Vitamin D Affects Survival Independently of Vascular Calcification in Chronic Kidney Disease

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Background and objectives: Cardiovascular disease is the main cause of mortality in chronic kidney disease (CKD) patients. Vitamin D might have beneficial effects on vascular health. The aim of this study was to determine the prevalence of vitamin D deficiency (25-hydroxyvitamin D [25D] ≤ 15 ng/ml) and insufficiency (25D levels between 16 and 30 ng/ml) in a cohort of patients at different CKD stages and the relationships between vitamin D serum levels, vascular calcification and stiffness, and the mortality risk.

Design, setting, participants & measurements: One hundred forty CKD patients (85 men, mean age 67 ± 12 yr; CKD stages 2 [8%], 3 [26%], 4 [26%], 5 [7%], and 5D [(33%]) were allocated for a prospective study. Serum levels of 25D and 1,25-dihydroxyvitamin D, aortic calcification score, and pulse wave velocity (PWV) were evaluated.

Results: There was a high prevalence of vitamin D deficiency (42%) and insufficiency (34%). Patients with 25D ≤ 16.7 ng/ml (median) had a significantly lower survival rate than patients with 25D >16.7 ng/ml (mean follow-up, 605 ± 217 d; range, 10 to 889; $P = 0.05$). Multivariate adjustments (included age, gender, diabetes, arterial pressure, CKD stage, phosphate, albumin, hemoglobin, aortic calcification score and PWV) confirmed 25D level as an independent predictor of all-cause mortality.

Conclusions: Vitamin D deficiency and insufficiency were highly prevalent in this CKD cohort. Low 25D levels affected mortality independently of vascular calcification and stiffness, suggesting that 25D may influence survival in CKD patients via additional pathways that need to be further explored.

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Vitamin D deficiency and insufficiency (characterized by 25-hydroxyvitamin D [25D] levels <15 ng/ml and between 16 and 30 ng/ml, respectively) are relatively common in the general population of non-intertropical countries and especially common in chronic kidney disease (CKD) patients (1). The kidney and certain vascular, immune, and gastrointestinal cells that express 1α-hydroxylase convert 25D to 1,25-dihydroxyvitamin D (1,25D, the active biologic form), which binds to the vitamin D receptor (VDR) and regulates gene transcription (2). There is much evidence to suggest that in addition to vitamin D's well-known actions on bone and mineral metabolism, it may also have pleiotropic effects on the immune and cardiovascular systems (3). It has been demonstrated that 1,25D suppresses the renin-angiotensin system and decreases cardiac myocyte hypertrophy (4). In addition, 1,25D's immunomodulatory and antiproliferative effects may decrease the risk of cancer (5). It has recently been reported that 25D is also capable of activating the VDR directly, albeit with 100-fold lower affinity, but compensated for by its 1,000-fold higher serum concentration when compared with 1,25D (6). Thus, low 25D levels may have a negative impact on various system functions via two mechanisms: (*1*) lower substrate availability for 1,25D production and (*2*) reduced VDR activation. Accordingly, low 25D levels have been linked to chronic heart disease (7), hypertension (8), and a higher incidence of cancer (9). Taken as a whole, these observations prompted a hypothesis whereby vitamin D deficiency could influence overall survival. Indeed, recent studies have repeatedly reported an association between higher mortality and low levels of 25D and (less consistently) 1,25D in both general and CKD populations (10–14).

CKD is associated with a high mortality rate, mainly as a result of cardiovascular disease (15). Vascular calcification and arterial stiffness are highly prevalent in this population and may play a pivotal role in the high cardiovascular mortality rate (16). Furthermore, CKD progression exposes patients to a particular risk of developing both 25D and 1,25D deficiencies (17,18). In animal models, exogenous administration of supraphysiological doses of VDR activators has been associated with both the incidence and progression of vascular calcification (19,20), whereas the administration of VDR activators at more

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physiologic doses (*i.e.*, just enough to correct secondary hyperparathyroidism) protect against aortic calcification (21). In a CKD setting, a recent study in adult hemodialysis patients described an association between 25D deficiency and arterial stiffness (13), whereas dialyzed children showed a bimodal association between 1,25D levels and vascular calcification and stiffness (22).

We therefore decided to determine the prevalence of vitamin D deficiency and insufficiency in a cohort of adult patients at different stages of CKD. Moreover, we investigated the relationships between vitamin D serum levels, vascular calcification and stiffness, and the risk of mortality in this population.

Materials and Methods

Patient Selection

Over an 18-mo period (from January 2006 to June 2007), a total of 140 Caucasian CKD patients were recruited from the nephrology department's outpatient clinic at Amiens University Hospital. All patients gave their informed, written consent. The study was approved by the local Institutional Review Board and was performed in accordance with the ethical principles of the Declaration of Helsinki.

Included patients had to be over the age of 40, with a confirmed diagnosis of CKD (defined as being on hemodialysis or having two previous, estimated creatinine clearances—calculated according to the Cockcroft and Gault formula [23]—with an interval of 3 to 6 mo and values <90 ml/min/1.73 m^2). CKD stage 5D patients had been receiving thrice-weekly hemodialysis for at least 3 mo. Exclusion criteria included the presence of chronic inflammatory disease, atrial fibrillation, complete heart block, abdominal aorta aneurysm, aortic and/or femoral artery prosthesis, primary hyperparathyroidism, kidney transplantation, and any acute cardiovascular event in the 3 mo before screening for inclusion.

Study Protocol

All patients were hospitalized for the day for laboratory blood tests, blood pressure (BP) measurement, a pulse wave velocity (PWV) determination, lateral lumbar x-ray, and multislice spiral CT scanning. For a given patient, all examinations were performed between 9:00 a.m. and 2:00 p.m. on the same day. Hemodialysis patients were preferentially seen on a dialysis-free day or the morning before the dialysis session. A patient interview focused on comorbidities and the personal disease history. The patients' medical charts were reviewed to record concomitant medications.

Laboratory

Blood samples were collected the morning before the other investigations. Selected variables were measured after the samples had been frozen and stored at −80°C. Serum calcium, phosphate, albumin, cholesterol, hemoglobin, creatinine (Scr), and C-reactive protein were analyzed in an on-site biochemistry laboratory using standard auto-analyzer techniques (Roche Diagnostics Modular IIP). Serum intact parathyroid hormone (iPTH 1 to 84) and 25D levels were determined using chemiluminometric immunoassays (respectively, the Liaison N-tact PTH CLIA and the Liaison 25OH Vitamin D TOTAL CLIA [which measures both D_2 and D_3]; Diasorin, Stillwater, MN). 1,25D serum levels were determined in an RIA with a 95% reference value for healthy volunteers of 25.1 to 66.1 pg/ml (^{125}I RIA Kit from Diasorin). Serum cystatin C levels were determined by immunonephelometry (N Latex Cystatin C; Dade Behring Marburg, Germany). To better describe glomerular filtration, we calculated the estimated glomerular filtration rate (GFR) combining Scr and CysC measurements for all nondialyzed patients according to the following, recently published (24) "CKD-epi" equation: $177.6 \times \text{Scr}^{-0.65} \times \text{CysC}^{-0.57} \times \text{Age}^{-0.20} \times$ (0.82 for female patients). For descriptive purposes, patients were then classified into CKD stages, according to the K/DOQI guidelines (25).

PWV Evaluation

Carotid-femoral PWV was determined automatically with a dedicated, validated device (Complior Colson; Createch Industrie, May, France), as described previously (26). PWV was evaluated by a trained physician with two pressure probes as formerly reported (27).

Abdominal Aorta Imaging with Plain Radiography

A technique similar to that described by Kauppila *et al.* was used to obtain images of the lower abdominal aorta and generate the aortic calcification score (28). All x-rays were reviewed by two investigators, and a consensus on the interpretation was reached in all cases.

Multislice Spiral CT Scan

All examinations were performed with a 64-detector CT scanner (Lightspeed VCT; GE Healthcare, Milwaukee, WI). The volume acquisition started at the aortic hiatus of the diaphragm and ended at the third lumbar vertebra. The scanning parameters were as follows: collimation, 64 × 0.625 mm; slice thickness, 0.625 mm; pitch, 1; gantry rotation speed, 0.5 s/rotation; tube voltage, 120 kV; tube current, 300 mA.

The volume acquisition was analyzed with commercially available software (Volume Viewer software, GE Healthcare). The abdominal aorta was segmented manually. To reduce errors caused by noise, a threshold of 160 UH was applied. The total calcification volume was calculated as the sum of all voxels in the remaining volume. The aortic calcification score was calculated as follows: [(total calcification volume) ÷ (aorta wall surface area) × 100)].

Survival

Death records were made prospectively, considering all patients that were included at least 1 yr before the study end date (June 30, 2008). Each medical chart was reviewed, and the cause of death was assigned by a physician on the basis of all of the available clinical information. For out-of-hospital deaths, the patient's general practitioner was interviewed to gain pertinent information on the cause.

Statistical Analyses

Data are expressed as mean ± SD, median and range, or frequencies, as appropriate. For analytical purposes, patients were divided in two groups according to the median 25D level (*i.e.*, 25D ≤ 16.7 ng/ml *versus* 25D > 16.7 ng/ml). Intergroup comparisons were performed using a χ^2 test for categorical variables and Student's *t*-test or a Mann-Whitney test for continuous variables. Pearson's correlation coefficient or Spearman's rank correlation were used to assess the relationships between 25D levels and multiple variables. A Kaplan-Meier actuarial method was used to estimate overall survival in the 25D groups. The log-rank test was used to compare the survival curves. Univariate and multivariate analyses of mortality were performed by using a Cox proportional hazards model of death as a function of 25D levels. In the multivariate analysis, the predefined, noncumulative models included those variables significantly associated with death in univariate analyses and those variables of prognostic importance according to published observations. The limited number of events (n = 25) prevented us from including more than three variables for each model. A *P* value

of ≤ 0.05 was considered to be statistically significant. All statistical analyses were performed using SAS software 9.1 (SAS Institute Inc., Cary, NC).

Results

Table 1 shows the demographic, clinical and biochemical characteristics of the 140 analyzed patients.

In terms of serum 25D levels, 76% of the patients display either vitamin D deficiency (42%) or insufficiency (34%). Figure 1 illustrates the distribution of 25D and 1,25D serum levels by CKD stage. Serum 25D levels were similar for all CKD stages, and serum 1,25D levels were significantly lower in stage 5D patients ($P = 0.035$). At enrolment, 36% of the patients were receiving vitamin D3 supplementation (either calcifediol [median dose, 14 μg/d; range, 5 to 32 μg/d] or cholecalciferol [median dose, 400 IU/d; range, 250 to 800 IU/d]), whereas only one individual was taking active vitamin D (α-calcidol) and bisphosphonate. When comparing patients grouped by study entry date (to evaluate for a possible seasonal bias), we did not find any significant difference in 25D levels between patients who entered during the summer/spring (n = 67) and those who entered during the autumn/winter (n = 73) (19.7 ± 12.3 *versus* 21.3 ± 14.8 ng/mL, respectively; $P = 0.669$). Moreover, there was no difference between these two seasonal groups in terms of the proportion of patients taking vitamin D3 supplementation (36% for summer/spring and 35% for autumn/winter; $P = 0.845$).

When comparing patients divided by the 25D median (25D ≤ 16.7 ng/ml *versus* 25D > 16.7 ng/ml), we found only that patients with 25D ≤ 16.7 ng/ml presented significantly lower levels of serum albumin and were less likely to receive vitamin D supplements than patients with 25D > 16.7 ng/ml.

Table 1. Main clinical characteristics and baseline data, as a function of vitamin D status

Characteristic	All (n = 140)	25D ≤ 16.7 ng/ml (n = 71)	25D > 16.7 ng/ml (n = 69)	*P*
Age, years	67 ± 12	68 ± 13	66 ± 12	0.299
Male gender, n (%)	85 (61)	42 (59)	43 (62)	0.702
Body mass index, kg/m^2	28 ± 6	28 ± 5	28 ± 7	0.646
Systolic BP, mmHg	153 ± 26	149 ± 27	157 ± 25	0.089
Diastolic BP, mmHg	81 ± 12	80 ± 12	82 ± 12	0.387
Diabetes, n (%)	59 (42)	32 (46)	27 (38)	0.317
Smoking habit, n (%)	55 (40)	24 (35)	31 (46)	0.221
Presence of CVD, n (%)	44 (31)	23 (32)	21 (30)	0.803
CKD stage, n (%)				0.864
2	11 (8)	6 (8.5)	5 (7)	
3	36 (26)	20 (28)	16 (23)	
4	37 (26)	18 (25)	19 (28)	
5	10 (7)	6 (8.5)	4 (6)	
5D	46 (33)	21 (30)	25 (36)	
Calcium, mMol/L	2.3 ± 0.2	2.3 ± 0.2	2.27 ± 0.19	0.390
Phosphate, mMol/L	1.3 ± 0.4	1.3 ± 0.5	1.3 ± 0.4	0.635
iPTH, pg/ml (median)	138 ± 138 (85)	150 ± 152 (99)	126 ± 122 (78)	0.537
Hemoglobin, g/L	12 ± 1.7	12 ± 1.9	12 ± 1.6	0.834
Albumin, g/L	37 ± 6	36 ± 6	38 ± 6	0.030
C-reactive protein, mg/L (median)	11 ± 23 (3.5)	13 ± 29 (3.5)	9 ± 15 (3.3)	0.812
Total cholesterol, mMol/L	4.9 ± 1.2	5.0 ± 1.2	4.7 ± 1.0	0.066
Aortic calcification score on CT, %[a] (median)	2.9 ± 3.0 (1.8)	3.1 ± 3.3 (1.9)	2.8 ± 2.7 (1.7)	0.910
Aortic calcification score on x-ray (scale 0-24)[bc] (median)	6.2 ± 6.5 (4.0)	6.2 ± 6.7 (4.0)	6.0 ± 6.5 (4.0)	0.812
PWV, m/s	14 ± 4	15 ± 4	14 ± 4	0.701
Vitamin D supplementation, n (%)	50 (36)	18 (25)	32 (46)	0.009
25-hydroxyvitamin D, ng/ml (median)	20.5 ± 13.6 (16.7)	10.8 ± 3.9 (10.7)	30.5 ± 12.9 (28.7)	

Data are means ± SD and sometimes (median) for variables with non-Gaussian distribution, or number (frequency) for binary variables. BP, blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; CKD, chronic kidney disease; iPTH, intact-parathyroid hormone; CT, computed tomography; PWV, pulse wave velocity.

[a]n = 129; [b]n =122; [c]by lateral lumbar x-rays, as previously described.

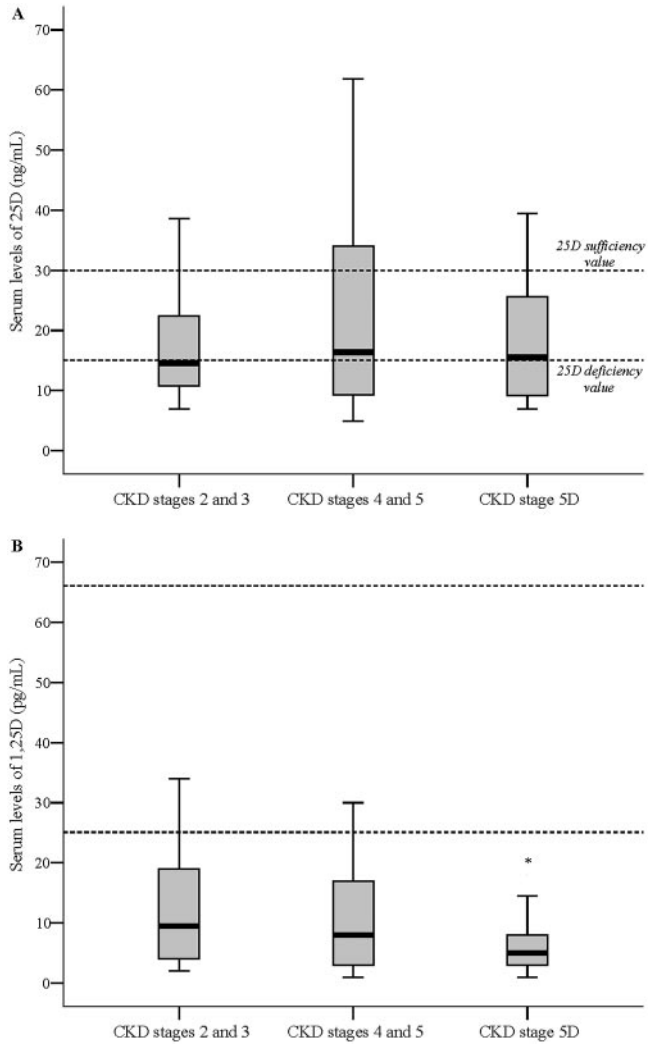

Figure 1. A) Serum levels of 25-hydroxyvitamin D (25D) and (B) serum levels of 1,25-dihydroxyvitamin D (1,25D) as a function of CKD stage (with dashed lines indicating the reference values). *$P < 0.05$ for CKD stage 5D *versus* other groups.

Table 2 describes the correlations between 25D serum levels and several variables. The only significant linear correlation was between 25D serum levels and albumin.

During the study period (mean follow-up period, 605 ± 217 d; median, 591; range, 10 to 889), 25 patients died (18 from cardiovascular causes, 5 from infectious diseases, and 2 from other causes). In the crude analysis (Figure 2), a 25D level of ≤16.7 ng/ml was a predictor of death ($P = 0.05$). Age (relative risk [RR] = 1.06; 95% CI, 1.02 to 1.10), CKD stage 5D (RR = 4.6; 95% CI, 1.98 to 10.66), smoking status (RR = 2.23; 95% CI, 1.03 to 5.25), albumin (RR = 0.94; 95% CI, 0.89 to 1.0), hemoglobin (RR = 0.68; 95% CI, 0.54 to 0.87), aortic calcification score on CT (RR = 1.3; 95% CI, 1.16 to 1.44), and aortic calcification score on an x-ray (RR = 1.1; 95% CI, 1.02 to 1.13) were also associated with the risk of death in a univariate Cox regression. There was no association between 1,25D levels and mortality in the study population.

Table 3 shows the predictive power of 25D for death when unadjusted or adjusted for multiple covariates. Lower serum 25D levels still had a significant effect on the risk of death after adjustment for age; gender; diabetes; smoking status; systolic and diastolic BP values; CKD stages; vitamin D supplementation; aortic calcification; PWV; and serum albumin, hemoglobin, and phosphate levels.

Table 2. Correlations between serum 25D levels (log transformed) and baseline clinical characteristics, nutritional status markers, mineral metabolism, and arterial calcification/function

Variable	*r*	*P*
Age	−0.034	0.380
Albumin	0.211	0.013
C-reactive protein	−0.021	0.799
Calcium	0.024	0.777
Phosphate	−0.119	0.162
1,25D	0.035	0.733
iPTH	−0.132	0.123
Systolic arterial pressure	0.081	0.343
Diastolic arterial pressure	0.067	0.432
Aortic calcification score, CT	−0.104	0.241
Aortic calcification score, x-ray	−0.031	0.738
PWV	−0.074	0.384

25D, 25-hydroxyvitamin D; 1,25D, 1,25-dihydroxyvitamin D; iPTH, intact parathyroid hormone; CT, computed tomography; PWV, pulse wave velocity.

Discussion

The present study revealed a high prevalence of 25D deficiency and insufficiency in a cohort of patients with CKD. Most important, there was a significant relationship between low 25D levels and mortality, even after adjustment for age, gender, the presence of diabetes, smoking status, BP, CKD stage, serum albumin, hemoglobin, PWV, and aortic calcification.

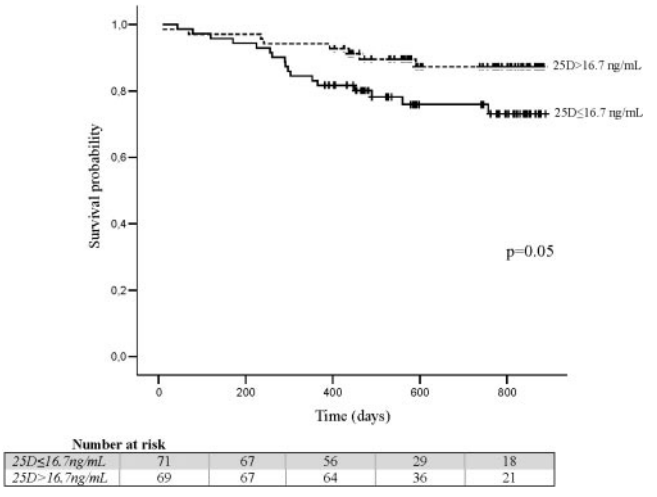

Figure 2. Crude patient survival as a function of vitamin D (25D) levels (≤16.7 ng/ml or >16.7 ng/ml).

Table 3. Cox regression models

Models of Patient Survival (event n = 25)	RR (95% CI)[a]	*P*
Model 0 (GF = 8.1; P = 0.004)		
unadjusted	0.571 (0.361 to 0.905)	0.013
Model 1 (GF = 22.4; P < 0.0001)		
age (y), DM, gender (masculine)	0.606 (0.391 to 0.942)	0.026
Model 2 (GF = 16.2; P = 0.003)		
albumin (g/L), hemoglobin (g/L), phosphate (mMol/L)	0.628 (0.409 to 0.963)	0.030
Model 3 (GF = 13.3; P = 0.004)		
systolic arterial pressure (mmHg), smoking habit (exposure)	0.536 (0.328 to 0.875)	0.011
Model 4 (GF = 22; P < 0.0001)		
vitamin D supplementation (exposure), CKD stage 5D	0.585 (0.379 to 0.902)	0.013
Model 5 (GF = 22; P < 0.0001)		
aortic calcification score on CT(log), PWV (m/s)	0.582 (0.368 to 0.923)	0.021

GF, goodness-of-fit; DM, diabetes mellitus; CT, computed tomography; PWV, pulse wave velocity.
[a]RR (95% CI); relative risk (95% confidence interval), summarizing the risk of a 10 ng/ml increase in the 25-hydroxyvitamin D levels for each noncumulative Cox regression model (unadjusted and then adjusted for the described variables).

CKD patients are at particular risk of 25D deficiency because of a range of factors that do not directly depend on renal function; these include reduced sun exposure, impaired production of the 25D precursor molecule, and reduced dietary intake (29). The present study evidenced a positive correlation between 25D and albumin levels, in accordance with previous observations (30–32). Reduced albumin levels are frequently related to malnutrition, because the latter also lowers levels of vitamin D binding protein (VBP) (6). Because vitamin D metabolites have low aqueous solubility and are mainly bound to plasma proteins (essentially VBP), low plasma protein levels could have contributed to the low observed 25D levels. However, the fact that we did not evaluate proteinuria prevents us from drawing further conclusions in this respect. In contrast to 25D, circulating levels of 1,25D chiefly depend on the ability of renal 1-α hydroxylase to convert 25D into 1,25D. This ability is decreased by (*1*) a reduction in the nephron mass, and (*2*) the hyperphosphatemia-induced increase in levels of the phosphaturic hormone FGF-23, which both inhibits the production of 1,25D and increases its degradation (33). Thus, as renal failure progresses and renal production of 1,25D decreases, 25D availability may constitute a rate-limiting step in the production of 1,25D by nonrenal tissues, where it may act either in autocrine or paracrine pathways (6). In accordance with other recently reported data (34), 25D deficiency was a better predictor of death than 1,25D in our cohort of CKD patients. The observed lack of a relationship between 1,25D levels and mortality in the study population may be due to the fact that most patients presented very low levels of 1,25D, and could have attenuated a potential risk. These low levels of 1,25D might result (at least in part) from the very low concentrations of 25 D (*i.e.*, the 1,25D precursor) observed in these patients. Nevertheless, this observation may most likely reflect 25D's advantageous characteristics as a biomarker (a more reproducible assay and a longer half-life) when compared with 1,25D, thus enabling more accurate measurement of exposure over time (6). In addition, it is possible that in CKD patients with markedly deficient renal production of 1,25D, the maintenance of adequate reserves of 25D may be especially important in maintaining VDR activation in cells that rely on autocrine pathways; this is the case for cultured human vascular smooth muscle cells (which have been shown to express functional 1-α hydroxylase [35]) and cultured human endothelial cells (in which the expression of the mRNA and protein for 1-α hydroxylase have been demonstrated [36]). It should be noted that the 25D assay used in the present study measures both ergocalciferol (D_2) and cholecalciferol (D_3). Hence, the observed high prevalence of vitamin D deficiency is unlikely to result from methodological limitations of the assay used here.

In addition, our current data did not show any association between 25D levels and aortic calcification or stiffness. In this regard, Shroff *et al.* have recently reported a bimodal association between vascular calcification and 1,25D levels in dialyzed children, but no association between 25D levels and the vascular measurements (22). London *et al.* observed that in adult hemodialysis patients, 1,25D and 25D levels were negatively correlated with aortic stiffness but not with vascular calcification (13). Our findings seem to suggest that 25D's effect on cardiovascular mortality may be independent of vascular calcification and stiffness. Hence, other negative effects of vitamin D deficiency on the cardiovascular system may be involved. It has been previously reported that left ventricular hypertrophy in hemodialysis patients is associated with lower survival (37). Interestingly, there is also experimental evidence that VDR gene deletion may induce myocardial hypertrophy and fibrosis (38). The absence of vitamin D-mediated signal transduction and genomic activation reportedly results in cardiomyocyte overstimulation and increased contractility, which ultimately lead to cardiomyocyte hypertrophy (39,40). In fact, low serum 1,25D concentrations have been implicated in poorer clinical outcomes for end-stage chronic heart failure patients (41). Furthermore, low levels of 25D have been associated with heart failure and sudden cardiac death in the general population (40,42). In peritoneal dialysis patients, Wang *et al.* reported an

association between low 25D levels and left ventricular dilation (43). One must bear in mind that the active form of vitamin D (1,25D) has been shown to operate as a negative hormonal regulator of the renin-angiotensin system, which plays an important role in the cardiovascular system by modulating BP and heart mass (4). In line with these observations, the administration of a VDR activator has been shown to attenuate left ventricular abnormalities in Dahl salt-sensitive rats exposed to a high-salt diet (44). Thus, our findings further corroborate the notion that vitamin D deficiency may affect cardiovascular system and mortality via several pathways other than an increase in vascular calcification or stiffness. Unfortunately, the fact that an echocardiographic evaluation was not performed in the present study prevented us from drawing further conclusions.

It is noteworthy that 28% of the deaths observed in our CKD cohort were attributed to noncardiovascular causes. Indeed, 25D deficiency in the general population has been also associated with higher mortality from noncardiovascular causes (namely colon, prostate, and breast cancer [45]) and augmented susceptibility to aggressive forms of specific infectious diseases (such as tuberculosis [46]). In line with these findings, observational studies in the general population have found that taking ordinary doses of vitamin D supplements is associated with a decrease in all-cause mortality (47). Moreover, two different cohort studies on hemodialysis patients demonstrated that oral (48) or injectable (49) doses of VDR activators increased overall survival. Unfortunately, in these latter studies, 25D status was not determined, but it was probably deficient in the North American cohort (49).

The present study's limitations include a relatively small cohort and limited follow-up, which may have affected the study power. Second, the conclusions may not apply to non-Caucasian populations living at different latitudes. In contrast, strong points include our assessment of vitamin D deficiency in patients with variable degrees of renal dysfunction, together with evaluation of important surrogate markers of cardiovascular health (namely, vascular calcification and PWV).

Our present findings are in line with recently published data that suggest (*1*) a high prevalence of 25D deficiency in CKD patients and (*2*) an association between 25D deficiency and mortality in the same population. Importantly, the effect of low 25D on mortality appeared to be independent of vascular calcification and stiffness, suggesting that 25D may influence survival in CKD patients via additional pathways that need to be further explored. The results highlight the imperative need for randomized clinical trials that evaluate the effects of vitamin D supplementation on mortality in CKD patients.

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Disclosures

None.

References

1. Holick MF: Vitamin D deficiency. *N Engl J Med* 357: 266–281, 2007
2. Hewison M, Burke F, Evans KN, Lammas DA, Sansom DM, Liu P, Modlin RL, Adams JS: Extra-renal 25-hydroxyvitamin D3–1alpha-hydroxylase in human health and disease. *J Steroid Biochem Mol Biol* 103: 316–321, 2007
3. Nagpal S, Na S, Rathnachalam R: Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* 26: 662–687, 2005
4. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP: 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 110: 229–238, 2002
5. Banerjee P, Chatterjee M: Antiproliferative role of vitamin D and its analogs–A brief overview. *Mol Cell Biochem* 253: 247–254, 2003
6. Dusso AS, Brown AJ, Slatopolsky E: Vitamin D. *Am J Physiol Renal Physiol* 289: F8–F28, 2005
7. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K: Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: Data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 167: 1159–1165, 2007
8. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC: Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 49: 1063–1069, 2007
9. Garland CF, Mohr SB, Gorham ED, Grant WB, Garland FC: Role of ultraviolet B irradiance and vitamin D in prevention of ovarian cancer. *Am J Prev Med* 31: 512–514, 2006
10. Melamed ML, Michos ED, Post W, Astor B: 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 168: 1629–1637, 2008
11. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Kinkeldei J, Boehm BO, Weihrauch G, Maerz W: Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* 168: 1340–1349, 2008
12. Inaguma D, Nagaya H, Hara K, Tatematsu M, Shinjo H, Suzuki S, Mishima T, Kurata K: Relationship between serum 1,25-dihydroxyvitamin D and mortality in patients with pre-dialysis chronic kidney disease. *Clin Exp Nephrol* 12: 126–131, 2008
13. London GM, Guerin AP, Verbeke FH, Pannier B, Boutouyrie P, Marchais SJ, Metivier F: Mineral metabolism and arterial functions in end-stage renal disease: Potential role of 25-hydroxyvitamin D deficiency. *J Am Soc Nephrol* 18: 613–620, 2007
14. Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, Mallamaci F, Zoccali C: Vitamin D levels and patient outcome in chronic kidney disease. *Kidney Int.* 75: 88–95, 2009
15. Foley RN, Parfrey PS, Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 32 5[Suppl 3]: S112–119, 1998
16. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM:

Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension* 38: 938–942, 2001
17. Ishimura E, Nishizawa Y, Inaba M, Matsumoto N, Emoto M, Kawagishi T, Shoji S, Okuno S, Kim M, Miki T, Morii H: Serum levels of 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25-hydroxyvitamin D in nondialyzed patients with chronic renal failure. *Kidney Int* 55: 1019–1027, 1995
18. LaClair RE, Hellman RN, Karp SL, Kraus M, Ofner S, Li Q, Graves KL, Moe SM: Prevalence of calcidiol deficiency in CKD: A cross-sectional study across latitudes in the United States. *Am J Kidney Dis* 45: 1026–1033, 2005
19. Bas A, Lopez I, Perez J, Rodriguez M, Aguilera-Tejero E: Reversibility of calcitriol-induced medial artery calcification in rats with intact renal function. *J Bone Miner Res* 21: 484–490, 2006
20. Haffner D, Hocher B, Muller D, Simon K, Konig K, Richter CM, Eggert B, Schwarz J, Godes M, Nissel R, Querfeld U: Systemic cardiovascular disease in uremic rats induced by 1,25(OH)2D3. *J Hypertens* 23: 1067–1075, 2005
21. Mathew S, Lund RJ, Chaudhary LR, Geurs T, Hruska KA: Vitamin D receptor activators can protect against vascular calcification. *J Am Soc Nephrol* 19: 1509–1519, 2008
22. Shroff R, Egerton M, Bridel M, Shah V, Donald AE, Cole TJ, Hiorns MP, Deanfield JE, Rees L: A bimodal association of vitamin D levels and vascular disease in children on dialysis. *J Am Soc Nephrol* 19: 1239–1246, 2008
23. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
24. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD III, Zhang YL, Greene T, Levey AS: Estimating GFR using serum cystatin C alone and in combination with serum creatinine: A pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis* 51: 395–406, 2008
25. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Am J Kidney Dis* 39 2[Suppl 1]: S1–S266, 2002
26. Zureik M, Temmar M, Adamopoulos C, Bureau JM, Courbon D, Thomas F, Bean K, Touboul PJ, Ducimetiere P, Benetos A: Carotid plaques, but not common carotid intima-media thickness, are independently associated with aortic stiffness. *J Hypertens* 20: 85–93, 2002
27. Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac AM, Target R, Levy BI: Assessment of arterial distensibility by automatic pulse wave velocity measurement: Validation and clinical application studies. *Hypertension* 26: 485–490, 1995
28. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW: New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis* 132: 245–250, 1997
29. K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. *Am J Kidney Dis* 42 [Suppl 3]: S1–S201, 2003
30. Del Valle E, Negri AL, Aguirre C, Fradinger E, Zanchetta JR: Prevalence of 25(OH) vitamin D insufficiency and deficiency in chronic kidney disease stage 5 patients on hemodialysis. *Hemodial Int* 11: 315–321, 2007
31. Corino A, D'Amelio P, Gancia R, Del Rizzo P, Gabasio S, Limone P, Isaia G: Hypovitaminosis D in internal medicine inpatients. *Calcif Tissue Int* 80: 76–80, 2007
32. Premaor MO, Alves GV, Crossetti LB, Furlanetto TW: Hyperparathyroidism secondary to hypovitaminosis D in hypoalbuminemic is less intense than in normoalbuminemic patients: A prevalence study in medical inpatients in southern Brazil. *Endocrine* 24: 47–53, 2004
33. Wetmore JB, Quarles LD: Calcimimetics or vitamin D analogs for suppressing parathyroid hormone in end-stage renal disease: Time for a paradigm shift? *Nat Clin Pract Nephrol* 5: 24–33, 2009
34. Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA, Jr., Tonelli M, Thadhani R: Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int* 72: 1004–1013, 2007
35. Somjen D, Weisman Y, Kohen F, Gayer B, Limor R, Sharon O, Jaccard N, Knoll E, Stern N: 25-hydroxyvitamin D3–1alpha-hydroxylase is expressed in human vascular smooth muscle cells and is upregulated by parathyroid hormone and estrogenic compounds. *Circulation* 111: 1666–1671, 2005
36. Zehnder D, Bland R, Chana RS, Wheeler DC, Howie AJ, Williams MC, Stewart PM, Hewison M: Synthesis of 1,25-dihydroxyvitamin D(3) by human endothelial cells is regulated by inflammatory cytokines: A novel autocrine determinant of vascular cell adhesion. *J Am Soc Nephrol* 13: 621–629, 2002
37. Silberberg JS, Barre PE, Prichard SS, Sniderman AD: Impact of left ventricular hypertrophy on survival in end-stage renal disease. *Kidney Int* 36: 286–290, 1989
38. Xiang W, Kong J, Chen S, Cao LP, Qiao G, Zheng W, Liu W, Li X, Gardner DG, Li YC: Cardiac hypertrophy in vitamin D receptor knockout mice: Role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab* 288: E125–E132, 2005
39. Mancuso P, Rahman A, Hershey SD, Dandu L, Nibbelink KA, Simpson RU: 1,25-Dihydroxyvitamin-D3 treatment reduces cardiac hypertrophy and left ventricular diameter in spontaneously hypertensive heart failure-prone (cp/+) rats independent of changes in serum leptin. *J Cardiovasc Pharmacol* 51: 559–564, 2008
40. Zittermann A, Schleithoff SS, Koerfer R: Vitamin D insufficiency in congestive heart failure: Why and what to do about it? *Heart Fail Rev* 11: 25–33, 2006
41. Zittermann A, Schleithoff SS, Gotting C, Dronow O, Fuchs U, Kuhn J, Kleesiek K, Tenderich G, Koerfer R: Poor outcome in end-stage heart failure patients with low circulating calcitriol levels. *Eur J Heart Fail* 10: 321–327, 2008
42. Pilz S, Marz W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, Boehm BO, Dobnig H: Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. *J Clin Endocrinol Metab* 93: 3927–3935, 2008
43. Wang AY, Lam CW, Sanderson JE, Wang M, Chan IH, Lui SF, Sea MM, Woo J Serum 25-hydroxyvitamin D status and cardiovascular outcomes in chronic peritoneal dialysis patients: A 3-y prospective cohort study. *Am J Clin Nutr* 87: 1631–1638, 2008
44. Bodyak N, Ayus JC, Achinger S, Shivalingappa V, Ke Q,

Chen YS, Rigor DL, Stillman I, Tamez H, Kroeger PE, Wu-Wong RR, Karumanchi SA, Thadhani R, Kang PM: Activated vitamin D attenuates left ventricular abnormalities induced by dietary sodium in Dahl salt-sensitive animals. *Proc Natl Acad Sci USA* 104: 16810–16815, 2007

45. Dizdar O, Harputluoglu H, Altundag K: Vitamin D intake and breast cancer risk in postmenopausal women. *Arch Intern Med* 167: 2532, 2007
46. Nnoaham KE, Clarke A: Low serum vitamin D levels and tuberculosis: A systematic review and meta-analysis. *Int J Epidemiol* 37: 113–119, 2008
47. Autier P, Gandini S: Vitamin D supplementation and total mortality: A meta-analysis of randomized controlled trials. *Arch Intern Med* 167: 1730–1737, 2007
48. Naves-Diaz M, Alvarez-Hernandez D, Passlick-Deetjen J, Guinsburg A, Marelli C, Rodriguez-Puyol D, Cannata-Andia JB: Oral active vitamin D is associated with improved survival in hemodialysis patients. *Kidney Int* 74: 1070–1078, 2008
49. Teng M, Wolf M, Ofsthun MN, Lazarus JM, Hernan MA, Camargo CA, Jr., Thadhani R: Activated injectable vitamin D and hemodialysis survival: A historical cohort study. *J Am Soc Nephrol* 16: 1115–1125, 2005