	14 (12.17%)	8 (14.81%)	
Current or former alcohol drinker, n (%) (n = 361)	2 (12.50%)	38 (21.11%)	30 (26.32%)	12 (23.53%)	0.557
Medical history					
Dialysis vintage, months (median)	93 (54, 126)	55 (29, 102)	45 (26, 88)	39 (26, 60)	**<0.004**
Type of dialysis, n (%)					0.613
Bicarbonate dialysis	12 (70.59%)	103 (52.82%)	48 (40.68%)	26 (45.61%)	
Hemofiltration (HF)	0 (0%)	14 (7.18%)	12 (10.17%)	6 (10.53%)	
Hemodiafiltration (HDF)	4 (23.53%)	47 (24.10%)	36 (30.51%)	15 (26.32%)	
Acetate free biofiltration (AFB)	1 (5.88%)	25 (12.82%)	19 (16.10%)	9 (15.79%)	
Other types of dialysis	0 (0%)	6 (3.08%)	3 (2.54%)	1 (1.75%)	
Previous kidney transplant, n (%)	2 (11.76%)	30 (15.38%)	19 (16.10%)	3 (5.26%)	0.218
Hypertension, n (%) IPA	9 (52.94%)	158 (81.03%)	92 (77.97%)	45 (78.95%)	0.061
Angina, n (%)	1 (5.88%)	30 (15.38%)	22 (18.64%)	11 (19.30%)	0.102
Myocardial infarction, n (%)	2 (11.76%)	31 (15.90%)	28 (23.73%)	12 (21.05%)	0.298
Atrial fibrillation, n (%)	2 (11.76%)	23 (11.79%)	19 (16.10%)	7 (12.28%)	0.735
Heart failure, n (%)	1 (5.88%)	18 (9.23%)	13 (11.02%)	7 (12.28%)	0.824
Diabetes mellitus, n (%)	1 (5.88%)	27 (13.85%)	31 (26.27%)	26 (45.61%)	**<0.001**
Peripheral vascular disease, n (%)					0.166
No	14 (82.35%)	129 (66.15%)	77 (65.25%)	33 (57.89%)	
Asymptomatic	2 (11.76%)	50 (25.64%)	29 (24.58%)	17 (29.82%)	
Intermittent claudication	1 (5.88%)	15 (7.69%)	9 (7.63%)	3 (5.26%)	
Amputation	0 (0%)	1 (0.51%)	3 (2.54%)	4 (7.02%)	
Cerebrovascular accident, n (%)					0.829
No	16 (94.12%)	177 (90.76%)	104 (88.14%)	49 (85.96%)	
Stroke	1 (5.88%)	9 (4.62%)	7 (5.93%)	3 (5.26%)	
Other type	0 (0%)	9 (4.62%)	7 (5.93%)	5 (8.78%)	

Table 1 (continued)

Variable	Patients underweight BMI < 18.5 (n = 17, 4.39%)	Patients normal weight 18.5 ≤ BMI < 25 (n = 195, 50.39%)	Patients overweight 25 ≤ BMI < 30 (n = 118, 30.49%)	Patients obese BMI ≥ 30 (n = 57, 14.73%)	p-Value
Routine biochemical profile					
Ca, mg/dL (median)	8.8 (8.6, 9.1)	9.2 (8.8, 9.6)	9.15 (8.8, 9.6)	9 (8.5, 9.4)	0.118
Ca, mg/dL, mean ± SD (not normal distributed)	8.90 ± 0.73	9.18 ± 0.71	9.21 ± 0.63	9.04 ± 0.66	0.183
P, mg/dL, mean ± SD (not normal distributed)	4.58 ± 1.38	4.80 ± 1.24	4.79 ± 1.30	4.80 ± 1.28	0.928
P, mg/dL (median)	4.2 (3.9, 5.4)	4.6 (3.72, 5.6)	4.65 (4, 5.4)	4.7 (4, 5.5)	0.822
Alkaline phosphatase, U/L (median)	90 (72, 202)	83 (69, 114)	83.5 (60, 110)	80 (61, 104)	0.089
PTH, pg/mL (median)	289 (130, 446)	244 (150, 384)	239.5 (132, 384)	217 (126, 355)	0.782
Albumin, g/dL (median)	3.5 (3.2, 3.8)	3.8 (3.5, 4.1)	3.9 (3.5, 4.1)	3.9 (3.5, 4.1)	0.114
CRP, mg/L (median)	2.25 (1.34, 14.2)	1 (0.39, 5)	2.9 (0.5, 6.7)	1.5 (0.68, 4.1)	**0.043**
KT/V, mean ± SD	1.34 ± 0.34	1.25 ± 0.28	1.26 ± 0.24	1.20 ± 0.24	0.279
Aluminium, mcg/L (median)	10 (7, 13)	12 (9, 22)	12 (8, 17)	12 (6.9, 25)	0.630
Total cholesterol, mg/dL (median)	172 (156, 185)	164 (134, 191)	170 (146, 197)	171 (146, 193)	0.191
Triglycerides, mg/dL (median)	141 (117, 158)	128 (97, 179)	162.5 (121, 221)	200 (147, 265)	**0.001**
HDL cholesterol, mg/dL (median)	45 (33, 53)	42 (34, 54)	39 (31, 47)	35 (30, 44)	**0.001**
LDL cholesterol, mg/dL (median)	97 (68, 120)	84.75 (68, 111.5)	96 (74, 120)	91 (65, 113)	0.223
25(OH)D, ng/mL (median)	22.2 (17.1, 36.8)	31 (20.4, 48.5)	28.75 (18, 38.9)	25.1 (19.1, 40.8)	0.098
BGP total, mcg/L (median)	204 (135, 437.8)	217 (119, 373)	152 (83.2, 251)	104 (62.7, 230)	**<0.001**
BGP undecarboxylated, ng/mL (median)	12.4 (4.6, 29.15)	11.96 (6.38, 18)	11.03 (4.04, 17.2)	8.1 (2.82, 12.84)	**0.009**
MGP total, nmol/L (median)	19.84 (11.15, 38.05)	18.9 (12.71, 29.24)	17.67 (12.7, 28.89)	20.01 (13.21, 34.71)	0.836
MGP decarboxylated, nmol/L (median)	683 (535, 1104)	533 (268, 908)	560.19 (309, 942)	683 (259, 1062)	0.458
Magnesemia, mg/dL (median)	2.45 (2.05, 2.85)	2.3 (2, 2.6)	2.3 (2.1, 2.8)	2.1 (2, 2.6)	0.488
(n = 139)	(n = 4)	(n = 67)	(n = 43)	(n = 25)	
'Magnesemia, mg/dL (median)' (not normal distributed by all group)	2.45 ± 0.54	2.44 ± 0.65	2.47 ± 0.50	2.25 ± 0.47	0.468
(n = 139)	(n = 4)	(n = 67)	(n = 43)	(n = 25)	

Significant differences ($p \leq 0.05$) are shown in bold.

Table 2: Therapy by BMI.

Drugs prescribed to patients	Patients underweight BMI < 18.5 (n = 17, 4.39%)	Patients normal weight 18.5 ≤ BMI < 25 (n = 195, 50.39%)	Patients overweight 25 ≤ BMI < 30 (n = 118, 30.49%)	Patients obese BMI ≥ 30 (n = 57, 14.73%)	p-Value
Warfarin, n (%)	1 (5.88%)	22(11.28%)	16(13.56%)	7(12.28%)	0.807
Steroid, n (%)	1 (5.88%)	10 (5.13%)	7 (5.93%)	3 (5.26%)	0.922
Thyroid hormones, n (%)	1 (5.88%)	19 (9.74%)	10 (8.47%)	10 (17.54%)	0.254
Antibiotics, n (%)	1 (5.88%)	6 (3.08%)	6 (5.08%)	3 (5.26%)	0.769
Antiepileptic, n (%)	0 (0.00%)	8 (4.10%)	4 (3.39%)	2 (3.51%)	0.852
Statin therapy, n (%)	2 (11.76%)	51 (26.15%)	45 (38.14%)	28 (49.12%)	**0.001**
Beta-blockers, n (%)	6 (35.29%)	71 (36.41%)	44 (37.29%)	23 (40.35%)	0.956
Antidiabetics, n (%)	0 (0%)	0 (0%)	1 (0.85%)	6 (10.53%)	**<0.001**
Insulin, n (%)	0 (0%)	20 (10.26%)	21 (17.80%)	17 (29.82%)	**0.001**
Anti-gastric, n (%)	14 (82.35%)	148 (75.90%)	88 (74.58%)	47 (82.46%)	0.630
Aluminium, n (%)	4 (23.53%)	49 (25.13%)	26 (22.03%)	17 (29.82%)	0.734
Calcium carbonate, n (%)	3 (17.65%)	62 (31.79%)	46 (38.98%)	21 (36.84%)	0.267
Calcium acetate, n (%)	0 (0%)	10 (5.13%)	3 (2.54%)	8 (14.04%)	**0.011**
Sevelamer, n (%)	6 (35.29%)	90 (46.15%)	47 (39.83%)	20 (35.09%)	0.386
Lanthanum, n (%)	3 (17.65%)	24 (12.31%)	19 (16.10%)	10 (17.54%)	0.668
Oral calcitriol, n (%)	7 (41.18%)	90 (46.15%)	55 (46.61%)	25 (43.86%)	0.965
Intravenous calcitriol, n (%)	0 (0%)	4 (2.05%)	5 (4.24%)	3 (5.26%)	0.448
Vitamin D analogues, n (%)	4 (23.53%)	43 (22.05%)	18 (15.25%)	12 (21.05%)	0.503
Calcimimetics, n (%)	3 (17.65%)	36 (18.46%)	21 (17.80%)	15 (26.32%)	0.556

Significant differences ($p \leq 0.05$) are shown in bold.

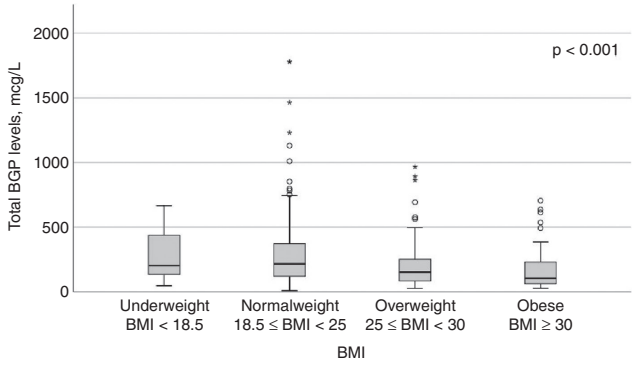

Figure 2: Total BGP levels by BMI.

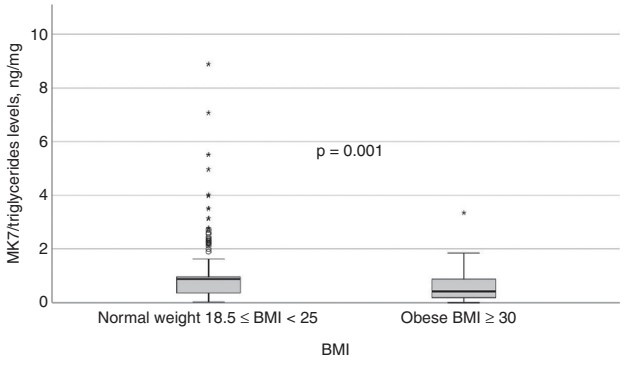

Figure 3: MK7/triglycerides levels in obese and normal weight patients.

bioavailability than other forms of vitamin K. MK7 is used as a dietary supplement secondary to its beneficial role in human health [15]. A prospective population-based study of 4807 subjects without clinical history of myocardial infarction, followed for 7 years, showed indeed that intake of menaquinone resulted in a significant risk reduction in coronary heart disease, all-cause mortality and severe aortic calcification [16].

Obesity prevalence has been reported in 30% of the US dialysis population, and it was found associated with increased risk for adverse outcomes, death and worsening of CKD [3, 14, 17–19]; it is also associated with earlier and progressive bone disorders, such as osteoporosis and fractures [19].

In hemodialysis patients, we previously reported higher prevalence of vitamin K deficiency (up to 35%) as compared to the general population [20–22]. In particular, lower MK7 levels was an independent predictor of iliac artery calcifications (OR 1.64), whereas MK4 deficiency was associated as predictors for aortic calcification. These findings support the role of vitamin K as an inhibitor of VC in the vessel wall [13, 23]. Indeed, the active MGP form (phosphorylated carboxylated: p-cMGP) proved to be effective in solubilizing circulating calcium crystals binding to a circulating fetuin-A complex. Moreover, p-cMGP has been demonstrated to inhibit the pro-osteoblastic transcription factor BMP-2 able to induce apoptosis

Table 3: Vitamin K status in patients.

Vitamers	Patients underweight BMI < 18.5 (n = 17, 4.39%)	Patients normal weight 18.5 ≤ BMI < 25 (n = 195, 50.39%)	Patients overweight 25 ≤ BMI < 30 (n = 118, 30.49%)	Patients obese BMI ≥ 30 (n = 57, 14.73%)	p-Value
K1, ng/mL (median)	0.40 (0.31, 0.53)	0.63 (0.36, 1.12)	0.63 (0.29, 0.98)	0.96 (0.51, 1.32)	**0.012**
K1/triglycerides, ng/mL (median)	0.24 (0.14, 0.40)	0.50 (0.25, 0.83)	0.40 (0.20, 0.70)	0.44 (0.20, 1.00)	**0.022**
MK4 ng/mL (median)	0.50 (0.07, 0.67)	0.56 (0.21, 0.67)	0.50 (0.23, 0.67)	0.48 (0.22, 0.67)	0.976
MK4/triglycerides ng/mL (median)	0.32 (0.05, 0.51)	0.43 (0.16, 0.51)	0.35 (0.13, 0.51)	0.31 (0.12, 0.51)	0.390
MK5 ng/mL (median)	1.00 (0.38, 1.18)	1.00 (0.54, 1.02)	1.00 (0.43, 1.01)	1.00 (0.40, 1.00)	0.581
MK5/triglycerides, ng/mL (median)	0.75 (0.36, 0.97)	0.75 (0.40, 0.75)	0.75 (0.27, 0.76)	0.51 (0.23, 0.75)	0.090
MK6 ng/mL (median)	0.44 (0.37, 0.63)	0.55 (0.24, 0.76)	0.46 (0.19, 0.63)	0.47 (0.13, 0.63)	0.514
MK6/triglycerides, ng/mL (median)	0.35 (0.26, 0.47)	0.44 (0.15, 0.61)	0.28 (0.11, 0.47)	0.24 (0.08, 0.47)	**0.017**
MK7 ng/mL (median)	0.98 (0.58, 1.57)	1.15 (0.47, 1.19)	1.06 (0.52, 1.20)	0.81 (0.32, 1.15)	0.431
MK7/triglycerides, ng/mL (median)	0.67 (0.39, 1.43)	0.87 (0.36, 0.96)	0.70 (0.31, 0.87)	0.42 (0.19, 0.87)	**0.005**

Significant differences ($p \leq 0.05$) are shown in bold.

Table 4: Linear regression model with outcome MK7/triglycerides (log-transformed) adjusted for BMI (log-transformed), HDL cholesterol (log-transformed), dialysis vintage (log-transformed), age, gender, MK4 (log-transformed) and decarboxylated MGP (log-transformed).

Variable	β	p-Value
Log BMI	−0.159	**0.003**
Log HDL cholesterol, mg/dL	0.123	**0.026**
Log dialysis vintage	−0.046	0.382
Age	−0.030	0.570
Gender	−0.064	0.232
Log MK4, ng/mg	0.235	**<0.001**
Log decarboxylated MGP, nmol/L	−0.083	0.106

Significant differences ($p \leq 0.05$) are shown in bold.

Table 5: Linear regression model with outcome total BGP (log-transformed) adjusted for BMI (log-transformed), age, gender, dialysis vintage (log-transformed), alkaline phosphatase (log-transformed), peripheral vascular disease and DM.

Variable	β	p-Value
Log BMI	−0.119	**0.012**
Age	−0.255	**<0.001**
Gender	0.077	0.086
Log dialysis vintage	0.103	**0.025**
Log alkaline phosphatase, U/L	0.277	**<0.001**
Peripheral vascular disease	−0.116	**0.016**
DM	−0.119	**0.016**

Significant differences ($p \leq 0.05$) are shown in bold.

and trans-differentiation of VSMCs into osteoblast-like cells [24]. BGP can exert a protective role on VCs through intriguing mechanism where BGP increases adiponectin secretion. The latter is an anti-inflammatory protein secreted by adipocytes and in the arterial wall, prevents the transdifferentiation of VSMCs into osteoblast-like cells in arterial media [25–27], thus protecting from VC development. In the MINOS study (774 men in osteoporosis), low total BGP levels have been shown to be a predictor of cardiovascular mortality, whereas higher total BGP concentrations are associated with lower abdominal aortic calcification progression rate and lower 10-year all-cause mortality (MINOS) [27].

In conclusion, we found an association between decreased vitamin K2 and obesity. Interventional studies with vitamin K supplementation in obese CKD subjects are warranted to confirm the potential role of low levels of vitamin K as a modifiable risk factor for the high morbidity and mortality in obese CKD patients.

Appendix

Laboratory determination

The Laboratory in Perugia determined vitamin K components by a simple, sensitive and selective reversed-phase high-performance liquid chromatography (HPLC) method, developed for the simultaneous determination of vitamin K in human plasma. Clear and well-separated chromatographic PK and MK profiles were obtained in healthy human and uremic plasma [13].

The adjustment for triglycerides concentration is particularly relevant for the assessment of vitamin K status. Vitamin K components are all liposoluble compounds that become part of chylomicrons after absorption from the gut and as such are transported to the liver. Vitamin K1 remains partly in the liver, whereas vitamin K2 is transferred to VLDL and LDL for transport and there is a close correlation

(r = 0.99) between triglycerides concentrations and vitamin K1 [13].

Uremic plasma is characterized by an increased level of plasma lipids and lipoproteins, which are interfering factors to chromatography. Thus, we adopted a liquid-liquid extraction and then a solid-phase extraction of human plasma using polymeric reversed-phase cartridges, achieving good reproducibility. The vitamers were measured by an electrochemical detector after post-column reduction with platinum on alumina powder and using the MK8 form as the internal standard. Quantitative recovery was obtained in the range of 80%–96% for PK and MK vitamers. Vitamin K values were corrected according to triglycerides levels [13].

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