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The Association of Vitamin D and Vitamin K Status with Subclinical Measures of Cardiovascular Health and All-Cause Mortality in Older Adults: The Hoorn Study

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ABSTRACT

Background: A low vitamin D and K status has been associated with increased cardiovascular disease (CVD) risk but the evidence of their combined effect on cardiovascular health is limited.

Objectives: Our study aimed to investigate the prospective association of vitamin D and K status with subclinical measures of cardiovascular health and all-cause mortality among a population of Dutch Caucasians.

Methods: We performed an observational prospective study on 601 participants of the Hoorn Study (mean ± SD age: 70 ± 6 y, 50.4% women, BMI: 27.2 ± 4.0 kg/m^2), of whom 321 underwent an echocardiogram in 2000–2001 and 2007–2009. Vitamin D and K status was assessed at baseline by serum 25-hydroxyvitamin D [25(OH)D] and plasma desphospho-uncarboxylated matrix-gla protein (dp-ucMGP)—high concentrations indicate low vitamin K status. Vital status was assessed from baseline until 2018. We studied the association of categories of 25(OH)D (stratified by the clinical cutoff of 50 mmol/L) and dp-ucMGP (stratified by the median value of 568 pmol/L) with echocardiographic measures using linear regression and with all-cause mortality using Cox regression, adjusted for confounders.

Results: Compared with markers of normal vitamin D and K status, markers of low vitamin D and K status were prospectively associated with increased left ventricular mass index (5.9 g/m$^{2.7}$; 95% CI: 1.8, 10.0 g/m$^{2.7}$). Participants with low vitamin D and K status were also at increased risk of all-cause mortality with an HR of 1.64 (95% CI: 1.12, 2.39) compared with normal vitamin D and K status.

Conclusions: A combination of low vitamin D and K status is associated with adverse cardiac remodeling and increased risk of all-cause mortality in men and women. Future studies should investigate whether vitamin D and K supplementation could help to improve cardiovascular health and to decrease CVD risk. *J Nutr* 2020;150:3171–3179.

Keywords: vitamin K, dp-ucMGP, vitamin D, echocardiography, mortality, cardiac structure

Introduction

Vitamin D is mainly known for its role in calcium metabolism (1). Vitamin D deficiency is associated with arterial hypertension, type 2 diabetes (T2D), and left ventricular (LV) hypertrophy (2, 3), and with a higher incidence of heart failure (HF) (4). Furthermore, a recent meta-analysis including 84 articles reported an inverse association between 25-hydroxyvitamin D [25(OH)D] concentrations and all-cause mortality (5). However, a meta-analysis of 21 randomized clinical trials did not show any significant effect of vitamin D supplementation on reduced rates of all-cause mortality or cardiovascular disease (CVD) events (6).

Vitamin K is mostly known for its function to activate hepatic coagulation factors, but has also a role in bone and cardiovascular health (7–9). In fact, vitamin K is responsible for the activation of vitamin K-dependent Gla-proteins in extrahepatic tissues, such as matrix Gla-protein (MGP). MGP is a powerful inhibitor of vascular calcification (8), and vitamin K deficiency increases the amount of nonfunctional MGP leading to increased arterial calcification and stiffness (8, 10). Further, a low vitamin K status was associated with incident CVD and CVD-related outcomes especially among high-risk populations, such as people with T2D, with the strongest association for HF (11, 12). However, several randomized trials

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have shown inconsistent effects of vitamin K supplementation on calcification (13, 14).

Previous studies have suggested a synergistic effect of vitamins D and K on cardiovascular health, because vitamin D promotes the production of vitamin K-dependent proteins (15–18). Intervention studies that evaluated the effect of combined vitamin D and vitamin K supplementation in postmenopausal women observed a benefit on vascular function (16), and observational studies demonstrated that low concentrations of both vitamins D and K are associated with hypertension and increased carotid intima-media thickness (19–21). Taken together, these studies indicate that the combined effect of vitamin D and vitamin K might be superior than the effect of either alone, but the evidence for their potential synergistic effect on cardiovascular health is still limited. The aim of this study was therefore to assess whether biomarkers of vitamin D and vitamin K status were synergistically and prospectively associated with subclinical measures of cardiovascular health and all-cause mortality. Moreover, we aimed to investigate whether estimates of cardiac structure and function acted as mediators in the relation between vitamin D and K status and mortality.

Methods

Study population

The Hoorn Study is a prospective observational study in Dutch Caucasian adults (n = 2484) and was initiated in 1989 (22). The current study was done in a subsample of 831 participants stratified by glucose tolerance status who had examinations in 2000–2001 (considered as the baseline) (22). The cohort has been followed since for registration of morbidity and mortality. Participants were excluded from the current study if they had missing data on survival status and on biomarkers of vitamin D and K status, as displayed in **Supplemental Figure 1**, resulting in a sample of 601 participants for survival analysis. After 7 y of follow-up (2007–2009), repeated echocardiograms were obtained in 438 participants (349 participants were lost to follow-up). For the prospective analysis on echocardiographic data, we selected a sample of 321 participants: exclusion of participants was based on the presence of missing data on biomarkers of vitamin D and K status and on echocardiography, as displayed in Supplemental Figure 1. We did not have complete data on all echocardiographic measures, which resulted in different analytic samples for each outcome measure: 250 participants for LV ejection fraction (LVEF), 252 for LV mass index ($LVMI^{2.7}$), and 270 for left atrial volume index maximal and minimal (LAVI max and LAVI min). All participants provided written informed consent. The study was approved by the local ethics committee of the VU University Medical Centre and complies with the Declaration of Helsinki.

Measurements of vitamin D and vitamin K status

A blood sample was drawn from all participants in an 8- to 12-h fasting state to measure biomarkers of vitamin D and vitamin K status at baseline. Samples were centrifuged at temperature of 4°C at 4000 rpm for 10 min and stored at −80°C until assessment of biochemical measures. For vitamin D status, serum 25(OH)D concentrations were determined by using a competitive binding protein assay (DiaSorin), which had an interassay CV of 10%–15%. To determine vitamin K status, the desphospho-uncarboxylated matrix-gla protein (dp-ucMGP) was measured in plasma samples by using a sandwich ELISA that uses antibodies against the dephosphorylated and uncarboxylated amino acid sequence (VitaK BV). The interassay variability for this assay was 9.9%. High concentrations of dp-ucMGP reflect a low vitamin K status (21).

Mortality

In 2018 the vital status of the entire cohort was assessed to determine all-cause mortality by linkage with the municipality register.

Echocardiography

An experienced ultrasound research technician performed the echocardiographic imaging at baseline and at follow-up, using the HP SONOS 5500 echocardiography system (2–4 MHz transducer) according to a standardized protocol consisting of 2-dimensional, M-mode, and pulsed-wave Doppler assessments. All echocardiograms were evaluated afterward by a senior cardiologist. LV systolic function was measured by LVEF: LV endocardial borders were manually traced at end-diastole and end-systole in the apical 4- and 2-chamber views and LV volumes and LVEF derived according to the biplane method of disks (modified biplane Simpson's rule). Cardiac structure was measured by $LVMI^{2.7}$, which was calculated by the American Society of Echocardiography recommended formula for estimation of LV mass from LV linear dimensions and indexed to height $(m)^{2.7}$ (23). As markers of LV diastolic function, left atrial volumes were measured at LV end-systole (LAVI max) and at LV end-diastole (LAVI min).

Covariates

Participants filled out questionnaires to obtain self-reported information on medication use, smoking status, education level, and physical activity. High education level was defined as higher vocational education or university, intermediate level as secondary education, and low level as elementary school, lower vocational training, or less. Time spent on outdoor activities (h/d) was assessed by a validated questionnaire as described elsewhere (22).

Glycated hemoglobin (HbA1c), fasting plasma glucose, and blood plasma lipid concentrations including total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured as described elsewhere (22). Serum intact parathyroid hormone was determined by an immunoradiometric assay (DiaSorin). Plasma B-type natriuretic peptide (BNP) was measured in spare frozen samples in EDTA-coated tubes that had been stored at −80°C and determined by using an immunoradiometric assay at baseline (Shionoria; inter- and intra-assay CVs < 10%) and by using a noncompetitive immunofluorometric assay at follow-up (TRIAGE, Biosite; intra-assay CV = 3.5%; total CV = 12.3%).

All participants underwent physical examination. BMI was calculated by dividing body weight by height squared (kg/m^2). Blood pressure (BP; mm Hg) was measured according to a standardized protocol and arterial hypertension was defined as systolic blood pressure (SBP) ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, and/or use of antihypertensive medication. As an estimate of kidney function, estimated glomerular filtration rate was calculated using serum creatinine values—determined using the Jaffé method—according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (24).

Statistical analysis

All analyses were performed using IBM SPSS Statistics for Windows version 22.0 (IBM Corp.) and R-Studio 3.4.2 (R Foundation for Statistical Computing). We aimed to test whether biomarkers of

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Abbreviations used: BNP, B-type natriuretic peptide; BP, blood pressure; CVD, cardiovascular disease; Dp-ucMGP, desphospho-uncarboxylated matrix-gla protein; HbA1c, glycated hemoglobin; HF, heart failure; IPW, inverse-probability weighting; LAVI, left atrial volume index; LV, left ventricular; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; MGP, matrix Gla-protein; SBP, systolic blood pressure; T2D, type 2 diabetes; 25(OH)D, 25-hydroxyvitamin D.

vitamin K and D status were synergistically associated with both estimates of cardiac structure and function and mortality. We therefore categorized participants into 4 categories based on the clinical cutoff value of 50 nmol/L for 25(OH)D and the median for dp-ucMGP, because no cutoff value is available yet. Because we analyzed 2 different subgroups of participants, we established analysis-specific vitamin K categories by using the median for each subgroup. The reference group was the group with 25(OH)D ≥ 50 nmol/L and dp-ucMGP < median, which was considered to have normal vitamin D and K status. Multiple linear regression analysis was used to study the associations between categories of vitamin D and K status and cardiac structure and function measures ($LVMI^{2.7}$, LVEF, LAVI max, and LAVI min) after 7 y of follow-up. Multivariable Cox proportional hazards models were used to estimate the HRs of categories of vitamin D and vitamin K status for all-cause mortality after 17 y of follow-up. Differences in survival between 25(OH)D and dp-ucMGP categories were graphically displayed using Kaplan–Meier curves followed by log-rank tests. For these analyses we used predefined models to adjust for potential confounders. A minimally adjusted model included age (y), sex, and glucose status (3 categories) (model 1) with the addition of the baseline echocardiographic measure and follow-up time for the linear regression analysis. The fully adjusted model in addition included BMI (kg/m^2), smoking (never/former/current), LDL-cholesterol concentrations (mmol/L), HbA1c concentrations (%), SBP (mm Hg), presence of CVD (yes/no), and antihypertensive medication use (yes/no). Sex and presence of CVD were assessed as effect modifiers by including interaction terms in the fully adjusted models, because of described sex differences in 25(OH)D concentrations (25) and because CVD treatment and self-care strategies could have already changed cardiac function. Likewise, interaction between biomarkers of vitamin D and vitamin K status as continuous variables was estimated by adding interaction terms to the fully adjusted models. All interaction terms were in addition included in the models with the main effects. Wald tests were used to assess whether the models with the interaction terms differed significantly from those without. A P value < 0.10 was considered statistically significant. We also assessed associations of dp-ucMGP as a continuous variable with echocardiographic measures and all-cause mortality in participants with normal and low vitamin D status. To avoid the influence of a few outliers with higher values of dp-ucMGP, these analyses were limited to participants with values of dp-ucMGP < 2000 pmol/L (25). Because a clinically established cutoff of vitamin D deficiency status exists, we decided not to analyze 25(OH)D as a continuous variable.

The role of cardiac structure and function measures as mediators in the relation between categories of vitamin D and K status and all-cause mortality was investigated in a subgroup of participants who underwent both baseline and follow-up examinations and for whom data on vital status were available (n = 320) (**Supplemental Figure 2**). For mediation analysis, the product-of-coefficients estimator was used to estimate the indirect effects, calculated as the product of a and b coefficients, where a measures the strength of the effect of vitamin D and K status on the mediator, and b is the effect of the mediator on mortality adjusting for vitamin D and K status (26). The 95% CIs of the indirect effects were then estimated with the Monte Carlo Method for Assessing Mediation by using the coefficient estimates and their associated asymptotic variances and covariance (27). A significant indirect effect indicates mediation by the mediation variable.

Sensitivity analyses were conducted by *1*) adjusting the final multiple linear regression and Cox regression models for changes over time in smoking, SBP, and BMI; *2*) excluding participants with incident CVD during follow-up; and *3*) creating categories of participants based on the currently proposed cutoff value for dp-ucMGP of 450 pmol/L (28). Finally, to investigate selection bias due to loss to follow-up, baseline characteristics of participants and dropouts were compared and a sensitivity analysis was performed using inverse-probability weighting (IPW) for both the linear regression and Cox regression models. In case of missing values for confounders (maximum 2.5%), multiple imputations were used combining 5 iterations into 1 imputation model. All reported P values were 2-sided and P values ≤ 0.05 were considered statistically significant.

Results

Study population

The mean ± SD age of the 601 participants was 70 ± 6 y, 50.4% of participants were women, and 18.5% had T2D. Those excluded in this study had a similar age, a higher proportion of T2D and hypertension, and a slightly worse cardiac structure and function (**Supplemental Table 1**).

The distribution of serum 25(OH)D was approximately normal with a mean value close to the clinical cutoff, equal to 53.7 ± 19.7 nmol/L, whereas plasma dp-ucMGP showed a skewed distribution with a median value of 568 pmol/L (IQR: 398–766 pmol/L). Participants with low vitamin D/low vitamin K status [25(OH)D ≤ 50 mmol/L and dp-ucMGP > 568 pmol/L] were older, had a higher BMI (28.6 compared with 25.9 kg/m^2), and a higher prevalence of T2D (25.8% compared with 10.2%) and of arterial hypertension (65.6% compared with 41.2%) than those with normal vitamin D/normal vitamin K status. In addition, the group with low vitamin D/low vitamin K status had a more prevalent cardiovascular drug use (16.1% compared with 10.2% for lipid-lowering agents and 47.0% compared with 21.1% for antihypertensive medications) and a higher BNP (23.4 compared with 12.1 pg/mL) than the reference group (**Table 1**).

Associations of vitamin D and vitamin K status with cardiac structure and function after 7 y of follow-up

Among 321 participants, the median follow-up time for changes in cardiac structure and function was 7.0 y (IQR: 6.0–8.0 y). Participants with low vitamin D/low vitamin K status had the least favorable cardiac structure compared with normal vitamin D and K status (**Table 2**). Low vitamin D/low vitamin K status was strongly prospectively associated with higher $LVMI^{2.7}$ (β: 7.0; 95% CI: 2.9, 11.1; model 1, Table 2) compared with the reference group. The association attenuated after adjustment (β: 5.9; 95% CI: 1.8, 10.0; model 2, Table 2), but remained significant. For $LVMI^{2.7}$, a significant interaction between biomarkers of vitamin D and K status as continuous variables was found (P-interaction = 0.014 for the fully adjusted model). No interaction was found between 25(OH)D and dp-ucMGP and the other echocardiographic variables. Categories of vitamin D and K status were not associated with LAVI max and LAVI min. We found no effect modification by presence of CVD in these associations (P < 0.10). We stratified the analyses for LVEF by sex because significant effect modification was present for the association between categories of vitamin D and K status and LVEF (P-interaction = 0.009 for the normal vitamin D/low vitamin K interaction dummy and P = 0.042 for the low vitamin D/normal vitamin K interaction dummy in the fully adjusted model). Compared with the reference category, men within the normal vitamin D/low vitamin K and within the low vitamin D/normal vitamin K group had no decline in LVEF after 7 y (β: 0.6; 95% CI: −0.8, 2.1; P = 0.409 and β: −0.01; 95% CI: −2.3, 2.3; P = 0.995, respectively; model 2, Table 2). Conversely, women within the normal vitamin D/low vitamin K group showed a tendency toward lower LVEF (β: −2.0; 95% CI: −3.8, −0.2, P = 0.032; model 2, Table 2). As regards the low vitamin D/normal vitamin K and the low vitamin D/low vitamin K groups, women did not have a significantly lower LVEF after 7 y (β: −2.1; 95% CI: −5.0, 0.7; P = 0.139 and β: −4.0; 95% CI: −11.3, 3.4, P = 0.285, respectively; model 2, Table 2). Men in the low vitamin D/low vitamin K category showed little or no reduction

TABLE 1 Baseline characteristics based on 25(OH)D and undercarboxylated Matrix-Gla-Protein status in 601 participants from the Hoorn Study[1]

	Normal vitamin D/ normal vitamin K[2]	Normal vitamin D/ low vitamin K	Low vitamin D/ normal vitamin K	Low vitamin D/ low vitamin K
Serum 25(OH)D, nmol/L	69.5 ± 14.1	67.0 ± 12.6	38.3 ± 7.5	34.5 ± 9.1
Plasma dp-ucMGP, pmol/L	395 [286–472]	726 [628–902]	390 [262–468]	809 [655–1004]
Demographics				
n	166	162	122	151
Age, y	67 ± 5	70 ± 6	70 ± 8	73 ± 7
Women	66 (39.8)	72 (44.4)	64 (52.5)	101 (66.9)
Education level[3]				
Low	93 (56.0)	99 (61.1)	65 (53.3)	88 (58.3)
Intermediate	54 (32.5)	53 (32.7)	44 (36.1)	50 (33.1)
High	19 (11.4)	10 (6.2)	13 (10.7)	13 (8.6)
Comorbidities				
Arterial hypertension	68 (41.2)	90 (55.6)	79 (64.8)	99 (65.6)
Prior CVD	76 (46.9)	91 (58.0)	59 (48.4)	96 (64.9)
Atrial fibrillation	2 (1.2)	7 (4.5)	0 (0.0)	8 (5.7)
Glucose status				
IGT	26 (15.7)	40 (24.7)	21 (17.2)	45 (29.8)
Diabetes	17 (10.2)	36 (22.2)	19 (15.6)	39 (25.8)
Medications				
Antihypertensive	35 (21.1)	70 (43.5)	36 (29.5)	70 (47.0)
Lipid-lowering agents	17 (10.2)	35 (21.7)	18 (14.8)	24 (16.1)
Insulin	8 (4.8)	17 (10.6)	10 (8.2)	9 (6.0)
Lifestyle				
Outdoor activities, h/d	0.9 [0.4–1.8]	0.8 [0.4–1.6]	0.9 [0.4–1.4]	0.6 [0.0–1.3]
Current smokers	35 (21.1)	13 (8.1)	24 (19.7)	19 (12.8)
Former smokers	80 (48.2)	84 (52.2)	48 (39.3)	59 (39.6)
Adiposity				
BMI, kg/m^2	25.9 ± 3.1	28.1 ± 4.0	26.3 ± 3.6	28.6 ± 4.6
Waist circumference, cm	90.9 ± 10.7	97.5 ± 11.2	92.2 ± 11.5	97.6 ± 11.9
Metabolic variables				
SBP, mm Hg	134 ± 16	142 ± 19	142 ± 20	151 ± 21
DBP, mm Hg	74 ± 9	77 ± 9	77 ± 10	78 ± 9
FPG, mmol/L	5.6 [5.2–6.2]	5.9 [5.5–6.5]	5.7 [5.4–6.4]	6.0 [5.5–6.5]
Plasma HbA1c, %	5.8 [5.4–6.1]	5.9 [5.6–6.3]	5.8 [5.5–6.1]	6.0 [5.6–6.3]
Plasma LDL-C, mmol/L	3.7 ± 0.9	3.6 ± 0.9	3.7 ± 0.9	3.6 ± 0.9
Plasma TGs, mmol/L	1.2 [0.9–1.7]	1.3 [1.0–1.7]	1.3 [1.0–1.7]	1.3 [1.0–1.9]
Serum PTH, pmol/L	4.9 [4.0–6.1]	5.7 [4.6–6.9]	5.6 [5.0–7.0]	6.4 [5.2–8.5]
eGFR, mL · min · 1.73 m^{-2}	83.9 [75.0–91.6]	77.9 [66.1–88.5]	84.8 [75.5–91.4]	80.7 [70.6–87.9]
Plasma BNP, pg/mL	12.1 [6.2–22.4]	20.8 [9.4–37.0]	16.3 [6.2–30.8]	23.4 [12.1–44.0]
Plasma CRP, mg/dL	1.6 [0.8–3.1]	2.5 [1.4–4.7]	1.6 [0.9–3.2]	2.6 [1.4–6.1]

[1]Values are *n* (%), means ± SDs, or medians [IQRs]. BNP, B-type natriuretic peptide; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; dp-ucMGP, desphospho-uncarboxylated matrix-gla protein; eGFR, estimated glomerular filtration rate; FPG: fasting plasma glucose; HbA1c, glycated hemoglobin; IGT, impaired glucose tolerance; LDL-C, LDL cholesterol; PTH, parathyroid hormone; SBP, systolic blood pressure; TG, triglyceride; 25(OH)D, 25-hydroxyvitamin D.
[2]Normal vitamin D stands for values of 25(OH)D > 50 mmol/L. Normal vitamin K stands for values of dp-ucMGP < 568 pmol/L.
[3]Education levels were defined as follows: low: elementary school, lower vocational training, or less; intermediate: secondary education; high: higher vocational education or university.

in LVEF after 7 y (β: −1.6; 95% CI: −7.4, 4.2; $P = 0.792$ for model 2, Table 2). When circulating dp-ucMGP was analyzed as a continuous variable in 318 participants with values of dp-ucMGP < 2000 pmol/L, a positive association with LVMI[2,7] was observed only in those with low vitamin D status (β: 2.6; 95% CI: 0.6, 4.5; $P = 0.010$; model 2, **Supplemental Table 2**).

Associations of vitamin D and vitamin K status with all-cause mortality after 17 y of follow-up

The mean follow-up time for survival analysis was 17.4 y (range: 17.0–18.0 y). After 17.4 y of follow-up, 310 of 601 participants had died (51.6%); all-cause mortality was the highest in the group with low vitamin D/low vitamin K status (68.2%), compared with the reference group with normal vitamin D/normal vitamin K status (38.6%). Differences in survival curves by combined vitamin D and vitamin K groups were significant for all-cause mortality: log-rank for quartiles $P < 0.001$ (**Figure 1**). The groups with low vitamin D/low vitamin K status had a significantly increased risk of mortality with an HR of 2.29 (95% CI: 1.67, 3.13; $P < 0.001$) compared with the reference group in a crude analysis. Adjustment for age, sex, and glucose status attenuated this association (HR: 1.60; 95% CI: 1.13, 2.26; $P = 0.008$; model 1, Table 3). Further adjustment for comorbidities and CVD risk factors

TABLE 2 Association of cardiac structure and function measures with 25(OH)D (≤/>50 mmol/L) and undercarboxylated Matrix-Gla-Protein (≤/>median) categories in 321 participants after 7 y of follow-up[1]

	Normal vitamin D/ normal vitamin K	Normal vitamin D/ low vitamin K	Low vitamin D/ normal vitamin K	Low vitamin D/ low vitamin K
LVMI$^{2.7}$, g/m$^{2.7}$	$n = 91$	$n = 73$	$n = 49$	$n = 39$
Baseline	38.0 ± 9.1	41.6 ± 11.7	39.0 ± 12.6	45.5 ± 14.4
Follow-up	38.1 ± 8.8	44.1 ± 12.3	39.2 ± 9.8	50.1 ± 15.1
Model 1,[3] β (95% CI)	Reference	1.2 (0.2, 2.3)	−0.5 (−2.3, 1.3)	7.0 (2.9, 11.1)
Model 2,[4] β (95% CI)	Reference	1.0 (−0.1, 2.1)	−0.5 (−2.3, 1.3)	5.9 (1.8, 10.0)
LVEF, %				
Men[5]	$n = 52$	$n = 36$	$n = 25$	$n = 16$
Baseline	62.3 ± 6.5	59.4 ± 7.9	64.2 ± 7.1	61.0 ± 9.7
Follow-up	51.9 ± 9.5	53.0 ± 10.2	53.3 ± 7.7	47.9 ± 11.4
Model 1,[3] β (95% CI)	Reference	0.9 (−0.5, 2.3)	0.6 (−1.7, 2.8)	−0.9 (−6.4, 4.5)
Model 2,[4] β (95% CI)	Reference	0.6 (−0.8, 2.1)	−0.01 (−2.3, 2.3)	−1.6 (−7.4, 4.2)
Women	$n = 40$	$n = 35$	$n = 28$	$n = 18$
Baseline	62.5 ± 10.2	66.0 ± 5.6	65.0 ± 5.5	62.8 ± 7.5
Follow-up	58.0 ± 9.8	52.8 ± 11.6	53.3 ± 10.2	53.2 ± 10.5
Model 1,[3] β (95% CI)	Reference	−2.0 (−3.7, −0.3)	−2.7 (−5.5, −0.01)	−4.5 (−11.4, 2.5)
Model 2,[4] β (95% CI)	Reference	−2.0 (−3.8, −0.2)	−2.1 (−5.0, 0.7)	−4.0 (−11.3, 3.4)
LAVI max, mL/m^2	$n = 95$	$n = 79$	$n = 55$	$n = 41$
Baseline	24.0 ± 7.4	24.0 ± 6.0	22.6 ± 4.9	27.8 ± 14.4
Follow-up	25.2 ± 10.5	24.7 ± 11.8	24.5 ± 10.4	32.0 ± 20.4
Model 1,[3] β (95% CI)	Reference	−0.5 (−1.5, 0.6)	−0.2 (−2.0, 1.5)	0.5 (−3.5, 4.5)
Model 2,[4] β (95% CI)	Reference	−0.9 (−2.0, 0.2)	−0.4 (−2.2, 1.4)	−0.8 (−4.9, 3.3)
LAVI min, mL/m^2	$n = 95$	$n = 79$	$n = 55$	$n = 41$
Baseline	13.5 ± 6.1	13.8 ± 5.4	12.4 ± 3.9	17.1 ± 12.7
Follow-up	12.6 ± 8.4	12.9 ± 8.0	13.0 ± 8.8	18.9 ± 20.5
Model 1,[3] β (95% CI)	Reference	−0.2 (−1.1, 0.6)	0.3 (−1.1, 1.7)	−0.1 (−3.3, 3.0)
Model 2,[4] β (95% CI)	Reference	−0.4 (−1.3, 0.4)	0.2 (−1.2, 1.6)	−0.3 (−3.6, 3.0)

[1] LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; 25(OH)D, 25-hydroxyvitamin D.
[3] Model 1 adjusted for age, sex, baseline echo index, follow-up time, and glucose status.
[4] Model 2 adjusted for the Model 1 variables plus BMI, glycated hemoglobin, LDL cholesterol, cigarette smoking, systolic blood pressure, antihypertensive medication use, and presence of cardiovascular disease.
[5] For the association between vitamin D and K status and LVEF, sex acted as an effect modifier. *P*-interaction = 0.009 for the normal vitamin D/low vitamin K interaction dummy and $P = 0.042$ for the low vitamin D/normal vitamin K interaction dummy in the fully adjusted model.

resulted in a similar risk estimate with an HR of 1.64 (95% CI: 1.12, 2.39; $P = 0.011$; model 2, Table 3). The other categories were not significantly associated with risk of all-cause mortality compared with the reference category with an HR of 1.29 (95% CI: 0.90, 1.85; $P = 0.171$) for the normal vitamin D/low vitamin K group and an HR of 1.12 (95% CI: 0.77, 1.63; $P = 0.537$) for the low vitamin D/normal vitamin K group, both in the fully adjusted model (Table 3). No significant interaction was observed between 25(OH)D and dp-ucMGP as continuous variables and all-cause mortality (*P*-interaction > 0.5). No effect modification by sex or presence of CVD was observed in these associations. We found a significantly increased risk of all-cause mortality for lower vitamin K status in 586 participants with values of dp-ucMGP < 2000 pmol/L, only in the category with low vitamin D status (HR: 1.23; 95% CI: 1.03, 1.46 for model 1 and HR: 1.19; 95% CI: 1.01, 1.41 for model 2, **Supplemental Table 3**), whereas no association was observed in the category of participants with normal vitamin D status.

Mediation analysis

None of the indirect effects estimated were significant, indicating no mediation by LVMI$^{2.7}$, LVEF, or LAVI max and LAVI min for any of the categories of vitamin D and K status (**Supplemental Table 4**).

Sensitivity analyses

Categorizing participants according to a cutoff of dp-ucMGP equal to 450 pmol/L for both linear regression and Cox regression attenuated associations between categories of vitamin D and K status and cardiac structure and function measures (**Supplemental Table 5**) and resulted in similar all-cause mortality risk estimates (**Supplemental Table 6**). In addition, excluding participants who developed CVD during follow-up ($n = 8$ for survival analysis and $n = 6$ for the prospective analysis on echocardiographic measures); adjusting for updated measurements of BMI, physical activity, and smoking status; or adjusting for selection bias due to loss to follow-up by using IPW did not materially change our findings (**Supplemental Tables 7, 8**).

Discussion

In this study, a combined low vitamin D and K status was prospectively associated with higher LVMI$^{2.7}$ values than for the reference group with normal vitamin D/normal vitamin K status. In women, the category with normal vitamin D/low vitamin K status showed a significantly depressed LVEF after 7 y of follow-up compared with adequate vitamin D and K status ($P = 0.032$), whereas this association was not significant ($P = 0.285$) for those with low vitamin D and K status.

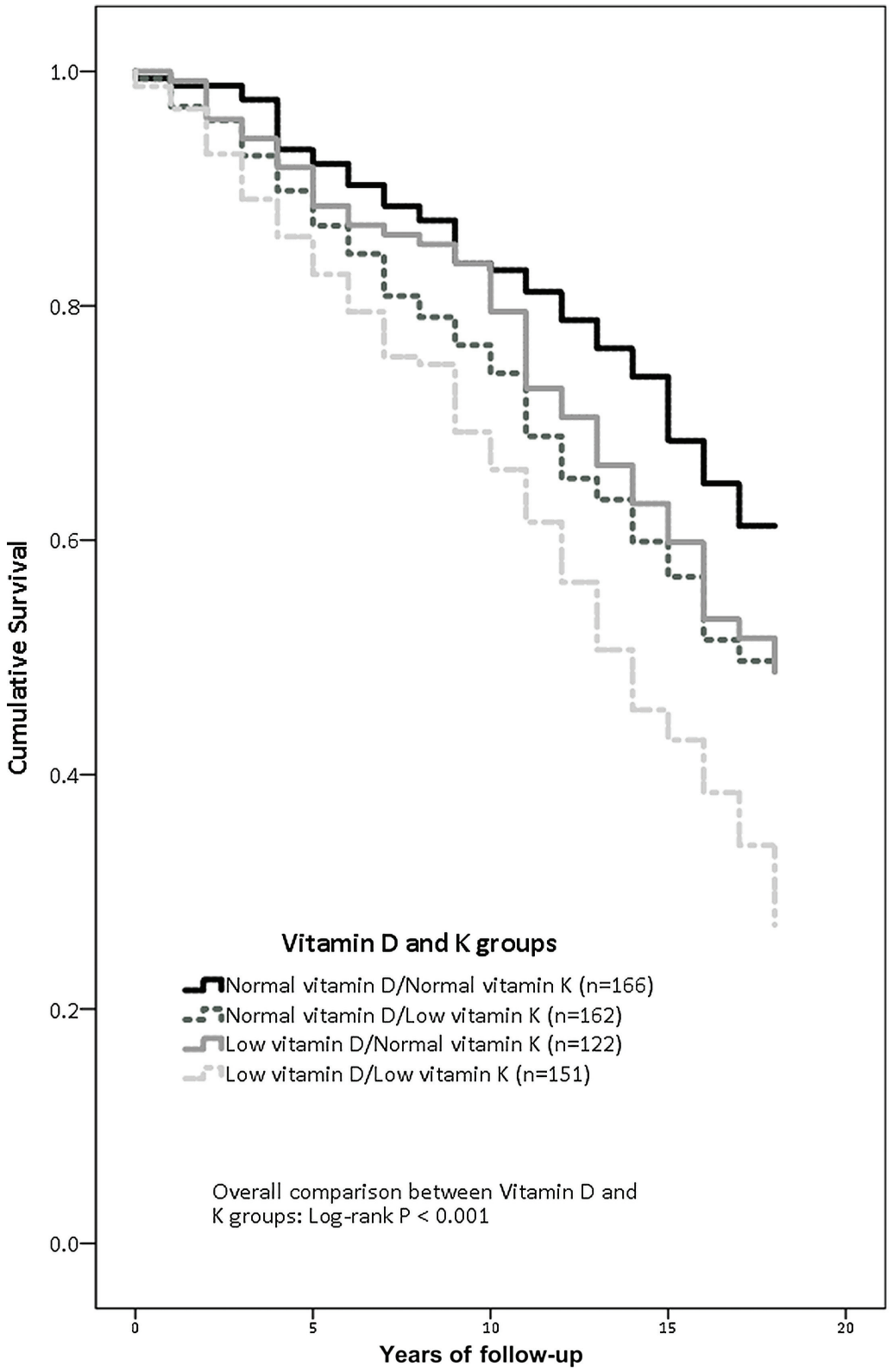

FIGURE 1 Kaplan–Meier curves for overall survival (log-rank $P < 0.001$), for 25-hydroxyvitamin D (≤/>50 mmol/L) and undercarboxylated Matrix-Gla-Protein (≤/>median) categories in 601 participants after 17 y of follow-up.

In men, none of the categories of vitamin D and K status showed a depressed LVEF. Participants with a combined vitamin D and vitamin K deficiency were at increased risk of all-cause mortality, compared with adequate vitamin D and K status. A significant association between circulating plasma dp-ucMGP as a continuous variable and both LVMI[2,7] and all-cause mortality was observed only in participants with low vitamin D status. The unfavorable effect of low vitamin D and

K status on survival was not mediated by cardiac structure and function measures.

Vitamin D and K status in relation to cardiac structure and function

The association between combined vitamin D and K status and echocardiographic measures has, to our knowledge, not been studied before. A combination of both low vitamin D and K status has been investigated in relation to other parameters of cardiovascular health such as BP and vascular stiffness, with results comparable with those of our study. In fact, combined low vitamin D and K status is associated with impaired carotid compliance and distensibility (16) and with systolic and diastolic hypertension (21). However, their joint effect on cardiac structure and function measures has yet to be established. In our study, only the category of participants with both low vitamin D and K status showed a significantly increased $LVMI^{2.7}$ after 7 y of follow-up. Moreover, a significant interaction between biomarkers of vitamin D and K and $LVMI^{2.7}$ was observed. The analysis of circulating plasma dp-ucMGP as a continuous variable confirmed this result, because only participants with low vitamin D status showed a significant association between vitamin K status and $LVMI^{2.7}$. The observed synergistic association of low vitamin D and K status with cardiac remodeling can be at least partially explained by the activity of the vitamin K–dependent protein MGP, which is a powerful inhibitor of soft tissue calcifications and has been associated with increased risk of CVD (11). MGP production is upregulated by vitamin D (29), and its carboxylation and consequent activation require vitamin K. Hence, if both vitamin D and K are deficient, the amount of the inactive form of MGP—dp-ucMGP—will increase. This will in turn lead to increased vascular calcification and arterial stiffness, which will raise cardiac afterload with higher risk of developing LV hypertrophy and cardiac remodeling (21). However, it is not yet clear whether the total amount of the inactive form of MGP, or the ratio of the active to the inactive form, has the more unfavorable impact on cardiovascular health (7, 30). Significant effect modification by sex was observed for the relation between categories of vitamin D and vitamin K status and LVEF. However, women with a combined low vitamin D and K status did not have a significantly lower LV systolic function than those with normal vitamin D and K status after 7 y of follow-up ($P = 0.285$). The association remained only significant for women within the category of normal vitamin D/low vitamin K ($P = 0.032$). A similar association was not present in men. This is in line with limited evidence of a beneficial effect of vitamin D and K supplementation on cardiovascular health in postmenopausal women (16). These results suggest a potential synergistic effect of vitamin D and K metabolism with other sex- and age-related unfavorable hormonal changes such as estrogen deficiency in determining increased CVD risk in postmenopausal women; however, more research is needed (31).

Vitamin D and K deficiency and mortality

After 17 y of follow-up, participants with low vitamin D/low vitamin K status had an increased risk of all-cause mortality (HR: 1.64; 95% CI: 1.12, 2.39; $P = 0.011$). However, the groups with normal vitamin D/low vitamin K and with low vitamin D/normal vitamin K status did not have a significantly higher risk estimate than the reference (HR: 1.29; 95% CI: 0.90, 1.85; $P = 0.171$ and HR: 1.12; 95% CI: 0.77, 1.63; $P = 0.537$, respectively). The joint association of low vitamin D and low vitamin K status with all-cause mortality has only been investigated once in a population of 461 stable kidney transplant recipients, indicating that combined vitamin D and K deficiency was associated with increased risk of death after 10 y of follow-up (HR: 2.33; 95% CI: 1.26, 4.30) (32). Accordingly, our results demonstrated that a joint vitamin D and K deficiency is associated with a higher risk of death than is deficiency of either of them alone. The analysis of plasma dp-ucMGP as a continuous variable confirmed this result, because a continuous association between vitamin K status and mortality was observed only in the setting of low vitamin D status.

Our analysis did not show mediation by cardiac function or structure measures of the association between vitamin D and K status and all-cause mortality. A plausible explanation for our results is the possible involvement of other unfavorable mechanisms, such as the activation of the renin–angiotensin–aldosterone system, with a consequent increase in pulse pressure and arterial stiffness, a reduced bone mass density and a higher risk of fractures, and increased coronary artery and vascular calcifications (21).

Strengths and limitations

To our knowledge, this is the first study to have investigated the synergistic association of vitamin D and K status with cardiac structure and function measures as well as with all-cause mortality in a general population. Strengths of this study include its prospective design with a 17-y follow-up time, the recording of cardiac structure and function measures over an extended period of time, and the detailed phenotyping of the participants that allowed us to adjust for multiple confounders. However, our study has several limitations that should be considered. Firstly, longitudinal echocardiographic data were available for a relatively small group of participants. Although we observed similar associations in our analyses after IPW, we cannot exclude some selection bias by the high loss to follow-up rates from baseline to the second cardiovascular assessment. Secondly, the definition of vitamin D and K status was based on a single measurement of biomarkers of vitamin D and K status performed at baseline, which may not adequately reflect the long-term status of the participants. Thirdly, we used a data-driven cutoff for vitamin K status, because currently there is no clinically accepted dp-ucMGP cutoff and this might induce difficulties in comparing results across studies. Reference intervals for plasma dp-ucMGP were recently defined in a population of healthy Caucasians, but the utilization of a different assay for the determination of dp-ucMGP does not allow a comparison between the studies (25). Although the distribution of dp-ucMGP in our population was similarly skewed, the median value of dp-ucMGP was higher than the one reported by other studies (21, 28). However, a sensitivity analysis using the proposed cutoff of 450 pmol/L did not substantially change our results. More research is needed to define optimal concentrations for vitamin K status.

Conclusions

In conclusion, this study showed that combined low vitamin D and low vitamin K status is synergistically associated with an unfavorable cardiac structure and with an increased risk of death. However, the association between vitamin D and K

TABLE 3 All-cause mortality risk estimates for 25(OH)D (≤/>50 mmol/L) and undercarboxylated Matrix-Gla-Protein (≤/>median) categories in 601 participants after 17 y of follow-up[1]

			Model 1[2]		Model 2[3]	
Vitamin D/vitamin K category	*n*	Events, *n* (%)	HR (95% CI)[4]	*P* value	HR (95% CI)	*P* value
Normal vitamin D/normal vitamin K[5]	166	64 (38.6%)	Reference		Reference	
Normal vitamin D/low vitamin K	162	83 (51.2%)	1.30 (0.94, 1.82)	0.117	1.29 (0.90, 1.85)	0.171
Low vitamin D/normal vitamin K	122	60 (49.2%)	1.13 (0.79, 1.61)	0.518	1.12 (0.77, 1.63)	0.537
Low vitamin D/low vitamin K	151	103 (68.2%)	1.60 (1.13, 2.26)	0.008	1.64 (1.12, 2.39)	0.011

[1]25(OH)D, 25-hydroxyvitamin D.
[2]Model 1 adjusted for age, sex, and glucose status.
[3]Model 2 adjusted for the Model 1 variables plus BMI, glycated hemoglobin, LDL cholesterol, cigarette smoking, systolic blood pressure, antihypertensive medication use, and presence of cardiovascular disease.
[4]HR is estimated with regard to the reference category of normal vitamin D/normal vitamin K.
[5]Normal vitamin D stands for values of 25(OH)D > 50 mmol/L. Normal vitamin K stands for values of desphospho-uncarboxylated matrix-gla protein < 568 pmol/L.

status and mortality cannot be explained by changes in cardiac function and structure measures. Future large-scale clinical studies should investigate whether combined vitamin D and K supplementation is an effective strategy to improve cardiac function and structure to prevent CVD.

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