ORIGINAL ARTICLE

Effect of vitamin K_2 on progression of atherosclerosis and vascular calcification in nondialyzed patients with chronic kidney disease stages 3–5

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KEY WORDS

atherosclerosis, chronic kidney disease, vascular calcification, vitamin K_2

ABSTRACT

INTRODUCTION Observational studies have shown that high dietary intake of vitamin K_2 is associated with reduced risk of coronary vascular disease and vascular calcification.

OBJECTIVES We assessed the effect of vitamin K_2 substitution on the progression of atherosclerosis and calcification in nondialyzed patients with CKD stages 3–5.

PATIENTS AND METHODS The study included 42 nondialyzed patients with CKD. The following measurements were taken at baseline and after 270 ±12 days of supplementation with vitamin K_2 at a dose of 90 µg (menaquinone, MK-7) together with 10 µg of cholecalciferol (K+D group) or 10 µg of cholecalciferol (group D): common carotid intima-media thickness (CCA-IMT), coronary artery calcification score (CACS), basic biochemical parameters, lipids, and calcification modulators: matrix Gla protein (MGP), desphosphorylated-uncarboxylated MGP (dp-ucMGP), osteoprotegerin (OPG), fetuin A, osteocalcin (OC), and fibroblast growth factor 23.

RESULTS The increase of CCA-IMT was significantly lower in the K+D group compared with the D group: from 0.95 ±0.2 mm to 1.01 ±0.3, $P = 0.003$ vs from 1.02 ±0.2 mm to 1.16 ±0.3, $P = 0.003$ (ΔCCA-IMT, 0.06 ±0.08 vs 0.136 ±0.05 mm, $P = 0.005$, respectively). The increase in CACS was slightly lower in the K+D group than in the D group (ΔCACS, 58.1 ±106.5 AU *vs* 74.4 ±127.1 AU, $P = 0.7$). In the K+D group, a significant decrease in the level of dp-ucMGP and total OC was observed.

CONCLUSIONS A 270-day course of vitamin K_2 administration in patients with CKD stages 3–5 may reduce the progression of atherosclerosis, but does not significantly affect the progression of calcification. Vitamin K_2 significantly changes the levels of calcification promoters and inhibitors: dp-ucMGP, OC, and OPG.

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Received: June 7, 2015.
Revision accepted: July 14, 2015.
Published online: July 15, 2015.
Conflict of interest: none declared.
Pol Arch Med Wewn. 2015; 125 (9): 631-640

INTRODUCTION Atherosclerosis and vascular calcification (VC) are common complications of chronic kidney disease (CKD) and significant risk factors for cardiovascular disease and mortality.[1,2]

VC is currently recognized as an actively regulated process dependent on the balance between its inducers and inhibitors.[3] Several studies have shown that arterial calcification resembles bone formation. In a uremic environment, vascular smooth muscle cells (VSMCs) express osteogenic proteins and deposit a mineralized bone-like matrix.[4] Two of these proteins, matrix Gla protein (MGP) and osteocalcin (OC), are principal regulators of tissue mineralization in the arterial wall and bone.[5] OC and MGP expression is upregulated by vitamin D and dependent on vitamin K for its calcium-binding capacity.[6] MGP is expressed by VSMCs within the arterial media of

the vessel wall. MGP needs to undergo posttranslational gammaglutamyl carboxylation to achieve full biologic activity. The carboxylation process is completely dependent on the availability of vitamin K, which is a cofactor of this process.[7]

Natural vitamin K consists of phylloquinone (vitamin K_1) and the menaquinones MK-4 through MK-10, all vitamin K_2. Both vitamin K_1 and K_2 catalyze the gammaglutamyl carboxylation of all vitamin K-dependent proteins. However, vitamin K_1 predominantly accumulates in the liver and is the most important substance for the activation of coagulation factors, while vitamin K_2 has a more widespread tissue distribution and is thus more specifically involved in the carboxylation of MGP.[8] Consequently, it exerts a major role in the calcification process. In cases of vitamin K deficiency, MGP is not activated and undercarboxylated MGP predominantly accumulates in the areas of VC, and is associated with both intimal and medial calcification.[9] Observational studies have shown that high dietary vitamin K_2 intake is associated with a reduced risk of coronary vascular disease and VC.[10,11] The second vitamin exerting multiple functions including a role in the calcification process is vitamin D.[12] Recent data have demonstrated a high prevalence of suboptimal levels of vitamins K and D in patients with CKD stages 3 to 5.[13]

The aim of this study was to assess the effect of supplementation of vitamin K_2 (menaquinone, MK-7) in combination with a low dose of cholecalciferol compared with cholecalciferol alone on the progression of atherosclerosis and coronary artery calcification (CAC) and on circulating levels of calcification regulators in nondialyzed CKD patients.

PATIENTS AND METHODS This prospective, randomized, and double-blind study was conducted between 2009 and 2012. Multislice computed tomography (CT) scanning of the thorax to assess the coronary calcification score (CACS) and ultrasonography of the common carotid artery with the measurement of intima-media thickness (CCA-IMT) were performed on the same day in 75 consecutive nondialyzed patients with CKD stages 3–5 from a single nephrology outpatient clinic who fulfilled the inclusion and exclusion criteria.

The inclusion criteria were as follows: age from 18 to 70 years old, a history of stable estimated glomerular filtration rate (eGFR <60 ml/min/1.73 m^2) over at least 6 months, not requiring dialysis. The exclusion criteria were as follows: a history of major cardiovascular complications (myocardial infarction, clinically significant arrhythmia including atrial fibrillation, congestive heart failure, stroke, peripheral vascular disease), history of thrombosis or coagulation disorders, treatment with oral anticoagulants, steroid and other hormonal therapies, and treatment with vitamin D or its analogs. Forty-two screened patients showed CACS values of 10 Agatston units (AU) or higher, and they were randomized to the study groups. All enrolled patients were nonsmoking Caucasians (22 men; mean age, 60 ±3.0 years, and 20 women, mean age, 56 ±1.5 years) and had pharmacologically well-controlled hypertension.

Information concerning medical history, medications, cardiovascular complications, and results of routine laboratory measurements was obtained from chart reviews. The causes of renal failure were chronic glomerulonephritis in 15 cases, diabetic nephropathy in 8, polycystic kidney disease in 4, hypertensive nephropathy in 5, tubulointerstitial nephritis in 3, and unknown in 7 patients.

The randomization cards were prepared on the assumption that twice the number of patients would be treated with both vitamins, K_2 and D, than with vitamin D alone (active vs control group). Patients were randomized to each group by computer. Twenty-nine patients in the K+D group received an oral dose of 90 μg of vitamin K_2 (menaquinone-7, MK-7) plus 10 μg of cholecalciferol per day for 270 ±12 days; 13 in group D received 10 μg of cholecalciferol alone (**FIGURE 1**). The tablets containing vitamin K_2+D or vitamin D were identical in size and appearance (both types of tablets were prepared by NattoPharma, Høvik, Norway). Patients from both groups were treated with statins because of hyperlipidemia. Four patients from group K+D and two from group D received calcium carbonate as a phosphate binder with doses unmodified throughout the study.

Anthropometric measurements were taken and fasting blood samples for biochemical, blood count, and coagulation tests were obtained at the time of randomization and at the end of treatment. Routine serum parameters, including creatinine, calcium, phosphate, parathyroid hormone, glucose, albumin, total protein, cholesterol, triglycerides, and high-density lipoprotein cholesterol levels were measured with routine laboratory methods, low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. The eGFR was calculated with a 4-variable Modification of Diet in Renal Disease (MDRD) equation. Serum and plasma samples were prepared after standard centrifugation and frozen at –80°C until measurements.

Circulating total MGP was measured using a sandwich enzyme immunoassay by an enzyme-linked immunosorbent assay (ELISA; USCN Life Science Inc, www.uscnk.com), while plasma desphosphorylated-uncarboxylated MGP (dp-ucMGP) was assessed using the ina*K*-tif MGP iSYS kit (Immunodiagnostic Systems; www.idsplc), which is a dual-antibody test based on the previously described sandwich ELISA (developed by VitaK, Maastricht University, The Netherlands), total serum OC by ELISA (Immunodiagnostic Systems; www.idsplc), serum osteoprotegerin (OPG) by ELISA (Immunodiagnostic Systems; www.idsplc), serum fetuin A by ELISA (Epitope Diagnostics,Inc., www.epitopediagnostics.com), serum 25-hydroxyvitamin D [25(OH)D] by a radioimmunoassay (IBL International; www.IBL-International.com), serum high-sensitive

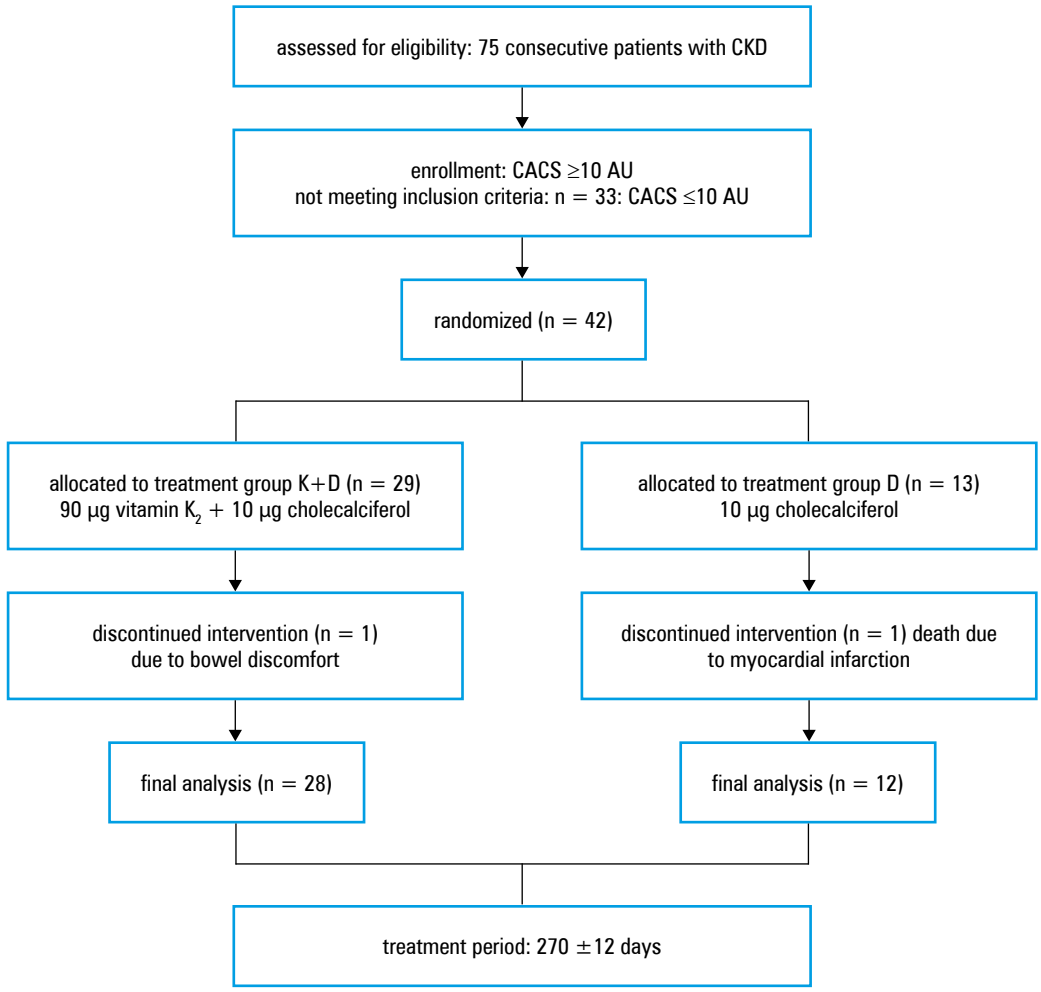

FIGURE 1 Schematic illustration of patient selection and randomization and study design
Abbreviations: AU, Agatston units; CACS, coronary artery calcium score; CKD, chronic kidney disease

C-reactive protein (hs-CRP) by ELISA (IBL International; www.IBL-International.com), plasma fibroblast growth factor 23 (FGF-23) was determined using a human FGF-23 ELISA kit (Immutopics, www.immutopicsintl.com).

Within 7 days after the end of active treatment, the CACS level was assessed by a multiscan CT and CCA-IMT—by ultrasonography.

Written informed consent was obtained from all subjects before entering the study, and the study protocol was approved by the local ethics committee.

Imaging procedures Multislice CT scanning of the thorax was performed using a General Electric Medical Systems Lightspeed 16 scanner to determine CAC. The acquisition parameters were as follows: 120 KVp, 350 mA, slice with 2.5 mm/8i. Data were reconstructed with a standard algorithm using a 512 × 512 matrix, 50-cm scan field of view, and 25 cm display field of view. The system was synchronized with the cardiac cycle to trigger scanning during the diastolic phase. All pixels with an intensity of 130 Hounsfield units or higher were counted, and the data were analyzed using the CardIQ Smart Score software (GE, Milwaukee, Wisconsin, United States). The CAC score (CACS) was determined using the Agatston scoring system, a CACS threshold of less than 10 AU was considered as indicating no calcification.

Ultrasonographic studies were performed with a "VIVID 7 PRO" apparatus (GE) using a 5–14-MHz linear high-resolution probe. Each patient was examined in the supine position in a semi-dark room by the same expert radiologist who was blinded to the purpose of the study, the results of CACS, and the allocation to the treatment group. CCA-IMT was defined as a low-level echo grey band that does not project into the arterial lumen and was measured at the diastolic phase as a distance between the leading edge of the first and second echogenic line. CCA-IMT was measured on the longitudinal views of the far wall of the distal segment of the common carotid artery, by means of a semiautomatic border-detection program 0.5, 1, and 2 cm below and above the bifurcation in a plaque-free arterial segment, and the mean value from all measurements from both carotids was used for statistical analysis.[13,14] The analysis was performed off-line on a workstation equipped with a dedicated software (EchoPac PC, GE Medical System).

Statistical analysis The results are presented as mean ± standard deviation. The χ^2 test was used for sex comparison. The Shapiro–Wilk test was used to confirm the normality of the distribution. For normal distributions, the *t* test for unpaired data was used to assess the significance of the differences between the means, and the

TABLE 1 Anthropometric and laboratory parameters at baseline and after 270 days of treatment with vitamin K_2+D (K+D group) or vitamin D alone (D group)

Parameter	Vitamin K+D (n = 28)			Vitamin D (n = 12)			*P* value[a]
	before treatment	after treatment	*P* value	before treatment	after treatment	*P* value	
BMI, kg/m²	30.3 ±4.6 30.8 (26.9–33.6)	29.8 ±4.1 30.4 (26.8–33.6)	0.3	28.7 ±5.2 28.3 (23.4–33.6)	28.5 ±4.9 28.4 (23.2–33.4)	0.2	0.4
serum creatinine, mg/dl	3.3 ±1.5 3.1 (2.2–3.5)	4.3 ±2.7 3.2 (2.5–5.5)	0.01	2.5 ±0.8 2.1 (1.8–3)	2.6 ±0.9 2.3 (1.7–3.2)	0.3	0.06
eGFR, ml/min/1.73 m²	22.2 ±9.8 19.5 (14–31)	18.7 ±11.2 17.0(9.5–25)	0.08	30.3 ±12.7 28.0 (23–39)	30.0 ±13.8 26.0 (21–44)	0.7	0.02
uric acid, mg/dl	6.8 ±1.4 6.4 (5.8–8.1)	6.5 ±1.3 6.4 (5.5–7.8)	0. 2	8.5 ±1.9 8.3 (7.6–8.8)	7.9 ±1.3 8.0 (6.9–8.5)	0.2	0.05
total cholesterol, mg/dl	208.5 ±66.7 186.5 (165–235)	218.9 ±56 194 (175–260)	0.5	167.5 ±32.9 160.0 (138–203)	186.8 ±40.2 187.5 (159–210)	0.06	0.2
triglycerides, mg/dl	215.2 ±121 175.0 (136–244)	198 ±113 170 (123.5–212)	0.4	140 ±48.8 120.0 (106–188)	149.8 ±52 135.0 (106–190)	0.5	0.3
LDL cholesterol, mg/dl	119.4 ±49.5 105 (82–143)	125.5 ±47.3 112.5 (92–160)	0.4	96.7 ±21.8 97.0 (78–123)	108 ±33.1 101.5 (76–123)	0.06	0.4
HDL cholesterol, mg/dl	53.1 ±15.9 47.5 (38–57)	57.2 ±28.1 47.5 (41–63)	0.9	45.8 ±10.0 43.0 (34–55)	51.3 ±12.2 55.5 (41–61)	0.02	0.4
calcium, mmol/l	2.4 ±0.1 2.4 (2.3–2.5)	2.4 ±0.2 2.4 (2.3–2.5)	0.4	2.4 ±0.1 2.4 (2.4–2.5)	2.5 ±0.2 2.4 (2.4–2.5)	0.2	0.1
phosphate, mmol/l	1.4 ±0.4 1.3 (1.2–1.4)	1.5 ±0.6 1.3 (1.2–1.8)	0.08	1.1 ±0.2 1.1 (0.9–1.1)	1.2 ±0.2 1.1 (1.1–1.4)	0.004	0.1
Ca × P, mmol²/l²	3.3 ±1.06 3.2 (2.7–3.4)	3.7 ±1.5 3.2 (2.8–4.2)	0.09	2.7 ±0.6 2.6 (2.2–2.7)	3.0 ±0.6 2.8 (2.6–3.6)	0.002	0.2
PTH, pg/ml	194 ±143.1 141 (77–298)	233 ±245.7 168 (74–246)	0.3	134 ±80.6 131.5 (56–182)	120.8 ±62.4 125.0 (79–147.7)	0.6	0.2
hemoglobin, g/dl	11.8 ±1.4 11.6 (10.9–12.7)	11.4 ±1.9 11.3 (10.3–12.6)	0.2	13.2 ±1.6 14.0 (11.6–14.6)	13.7 ±1.8 14.0 (12.1–14.8)	0.02	0.001
prothrombin time, s	13.2 ±0.4 13.2 (12.9–13.3)	12.9 ±0.6 31.5 (21.6–34.4)	0.8	13.1 ±05 12.8 (12.7–13.5)	13.0 ±0.4 13.0 (12.8–13.5)	0.7	0.9
25(OH)D, ng/ml	20.8 ±9.8 20.4 (12.6–27.5)	32.1 ±12.1 31.5 (21.6–34.4)	0.004	24.8 ±12.9 20.2 (14.7–33.6)	33.4 ±11.7 28.9 (24.1–38.8)	0.03	0.8
MGP, pg/ml	639.6 ±187 595.1 (533.6–831.2)	742.8 ±249.1 684 (555.6–888.4)	0.06	640.7 ±195.4 560.3 (516.3–718.2)	615 ±165.9 594.9 (489.1–680.0)	0.6	0.1
dp-ucMGP, pmol/l	1077.1 ±507.7 1004 (590–1670)	961.5 ±506.7 812 (510–1580)	0.02	793.9 ±400.3 715(467–1190)	820.7 ±565.2 710 (490–1119)	0.7	0.5
FGF-23, pg/ml	41.3 ±120 12.8 (9.0–23.3)	71.5 ±163 18.05 (9.4–43.7)	0.06	16.7 ±15 12.5 (6.8–18.6)	13.3 ±8.4 10.1 (7.4–22)	0.3	0.3
OC, ng/ml	63.3 ±41.4 60.2 (43.1–75.7)	56.5 ±42.0 54.7 (29.9–83.5)	0.04	40.8 ±54 29.3 (19.4 –64.3)	58 ±43 50.2 (38.8–78.3)	0.03	0.9
OPG, pg/ml	5.7 ±2.2 4.7 (4.6–6.4)	6.3 ±2.2 5.8 (4.7–7.3)	0.02	4.7 ±1.8 4.2 (3.3–6.1)	5.1 ±1.7 5.7 (3.5–6.5)	0.08	0.1
fetuin A, ng/ml	111.4 ±43 112.2 (90.4–132.2)	113.4 ±37 108.9 (87.1–136.6)	0.7	110.6 ±34.8 103.7 (90.4–129.9)	120.4 ±30.2 120.0 (84–149)	0.4	0.6
hs-CRP, µg/ml	6.6 ±4.8 4.5 (3.3–9.8)	7.9 ±5.9 6.4 (2.6–10.3)	0.19	4.5 ±4.9 2.0 (1.1–7.7)	6.9 ±5.6 5.7 (3.0–10.1)	0.08	0.6

Data are presented as mean ± standard deviation or median (interquartile range).

a between treatment groups (vitamin K+D vs vitamin D) after treatment

Conversion factors to SI units are as follows: for creatinie, 88.4; uric acid, 59.48; cholesterol, 0.02586; triglycerides, 0.0114; and hemoglobin, 0.6206

Abbreviations: BMI, body mass index; Ca × P, calcium–phosphorus product; CCA-IMT, common carotid artery intima media-thickness; dp-ucMGP, desphosphorylated-uncarboxylated MGP; eGRF, estimated glomerular filtration rate; FGF-23, fibroblast growth factor 23; HDL, high-density lipoprotein; hs-CRP, high-sensitive C-reactive protein; 25(OH)D, 25-hydroxyvitamin D; LDL, low-density lipoprotein; MGP, matrix Gla protein; OC, osteocalcin; OPG, osteoprotegerin; PTH, parathormone

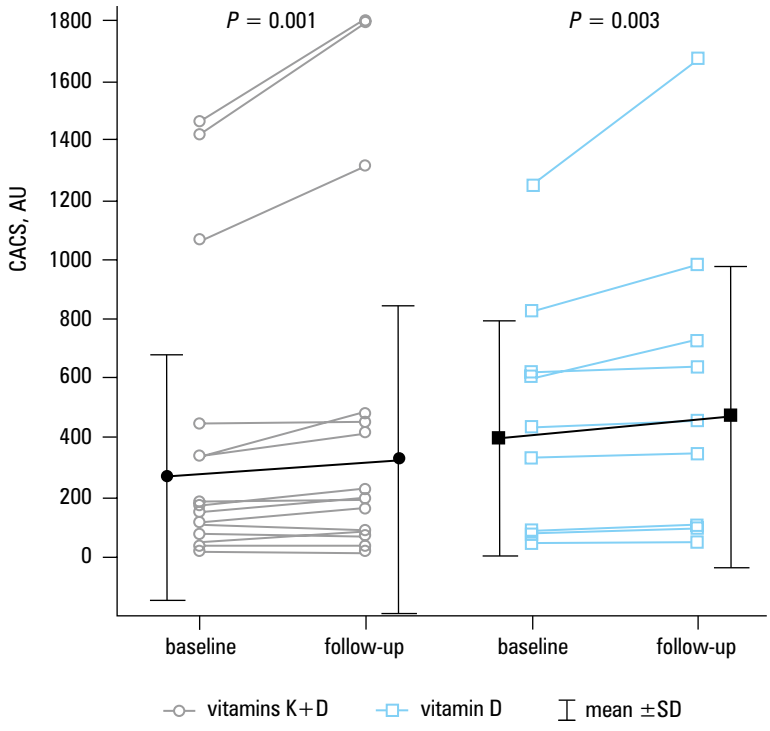

FIGURE 2 Coronary artery calcification score before and after 270 ±12 days of treatment with vitamin K_2 and vitamin D (K+D group) or vitamin D alone (D group)
Abbreviations: SD, standard deviation; others, see **FIGURE 1**

TABLE 2 The change in coronary artery calcification score and carotid artery intima media thickness from baseline to day 270 of treatment with vitamin K_2+D (K+D group) or vitamin D alone (D group)

Parameter	Vitamin K+D (n = 28)	Vitamin D (n = 12)	*P* value
ΔCACS, AU	58.1 ±106.5	74.4 ±127.1	0.7
	11.0 (0–55.5)	20.5 (8.0–119.0)	0.2
	ANCOVA		0.3
	16.7% ±23.3%	15.9% ±12.9%	0.9
	13.9 (0.0–26.7)	11.1 (4.8–19.7)	0.8
ΔCCA-IMT, mm	0.06 ±0.08	0.136 ±0.05	0.005
	0.000 (0.000–0.1)	0.100 (0.1–0.2)	0.004
	ANCOVA		0.007
	6.0% ±7.1%	13.8% ±4.9%	0.003
	0.0 (0.0–12.5)	13.3 (10.0–18.2)	0.009

Data are presented as mean ± standard deviation or median (interquartile range).

Abbreviations: ANCOVA, analysis of covariance; ΔCACS, change in coronary artery calcification score; ΔCCA-IMT, change in carotid artery intima media thickness

Bonferroni correction was applied for multiple comparisons. Pearson's linear regression equations were used to determine the power of association between continuous variables, while the Spearman rank correlation coefficient was calculated for variables with nonnormal distribution. Depending on data distribution, the comparison of follow-up data versus baseline was performed using the *t* test for dependent variables or Wilcoxon test, for independent variables. The Wilcoxon test was used to compare the results before and after the treatment in the same group, and the Mann–Whitney test to compare the results between the 2 treated groups. The analysis of covariance (ANCOVA) was used to adjust the follow-up CCA-IMT and CACS for baseline values. A subsequent stepwise linear regression analysis was performed to identify independent determinants of changes in CCA-IMT and CACS according to a gradual modeling approach. A *P* level of less than 0.05 was considered statistically significant.

RESULTS At baseline, 42 of 75 initially screened patients fulfilled the inclusion criterion of a CACS of 10 AU or higher. Compared with patients in the vitamin D group (5 women, 8 men; mean age, 55.4 ±15.2 years), patients randomized to the K+D group (14 women, 15 men; mean age, 59.4 ±9.6 years) demonstrated a lower baseline eGFR (22.4 ±10.1 ml/min/1.73m^2 vs 30.2 ±12.6 ml/min/1.73m^2, $P < 0.02$); lower serum uric acid levels (6.8 ±1.7 vs 8.5 ±1.9 mg/dl, $P < 0.004$); higher serum phosphate (1.4 ±0.4 vs 1.1 ±0.2 mmol/l, $P < 0.03$); higher calcium × phosphate product (3.3 ±1.06 vs 2.7 ±0.6 mmol^2/l^2, $P < 0.03$); and lower hemoglobin levels (11.7 ±1.3 vs 13.2 ±1.7 g/l, $P < 0.004$). Other initial routine laboratory parameters were not significantly different between the groups.

Forty patients completed the study. The anthropometric and laboratory parameters of the patients from both groups at the beginning and at the end of the treatment are shown in **TABLE 1**. Two patients were withdrawn during the study: 1 patient from the vitamin K+D group discontinued the treatment owing to bowel discomfort in the fifth week of the study and 1 patient from the D group died in the second month due to myocardial infarction (this patient had diabetes mellitus and a very high CACS, 1902 AU). Two patients from the vitamin K+D group started dialysis therapy during the intervention period (the first one 212 days after the start of the study and the second one after 234 days; their data were included into the final analysis).

The CACS significantly increased in both groups at the end of the treatment period: in the vitamin K+D group from 267.6 ±414.2 to 325.7 ±516.9, $P < 0.001$, and in the vitamin D group from 398.6 ±393.2 to 473 ±507.7, $P < 0.003$ (**FIGURE 2**). The change of the CACS was slightly lower in the vitamin K+D group than in the vitamin D group (**TABLE 2**). While a decrease of the CACS was noticed in 5 patients from the vitamin K+D group (5.4 ±5.2 AU), the CACS did not change in 2 patients. The scope of changes ranged from –11.8 to 380 AU in patients from the vitamin K+D group. In patients treated with vitamin D alone, the CACS increased from 4 to 426.5 AU. When patients with a CACS of 1000 AU or higher were excluded from the analysis, the differences in ΔCACS between patients treated with vitamin K+D and those receiving vitamin D alone showed borderline significance: 18.2 ±29.1 AU vs 39.2 ±49.8, respectively ($P = 0.06$).

A significantly lower increase of CCA-IMT during the intervention period was noticed in the

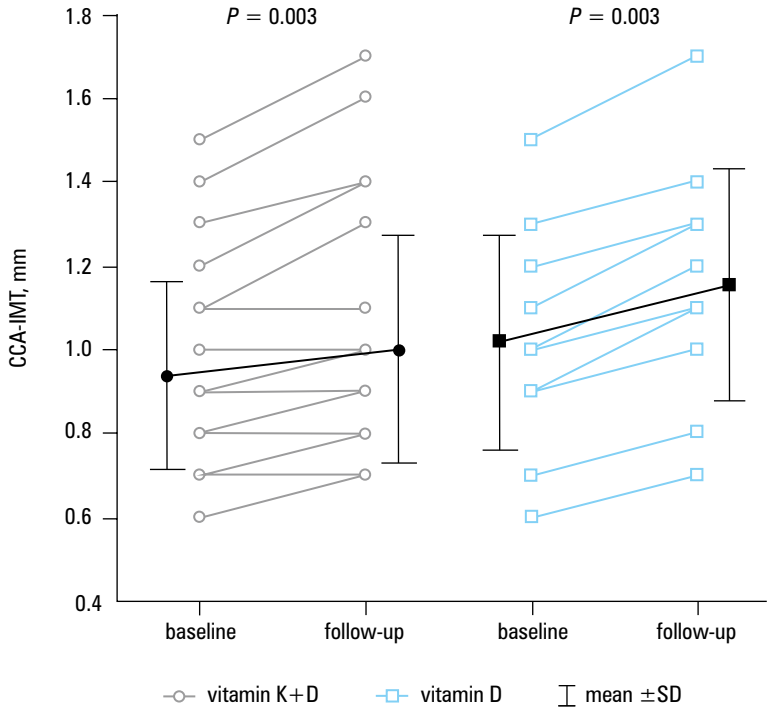

FIGURE 3 Common carotid intima-media thickness before and after 270 ±12 days of treatment with vitamin K_2 and vitamin D (K+D group) or vitamin D alone (D group) Abbreviations: see TABLE 1 and FIGURE 2

TABLE 3 Determinants affecting the change of common carotid artery intima-media thickness in a stepwise multivariate linear regression analysis in the whole study group

Variable	β	B ±SEM	B	B ±SEM	*P* value
CACS	0.486473	0.117017	0.000093	0.000022	0.0003
allocation to the treatment group	0.324939	0.123649	0.053876	0.020502	0.01
age	0.281064	0.128746	0.001903	0.000872	0.04
OPG	–0.219077	0.119218	–0.008118	0.004418	0.08
total cholesterol	–0.163047	0.120308	–0.000212	0.000157	0.2
FGF-23	0.154598	0.114646	0.000118	0.000088	0.2
hs-CRP	–0.126798	0.118165	–0.002016	0.001878	0.3

Abbreviations: SEM, standard error of the mean; others, see FIGURE 1 and TABLE 1

vitamin K+D group from 0.95 ±0.2 to 1.01 ±0.3, P <0.003 than in the D group: from 1.02 ±0.2 to 1.16 ±0.3, P <0.003 (FIGURE 3, TABLE 2). After 9 months of vitamin K_2 supplementation, a significant decrease of dp-ucMGP was observed. This effect was not observed in the vitamin D group. A significant increase of serum OPG levels was found in the vitamin K+D group. A significant increase of serum OC concentrations was observed in patients treated with vitamin D alone, in contrast to the vitamin K+D group, in which the serum concentration of OC decreased. A borderline increase in the FGF-23 level in K+D patients during the treatment was observed, while in the vitamin D group, it did not change. The changes in laboratory parameters between the study groups at the end of the study are presented in TABLE 1.

A strong linear correlation was noted between changes over time of ΔCACS and ΔCCA-IMT in patients from the vitamin K+D group (r = 0.65, P = 0.0004, FIGURE 4). Significant correlations between serum phosphorus and ΔCACS (r = 0.47, P = 0.01), similar to the relationship between the calcium–phosphate index and ΔCACS (r = 0.47, P = 0.01) were observed only in the vitamin K+D group. No significant correlation between calcification modulators and CACS and CCA-IMT was found.

In a stepwise multivariate linear regression analysis performed for patients from both groups together, the CACS, allocation to the treatment group, and age were found to be independent determinants of the ΔCCA-IMT (TABLE 3). The ΔCACS was dependent on the CACS, hs-CRP, 25(OH)D, phosphate, triglyceride, FGF-23, LDL cholesterol, and OC levels (TABLE 4). The allocation to the treatment group had no effect on ΔCACS in this analysis.

DISCUSSION To the best of our knowledge, this study is the first to assess the effect of vitamin K_2 administration on atherosclerosis and calcification progression in CKD patients. It showed that the progression of CCA-IMT is significantly slower in patients treated with both vitamin K_2 and cholecalciferol compared with patients receiving vitamin D alone. Therefore, since all patients received the same dose of vitamin D, but only in one of the arms vitamin K_2 was administered, by comparing the treatment results from both groups, we could assume that the differences between the groups were dependent mainly on the effects of vitamin K_2. This is consistent with a previous observation of Gast et al[15] that high dietary intake of menaquinones may protect against the development of coronary heart disease. Shanahan et al[16] showed that MGP mRNA was present in the normal media, but its expression was the greatest in the atheromatous intima. The immunohistochemical data reported by Schurgers et al[9] demonstrated that undercarboxylated MGP is abundantly present in atherosclerotic intima and in media sclerosis, suggesting local vitamin K deficiency and impaired protection attributable to poor MGP carboxylation.[9]

A tendency to slow the progression of CAC in patients who received both vitamins K_2 and D was observed in the present study, mostly in patients with less advanced baseline calcification. However, the change between both treated groups was relatively small, probably due to a short follow-up period, insufficient number of patients, and a wide range of CACS at baseline. Furthermore, in this study, although regression and stabilization of CACS was noticed in a few patients supplemented with MK-7, such effect was not observed in the vitamin D group. Previously, Shea et al[17] showed that the supplementation of vitamin K_1 for 3 years slowed the progression of VC in elderly people with preexisting calcification. A number of studies concluded that these 2 processes, atherosclerosis and VC, are different pathologies,[18] while others showed that they were closely related and VC represented a more advanced atherosclerotic

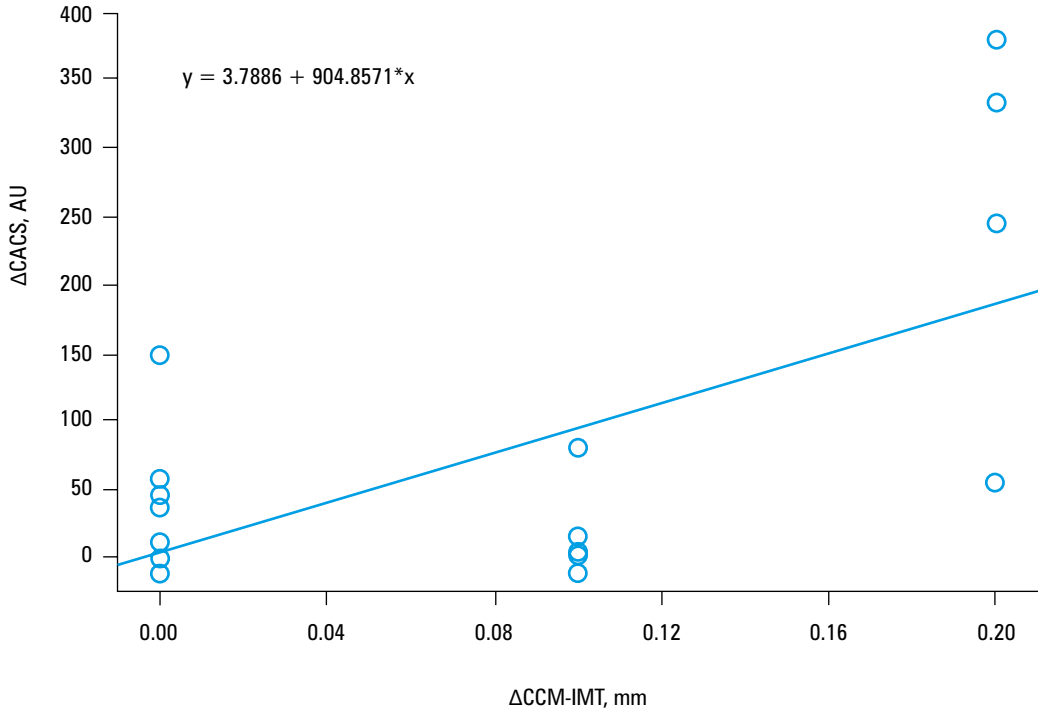

FIGURE 4 Correlation between the change of coronary artery calcification score (ΔCACS) and the change of common carotid intima-media thickness (ΔCCA-IMT) over time of vitamin K_2 and D administration ($r = 0.65$, $P = 0.0004$)
Abbreviations: **TABLE 2**

process that involved both layers.[19-21] Our study showed a strong correlation between the changes of CACS and CCA-IMT. Considering the common background of these 2 pathologies, we suspect that supplementing vitamin K_2 for a longer time could result in a more distinct inhibition of calcification process.

Deficiency of carboxylated MGP may contribute substantially to the development and progression of arterial calcification. Areas of calcification in vascular tissue are associated with accumulation of unMGP species, which has also been found to precede the development of clinically overt calcification in children on dialysis.[22] In our study, the serum level of dp-ucMGP decreased significantly during vitamin K_2 supplementation. The substitution of vitamin K_2 could possibly cause an increase in MGP carboxylation in the vascular wall and slow down the progression of atherosclerosis.

Previous studies evaluating the association of OC with VC and cardiovascular disease have provided conflicting results. One study demonstrated that higher OC levels are associated with lower vascular stiffness and CCA-IMT,[23] whereas another showed that higher OC levels are associated with more advanced VC in animal models.[24] Parker et al[25] observed no association of OC and aortic calcification in postmenopausal women. In our study, the OC level decreased during the substitution of vitamin K_2 with vitamin D. This phenomenon seems to be dependent on vitamin K_2 concentration as the OC level was found to increase in patients from the reference group receiving vitamin D alone.

OPG has been proposed as a protective factor against VC.[26] In our study, the serum OPG increased during supplementation with vitamin K_2. Our findings confirm the previous experimental[27] and human studies[28] which found that 15 mg/24 h of menatetrenone (vitamin K_2) prevents the reduction of serum OPG levels in patients treated with glucocorticoids. OPG has previously been reported to prevent calcification during the administration of warfarin and vitamin D.[29]

No significant changes in the level of fetuin A and mineral parameters including FGF-23 during vitamin K_2 substitution was noted in the present study. Nagasawa et al[30] showed a decrease in total and LDL cholesterol levels after 6 months of supplementation with 45 μg of vitamin K in patients on peritoneal dialysis. We did not observe any significant effect of vitamin K_2 substitution on lipid levels, but all our study patients were treated with statins before and during vitamin substitution.

The limitations of our study include a small sample size, resulting in the statistical power that does not allow clinically relevant conclusions to be drawn. A follow-up interval of 270 days may have been too short to detect the differences in the progression of atherosclerosis and VC, which develop over many years. On the other hand, the development of vascular wall changes in CKD patients is faster than in the general population.[31] Our study group was quite homogenous, but the treatment groups were different in baseline eGFR, uric acid, phosphate and calcium × phosphate products, and hemoglobin level. The baseline values of CACS and CCA-IMT were not significantly different between the vitamin K+D and vitamin D groups but were slightly lower in the former, hence the effect of the baseline values on our final observation cannot be excluded. The power of the test used to compare the effect of vitamin K+D versus vitamin D alone on the ΔCACS and ΔCCA-IMT was insufficient due to the small group of patients and a wide range of baseline values. Furthermore, only 1 dose of MK-7 was studied and the vitamin K concentration was not

TABLE 4 Determinants affecting the change of coronary artery calcification score analyzed in a stepwise multivariate linear regression analysis in the whole study group

Variable	B	B ±SEM	B	B ±SEM	*P* value
CACS	0.893942	0.048580	0.245	0.01332	0.000
hs-CRP	0.179852	0.046419	4.119	1.06316	0.001
25(OH)D	0.174530	0.052890	1.784	0.54050	0.003
phosphate	0.233145	0.083932	67.625	24.34490	0.01
triglyceride	0.191224	0.070772	0.208	0.07708	0.01
FGF-23	–0.188738	0.075961	–0.208	0.08387	0.02
LDL cholesterol	–0.174231	0.072816	–0.453	0.18925	0.02
OC	0.141978	0.065692	0.300	0.13881	0.04
age	–0.078647	0.055006	–0.767	0.53647	0.2
OPG	–0.065404	0.051691	–3.492	2.75981	0.2
MGP	0.042400	0.049333	0.025	0.02906	0.4

Abbreviations: see **FIGURE 1** and **TABLE 1**

measured before and after the treatment; however, the patient's compliance may be confirmed by the change of dp-ucMGP and 25(OH)D levels observed in both groups. In addition, we did not notice any significant differences between the treated groups over time; we only found differences within the groups and between the groups at baseline and at final measurement. Another limitation of the study is that we did not distinguish between different forms of OC (carboxylated, uncarboxylated), which made the interpretation of the results more difficult.

In conclusion, a 270-day course of vitamin K_2 administration (90 μg) may reduce the progression of atherosclerosis in nondialysis subjects with CKD stages 3–5, but does not have a significant effect on CAC progression. Larger studies are needed to confirm whether vitamin K_2 needs to be supplemented in CKD patients for the prevention of atherosclerosis and VC. The mechanisms by which vitamin K_2 may exert a protective effect on the progression of vessel damage are still uncertain, but may be associated with the effect of MK-7 on the regulators of calcification, including the impact on the MGP carboxylation process.

Contribution statement IK designed and performed the study, analyzed the data, and wrote the paper. AM-Z, PG, and MK performed the study. LS performed the study and contributed to the writing of the paper. CV designed the study and analyzed the data. KM designed the study and analyzed the data. MN designed the study, analyzed the data, and wrote the paper. All authors provided intellectual content of critical importance to the study as well as edited and approved the final version of the manuscript.

Acknowledgments The authors gratefully acknowledge Hogne Vik, MD, PhD (CEO, NattoPharma, Norway) for supplying MenaQ7 and for preparation of the vitamin K_2+cholecalciferol and cholecalciferol-alone tablets. This study was a part of a habilitation thesis (Kurnatowska I. Medical University, Łódź 2013). This study was partially supported by the Medical University of Lodz, grant No. 503/1-151-02/503-01, and was registered in *ClinicalTrials.gov Identifier: NCT01101698*.

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ARTYKUŁ ORYGINALNY

Wpływ witaminy K_2 na postęp zmian miażdżycowych i zwapnienia naczyń u niedializowanych chorych w 3.–5. okresie przewlekłej choroby nerek

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SŁOWA KLUCZOWE

miażdżyca, przewlekła choroba nerek, witamina K_2, zwapnienie naczyń

STRESZCZENIE

WPROWADZENIE Badania obserwacyjne wskazują, że duża zawartość witaminy K_2 w diecie zmniejsza ryzyko wystąpienia choroby wieńcowej oraz zwapnienia naczyń.

CELE Oceniano wpływ podawania witaminy K_2 na postęp zmian miażdżycowych i zwapnienia tętnic wieńcowych u niedializowanych chorych w 3.–5. okresie przewlekłej choroby nerek (PChN).

PACJENCI I METODY W badaniu udział wzięło 42 pacjentów niedializowanych z PChN. Wyjściowo oraz po 270 ±12 dniach podawania 90 µg witaminy K_2 (menaquinone-7) łącznie z 10 µg cholekalcyferolu (grupa K+D) lub tylko 10 µg cholekalcyferolu (grupa D) zostały zmierzone: ultrasonograficznie grubość kompleksu błony środkowej i wewnętrznej (*common carotid intima-media thickness* – CCA-IMT), wskaźnik uwapnienia tętnic wieńcowych (*coronary artery calcification score* – CACS), podstawowe parametry biochemiczne, lipidy oraz modulatory zwapnień – białko Gla macierzy (*matrix Gla protein* – MGP), defosforylowana-niekarboksylowana postać MGP (dp-ucMGP), osteoprotegeryna (OPG), fetuina A, osteokalcyna (OC) oraz czynnik wzrostu fibroblastów 23.

WYNIKI Wzrost CCA-IMT był znamiennie mniejszy w grupie K+D w porównaniu z grupą D i wynosił odpowiednio: od 0,95 ± 0,2 mm do 1,01 ±0,3; p = 0,003 *vs* od 1,02 ±0,2 mm do 1,16 ±0,3, p = 0,003 (ΔCCA-IMT: 0,06 ±0,08 *vs* 0,136 ±0,05; p = 0,005). Zwiększenie CACS było nieznacznie mniejsze w grupie K+D niż w grupie D (ΔCACS: 58,0 ± 106,5 j.A. *vs* 74,4 ± 127,0 j.A.; p = 0,7). U pacjentów z grupy K+D stwierdzono znamienne zmniejszenie stężenia dp-ucMGP i OC oraz zwiększenie OPG.

WNIOSKI 270-dniowe stosowanie witaminy K_2 u chorych w 3.–5. okresie PChN może zmniejszyć postęp zmian miażdżycowych, nie wpływa zaś istotnie na postęp zwapnienia tętnic wieńcowych. Witamina K_2 znamiennie wpływa na stężenia czynników pobudzających i hamujących wapnienie naczyń: białko dp-ucMGP, OC oraz OPG.

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Praca wpłynęła: 07.06.2015.
Przyjęta do druku: 14.07.2015.
Publikacja *online*: 17.07.2015.
Nie zgłoszono sprzeczności interesów.
Pol Arch Med Wewn. 2015; 125 (9): 631-640