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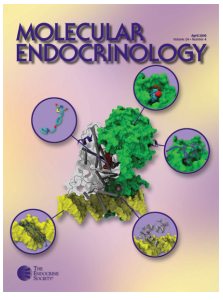

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Department of Public Health, Social and Preventive Medicine (S.P., W.M.), Mannheim Medical Faculty, University of Heidelberg, 68135 Mannheim, Germany; Department of Internal Medicine (S.P., A.F.-P., H.P.D., H.D.), Division of Endocrinology and Nuclear Medicine, Medical University of Graz, 8010 Graz, Austria; Synlab Center of Laboratory Diagnostics (W.M.), 69037 Heidelberg, Germany; LURIC Study Nonprofit LLC (B.W., U.S.), Freiburg, Germany; and Department of Internal Medicine I (B.O.B.), Division of Endocrinology and Diabetes, Ulm University, 89070 Ulm, Germany

Context: Vitamin D has been shown to influence cardiac contractility and myocardial calcium homeostasis.

Objectives: We aimed to elucidate whether insufficient vitamin D status is associated with heart failure and sudden cardiac death (SCD).

Design, Setting, and Participants: We measured 25-hydroxyvitamin D [25(OH)D] levels in 3299 Caucasian patients who were routinely referred to coronary angiography at baseline (1997–2000).

Main Outcome Measures: The main outcome was cross-sectional associations of 25(OH)D levels with measures of heart failure and Cox proportional hazard ratios for deaths due to heart failure and for SCD according to vitamin D status.

Results: 25(OH)D was negatively correlated with N-terminal pro-B-type natriuretic peptide and was inversely associated with higher New York Heart Association classes and impaired left ventricular function. During a median follow-up time of 7.7 yr, 116 patients died due to heart failure and 188 due to SCD. After adjustment for cardiovascular risk factors, the hazard ratios (with 95% confidence intervals) for death due to heart failure and for SCD were 2.84 (1.20–6.74) and 5.05 (2.13–11.97), respectively, when comparing patients with severe vitamin D deficiency [25(OH)D $<$25 nmol/liter)] with persons in the optimal range [25(OH)D $\geq$75 nmol/liter]. In all statistical analyses, we obtained similar results with 25(OH)D and with 1,25-dihydroxyvitamin D.

Conclusions: Low levels of 25(OH)D and 1,25-dihydroxyvitamin D are associated with prevalent myocardial dysfunction, deaths due to heart failure, and SCD. Interventional trials are warranted to elucidate whether vitamin D supplementation is useful for treatment and/or prevention of myocardial diseases. **(*J Clin Endocrinol Metab* 93: 3927–3935, 2008)**

Worldwide, the prevalence of vitamin D deficiency is as high as almost 50% among the elderly. A recent meta-analysis showing that vitamin D supplementation significantly decreased all-cause mortality raised the public health interest in vitamin D (1–4). The classic role of vitamin D for maintaining bone health was recently extended by reports linking vitamin D deficiency to various other diseases, including arterial hypertension and diabetes mellitus (5, 6). It also turned out that the myo-

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Abbreviations: CAD, Coronary artery disease; CI, confidence interval; CRP, C-reactive protein; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; LV, left ventricular; NT-pro-BNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; 1,25(OH)2D, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; SCD, sudden cardiac death.

cardium is an important target tissue for vitamin D-mediated effects on a genomic and nongenomic level (7–11).

Cardiomyocytes express the vitamin D receptor, and studies in rodents have shown that vitamin D protects against cardiac hypertrophy and myocardial dysfunction (7–11). Vitamin D suppresses systemic and local activation of the renin-angiotensin system and reduces the expression of genes associated with myocardial hypertrophy including those of the natriuretic peptides (7–11). Vitamin D treatment of hemodialysis patients was recently associated with a regression of cardiac hypertrophy and was accompanied by a reduction of QTc dispersion, which is a risk factor for sudden cardiac death (SCD) (9, 12, 13). Our previous results from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study show that low levels of 25-hydroxyvitamin D [25(OH)D] are an independent risk factor for total, overall cardiovascular and cancer mortality in patients referred to coronary angiography (14, 15).

In this work we present further data from 3299 LURIC probands to extend the rare cross-sectional knowledge about vitamin D metabolites and heart failure (16, 17) and to address the so-far unknown predictive value of vitamin D status for future deaths due to heart failure and SCD. Our analyses include measures of 25(OH)D and of 1,25-dihydroxyvitamin D [1,25(OH)2D], the latter of which possesses a higher affinity for the vitamin D receptor but circulates in lower concentrations than 25(OH)D (18, 19). 25(OH)D is converted to 1,25(OH)2D by 1α-hydroxylation in renal and several extrarenal tissues so that 25(OH)D rather than 1,25(OH)2D serum levels are considered a better predictor of overall vitamin D status.

Subjects and Methods

Study population

The LURIC study, a prospective cohort study of patients referred to coronary angiography, was designed to investigate environmental and genetic risk factors for cardiovascular diseases (20). The baseline examination was performed between July 1997 and January 2000 at a single tertiary care center in southwest Germany (Herzzentrum Ludwigshafen) and included 3316 study participants. Serum concentrations of 25(OH)D and 1,25(OH)2D were available in 3299 and 3315 study participants, respectively. Inclusion criteria were the availability of a coronary angiogram, clinical stability with the exception of acute coronary syndromes, and Caucasian origin, to limit genetic heterogeneity. Patients with a history of malignancy within the past 5 yr, any acute illness other than acute coronary syndrome, and any predominant noncardiac disease were excluded from the study. Informed written consent was obtained from all study participants, and the ethics committee at the "Ärztekammer Rheinland-Pfalz" (Mainz, Germany) approved the study.

Baseline examination

Detailed descriptions of the baseline examination in LURIC have been published previously (20). Angiographic coronary artery disease (CAD) was defined as the occurrence of at least one stenosis of at least 50% of at least one of 15 coronary segments, using the maximal luminal narrowing estimated by visual analysis. Contrast ventriculography was used for a semiquantitative grading of left ventricular (LV) function into "normal," "minimally," "moderately," or "severely impaired." LV ejection fraction, calculated from the right anterior oblique was available in 1360 of our study participants and correlated highly significantly with the semiquantitative assessed LV function (Spearman's correlation coefficient = -0.84; $P < 0.001$), suggesting that the semiquantitative grading provides a reliable estimate of LV function. Diabetes mellitus was diagnosed if the fasting glucose was greater than 7.0 mmol/liter or the 2-h value in an oral glucose tolerance test was greater than 11.1 mmol/liter and in patients already receiving antidiabetic medication. Arterial hypertension was diagnosed if the mean systolic and diastolic blood pressures out of five measurements exceeded 140 and/or 90 mm Hg or if there was a clinically significant history of hypertension. Pulse pressure, which is considered a cardiovascular risk factor (21), was calculated as the difference between the systolic and diastolic blood pressure. Physical activity level was estimated by a nonvalidated questionnaire and was categorized into "below average" (not very active), "average" (usual office work), and "above average" (heavy work or sports).

Measurements

Venous blood sampling was performed in the morning before coronary angiography, and routine laboratory parameters including 25(OH)D and 1,25(OH)2D were immediately measured on a daily basis as previously published (20). Remaining blood samples were snap-frozen for further determinations and stored at -80 C until analysis. Serum concentrations of 25(OH)D were measured by a RIA (DiaSorin, Antony, France, and Stillwater, MN) with intra- and interassay coefficients of variation of 8.6 and 9.2%, respectively. In 100 randomly chosen samples, we determined 25(OH)D by liquid chromatography tandem mass spectrometry with isotopic labeled internal standard and two fragments *m/z* 401.4/382.2 (quantifier) and 401.4/365.3 (qualifier) and found a highly significant correlation between the 25(OH)D levels obtained by RIA and liquid chromatography tandem mass spectrometry ($r = 0.875$; $P < 0.001$). Serum levels of 1,25(OH)2D were analyzed by RIA (Nichols Institute Diagnostika GmbH, Bad Nauheim, Germany) on a Berthold LB2014 multicrystal counter. N-terminal pro-B-type natriuretic peptide (NT-pro-BNP), a marker of ventricular dysfunction and cardiovascular risk (22), and PTH were determined by ElectroChemiLuminescence on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). C-reactive protein (CRP) was measured by immunonephelometry (N High Sensitivity CRP, Dade Behring, Marburg, Germany). Glomerular filtration rate (GFR) was calculated according to the abbreviated Modification of Diet in Renal Disease (MDRD) study equation (23).

Follow-up

Information on vital status was continuously obtained from local person registries. Death certificates were reviewed to classify the deceased into those who died from cardiovascular and noncardiovascular causes. Cardiovascular death was further categorized into SCD, fatal myocardial infarction, death due to heart failure, death after intervention to treat CAD, stroke, and other deaths due to heart disease. SCD was defined as sudden unexpected death either within 1 h of symptom onset or within 24 h of having been observed alive and symptom free (13, 24). SCD was not adjudicated in persons whose sudden death was most likely attributable to a noncardiac disease and in deceased patients who suffered from any predominant noncardiac and terminal disease (*e.g.* cancer) so that their death was not unexpected. Two experienced physicians that were blinded to any data of the study probands except for the information from the death certificates independently classified the causes of death. In the case of a disagreement concerning the classification, it was discussed and the final decision was made by one of the principal investigators of LURIC, who was also blinded to any data except the death certificates.

Statistical analyses

Based on 25(OH)D concentrations, we formed four vitamin D categories according to widely used cutoff values (2, 3, 25): severe vitamin D deficiency, less than 25.00 nmol/liter (<10.00 ng/ml); moderate vitamin D deficiency, 25.00–49.99 nmol/liter (10.00–19.99 ng/ml); vitamin

D insufficiency, 50.00–74.99 nmol/liter (20.00–29.99 ng/ml); and vitamin D optimal range, at least 75.00 nmol/liter (≥30.00 ng/ml). Quartiles of 1,25(OH)2D were calculated according to the 1,25(OH)2D concentrations of the whole study population. All continuous parameters following a nonnormal distribution were logarithmically transformed before being used in parametric procedures. Categorical data are presented as percentages and depending on their distribution, continuous parameters are shown as means with SD values (normal distribution) and as medians with interquartile ranges (skewed distribution). Comparisons between groups were performed by analysis of (co-) variance [AN(C)OVA] with *P* for linear trend for continuous parameters and with χ^2 test with *P* for linear-by-linear test for categorical variables. Simple correlation analyses and multiple linear regression analyses including several cardiovascular risk factors were performed to examine whether vitamin D metabolites were associated with NT-pro-BNP. To reduce statistical bias due to seasonal variation of vitamin D, we also calculated z-values of logarithmically transformed 25(OH)D and 1,25(OH)2D levels based on their means and SD values within each month of blood sampling (formula for z-values: X-mean/SD). Hazard ratios (HRs) with 95% confidence intervals (CI) for mortality due to heart failure, SCD, and myocardial infarction were calculated with Cox proportional hazard models. We used the categories with the highest 25(OH)D and 1,25(OH)2D concentrations as the reference and adjusted for several possible confounders. To account for the seasonal variation of vitamin D, we adjusted our mortality analyses for a variable that ranks all months of a year according to their median 25(OH)D or 1,25(OH)2D concentrations, which were calculated from the values of the blood samples that were drawn within the respective month. We also formed quartiles based on the 25(OH)D and 1,25(OH)2D concentrations from each month of blood draw, which is another approach to account for the seasonal fluctuation of vitamin D. We tested for interactions of arterial hypertension, angiographic CAD, and sex by adding product terms of these parameters with 25(OH)D groups to our analyses. Backward LR selection method (*P* values for inclusion and exclusion were 0.05 and 0.10, respectively) was used for the multivariable adjusted models, and the results of the final steps are shown. A *P* value <0.05 was considered statistically significant. Data were analyzed using SPSS 15.0 statistical package (SPSS Inc., Chicago, IL).

Results

Clinical and laboratory parameters at baseline according to 25(OH)D groups are shown in Table 1. Main findings were that groups with lower 25(OH)D concentrations included more females and patients with diabetes mellitus, arterial hypertension, higher age, lower physical activity level, higher New York Heart Association (NYHA) classes, and impaired LV function, whereas the prevalence of CAD was not significantly different between the groups (Table 1). Baseline characteristics according to 1,25(OH)2D quartiles are presented in the supplemental Table 1 (published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). We have already reported about a positive association between 25(OH)D and 1,25(OH)2D levels in our previous work (14), and these data are slightly extended by analyses of the present paper: from patients with severe vitamin D deficiency, 45.5% had 1,25(OH)2D concentrations within the lowest 1,25(OH)2D quartile; and from patients with optimal vitamin D levels, 45.7% had 1,25(OH)2D concentrations within the highest 1,25(OH)2D quartile. The Pearson correlation coefficient of logarithmically transformed 25(OH)D and 1,25(OH)2D levels was 0.39 ($P < 0.001$) in the entire study cohort. In analyses stratified by vitamin D groups, this correlation coefficient was 0.21 ($P < 0.001$) in patients with severe vitamin D deficiency, 0.19 ($P < 0.001$) in patients with moderate vitamin D deficiency, 0.13 ($P < 0.001$) in patients with vitamin D insufficiency, and −0.01 ($P = 0.832$) in patients with optimal vitamin D levels.

Associations of vitamin D with heart failure

NT-pro-BNP was significantly correlated with 25(OH)D (Spearman correlation coefficient $R = -0.190$; $P < 0.001$) and with 1,25(OH)2D ($R = -0.253$; $P < 0.001$). In multiple linear regression analyses including age, sex, body mass index, physical activity level, low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)-cholesterol, triglycerides, diabetes mellitus, active smoking status, arterial hypertension, GFR, and CRP, 25(OH)D (β coefficient = −0.082) and 1,25(OH)2D (β coefficient = −0.180) remained independently associated with NT-pro-BNP ($P < 0.001$ for both).

In groups according to LV function, 25(OH)D and 1,25(OH)2D decreased with impaired LV function as evaluated by ANOVA with *P* for trend ($P < 0.001$ for both), and this association remained significant after multivariable adjustments (same covariates as in the above-mentioned multiple regression analyses) in AN(C)OVA ($P < 0.001$ for both). Higher NYHA classes were also associated with lower levels of 25(OH)D and 1,25(OH)2D ($P < 0.001$ for both), but these associations lost their significance in AN(C)OVA [$P = 0.214$ for 25(OH)D and $P = 0.058$ for 1,25(OH)2D]. However, when physical exercise level was tentatively removed from the covariate list, both vitamin D metabolites remained significantly decreased in higher NYHA classes [$P < 0.001$ for 25(OH)D, and $P = 0.002$ for 1,25(OH)2D)].

In accordance with results from other studies, we observed a seasonal variation of 25(OH)D in LURIC with the lowest median concentrations in February (32.2 nmol/liter) and the highest in August (56.4 nmol/liter) (2, 3, 14, 15, 19, 26). We accounted for this seasonal variation by the use of z-values for 25(OH)D and 1,25(OH)2D that were significantly associated with NT-pro-BNP and impaired LV function as evaluated by the above-described AN(C)OVAs, correlation, and regression analyses ($P < 0.001$ for all). Z-values for 25(OH)D and 1,25(OH)2D were significantly associated with higher NYHA classes as evaluated by ANOVA ($P < 0.001$ for both), and these results remained significant after multivariable adjustments including physical activity level [$P = 0.005$ for z-values of 25(OH)D and $P = 0.010$ for z-values of 1,25(OH)2D].

Vitamin D and fatal cardiovascular events

Eighteen persons were lost during follow-up; in 24 study participants we did not obtain the death certificates, and they were thus excluded from the mortality analyses. After a median follow-up time of 7.7 yr, 760 patients with available 25(OH)D levels died, 188 from SCD (25% of all deaths), and 116 due to heart failure (15%).

Unadjusted HRs (with 95% CI) for death due to heart failure and SCD in the group with severe vitamin D deficiency compared with the optimal range group were 4.13 (1.77–9.62) and 5.98

TABLE 1. Baseline characteristics according to 25(OH)D concentrations

	Groups				
	Severe deficiency, <25.00[a] (n = 802)	**Moderate deficiency, 25.00–49.99[a] (n = 1362)**	**Insufficiency, 50.00–74.99[a] (n = 796)**	**Optimal range, ≥75.00[a] (n = 339)**	***P* value**
Age (yr)	66.0 (58.3–72.9)	64.0 (56.0–70.8)	61.8 (55.6–68.4)	61.0 (54.8–66.8)	<0.001
Females (%)	43.0	27.2	26.0	22.7	<0.001
Body mass index (kg/m²)	27.1 (24.3–30.0)	27.3 (24.9–30.0)	26.8 (24.7–29.4)	26.8 (24.7–29.0)	0.074
Diabetes mellitus (%)	40.3	34.4	25.4	18.6	<0.001
HbA1c (%)	6.1 (5.7–7.0)	6.0 (5.6–6.6)	5.9 (5.5–6.4)	5.8 (5.5–6.3)	<0.001
Arterial hypertension (%)	74.8	74.0	70.4	67.6	0.003
Blood pressure (mm Hg)					
Systolic	141 ± 25	142 ± 24	141 ± 22	139 ± 23	0.117
Diastolic	79 ± 12	81 ± 12	82 ± 11	81 ± 11	<0.001
Pulse pressure	61 (47–75)	59 (47–72)	56 (46–70)	55 (45–68)	<0.001
Active smokers (%)	23.1	18.6	19.0	18.0	0.035
Blood lipids (mmol/liter)					
LDL-cholesterol	2.90 (2.32–3.57)	2.98 (2.46–3.55)	2.98 (2.46–3.57)	3.00 (2.51–3.63)	0.048
HDL-cholesterol	0.93 (0.78–1.11)	0.96 (0.80–1.17)	1.01 (0.85–1.19)	1.01 (0.85–1.24)	<0.001
Trigylycerides	1.67 (1.23–2.26)	1.68 (1.23–2.27)	1.63 (1.21–2.27)	1.59 (1.27–2.24)	0.708
GFR (ml/min per 1.73 m²)	80 (67–93)	81 (70–92)	81 (70–92)	81 (69–92)	0.023
CRP (mg/liter)	5.1 (1.7–10.7)	3.2 (1.2–7.7)	2.8 (1.2–7.5)	2.8 (1.2–6.8)	<0.001
CAD (%)	70.8	68.4	65.4	69.2	0.129
NT-pro-BNP (ng/ml)	416 (165–1508)	284 (103–801)	232 (93–749)	197 (83–507)	<0.001
NYHA class (%)					
NYHA 1	44.9	51.2	56.4	62.2	<0.001
NYHA 2	27.9	30.5	28.6	27.1	<0.001
NYHA 3	22.4	15.9	11.9	9.4	<0.001
NYHA 4	4.7	2.4	3.0	1.2	<0.001
LV function (%)					
Normal	63.9	71.2	72.5	78.2	<0.001
Minimally impaired	10.5	10.8	11.4	10.7	<0.001
Moderately impaired	15.3	11.8	12.0	6.5	<0.001
Severely impaired	10.3	6.2	4.1	4.6	<0.001
Physical activity level (%)					
Below average	38.1	24.8	20.3	16.2	<0.001
Average	54.2	57.0	52.3	45.0	<0.001
Above average	7.8	18.2	27.4	38.8	<0.001
Medication use (%)					
ACE inhibitor	61.3	52.9	47.1	50.7	<0.001
β-Blocker	61.1	63.8	64.8	64.3	0.435
Diuretics	39.0	29.2	21.1	17.4	<0.001
PTH (ng/liter)	35 (26–47)	30 (22–39)	27 (20–35)	25 (19–32)	<0.001
Serum calcium (mmol/liter)	2.31 (2.24–2.38)	2.33 (2.26–2.40)	2.33 (2.28–2.40)	2.34 (2.28–2.40)	<0.001

Continuous data are presented as means ± SD or median (interquartile range), and categorical data are shown as percentages. ANOVA with *P* for trend and χ^2 test were used. ACE, angiotensin converting enzyme; HbA1c, glycosylated hemoglobinA1c.

[a] 25(OH)D concentration (nmol/liter).

(2.60–13.74), respectively. These HRs remained significant, even after controlling for various possible confounders including cardiovascular risk factors, the use of medications, parameters of mineral metabolism, and physical activity levels (Table 2).Cox proportional hazard models for quartiles of 1,25(OH)2D also showed that persons in the first (lowest) quartile were at increased risk for deaths due to heart failure and for SCDs when compared with those in the fourth quartile (Table 3). In patients without angiographic CAD and without arterial hypertension, we recorded only 20 and 25 deaths due to heart failure and 39 and 43 SCDs, respectively. We therefore used a combined endpoint of these two fatal cardiovascular events in tests for interactions. In the fully adjusted model (model 3 in Table 2), there was no significant interaction with arterial hypertension ($P = 0.250$), whereas the interaction with angiographic CAD was close to reaching statistical significance ($P = 0.059$). Thus, we performed subgroup analyses of patients with and without CAD. Fully adjusted HRs were 3.75 (1.89–7.44) for patients with CAD and 5.56 (1.30–23.75) for patients without CAD when comparing the severe vitamin D-deficient with the optimal range group. We also calculated HRs for the combined endpoint of SCD and death due to heart failure for patients who were both severe vitamin D deficient and had 1,25(OH)2D concentrations within the lowest 1,25(OH)2D quartile (n = 365) when compared with those who had an optimal vitamin D status plus a 1,25(OH)2D concentration within the highest 1,25(OH)2D

TABLE 2. HRs with 95% CIs for death due to heart failure and SCD according to 25(OH)D groups

	Groups			
	Severe deficiency, <25.00[a]	**Moderate deficiency, 25.00–49.99[a]**	**Insufficiency, 50.00–74.99[a]**	**Optimal range, ≥75.00[a]**
Death due to heart failure				
No. of study participants at risk	789	1346	786	336
No. of deaths	50 (6.3%)	47 (3.5%)	13 (1.7%)	6 (1.8%)
Median follow-up time (yr)	7.75	7.83	7.67	7.50
Unadjusted	4.13 (1.77–9.62)	2.04 (0.87–4.76)	0.94 (0.36–2.48)	1.0 reference
Model 1	4.98 (2.08–11.91)	2.27 (0.96–5.34)	0.97 (0.37–2.55)	1.0 reference
Model 2	3.49 (1.48–8.23)	1.77 (0.75–4.15)	0.88 (0.33–2.31)	1.0 reference
Model 3	2.84 (1.20–6.74)	1.55 (0.66–3.65)	0.90 (0.34–2.37)	1.0 reference
Model 3 plus additional				
PTH	2.61 (1.02–6.68)	1.67 (0.66–4.22)	0.94 (0.33–2.68)	1.0 reference
Serum calcium	2.84 (1.20–6.74)	1.55 (0.66–3.65)	0.90 (0.34–2.37)	1.0 reference
Physical activity level	2.63 (1.07–6.44)	1.40 (0.59–3.32)	0.80 (0.30–2.12)	1.0 reference
SCD				
No. of study participants at risk	789	1346	786	336
No. of deaths	74 (9.4%)	72 (5.3%)	36 (4.6%)	6 (1.8%)
Median follow-up time (yr)	7.75	7.83	7.67	7.50
Unadjusted	5.98 (2.60–13.74)	3.11 (1.35–7.15)	2.61 (1.10–6.19)	1.0 reference
Model 1	7.47 (3.20–17.43)	3.53 (1.53–8.15)	2.69 (1.13–6.39)	1.0 reference
Model 2	6.41 (2.73–15.08)	3.07 (1.33–7.10)	2.52 (1.06–5.97)	1.0 reference
Model 3	5.05 (2.13–11.97)	2.58 (1.11–5.97)	2.52 (1.06–6.00)	1.0 reference
Model 3 plus additional				
PTH	4.37 (1.84–10.40)	2.38 (1.03–5.53)	2.45 (1.03–5.82)	1.0 reference
Serum calcium	5.05 (2.13–11.96)	2.58 (1.11–5.98)	2.52 (1.06–6.00)	1.0 reference
Physical activity level	5.35 (2.09–13.67)	2.77 (1.11–6.92)	2.70 (1.05–6.91)	1.0 reference

Model 1 was adjusted for the month of blood sampling. Model 2 was additionally adjusted for age and sex. Model 3 was additionally adjusted for body mass index, active smokers, diabetes mellitus, arterial hypertension, GFR, LDL- and HDL-cholesterol, triglycerides, CRP, CAD, ACE (angiotensin converting enzymes) inhibitors, diuretics, and β-blockers.

[a] 25(OH)D concentration (nmol/liter).

quartile (n = 155).The unadjusted HR (with 95% CI) for patients who had low values for both forms of vitamin D was 7.35 (2.96–18.21), and the multivariable adjusted HR (according to model 3 in Table 2) was 4.40 (1.74–11.15). For fatal myocardial infarction (n = 90), the fully adjusted HR (model 3 in Table 2) was 1.54 (0.67–3.58) in patients with severe vitamin D deficiency when compared with the group with optimal vitamin D levels.

Using quartiles based on the 25(OH)D values from each month of blood draw, the unadjusted and fully adjusted HRs were 4.33 (2.40–7.80) and 2.93 (1.59–5.39) for deaths due to heart failure and 3.81 (2.37–6.13) and 2.68 (1.65–4.38) for SCDs, respectively. HRs for quartiles based on the 1,25(OH)2D concentrations within each month of blood sampling revealed similar results (data not shown). Albumin concentrations were available in 2880 persons only, but in all our Cox regression analyses, the inclusion of albumin as a covariate did not significantly attenuate the HRs or the *P* values (data not shown). Furthermore, we obtained similar results for all Cox regression analyses when using corrected calcium instead of absolute calcium concentrations (data not shown).

Interestingly, we noticed that in the entire study population [including also patients without available 25(OH)D levels], more SCDs and deaths due to heart failure occurred in the months from December to May, which were the 6 months with the lowest median 25(OH)D levels at baseline, compared with the months from June to November: 101 *vs.* 91 SCDs, and 62 *vs.* 54 deaths due to heart failure.

The results of all AN(C)OVAs and regression analyses did not materially change when males and females were analyzed separately. There was no significant interaction of 25(OH)D and 1,25(OH)2D groups with sex in our Cox regression analyses (data not shown).

Discussion

In patients referred to coronary angiography, 25(OH)D and 1,25(OH)2D levels were negatively correlated with NT-proBNP and were inversely associated with impaired LV function and higher NYHA classes. During a median follow-up time of 7.7 yr, low levels of 25(OH)D and 1,25(OH)2D were a significant risk factor for mortality due to heart failure and for SCDs.

Our finding that heart failure patients exhibit "insufficient" vitamin D concentrations confirms and extends results of previous smaller studies (16, 17). However, one may argue that low vitamin D levels in heart failure patients are simply a consequence of a disease-related sedentary lifestyle with reduced outdoor activities and subsequent limited UVB-induced vitamin D production in the skin (17). We acknowledge that limited mobility is a risk factor for the development of vitamin D deficiency,

TABLE 3. HRs with 95% CIs for death due to heart failure and SCD according to vitamin 1,25(OH)2D quartiles

	1st quartile, <66.6	2nd quartile, 66.6–86.3	3rd quartile, 86.4–111.5	4th quartile, >111.5
Death due to heart failure				
No. of study participants at risk	818	829	809	817
No. of deaths	54 (6.6%)	24 (2.9%)	23 (2.8%)	15 (1.8%)
Median follow-up time (yr)	7.67	7.83	7.67	7.75
Unadjusted	4.18 (2.36–7.42)	1.67 (0.88–3.19)	1.60 (0.84–3.07)	1.0 reference
Model 1	4.25 (2.37–7.60)	1.69 (0.88–3.23)	1.61 (0.84–3.09)	1.0 reference
Model 2	3.77 (2.11–6.71)	1.54 (0.81–2.94)	1.52 (0.79–2.91)	1.0 reference
Model 3	2.78 (1.55–4.97)	1.20 (0.64–2.30)	1.36 (0.71–2.62)	1.0 reference
Model 3 plus additional				
PTH	2.89 (1.55–5.39)	1.34 (0.68–2.65)	1.55 (0.78–3.07)	1.0 reference
Serum calcium	2.78 (1.55–4.97)	1.20 (0.63–2.30)	1.36 (0.71–2.62)	1.0 reference
Physical activity level	2.64 (1.47–4.72)	1.19 (0.62–2.28)	1.34 (0.69–2.59)	1.0 reference
SCD				
No. of study participants at risk	818	829	809	817
No. of deaths	70 (8.6%)	51 (6.2%)	39 (4.8%)	32 (3.9%)
Median follow-up time (yr)	7.67	7.83	7.67	7.75
Unadjusted	2.45 (1.61–3.72)	1.63 (1.05–2.54)	1.26 (0.79–2.01)	1.0 reference
Model 1	2.55 (1.66–3.90)	1.67 (1.07–2.61)	1.27 (0.80–2.03)	1.0 reference
Model 2	2.23 (1.46–3.41)	1.51 (0.97–2.35)	1.20 (0.75–1.91)	1.0 reference
Model 3	1.75 (1.14–2.68)	1.26 (0.81–1.98)	1.14 (0.71–1.82)	1.0 reference
Model 3 plus additional				
PTH	1.70 (1.10–2.63)	1.31 (0.83–2.06)	1.12 (0.69–1.80)	1.0 reference
Serum calcium	1.75 (1.14–2.68)	1.27 (0.81–1.98)	1.14 (0.71–1.82)	1.0 reference
Physical activity level	1.76 (1.14–2.70)	1.18 (0.75–1.86)	1.15 (0.72–1.85)	1.0 reference

Model 1 was adjusted for the month of blood sampling. Model 2 was additionally adjusted for age and sex. Model 3 was additionally adjusted for body mass index, active smokers, diabetes mellitus, arterial hypertension, GFR, LDL- and HDL-cholesterol, triglycerides, CRP, CAD, ACE (angiotensin converting enzymes) inhibitors, diuretics, and β-blockers.

[a] 1,25(OH)2D concentration (pmol/liter).

but in our study the association between vitamin D metabolites and LV dysfunction as well as deaths due to heart failure remained significant after multivariable adjustments also including physical activity level. Z-values and "absolute" concentrations of 25(OH)D and 1,25(OH)2D were also inversely associated with higher NYHA classes after adjustment for cardiovascular risk factors, but this association lost its significance for absolute 25(OH)D and 1,25(OH)2D levels after additional adjustment for physical activity level. This result might be attributed to the fact that reduced physical activity is also a consequence of heart failure and is closely related to the NYHA classification and may therefore indeed represent an inadequate covariate in the above-mentioned analyses. In this context, it should also be considered that vitamin D deficiency itself causes muscle weakness with subsequent limited mobility that was shown to be restored by vitamin D supplementation (27, 28).

Animal models show that vitamin D deficiency is associated with myocardial hypertrophy and fibrosis and aberrant cardiac contractility and relaxation (8–11, 29). Furthermore, paricalcitol, an activated vitamin D compound, reduced myocardial hypertrophy and dysfunction in a rat model of hypertension and heart failure (9). This is supported by observations that vitamin D supplementation improved ventricular function and reduced myocardial hypertrophy in hemodialysis patients (12).

Possible causal mechanisms that may link vitamin D deficiency to heart failure may involve the regulatory effects of 1,25(OH)2D on myocardial gene expressions. Several genes that are up-regulated in the course of myocardial hypertrophy have been found suppressed by 1,25(OH)2D treatment in rats (11). Vitamin D has also been shown to reduce the activation of the systemic and cardiac renin-angiotensin system, which contributes to hypertension and cardiac hypertrophy (10). Furthermore, 1,25(OH)2D may be cardioprotective by its anti-inflammatory effects that were confirmed in an interventional trial with 1,25(OH)2D in heart failure patients (30). Myocardial calcium homeostasis, which is crucial for the contractility and electrophysiology of the heart, is also partially regulated by 1,25(OH)2D, mediated by its influence on ion channels and enzymatic reactions (31–35). The precise mechanisms remain to be clarified further, but it appears that sufficient vitamin D status is important to prevent myocardial hypercontractility and to maintain diastolic function (8, 9).

To the best of our knowledge, this is the first study to show that low levels of 25(OH)D and 1,25(OH)2D are independent predictors of SCD. A recent report that 1,25(OH)2D treatment reduced the QTc dispersion in hemodialysis patients may suggest that this relationship might be causal (12). The underlying pathways are still unclear, and it can thus only be speculated whether the regulatory effects of 1,25(OH)2D on calcium-dependent processes in the myocardium are relevant for the pathogenesis of SCD (31–36).

It is important to note that a low vitamin D status was not closely associated with prevalent CAD and that 25(OH)D and 1,25(OH)2D (data not shown) were not significantly associated

with fatal myocardial infarction after multivariable adjustments. This may suggest that a sufficient vitamin D status may be more important for the physiology of the cardiomyocytes and less for the coronary circulation, although we are of course aware that CAD and myocardial diseases are closely related to each other. Interestingly, the risk for the combined endpoint of death due to heart failure and SCD was even higher for study participants without CAD than for those with CAD, suggesting that vitamin D deficiency might be more closely related to the pathogenesis of "nonischemic" myocardial diseases compared with those with an ischemic origin. However, 1,25(OH)2D has been shown to exert several protective effects on atherogenesis and vascular calcification, and in a case-control study 25(OH)D levels were significantly reduced in patients with myocardial infarction (37–40). Moreover, recent data from 1739 Framingham Offspring Study participants without cardiovascular disease at baseline showed that after a follow-up time of 5.4 yr, in which 120 nonfatal and fatal cardiovascular events occurred, low vitamin D levels were independently associated with an increased risk of cardiovascular events (41). These data are in line with our results. However, in the LURIC population we did not find an interaction between 25(OH)D levels and systemic hypertension, whereas in the Framingham Offspring Study the association between cardiovascular events and 25(OH)D levels was confined to people with arterial hypertension (41). Furthermore, our data are in line with the findings by Visser *et al.* (42) that suggest an association of 25(OH)D and mortality in older persons.

Our findings are limited because the LURIC cohort consists of Caucasian patients from a single geographic with an indication for coronary angiography, and our results may therefore not be generalizable to persons with lower cardiovascular risk, the general population, non-Caucasian populations, or persons from other latitudes. On the other hand, inclusion only of Caucasian patients and the setting in a single geographic area may be regarded as a strength of our work because this may reduce a possible bias of differences in seasonal vitamin D variations that are greater in northern than in southern latitudes and that are attenuated in Blacks when compared with Whites (43, 44). Other limitations are that we used a nonvalidated questionnaire for the assessment of physical activity and that each patient had only a single blood draw, and not serial measurements of vitamin D metabolites that would provide a better estimate of the vitamin D status of each individual. Furthermore, our assumption made for the seasonal adjustments that an individual with a high vitamin D percentile during summer would also be within a high percentile during winter is not necessarily validated. Our analyses were adjusted for several possible confounders, but statistical adjustments can never exclude the existence of other unconsidered or unmeasured confounders, and we can therefore not rule out that an insufficient vitamin D status is only an indicator for other factors that contribute to heart failure and/or SCD. Thus, we cannot draw a final conclusion about whether the predictive value of vitamin D deficiency for deaths due to heart failure and SCD reflects a causal relationship, but in view of the currently available data including the meta-analysis showing that vitamin D supplementation significantly decreases total mortality, there exists an urgent need for interventional trials with vitamin D supplementation for the treatment and/or prevention of myocardial diseases. Importantly, future trials should use adequate high doses of vitamin D and consider the recently reviewed safety of vitamin D intake and the fact that vitamin D doses in previous trials were often too low to reach the desirable 25(OH)D level of at least 75 nmol/liter (30 ng/ml) (4, 45–48). Concerning 1,25(OH)2D status, it is important to note that mineral metabolism and renal function have a significant impact on circulating 1,25(OH)2D levels (2). For patients with impaired renal function and subsequently reduced renal 1α-hydroxylase activity, the currently most effective approach to increase the 1,25(OH)2D status is supplementation of 1,25(OH)2D (or its analogs), which has been shown to decrease mortality in patients with renal failure (49). For patients with preserved renal function, there exist controversial data about the impact of vitamin D supplementation on 1,25(OH)2D levels (50, 51). Toward this, our finding that the correlation between 25(OH)D and 1,25(OH)2D levels becomes stronger with decreasing 25(OH)D concentrations supports the hypothesis that in patients with severe vitamin D deficiency, substrate availability of 25(OH)D may be a rate-limiting factor for the renal conversion (1α-hydroxylation) of 25(OH)D to 1,25(OH)2D (14). Accordingly, it might be speculated that in patients with severe vitamin D deficiency, supplementation of vitamin D could increase both 25(OH)D as well as circulating 1,25(OH)2D levels. In addition, it should also be emphasized that, irrespective of renal function, vitamin D supplementation increases the local production of 1,25(OH)2D by 1α-hydroxylase activity in various extrarenal tissues (19, 37, 52).

In summary, we presented evidence that in patients referred to coronary angiography, 25(OH)D and 1,25(OH)2D are associated with heart failure in cross-sectional analyses and predict future deaths due to heart failure and SCDs even after correcting for possible confounders including PTH, calcium, physical activity, and kidney function. These data strongly indicate that the maintenance of an optimal vitamin D status may be a promising approach for the prevention and/or therapy of myocardial diseases, warranting confirmation in interventional trials with vitamin D supplementation.

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Address all correspondence and requests for reprints to: Stefan Pilz, Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria. E-mail: stefan.pilz@chello.at.; or Harald Dobnig, Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria. E-mail: harald.dobnig@meduni-graz.at.

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