Original Article

Joint Association of Low Vitamin D and Vitamin K Status With Blood Pressure and Hypertension

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Abstract—Low vitamin D and K status are both associated with an increased cardiovascular risk. New evidence from experimental studies on bone health suggest an interaction between vitamin D and K; however, a joint association with vascular health outcomes is largely unknown. To prospectively investigate whether the combination of low vitamin D and K status is associated with higher systolic and diastolic blood pressure in 402 participants and with incident hypertension in 231 participants free of hypertension at baseline. We used data from a subsample of the Longitudinal Aging Study Amsterdam, a population-based cohort of Dutch participants aged 55 to 65 years. Vitamin D and K status were assessed by 25-hydroxyvitamin D and dp-ucMGP (dephosphorylated uncarboxylated matrix gla protein) concentrations (high dp-ucMGP is indicative for low vitamin K status) in stored samples from 2002 to 2003. Vitamin D and K status were categorized into 25-hydroxyvitamin D <50/≥50 mmol/L and median dp-ucMGP <323/≥323 pmol/L. During a median follow-up of 6.4 years, 62% of the participants (n=143) developed hypertension. The combination of low vitamin D and K status was associated with increased systolic 4.8 mm Hg (95% confidence interval, 0.1–9.5) and diastolic 3.1 mm Hg (95% confidence interval, 0.5–5.7) blood pressure compared with high vitamin D and K status (*P* for interaction =0.013 for systolic blood pressure and 0.068 for diastolic blood pressure). A similar trend was seen for incident hypertension: hazard ratio=1.62 (95% confidence interval, 0.96–2.73) for the low vitamin D and K group. The combination of low vitamin D and K status was associated with increased blood pressure and a trend for greater hypertension risk. (*Hypertension*. 2017;69:00-00. DOI: 10.1161/HYPERTENSIONAHA.116.08869.) ● Online Data Supplement

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Prospective studies have shown that both low vitamin D measured as low 25-hydroxyvitamin D [25(OH)D] concentrations and low vitamin K status measured as high dp-ucMGP (dephosphorylated uncarboxylated matrix Gla protein) concentrations are associated with an increased risk of vascular disease and adverse cardiovascular events.[1–3] Both vitamin D and K are involved in bone metabolism and potentially to mineral deposition in the vasculature, which in turn could lead to calcification in valves and vessels. Vascular calcification in different beds is linked with vascular stiffness and increased blood pressure[4,5] and is an important predictor of coronary heart disease.[5]

Vitamin K is involved in vascular calcification through its role as a cofactor in the carboxylation, that is, activation, of the calcification inhibitor MGP (matrix Gla protein). A loss-of-function mutation of the *MGP* gene has been shown to cause rapid calcification of arteries and, consequently, death in mice.[6] Studies showed that vitamin K deficiency, induced by vitamin K antagonists, may lead to under-carboxylated MGP and increased vascular calcification.[7] Under-carboxylated or inactive MGP, measured as high dp-ucMGP concentrations, is a marker of low vitamin K status and has a high affinity to bind calcium, thereby, stimulating the incorporation into extracellular matrix of soft tissues.[8] High levels of dp-ucMGP have been associated with increased vascular calcification[2] and cardiovascular disease risk,[9] but not in all populations.[10]

Previous prospective studies have indicated that lower vitamin D concentrations are associated with greater hypertension risk.[11] At physiological levels, vitamin D can upregulate the expression of MGP in vascular smooth muscle cells, which, in turn, could increase the demand for vitamin K and potential benefits of MGP on vascular health.[12–16] A randomized controlled trial in postmenopausal women found that supplementation of vitamin K_1, vitamin D, and minerals prevented a decrease in elastic properties of the carotid artery after 3 years compared with vitamin D and minerals or placebo alone.[17] This suggests that adequate levels of both vitamin D and vitamin K are needed for optimal vascular health. However, the combined

association of vitamin D and vitamin K on vascular health is currently unknown. Previous analysis in our study population have indicated that both 25(OH)D and dp-ucMGP were associated with cardiovascular disease risk.[18,19]

We now investigated the joint association of serum 25(OH)D and plasma dp-ucMGP, with systolic and diastolic blood pressure, and the incidence of hypertension in older adults in the Netherlands.

Materials and Methods

Study Design and Population

The Longitudinal Aging Study Amsterdam (LASA) started in 1992 with the goal to determine predictors and consequences of ageing focusing on physical, cognitive, emotional, and social aspects in a Dutch population. A detailed description of the cohort sampling and data collection procedures has been provided elsewhere.[20] In short, LASA personnel used registers of 11 municipalities in 3 geographical areas in the Netherlands to recruit males and females aged 55 to 85 years.

The sample was randomly selected from municipal registries in 1992, with an oversampling of the oldest old and older men. The initial response rate was 60% (n=3805).

For the current study, we used data from second LASA cohort, which started in 2002/03 with adults aged 55 to 65 years. Altogether, 1002 males and females were recruited for the baseline examination using the same procedures as in the first LASA cohort. Follow-up took place in 3 cycles: 2005/06, 2008/09, and 2011/12.

For the present analysis, we excluded participants with a missing baseline blood sample (n=254), or vitamin D and vitamin K measurements (n=53), or using antihypertensive medication at baseline (n=188). For the blood pressure analysis, we excluded participants with missing information on all follow-up blood pressure measurements (n=64) or with missing information on medication use for all measurements (n=39) or using vitamin K antagonists (n=2), resulting in 402 participants for the final analysis. For participants who started using antihypertensive mediation during follow-up, we only included blood pressure measurements up to the point of antihypertensive medication use.

For the incident hypertension analysis, we further excluded participants with prevalent hypertension at baseline based on blood pressure (n=171), resulting in a sample of 231 participants.

All participants provided written informed consent. The study was conducted according to the Declaration of Helsinki and has been approved by the Medical Ethical Committee of the VU University Medical Center Amsterdam.

Measurements of Vitamin D and K

LASA study personnel collected morning blood samples during a medical interview in a nonfasted state, and samples were shipped to the VU University Medical Center in 2002 to 2003.

Blood samples were centrifuged and stored at −80°C until analysis in 2010 to 2011. For vitamin D status, serum 25(OH)D concentrations were determined by the Endocrine Laboratory of the VU University Medical Center using a radioimmunoassay (DiaSorin, Stillwater, MN). The interassay coefficient of variation was 10.0%.

For vitamin K, plasma dp-ucMGP concentrations were determined using a sandwich ELISA with the capture of antibody directed against the nonphosphorylated MGP sequence 3 to 15 (mAb-dpMGP; VitaK BV, Maastricht, the Netherlands). High levels of dp-ucMGP reflect a low vitamin K status.[21] The interassay coefficient of variation was 9.9%.

Ascertainment of Blood Pressure and Incident Hypertension

During baseline and 3 follow-up visits, systolic and diastolic blood pressure was taken after 5 minutes of rest at the upper left arm with participants in a seated position, using an oscillometric monitor for a total of 4 times taken 1-minute apart (model HEM-706; Omron Corporation, Tokyo, Japan). We used the last 3 tests to calculate mean blood pressure. We defined hypertension as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or use of blood pressure–lowering medication.[22] Trained LASA interviewers assessed medication use during each medical interview by medication inventory of all medicines that were currently taken by the participant, including name, dose, frequency of intake, and duration of use. Medications were recorded into official international ATC (anatomical therapeutic chemical classification system) codes; antihypertensive drugs were classified with the codes C01 to C10 (cardiovascular system).

Other Measurements

LASA interviewers obtained comprehensive data on participants' demographics, anthropometrics, and comorbid conditions. Height was measured to the nearest 0.1 cm using a stadiometer. Weight was measured without clothes and shoes to the nearest 0.1 kg using a calibrated bathroom scales (Seca, model 100; Lameris, Utrecht, the Netherlands). Body mass index (BMI) was calculated by dividing body weight (kg) by height (m) squared (kg/m²).

A self-administered questionnaire was used to assess participants self-reported education level, smoking status, alcohol use, and physical activity level. Level of education was reported on a 9-category scale. We distinguished education into 3 categories: low (elementary school or less), medium (lower vocational or general intermediate education) and high (intermediate vocational education, general secondary school, higher vocational education, college, or university). Smoking status was categorized as never, former, and current smoker. Alcohol use, based on the number of days and the amount of alcohol used per week, was characterized into 3 categories: none/light (0–3 glasses/week), moderate (4–7 glasses/week), excessive or very excessive (≥8 glasses/week) according to the Garretsen index.[23] Physical activity was assessed using the validated LASA Physical Activity Questionnaire in minutes per day by multiplying the frequency and duration of each activity in the previous 2 weeks and summing these values across activities. Total physical activity was divided into tertiles: low, moderate, and high.[24]

Season of blood collection was calculated from baseline blood collection date using meteorologic seasons and divided into autumn, winter, and spring/summer (combined because of few summer measurements [n=5]). Total cholesterol was determined from fasting serum samples using enzymatic colorimetry method with a Hitachi 747 analyzer (Roche diagnostics, Mannheim, Germany). Furthermore, we estimated glomerular filtrate rate, derived from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration 2009 equation (21), as an indicator of kidney function. Parathyroid hormone was measured with a second-generation immunoradiometric assay that measures intact parathyroid hormone (Incstar Corp., Stillwater, MN) with an interassay coefficient of variation of 5%. All biochemical analyses were performed by the Endocrine Laboratory of the VU University Medical Center Amsterdam.

Statistical Methods

We categorized serum 25(OH)D and dp-ucMGP into 4 categories based on the clinical cutoff value of 50 nmol/L 25(OH)D and median dp-ucMGP (323 pmol/L) because there is currently no cutoff value defined for high dp-ucMGP. The reference group was the group with 25(OH)D ≥50 nmol/L and dp-ucMGP <323 pmol/L considered high vitamin D status and high vitamin K status. Baseline characteristics are presented as mean and standard deviation for continuous variables and number and percentage for the categorical variables vitamin D and K combined categories. Skewed variables are presented as median and interquartile range.

To investigate the association between 25(OHD) and dp-ucMGP categories with systolic and diastolic blood pressure, we used a generalized estimating equation model to estimate regression coefficients and 95% confidence intervals. For the incident hypertension analyses, we reported unadjusted incidence rates of hypertension per 100 person-years. Risk time was calculated from the baseline examination until the examination at which hypertension was first diagnosed,

the last examination before loss to follow-up or the last LASA cycle 2011 to 2012. We used Cox proportional hazard models to estimate hazard ratios (HRs) and 95% confidence intervals for 25(OH)D and dp-ucMGP categories with incident hypertension. Given the high incidence of hypertension in our study population (62%), a Cox regression model is preferred because it takes risk time into account, and other methods may present difficulties when the outcome is prevalent, experience the risk of overdispersion, or require the estimation of additional parameters for bands of time.[25]

We used nested models to adjust for potential confounders. A minimally adjusted model included age (year) and sex (model 1). The fully adjusted model included age and sex, as well as BMI (kg/m²), alcohol consumption (none-light/moderate/excessive), smoking (never/former/current), education (low/medium/high), season of blood collection (3 categories), total cholesterol (nmol/L) concentrations, and estimated glomerular filtration rate (mL/min per 1.73 m2). Further, we also added parathyroid hormone (PTH) as a potential mediator (pmol/L). We imputed missing data for 16 respondents with at least 1 baseline characteristic missing using 5 iterations using Rubin's rule.[26]

To determine the joint association between vitamin D and vitamin K with blood pressure and incident hypertension, we estimated the presence of interaction. For the association between vitamin D and vitamin K with blood pressure, we added vitamin D (2 groups) and vitamin K (2 groups) to the generalized estimating equation model plus the product terms for each category (3 dummies), calculating the Likelihood ratio test. For the Cox regression, interaction between vitamin D and vitamin K status was estimated by adding the product terms for vitamin D and vitamin K. A *P* value <0.10 was considered statistically significant. We also calculated interaction on a continuous scale. In linear regression (generalized estimating equation), the regression coefficient of the product term reflects interaction as departure from additivity. In the Cox regression, the product term reflects interaction as departure from multiplicativity (multiplicative scale versus additive scale). However, It has been argued that interaction estimated as departure from additivity better reflects biological interaction.[27]

Interaction on an additive scale for relative risks from Cox regression can be estimated by calculating the Relative Excess Risk due to Interaction. Relative Excess Risk due to Interaction=relative risk (RR) A+B+ − RRA+B− − RRA-B+ + 1; A=vitamin D group; B=vitamin K group. A Relative Excess Risk due to Interaction of 0 reflects the absence of interaction, meaning no evidence of additive interaction.[27] We performed data analysis using IBM SPSS Statistics 23.0 for Windows 7 (SPSS Inc Chicago, IL), and all *P* values are 2-sided.

Results

Study Population

Among the 402 included LASA participants at baseline, the mean age was 59.7±2.9 years, and 55% (n=222) of participants were female. The mean BMI was 26.4±3.7 kg/m² and 27% were current smokers and 42% had a lower education level.

Excluded participants with a vitamin D and K sample (n=293) had a higher BMI (28.2 versus 26.4 kg/m²), had lower 25(OH)D (54 versus 59 nmol/L), had higher dp-ucMGP (442 versus 349 pmol/L) concentrations, used more medication (74% versus 45%), and had lower estimated glomerular filtration rate values (81 versus 84 mL/min per 1.73 m²; *P*<0.05).

Serum 25(OH)D and dp-ucMGP Concentrations

Plasma 25(OH)D and dp-ucMGP levels were approximately normally distributed, with mean concentrations of 59±21 nmol/L and 349±209 pmol/L, respectively.

Participants in the combined low vitamin D and low vitamin K status group (25(OH)D <50 nmol/L and dp-ucMGP ≥323 pmol/L) represented 19% of the sample and had higher BMI, waist circumference, and PTH concentrations compared with the reference group (25(OH)D ≥50 nmol/L and dp-ucMGP <323 pmol/L; Table 1). Characteristics for plasma 25(OH)D dp-ucMGP categories separately are available in Table S1 in the online-only Data Supplement.

Associations of Vitamin D and Vitamin K With Blood Pressure

Systolic and diastolic blood pressure were normally distributed with mean measurements of 137±23 and 83±12 mm Hg at baseline, respectively. Systolic blood pressure was strongly correlated with diastolic blood pressure (correlation coefficient =0.82). Both systolic and diastolic blood pressures were highest in the low vitamin D and low vitamin K status group (Table 2). The average increase in systolic and diastolic blood pressure during follow-up was 7.4±17.3 and 2.5±9.7.

In the generalized estimating equation model, after adjusting for age and sex, participants in the group of low vitamin D and low vitamin K status had a 6.5 mm Hg (1.9–11.1 mm Hg) higher systolic blood pressure compared with the reference group, with high vitamin D and high vitamin K status (25(OH)D≥50 mmol/L and dp-ucMGP<323 pmol/L). After further adjustment for potential confounders (model 2), the results remained significant with a 4.8 mm Hg (0.1–9.5 mm Hg) difference between the low vitamin D and low vitamin K status group compared with the reference group. Similarly, diastolic blood pressure was significantly higher (4.2 mm Hg (1.5–6.8 mm Hg) in the low vitamin D and low vitamin K status group compared with the reference, which attenuated in the fully adjusted model to a 3.1 mm Hg (0.5–5.7 mm Hg) difference, but remained statistically significant. Diastolic blood pressure was also significantly higher in the group with high vitamin D and low vitamin K status: 2.8 mm Hg (0.5–5.0 mm Hg) compared with the reference group. No other significant associations between vitamin D and K groups were observed in relation to blood pressure. For systolic blood pressure, the *P* value for interaction was 0.013 for the fully adjusted model and 0.068 for diastolic blood pressure. Adjusting for PTH as a potential mediator slightly attenuated the results 4.5 mm Hg (−0.3 to 9.2 mm Hg) and 2.6 mm Hg (0.0–5.3 mm Hg) for systolic and diastolic blood pressure, respectively, comparing the low vitamin D and K category compared with the reference group. We additionally adjusted for waist circumference as a measure of central obesity; however, the associations were numerically similar (4.9 [0.2–9.7] mm Hg and 3.2 [0.6–5.7] mm Hg) for systolic and diastolic blood pressure, respectively.

Associations of Vitamin D and Vitamin K With Incident Hypertension

During a median follow-up time of 6.4 years (range 2.0–9.7), 143 participants (62%) developed hypertension (Table 3). The associations between vitamin D and vitamin K categories and incident hypertension showed similar patterns as for blood pressure. After adjustment for sex and age, participants in the low vitamin D and K status group had a 69% higher risk of developing hypertension compared with the participants in the high vitamin D and vitamin K status group: HR 1.69 (1.06–2.68). However, after further adjustments for potential confounders, the association remained similar, but was no longer

Table 1.   Baseline Characteristics of 402 LASA Participants Stratified by Combined 25-Hydroxyvitamin D and Dephosphorylated Uncarboxylated Matrix Gla Protein Categories

Baseline Characteristics	Categories of 25(OH)D (nmol/L) and dp-ucMGP status (pmol/L)			
	25(OH)D≥50/ dp-ucMGP<323	25(OH)D<50/ dp-ucMGP<323	25(OH)D≥50/ dp-ucMGP≥323	25(OH)D<50/ dp-ucMGP≥323
Demographics				
N	130	72	125	75
Age, y	59.6±3.0	59.7±2.8	59.5±2.8	60.1±3.0
Female	68 (52%)	42 (58%)	73 (58%)	39 (52%)
Season				
Fall	40 (31%)	27 (38%)	41 (33%)	24 (32%)
Winter	59 (45%)	31 (43%)	57 (46%)	37 (49%)
Spring/summer	31 (24%)	14 (19%)	27 (22%)	14 (19%)
Alcohol use				
Light	70 (54%)	39 (54%)	62 (50%)	40 (53%)
Moderate	49 (38%)	22 (31%)	55 (44%)	24 (32%)
Excessive	11 (9%)	11 (15%)	8 (6%)	11 (15%)
Smoking				
Never	33 (26%)	15 (23%)	37 (28%)	20 (24%)
Former	67 (52%)	22 (31%)	64 (51%)	36 (48%)
Current	28 (22%)	33 (46%)	26 (21%)	21 (28%)
Physical activity				
Low	40 (31%)	29 (40%)	35 (28%)	31 (41%)
Medium	44 (34%)	24 (33%)	46 (37%)	22 (29%)
High	46 (35%)	19 (26%)	44 (35%)	22 (29%)
Education				
Low	54 (42%)	30 (42%)	53 (42%)	32 (43%)
Middle	52 (40%)	23 (32%)	45 (36%)	18 (24%)
High	24 (19%)	19 (26%)	27 (22%)	25 (33%)
Adiposity				
BMI, kg/m^2	25.5±3.3	25.8±4.2	26.9±3.4	27.7±4.0
Waist circumference, cm	92.0±10.3	92.3±11.4	95.3±10.2	97.5±11.2
Waist/Hip	0.91±0.07	0.91±0.08	0.92±0.07	0.93±0.09
Metabolic variables				
Total cholesterol, nmol/L	5.9±0.9	5.7±1.0	6.0±0.94	6.0±1.0
Systolic blood pressure, mmol/L	133±20	133±24	135±21	139±21
Diastolic blood pressure, mmol/L	76±8	77±8	77±9	75±10
eGFR, mL/min per 1.73 m2	83.8±15.0	86.7±14.0	82.7±15.5	84.6±16.8
Parathyroid hormone, pmol/L	5.1±1.6	6.0±1.7	5.7±2.3	6.6±2.4
25(OH)D, nmol/L	71±15.8	38±9.3	72±14.9	39±8.4
dp-ucMGP, pmol/L	208±72	217±71	480±164	480±244
Medication use				
Diuretics	0 (0%)	3 (2%)	1 (1%)	3 (4%)
Lipid lowering	6 (5%)	6 (5%)	3 (4%)	5 (7%)

Values are mean±SD or n (%). 25(OH)D indicates 25-hydroxyvitamin D; BMI, body mass index; dp-ucMGP, dephosphorylated uncarboxylated matrix Gla protein; and LASA, Longitudinal Aging Study Amsterdam.

Table 2. Regression Coefficients of Multiple Generalized Estimating Equation Analysis for Diastolic and Systolic Blood Pressure and 25-Hydroxyvitamin D and Dephosphorylated Uncarboxylated Matrix Gla Protein Categories During 6.4 Years of Follow-Up in 402 LASA Participants

Outcome Measure	25(OH)D≥50/dp-ucMGP<323, N=130	25(OH)D<50/dp-ucMGP<323, N=72	25(OH)D≥50/dp-ucMGP≥323, N=125	25(OH)D<50/dp-ucMGP≥323, N=75
Systolic blood pressure, mm Hg				
Model 1	Reference (0.0)	2.4 (−3.3 to 8.2)	4.4 (0.1 to 8.6)	6.5 (1.9 to 11.1)
Model 2	Reference (0.0)	2.9 (−2.4 to 8.2)	3.6 (−0.6 to 7.8)	4.8 (0.1 to 9.5)
Diastolic blood pressure, mm Hg				
Model 1	Reference (0.0)	1.6 (−1.5 to 4.7)	3.1 (0.9 to 5.3)	4.2 (1.5 to 6.8)
Model 2	Reference (0.0)	1.4 (−1.4 to 4.3)	2.8 (0.5 to 5.0)	3.1 (0.5 to 5.7)

Regression coefficients can be interpreted as difference in mm Hg blood pressure expressed as β and 95% confidence intervals. Low dp-ucMGP indicates high vitamin K status and high dp-ucMGP indicates low status. Model 1 adjusted for age and sex. Model 2 adjusted for age (y), sex, BMI (kg/m^2), alcohol consumption (none-light/moderate/excessive), smoking (never, former, current), education (low/medium/high), season of blood collection (fall/winter/spring-summer), total cholesterol (nmol/L), and estimated glomerular filtration rate (mL/min per 1.73 m^2). 25(OH)D indicates 25-hydroxyvitamin D; BMI, body mass index; dp-ucMGP, dephosphorylated uncarboxylated matrix Gla protein; and LASA, Longitudinal Aging Study Amsterdam.

statistically significant: HR 1.62 (0.96–2.73). The HRs for the other categories were 1.28 (0.77–2.13) for 25(OH)D<50/dp-ucMGP<323 and 1.29 (0.84–1.99) for 25(OH)D≥50/dp-ucMGP≥323. Adjusting for PTH as a potential mediator further attenuated the results to HR=1.59 (0.93–2.73) for the 25(OH)D<50/dp-ucMGP<323 category.

The *P* for interaction on a multiplicative scale was 0.226. For incident hypertension, the relative excess risk because of interaction for model 1 is 1.69−1.28−1.29+1=0.12 and for model 2 is 1.62−1.42−1.37+1=−0.17.

No interaction was observed between 25(OH)D and dp-ucMGP as continuous variables for systolic and diastolic blood pressure, as well as for incident hypertension, as presented in Table S2 (*P* for interaction >0.262).

Discussion

In the current study, we reported associations of serum 25(OH)D and dp-ucMGP with blood pressure and incident hypertension in a population-based cohort with serial follow-up in older men and women. The combination of low vitamin D (25(OH)D<50 nmol/L) and low vitamin K (dp-ucMGP≥323 pmol/L) status was associated with increased systolic and diastolic blood pressure, with a similar trend for incident hypertension, albeit not statistically significant. An additive effect of 25(OH)D and dp-ucMGP on blood pressure was evident based on the interaction tests; however, we did not find a statistically significant interaction between vitamin D and K for incident hypertension based on the relative excess risk because of interaction, which was approximately zero, indicating no evidence of additive interaction. No interaction was observed between 25(OH)D and dp-ucMGP as continuous variables, meaning there might be a threshold association for both 25(OH)D and dp-ucMGP as observed in the categorical associations.

To our knowledge, the importance of optimal vitamin D and K concentrations on blood pressure and incident hypertension has, thus, far only been studied in isolation. Previous prospective studies have reported lower plasma vitamin D concentrations to be associated with higher hypertension risk[11] and higher dp-ucMGP concentrations to be associated

Table 3. Associations of Serum 25-Hydroxyvitamin D and Dephosphorylated Uncarboxylated Matrix Gla Protein Categories With Risk of Hypertension After 6.4 Years of Follow-Up Among 231 LASA Participants

Outcome Measure	25(OH)D≥50/dp-ucMGP<323, N=76	25(OH)D<50/dp-ucMGP<323, N=39	25(OH)D≥50/dp-ucMGP≥323, N=70	25(OH)D<50/dp-ucMGP≥323, N=46
Person-years, y	476	244	423	276
Incidence rate per 100 py	8.4	10.3	10.4	12.3
No of cases, %	40 (52%)	25 (64%)	44 (63%)	34 (74%)
Model 1	Reference (1.0)	1.28 (0.77–2.13)	1.29 (0.84–1.99)	1.69 (1.06–2.68)
Model 2	Reference (1.0)	1.42 (0.83–2.41)	1.37 (0.86–2.19)	1.62 (0.96–2.73)

Hazard ratios and 95% confidence intervals derived from Cox proportional hazards models. Low dp-ucMGP indicates high vitamin K status and high dp-ucMGP indicates low status. Model 1 adjusted for age and sex. Model 2 adjusted for age (y), sex, BMI (kg/m^2), alcohol consumption (none-light/moderate/excessive), smoking (never, former, current), education (low/medium/high), season of blood collection (3 categories), total cholesterol (nmol/L) and estimated glomerular filtration rate (mL/min per 1.73 m^2). 25(OH)D indicates 25-hydroxyvitamin D; BMI, body mass index; dp-ucMGP, dephosphorylated uncarboxylated matrix Gla protein; and LASA, Longitudinal Aging Study Amsterdam.

with arterial stiffness[28] and higher risk of coronary artery calcification.[29] The first study that explored the combined effect of vitamin D and K supplementation on cardiovascular health was a double-blind placebo controlled trial of 181 postmenopausal women aged 50 to 60 years and revealed that supplementation of vitamin K_1, vitamin D, and minerals was superior to vitamin D plus minerals alone in preventing a decrease of elastic properties of the carotid artery over 3-year follow-up.[17] However, in this experiment,[17] vitamin D and K was combined with calcium, magnesium, and zinc, making it difficult to solely pinpoint the effect to vitamin D and K. Our results appear in line with this trial because a combined low vitamin D and vitamin K status was associated with increased blood pressure compared with those with a high vitamin D and K status. We also provide evidence for an additive effect of vitamin D and vitamin K because the interaction for blood pressure was significant. A similar trend was seen for low vitamin D and K status with incident hypertension; however, it should be noted that the power to test interaction is limited in small sample sizes (eg, <250 subjects) and that type 1 error rates could be high, which might explain the difference in interaction tests between blood pressure and hypertension.[30]

The hypothesized joint association between vitamin D and K on cardiovascular health is further supported by evidence on bone health outcomes. In a recent case–control study of 184 older Norwegian men and women, low phylloquinone and low 25(OH)D concentrations were synergistically associated with a higher risk of hip fractures.[31] Further, multiple human trials have demonstrated a synergistic effect of vitamin D and K on bone health.[32,33] Only the combination of vitamin D and K supplementation resulted in reduced bone loss. It should be noted that in the Postmenopausal Health Study II, all intervention groups significantly increased total-body bone mineral density compared with the control group; however, only the vitamin D and K combination groups increased lumbar spine bone mineral density. In the study of Ushiroyama et al,[33] postmenopausal women with osteopenia and osteoporosis with low vitamin D values were included, and no compliance markers of circulating measures of vitamin D and vitamin K were taken into account. Other studies using solely vitamin K supplementation did not find effects on bone density.[34–36] These limited studies indicate that the combined effect of vitamin D and K might be superior than using either vitamin alone. Our study provides evidence that this combined effect may also be present for vascular outcomes. This underscores the need for a concomitant adequate intake/supply of vitamin D and vitamin K.

The potential mechanism to explain the joint association of vitamin D and K is via stimulation of the γ-carboxylase system of vitamin K–dependent proteins. Two of these proteins are Gla-containing proteins osteocalcin and MGP, which play a role in the development of coronary artery disease.[8,37] Osteocalcin has been shown to have a regulatory role in bone mineralization and calcium ion homeostasis,[38] with the potential to prevent calcium build-up in soft tissues, thereby, preventing vascular calcification.[39] Similarly, MGP limits calcium incorporation into extracellular matrix of soft tissues, thus, acting as a potent inhibitor of soft tissue calcification.[8,38] Activation of osteocalcin and MGP is dependent on vitamin K, which acts

as a cofactor in the carboxylation cycle. Low expression or inactivation of vitamin K–dependent proteins can lead to vascular stiffness, which can increase blood pressure and incident hypertension risk.[4] Circulating vitamin D has been shown to increase MGP mRNA expression in humans.[40] It has also been demonstrated that vitamin K promotes 1,25-dihyroxyvitamin D–stimulated osteocalcin accumulation and mineralization.[41] In addition, in an animal model, the coadministration of vitamin K and 1,25-dihydroxyvitamin D increased levels of bone anabolic markers, including osteocalcin in a time-dependent manner,[42] while these effects were not visible with the separate vitamins, as demonstrated by randomized trials.[43,44] In addition, the results attenuated further after adjusting for PTH, meaning that there might be potential mediation by PTH; however it remains unclear if dp-ucMGP is related to PTH. Overall, from experimental studies, it is clear that vitamin D and K can mutually enhance each other, which could explain a joint association of these vitamins on cardiovascular health.

Our study has some limitation that should be addressed. Because of the high number of exclusions, the current study sample was relatively small varying between 231 and 402 participants. Excluded participants had poorer health as observed from higher BMI, lower vitamin status, and more medication use, which could have impacted our results, although this would most likely dilute the observed associations. Furthermore, the sample size for the occurrence of hypertension was relatively small, which probably explains the borderline significant results for the combined low vitamin D and K status and non-significant interactions for incident hypertension. Future studies with larger sample sizes are needed to confirm our results. Furthermore, the use of data-driven cutoff points for vitamin K status can cause difficulties comparing results across studies. In our study, the median dp-ucMGP was 323 pmol/L, while other studies report higher median concentrations (466–525 pmol/L).[45,46] Currently, there is no clinically accepted dp-ucMGP cutoff point for healthy populations. More research is needed to define optimal concentrations for vitamin K status using studies with a broad range of dp-ucMGP concentration with long-term follow-up of clinical outcomes.

Our study has also some strengths. We assessed joint associations of 25(OH)D and dp-ucMGP in relation to both blood pressure and incident hypertension in a population free of baseline hypertension and vitamin K antagonist use, minimalizing potential bias and misclassification. The LASA cohort is a nationally representative sample with a substantial follow-up time, repeated measured blood pressure and medication information, and a large number of covariates to adjust for potential confounding. Furthermore, we used blood samples to assess vitamin K status by measuring dp-ucMGP, which has been shown to be a valid biomarker of vitamin K status and reflects menaquinone intake in a dose-response manner.[21] The majority of the previous studies assessed vitamin K intake with a food-frequency questionnaire, which has well known limitations.[3,47]

In conclusion, the combination of low vitamin D and low vitamin K status was associated with increased systolic and diastolic blood pressure in a cohort of older men and women with a similar trend for incident hypertension risk compared with high vitamin D and vitamin K status.

Perspective

This is the first study investigating joint associations of vitamin D and vitamin K in relation to cardiovascular health. An additive effect of vitamin D and vitamin K status on blood pressure was evident based on the interaction tests; however, we did not find a statistically significant interaction between vitamin D and K for incident hypertension. The combination of low vitamin D and K status was associated with increased blood pressure and might play a role in development of hypertension. Further prospective studies are needed to better understand the clinical implications of this relationship to promote cardiovascular health.

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Disclosures

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American Heart Association.

Novelty and Significance

What Is New?

- This is the first study that prospectively investigates whether the combination of low vitamin D and K status is associated with higher systolic and diastolic blood pressure in 402 participants and with incident hypertension in 231 participants free of hypertension at baseline in a population-based cohort with serial follow-up in older men and women.

What Is Relevant?

- The combination of high vitamin D and K status was associated with increased systolic (4.8 mm Hg; 95% confidence interval, 0.1–9.5) and diastolic (3.1 mm Hg; 95% confidence interval, 0.5–5.7) blood pressure compared with low vitamin D and K status (*P* for interaction =0.013 for systolic blood pressure and 0.068 for diastolic blood pressure).

- A similar trend was seen for incident hypertension: hazard ratio =1.62 (95% confidence interval, 0.96–2.73) for the low vitamin D and K group.

Summary

During a median follow-up of 6.4 years, 62% of the participants (n=143) developed hypertension. The combination of low vitamin D and K status was associated with increased blood pressure and a trend for greater hypertension risk after adjustment for potential confounders.

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The Joint Association of Low Vitamin D and Vitamin K Status with Blood Pressure and Hypertension

Running title: vitamin D and K status with blood pressure

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Supplemental tables

Table S1. Baseline characteristics of 402 LASA participants stratified by categories of 25-hydroxyvitamin D and dephosphorylated uncarboxylated matrix Gla protein

	Categories of 25(OH)D (nmol/L) and dp-ucMGP status (pmol/L)			
	25(OH)D≥50/	25(OH)D <50/	dp-ucMGP<323	dp-ucMGP≥323
Demographics				
N	255	147	202	200
Age (y)	59.5 ± 2.9	59.9 ± 2.9	59.7 ± 2.9	59.7 ± 2.9
Female	141 (55%)	81 (55%)	110 (55%)	112 (56%)
Season				
Fall	81 (32%)	51 (35%)	67 (33%)	65 (33%)
Winter	116 (46%)	68 (46%)	90 (45%)	94 (47%)
Spring/Summer	58 (23%)	58 (23%)	45 (22%)	41 (20%)
Alcohol use				
Light	79 (54%)	132 (52%)	109 (54%)	102 (51%)
Moderate	46 (31%)	104 (41%)	71 (35%)	79 (40%)
Excessive	22 (15%)	19 (8%)	22 (11%)	19 (9%)
Smoking				
Never	70 (28%)	35 (24%)	52 (26%)	53 (27%)
Former	131 (51%)	58 (40%)	89 (44%)	100 (50%)
Current	54 (21%)	54 (37%)	61 (30%)	47 (23%)
Physical activity				
Low	75 (29%)	60 (41%)	69 (34%)	66 (33%)
Medium	90 (35%)	46 (31%)	68 (34%)	68 (34%)
High	90 (35%)	41 (28%)	65 (32%)	66 (33%)
Education				
Low	107 (42%)	62 (42%)	84 (42%)	85 (42%)

Middle	97 (38%)	41 (28%)	75 (37%)	63 (32%)
High	51 (20%)	44 (30%)	43 (21%)	52 (26%)
Adiposity				
BMI (kg/m^2)	26.2 ± 3.4	26.7 ± 4.2	25.6 ± 3.6	27.2 ± 3.6
Waist circumference (cm)	93.6 ± 10.5	94.9± 11.6	92.1 ± 10.7	96.1 ± 10.6
Waist/Hip	0.92 ± 0.07	0.92 ± 0.08	0.91 ± 0.07	0.93 ± 0.08
Metabolic Variables				
Total cholesterol (nmol/L)	6.0 ± 0.9	5.9± 1.0	5.9 ± 0.9	6.0 ± 1.0
Systolic blood pressure (mmol/L)	134±21	136±22	134±22	136±21
Diastolic blood pressure (mmol/L)	82±11	82±12	81±10	82±12
eGFR (ml/min/1.73m^2)	83.3 ± 15.2	85.6 ± 15.4	84.9 ± 14.7	83.4 ± 16.0
Parathyroid hormone(pmol/L)	5.4 ± 2.0	6.3 ± 2.1	5.4 ± 1.7	6.0 ± 2.4
25(OH)D (nmol/L)	71 ± 15.3	38 ± 8.7	59 ± 21	59 ± 20
dp-ucMGP (pmol/L)	341 ± 224	351 ± 185	211 ± 72	480 ± 197
Medication use				
Diuretics	3 (1.2%)	4 (2.7%)	1 (0.5%)	6 (3.0%)
Lipid-Lowering	12 (4.7%)	8 (5.4%)	9 (4.5%)	11 (5.5%)

Values are mean ± SD or n (%)

Table S2 Continuous associations of 25(OH)D and dp-ucMGP with blood pressure and hypertension

	Model 1	Model 2
Systolic blood pressure (mmHg)		
Vitamin D per 10 nmol/L increment	0.11 (-1.80, 2.01)	-0.13 (-1.87,1.61)
Vitamin K per 50 pmol/L increment	0.74 (-1.00, 2.49)	0.64 (-0.88, 2.15)
P-for interaction	0.679	0.728
Diastolic blood pressure (mmHg)		
Vitamin D per 10 nmol/L increment	-0.53(-1.65,0.60)	-0.49 (-1.49, 0.52)
Vitamin K per 50 pmol/L increment	-0.16 (-1.18,0.85)	-0.32 (-1.22, 0.57)
P-for interaction	0.405	0.262
Incident hypertension		
Vitamin D per 10 nmol/L increment	1.01 (0.89, 1.15)	1.02 (0.89, 1.16)
Vitamin K per 50 pmol/L increment	1.05 (0.95, 1.16)	1.06 (0.96, 1.18)
P-for interaction	0.650	0.536

Model 1 adjusted for age and sex

Model 2 adjusted for age (y), sex, BMI (kg/m2), alcohol consumption (none-light/moderate/excessive), smoking (never, former, current), education (low/medium/high), season of blood collection (3 categories), total cholesterol (nmol/L) and estimated glomerular filtration rate (ml/min/1.73m2)