Low Levels of Serum 25-Hydroxyvitamin D Are Associated with Increased Risk of Myocardial Infarction, Especially in Women: Results from the MONICA/KORA Augsburg Case-Cohort Study

Mahir Karakas, Barbara Thorand, Astrid Zierer, Cornelia Huth, Christa Meisinger, Michael Roden, Wolfgang Rottbauer, Annette Peters, Wolfgang Koenig, and Christian Herder

Department of Internal Medicine II-Cardiology (M.K., W.R., W.K.), University of Ulm Medical Center, D-89081 Ulm, Germany; Helmholtz Zentrum München (B.T., A.Z., C.Hu., C.M., A.P.), German Research Center for Environmental Health, Institute of Epidemiology II, D-85764 Neuherberg, Germany; and Institute for Clinical Diabetology (M.R., C.He.), German Diabetes Center, Leibniz Center for Diabetes Research, and Department of Metabolic Diseases (M.R.), Heinrich Heine University Düsseldorf, D-40225 Düsseldorf, Germany

Context and Objective: A growing body of evidence suggests that vitamin D deficiency may adversely affect the cardiovascular system. Therefore, we thought to prospectively assess the association between serum 25-hydroxyvitamin D, the most commonly used index of vitamin D status, and incident coronary heart disease.

Design, Setting, and Patients: We measured serum levels of 25[OH]D in 1783 healthy middle-aged subjects (964 men, 819 women) in the population-based Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg studies. A total of 298 coronary heart disease cases were identified over a mean follow-up period of 11 yr.

Results: After adjustment for age, survey, and season of blood sampling, the hazard ratio (HR) and 95% confidence interval comparing tertile extremes of serum levels of 25[OH]D was 0.32 (0.16–0.65) (P for trend = 0.001) in women and 0.56 (0.38–0.82) (P for trend = 0.005) in men. Further adjustment for traditional cardiovascular risk factors slightly attenuated the association in women [HR 0.39 (0.18–0.84); P for trend = 0.013], whereas it became nonsignificant in men [HR 0.76 (0.49–1.17); P for trend = 0.215]. After additional adjustment for C-reactive protein, IL-6, soluble intercellular adhesion molecule-1, and interferon-γ-inducible protein-10, the association still remained significant in women [HR 0.42 (0.19–0.93); P for trend = 0.028], and it was further reduced in men [HR 0.84 (0.52–1.35); P for trend = 0.461].

Conclusion: Our findings suggest that higher vitamin D levels are associated with decreased risk of coronary heart disease. This effect is more pronounced in women than in men. Further clinical and experimental studies are needed to investigate the sex differences and whether vitamin D supplementation could contribute to the prevention of coronary heart disease. (***J Clin Endocrinol Metab*** **98: 272–280, 2013)**

Abbreviations: AUC, Area under the curve; CHD, coronary heart disease; CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; HDL-C, high-density lipoprotein-cholesterol; IP-10, interferon-γ-inducible protein 10; KORA, Kooperative Gesundheitsforschung in der Region Augsburg (Cooperative Health Research in the Region of Augsburg); MI, myocardial infarction; MONICA, Monitoring of Trends and Determinants in Cardiovascular Diseases; 25[OH]D, 25-hydroxyvitamin D; S, survey; S1, MONICA/KORA baseline survey 1 conducted in 1984–1985; S2, MONICA/KORA baseline survey 2 conducted in 1989–1990; S3, MONICA/KORA baseline survey 3 conducted in 1994–1995; SAS, Statistical Analysis System; SCD, sudden cardiac death; sICAM-1, soluble intercellular adhesion molecule-1; VDR, vitamin D receptor.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2012–2368 Received May 31, 2012. Accepted October 5, 2012.
First Published Online November 12, 2012

A growing body of evidence suggests that vitamin D deficiency may adversely affect the cardiovascular system, but data from longitudinal studies are scarce (1). Circulating 25-hydroxyvitamin D (25[OH]D), the most commonly used index of vitamin D status, is converted to the active hormone 1,25-dihydroxyvitamin D3, and exerts its physiological effects through the vitamin D receptor (VDR), which is present in various cells of the cardiovascular system, including cardiomyocytes, vascular smooth muscle cells, and endothelial cells (2–4). Active vitamin D inhibits *in vitro* cell proliferation, induces differentiation and apoptosis, improves vascular compliance, decreases PTH levels, improves glycemic control, and thus may protect against coronary heart disease (CHD) (5). Vitamin D also exerts antiinflammatory effects. In monocytes of patients with diabetes, vitamin D reduces levels of TNF-α as well as IL-6, IL-1, and IL-8, whereas the inhibitory effect on TNF-α is mediated by decreased activity of nuclear factor-κB and increased activity of its inhibitor (6).

Although results from the aforementioned experimental studies seem to be fairly consistent, data from clinical and epidemiological studies remain controversial. Whereas several studies investigating the association between circulating levels of vitamin D and cardiovascular outcomes and total mortality yielded fairly strong inverse associations, others found much weaker associations or no effect (7–12).

Therefore, we prospectively assessed the association between serum 25[OH]D concentrations and incident CHD [fatal and nonfatal myocardial infarction (MI) and sudden cardiac death (SCD)] in a large prospective, population-based cohort of middle-aged men and women from southern Germany.

Materials and Methods

Study population

The Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region of Augsburg (KORA) studies served as the database for this prospective case-cohort study in initially healthy, middle-aged men and women (13). Briefly, three independent population-based MONICA Augsburg surveys (S), with a total number of 13,427 participants (6,725 men and 6,702 women) aged 25–64 (S1) or 25–74 yr (S2-S3), were conducted in 1984–1985 (S1), 1989–1990 (S2) and 1994–1995 (S3) and all subjects were prospectively followed until the end of 2002 within the framework of KORA. The case-cohort design used in the present study has been described previously in detail (14).

Due to the low incidence of CHD under the age of 35 yr, the present study was limited to 10,718 persons (5,382 men and 5,336 women) between 35 and 74 yr of age at baseline who participated in at least one of the three surveys. After exclusion of 1187 subjects with missing blood samples and 231 participants with self-reported, prevalent CHD, the source population for the present study comprised 9300 subjects (4507 men and 4793 women). From the source population, a random subsample was selected stratifying by sex and survey. Furthermore, we aimed to include all incident cases that occurred during the follow-up time. After exclusions due to missing values, prevalent CHD *etc.*, the final study sample comprised 1783 participants aged 35–74 yr (225 men/73 women with and 739 men/746 women without incident CHD). All participants provided written informed consent, and the study was approved by the Ethics Committee of the Bavarian Chamber of Physicians.

Assessment of risk factors for cardiovascular disease

Trained medical staff collected information on sociodemographic variables, smoking habits, leisure time physical activity, alcohol consumption, and parental history of CHD at baseline through standardized interviews. In addition, standardized medical examinations including collection of a nonfasting venous blood sample were performed at baseline. All assessment procedures and standard laboratory methods have been described elsewhere (15). In 2010, serum samples continuously stored at −80 C or below were used to analyze baseline levels of 25[OH]D. Serum levels of 25[OH]D were measured by the OCTEIA 25-hydroxyvitamin D assay from IDS (Frankfurt, Germany). The intra- and interassay coefficients of variation were 3.3 and 6.3%, respectively. Because vitamin D levels vary during the year, all analyses were adjusted for season of blood sampling. Serum levels of IL-6, interferon-γ-inducible protein 10 (IP-10), E-selectin, IL-18, IL-8, macrophage migration inhibitory factor, monocyte chemotactic protein-1, regulated upon activation, normal T cell expressed, and secreted, leptin, adiponectin, myeloperoxidase, oxidized low-density lipoprotein-cholesterol, TGF-β, and soluble intercellular adhesion molecule-1 (sICAM-1) were determined as previously described using ELISAs and bead-based multiplex assays (16, 17). C-reactive protein (CRP) concentrations were measured with a high-sensitivity immunoradiometric assay or a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany) as previously described in more detail (16). CRP levels and distributions were comparable between both assays. All the analyses were run in a blinded fashion.

Ascertainment of CHD at follow-up

A combined end point that included incident fatal/nonfatal MI and SCD occurring before the age of 75 yr was used as the outcome variable and was identified through the MONICA/KORA Augsburg coronary event registry and through follow-up questionnaires for subjects who had moved out of the study area. Until December 2000, the diagnosis of a major, nonfatal MI event was based on the MONICA algorithm taking into account symptoms, cardiac enzymes, and electrocardiogric changes. Since January 1, 2001, all patients with MI diagnosed according to European Society of Cardiology and American College of Cardiology criteria were included. Deaths from MI were vali-

dated by autopsy reports, death certificates, chart reviews, and information from the last treating physician.

Statistical methods

Using the Statistical Analysis System (SAS) procedure SURVEYMEANS (SAS Institute, Cary. NC), which estimated SEs appropriate to the sampling scheme, means or proportions for baseline demographic and clinical characteristics were computed. For categorical variables, tests were carried out using Wald χ^2 test based on the SAS procedure SURVEYFREQ. Associations between continuous variables were performed as *t* tests on regression coefficients based on the SAS procedure SURVEYREG. In case of nonnormality, tests were carried out with log-transformed variables, and results were presented as geometric means with antilogs of SEs of the adjusted log means. Pearson correlation coefficients were calculated in the random cohort sample to assess univariate associations between inflammatory markers and 25[OH]D. Weighing was performed using the survey- and sex-specific sampling weights. Cox proportional hazards analyses were used to assess the association between 25[OH]D and incident CHD. For comparison of hazard ratios (HRs), sex-specific tertiles of 25[OH]D were coded with the bottom tertile as the reference category.

Because of the case-cohort design, correction of the variance estimation was required. We used an approach developed by Barlow (18) based on the sampling weights to give robust variance estimation. Cox proportional hazards models with different degrees of adjustment were calculated (see footnotes of the tables). For test for trends, tertiles were coded by their median values. Results are presented for each tertile as HR together with its 95% confidence interval (CI). Interactions between tertiles of 25[OH]D with sex, smoking status, and overweight were exam-

TABLE 1. Baseline demographic, clinical, and laboratory characteristics of participants with and without incident CHD during follow-up for men and women

	Men			Women		
Characteristic	**CHD cases**	**Noncases**	***P* value[a]**	**CHD cases**	**Noncases**	***P* value[a]**
Number	225	739		73	746	
Age (yr)[a]	56.8 (0.53)	51.9 (0.42)	<0.001	57.7 (0.76)	52.5 (0.39)	<0.001
Education (<12 yr) (%)	76.2 (0.03)	66.1 (0.02)	0.003	84.9 (0.04)	84.5 (0.01)	0.932
Smoking status (%)			0.001			0.290
Current smoker	44.9 (0.03)	30.9 (0.02)		26.9 (0.05)	19.5 (0.01)	
Former smoker	34.5 (0.03)	39.5 (0.02)		17.4 (0.04)	15.4 (0.01)	
Never smoker	20.6 (0.03)	29.7 (0.02)		55.7 (0.06)	65.0 (0.02)	
Frequency of exercise (%)			0.001			0.002
Inactive[b]	70.4 (0.03)	58.3 (0.02)		80.8 (0.05)	64.2 (0.02)	
Alcohol consumption[c] (%)			0.172			0.022
0 g/d	19.5 (0.03)	16.6 (0.01)		58.6 (0.06)	43.3 (0.02)	
<40/20 g/d	43.0 (0.03)	50.2 (0.02)		30.1 (0.05)	35.6 (0.02)	
≥40/20 g/d	37.5 (0.03)	33.2 (0.02)		11.4 (0.04)	21.1 (0.02)	
Body mass index (kg/m^2)	27.9 (0.26)	27.4 (0.14)	0.128	29.5 (0.56)	26.7 (0.17)	<0.001
Waist to hip ratio[a,d]	0.95 (0.01)	0.93 (<0.01)	<0.001	0.84 (0.01)	0.81 (<0.01)	0.001
Parental history of MI (%)			0.018			0.465
Positive	23.5 (0.03)	17.4 (0.01)		21.4 (0.05)	21.7 (0.02)	
Unknown	26.8 (0.03)	21.9 (0.02)		26.9 (0.05)	20.3 (0.01)	
Negative	49.7 (0.03)	60.7 (0.02)		51.7 (0.06)	58.0 (0.02)	
Actual hypertension[e] (%)	65.0 (0.03)	44.3 (0.02)	<0.001	69.8 (0.05)	38.8 (0.02)	<0.001
Systolic BP (mm Hg)[a]	141.2 (1.29)	135.7 (0.69)	<0.001	144.9 (2.66)	131.6 (0.76)	<0.001
Diastolic BP (mm Hg)[a]	83.0 (0.81)	83.6 (0.42)	0.492	83.9 (1.70)	79.8 (0.40)	0.017
Current HRT (%)[f]				4.5 (0.03)	10.9 (0.02)	0.038
Ratio TC/HDL[a]	5.71 (0.13)	5.06 (0.07)	<0.001	5.13 (0.20)	4.03 (0.05)	<0.001
CRP (mg/liter)[g]	2.42 (1.08)	1.46 (1.04)	<0.001	2.84 (1.14)	1.43 (1.04)	<0.001
IL-6 (pg/ml)[g]	2.86 (1.07)	2.07 (1.04)	<0.001	3.59 (1.09)	1.91 (1.04)	<0.001
sICAM-1 (ng/ml)[a]	885.3 (21.0)	799.9 (12.0)	<0.001	892.3 (35.7)	733.1 (9.8)	<0.001
IP-10 (pg/ml)[g]	255.8 (1.05)	218.3 (1.03)	0.010	271.0 (1.08)	229.5 (1.03)	0.039
25[OH]D (nmol/liter)[g]	37.7 (1.03)	43.9 (1.02)	<0.001	31.9 (1.05)	39.7 (1.01)	<0.001

Data are weighted percentages for categorical variables, The *t* test was used for comparisons of continuous variables and the χ^2 test for comparisons categorical variables. BP, Blood pressure; HRT, hormone replacement therapy; TC, total cholesterol.

[a] Weighted means (SE) for normally distributed continuous variables.

[b] Physical activity was assessed by a four-level graded scale assessing sports activities during summer and winter time (0, <1, 1–2, >2 h/wk).

[c] Men: 0, greater than 0 to 39.9 g/d, 40 g/d or greater; women: 0, greater than 0 to 19.9 g/d, 20 g/d or greater.

[d] Only measured in participants of S2 and S3 (cases: n = 196; noncases: n = 975). Weights: cases = all cases/nonmissing cases; noncases = 1/sampling fraction with sampling fraction = subcohort/full cohort without cases for each sex and survey.

[e] Actual hypertension was defined as BP values 140/90 mmHg or greater and/or use of antihypertensive medication given that the subjects were aware of being hypertensive.

[f] Only for women aged 50 yr old or older (cases: n = 63, noncases: n = 426) with no current use of oral contraceptives.

[g] Weighted geometric means with (antilog of SE of log means) for skewed continuous variables.

ined using likelihood ratio tests. Variance inflation factors were calculated to assess collinearity. Inflation factors were below 5, indicating no collinearity problems. The accuracy of the different models to assess 10-yr CHD risk were estimated by three measures: 1) the area under the receiver-operating characteristic curve (AUC; also known as C-statistic or C-index) using survival probabilities within 10 yr estimated by a modified Kaplan-Meier method to account for censored observations and the weighting scheme appropriate to the case-cohort design; the AUC differences between two models are given as ΔAUC (19); 2) the integrated discrimination improvement statistics, which can be viewed as the difference of the R_2 statistic between two models, *i.e.* the difference in the proportion of variance explained by the two models; and 3) the net reclassification index using the categories less than 10%, 10–20%, and greater than 20% (20). For interaction analyses, a $P < 0.10$ was considered to be statistically significant, whereas for all analyses, a $P < 0.05$ was considered to be statistically significant. All statistical evaluations were performed using the SAS software package (version 9.2; SAS Institute).

Results

A total of 298 CHD cases (225 men, 73 women) were identified during average follow-up of 11 yr. Baseline demographic, clinical, and laboratory characteristics of the study population are shown in Table 1. Subjects with incident CHD were older, were less active, and showed a higher waist to hip ratio compared with noncases. Furthermore, cases more frequently had hypertension, whereas differences in parental history of MI, smoking status, and educational levels were observed only in men and significant differences in alcohol consumption between the two groups only in women. As expected, total cholesterol to high-density lipoprotein cholesterol (HDL-C) ratio was considerably higher in cases compared with noncases. Furthermore, concentrations of CRP, IL-6, sICAM-1, and IP-10 were higher in CHD cases than in noncases. Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org, shows the of 25[OH]D levels in men and women. Geometric mean of 25[OH]D in cases was 37.7 (1.03) nmol/liter for men and 31.9 (1.05) nmol/liter for women and in noncases it was 43.9 (1.02) nmol/liter for men and 39.7 (1.01) nmol/liter for women. Thus, women showed significantly lower 25[OH]D concentrations than men.

Significant inverse correlations with correlation coefficients $|r| > 0.1$ in either men or women between 25[OH]D, and markers of inflammation were observed for sICAM-1, IL-6, IP-10, and CRP (Table 2). Therefore, these four markers were selected from a panel of 13 markers of inflammation and considered as potential mediating factors.

TABLE 2. Weighted Pearson correlation coefficients between 25[OH]D and selected biomarkers for CHD in the randomly drawn subcohort for men and women

	25[OH]D (log) Men (n = 811)		25[OH]D (log) Women (n = 766)	
Characteristics	**R**	***P* value**	**R**	***P* value**
Log Il-6	−0.114	0.001	−0.086	0.017
Log CRP	−0.079	0.025	−0.110	0.002
IP-10	−0.108	0.002	−0.081	0.025
sICAM-1	−0.178	<0.001	−0.091	0.012

Table 3 shows the results of Cox proportional hazards analyses, in which the association of baseline levels of 25[OH]D with incident CHD was assessed. Interaction analyses using likelihood ratio tests showed relevant interaction of 25[OH]D with sex ($P = 0.06$ in model 2); therefore, the presentation of tables has been stratified by sex throughout. In the basic model, which adjusted for age, survey, and season of blood sampling, the HR and 95% CI comparing tertile extremes of serum levels of 25[OH]D were 0.32 (0.16–0.65) (P for trend = 0.001) in women, and 0.56 (0.38–0.82) (P for trend = 0.005) in men (model 1). Further adjustment for traditional cardiovascular risk factors (including body mass index, smoking, physical activity, alcohol intake, systolic blood pressure, total cholesterol to HDL-C ratio, and parental history of MI) slightly attenuated the association in women [HR 0.39 (0.18–0.84); P for trend = 0.013] and more strongly in men in whom it became nonsignificant [HR 0.76 (0.49–1.17); P for trend = 0.215] (model 2). After additional adjustment for CRP, IL-6, sICAM-1, and IP-10, the inflammatory markers with correlation coefficients $|r| > 0.1$ in either men or women relative to 25[OH]D, the effect remained significant in women [HR 0.42 (0.19–0.93); P for trend = 0.028], whereas it was further reduced in men [HR 0.84 (0.52–1.35); P for trend = 0.461] (model 3). Additional adjustment for prevalent diabetes in models 2 and 3 did not attenuate the effects. Also, using crude categories of vitamin D (>50, 25–50, <25 nmol/liter) did not show a relevant change from the above reported results (data not shown).

Furthermore, Table 3 shows, that the predictive accuracy in the diagnosis of incident CHD, as quantified by the area under the receiver-operating characteristic curve (AUC), in women increased by 25[OH]D in all models, although the absolute incremental value in the widely adjusted model 2 was only modest (0.832 *vs.* 0.843). This was confirmed by two other measures for accuracy (integrated discrimination improvement and net reclassification index). By contrast, C statistics showed virtually no

TABLE 3. Sex-stratified[a] hazard ratios (95% CI) for incident CHD according to baseline concentrations of 25[OH]D

	Tertiles of 25[OH]D			*P* for trend	AUC without vitamin D	AUC with vitamin D	ΔAUC
	T1	T2	T3				
	Median [lower-upper limit] (nmol/liter)						
Men	27.0 [5.08; 35.02]	43.5 [35.03; 54.13]	66.9 [54.14; 153.92]				
	HR	HR (95% CI)	HR (95% CI)				
Model 1[b]	1.0	0.53 (0.36–0.78)	0.56 (0.38–0.82)	0.005	0.762	0.770	0.008
Model 2[c]	1.0	0.60 (0.40–0.91)	0.76 (0.49–1.17)	0.215	0.800	0.801	0.001
Model 3[d]	1.0	0.66 (0.43–1.02)	0.84 (0.52–1.35)	0.461	0.810	0.810	0.000
	Median [lower-upper limit] (nmol/liter)						
Women	26.4 [9.87; 33.15]	39.6 [33.16; 47.69]	58.5 [47.70; 127.69]				
	HR	HR (95% CI)	HR (95% CI)				
Model 1[b]	1.0	0.68 (0.39–1.17)	0.32 (0.16–0.65)	0.001	0.770	0.788	0.018
Model 2[c]	1.0	0.69 (0.37–1.28)	0.39 (0.18–0.84)	0.013	0.832	0.843	0.011
Model 3[d]	1.0	0.67 (0.35–1.29)	0.42 (0.19–0.93)	0.028	0.862	0.869	0.007

[a] Interaction analyses using likelihood ratio tests showed significant interaction of 25[OH]D with sex in model 3 ($P < 0.05$), whereas no statistically significant interaction was found in model 2 ($P = 0.06$) and in model 1 ($P > 0.1$).

[b] Adjustment for age, sex, season, and survey.

[c] Additional adjustment for body mass index, smoking, physical activity, alcohol, systolic blood pressure, total cholesterol/HDL, or parental history of MI.

[d] Additional adjustment for CRP, IL-6, sICAM-1, and IP-10.

incremental benefit for the addition of 25[OH]D in men (data not shown).

Supplemental Fig. 1 shows the distribution of 25[OH]D levels in men and women. The 25[OH]D levels were somewhat lower in women, the median was 43.5 nmol/liter in men and 39.6 nmol/liter in women.

Discussion

In this prospective, population-based study in initially healthy middle-aged men and women from the general population we found an independent inverse association between vitamin D and the risk of incident CHD after adjustment for various established cardiovascular risk factors. Risk of incident CHD was more than halved in women with 25[OH]D levels above 47.7 nmol/liter in the fully adjusted model 3, which clearly is overadjusted, taking into account that in this setting inflammatory parameters may reflect mediators of disease instead of confounding factors. Risk in men was lower, which may be due to the statistically significant higher vitamin D levels ($P < 0.001$). Also, the nonlinear decrease in risk in men could be attributable to certain overadjustment in models 2 and 3. Although the incremental value in AUC observed with vitamin D was small, it might yet be clinically relevant, given the relative insensitivity of the AUC for detecting moderately sized effects. For example, even widely established cardiovascular risk factors such as systolic blood pressure and cholesterol are associated with only small incremental gains in the AUC for the prediction of cardiovascular events (21).

Pathophysiological role of vitamin D in atherogenesis

The fact that there is a higher rate of CHD in northern than in southern Europe, and data from the United States that the higher the altitude of residence and hence the greater the sunlight intensity, the lower is the risk of CHD, suggest that susceptibility to CHD is affected by duration of exposure to sunlight (22). In addition, the only dietary change that consistently protects against CHD is increased consumption of fish, which contains large amounts of vitamin D (23). Although we cannot establish causality in our prospective observational study, multiple potential mechanisms can be considered for the observed protective effect of vitamin D. Major direct beneficial key mechanisms of vitamin D are the inhibition of exaggerated proliferation of cardiomyocytes by suppression of protooncogenes such as c-myc, down-regulation of several components of the renin-angiotensin system in the kidney, suppression of gene expression of natriuretic peptides by liganded VDR, and down-regulation of matrix metalloproteinase-2 and matrix metalloproteinase-9 with simultaneous up-regulation of tissue inhibitor of metalloproteinase-1 and tissue inhibitor of metalloproteinase-3 (24–28). Furthermore, in a cross-sectional study of 280 patients with type 2 diabetes,

25[OH]D status was significantly associated with brachial artery flow-mediated dilatation and circulating endothelial progenitor cells (CD133+/KDR+), suggesting that vitamin D deficiency might contribute to atherosclerotic disease by depletion of endothelial progenitor cells and endothelial dysfunction (29). This finding was confirmed in mainly nondiabetic individuals in an almost simultaneously published report (30). Here the authors evaluated 25[OH]D levels, endothelial function as assessed by brachial artery flow-mediated dilation, microvascular function as assessed by digital reactive hyperemia index, and arterial stiffness in 554 subjects and clearly showed that vitamin D deficiency is associated with increased arterial stiffness and endothelial dysfunction in the conductance and resistance blood vessels, irrespective of traditional cardiovascular risk burden. Indirect effects of vitamin D on the myocardium are mainly mediated through PTH. Elevated levels of PTH are observed in patients with vitamin D deficiency to maintain normal calcium levels and have been associated with hypertension, diabetes, dyslipidemia, and increased risk of cardiovascular diseases (31). Recently Takeda *et al.* (32) published the first animal study showing the beneficial actions of vitamin D in the treatment of atherosclerosis. Using a mouse model, they demonstrated that orally administered 25[OH]D led to a marked reduction in atherosclerotic lesion formation by changing the function and differentiation of dendritic cells and regulatory T cells with subsequent increased IL-10 and decreased IL-12 mRNA expression. In a subset of experiments, they were able to demonstrate similar immune mechanisms involved in atherosclerosis compared with other autoimmune disorders. The cell types investigated were major players in immune reactions in autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, lupus, and osteoporosis, diseases in which vitamin D supplementation may be beneficial or is even currently prescribed (33).

Potential sex-specific role of vitamin D in atherogenesis

Because we observed a stronger inverse association in women, how can the sex-specific effect be explained? Several previous studies have already reported sex differences, assuming that various hormones, especially estrogen, may result in differential responsiveness to vitamin D in men and women. This may arise from increased VDR gene transcript levels, protein expression, and endogenous vitamin D bioactivity, leading to more pronounced antiproliferative and antiapoptotic effects in women (34, 35). As a consequence, Correale *et al.* (36) could demonstrate stronger vitamin D-dependent immunomodulatory effects in women, like inhibition of self-reactive T cell proliferation and reduction in interferon-γ- and IL-17-secreting cell numbers. They also found fewer CYP24A1 transcripts encoding the vitamin D-inactivating enzyme as well as greater binding and internalization of vitamin D-binding protein in women, a transporter for vitamin D and its metabolites. Interestingly, 17-β estradiol reproduced these effects on self-reactive T cells and macrophages from male subjects, suggesting a functional synergy between vitamin D and 17-β estradiol, mediated through estrogen receptor-α. Taken together, this implies that in women a proper vitamin D supply exerts more beneficial effects on human metabolism than in men, most probably due to the potentiating effect of estrogen and its derivatives.

Accordingly, in a recent publication, we demonstrated the beneficial effects of elevated levels of 25[OH]D on incident diabetes in the MONICA/KORA population, and stratified analyses revealed stronger associations in younger ($<$52 yr) than in older ($\geq$52 yr) women (37). Due to low numbers of women younger than 52 yr (statistical age of menopause), we were not able to perform age-stratified analyses in this study. Because menopause is associated with dramatic changes in hormone levels, bone mineral metabolism, cardiovascular physiology, and overall physiology, the statistically supported finding that the relationship between vitamin D and CHD in our study is stronger in women might also be attributable to menopause and postmenopausal women only.

Vitamin D and risk of CHD

The present report represents a large single-center study assessing the association of 25[OH]D with incident CHD. Three recent studies have addressed this issue: in the Framingham Offspring prospective study, involving a total of 1739 participants, 120 subjects developed incident CHD after a mean follow-up of 5.4 yr (38). The study yielded a HR of 1.81 (95% CI 1.03–3.18; $P = 0.01$) comparing extreme tertiles of vitamin D in a three-category model with the highest tertile as the reference category. However, this study has several shortcomings. In addition to the relatively low number of end points, the authors missed eminent adjustments, especially for season that is well known to be associated with variable vitamin D levels due to different sun exposure in summer and winter. More recently, in a study based on the MINI-Finland Health Survey, vitamin D was measured in 6219 middle-aged men and women (39). During a median follow-up of 27.1 yr, 640 CHD deaths were identified. In contrast to the Framingham study, the authors could not confirm an independent association of vitamin D with CHD deaths after adjustment for season and traditional cardiovascular risk factors (HR comparing extreme quintiles: 0.91; 95% CI 0.70 to 1.18; $P = 0.20$). Contrary to this finding, in the

Cardiovascular Health Study, each 10-ng/ml lower vitamin D concentration was associated with a 9% (95% CI 2–17%) greater relative hazard of mortality and a 25% (95% CI 8–44%) greater relative hazard of MI (40). As a limitation, this finding is applicable only to older adults because only men and women aged 65 yr and older were enrolled. Most recently, in accordance with our data, preliminary results from the National Health and Nutrition Examination Survey III study suggested that vitamin D deficiency is linked to fatal stroke: over a median of 14 yr, Caucasians with levels of 25-hydroxyvitamin D below 15 ng/ml (37.5 nmol/liter) had twice the risk of dying from stroke compared with those with higher levels (HR 2.13; 95% CI 1.01–4.50) (41). Yet results had not been adjusted for season of blood draw.

Our findings provide additional evidence for a significant role of vitamin D in the pathophysiology of CHD. It represents the first prospective study on 25[OH]D and CHD risk that included a large panel of markers of subclinical inflammation. Inclusion of these markers in the regression models indicated that subclinical inflammation could be one mediating factor linking a low vitamin D status with the development of CHD. Even after extensive adjustments including markers of inflammation, the present study, involving 1783 men and women, indicates that low levels of circulating 25[OH]D predict future risk of CHD, especially in women, thereby further strengthening a potential role for vitamin D in atherosclerosis. Furthermore, the study suggests a modulating role of sex hormones, which needs to be clarified in further studies.

Of special note, according to The Endocrine Society Clinical Practice Guidelines 2011, greater than 50% of our population are considered as vitamin D deficient (42). Although in this study the median 25[OH]D level was 43.5 nmol/liter in men and 39.6 nmol/liter in women, according to current guidelines, vitamin D deficiency is defined as a 25[OH]D concentration below 50 nmol/liter (20 ng/ml).

Limitations and strengths of the study

This study has several limitations that need to be addressed. First, the number of female cases was considerably lower than male cases. Second, 25[OH]D was measured at a single time point. However, in a prior study a strong correlation between two measurements of 25[OH]D taken 3 yr apart was shown (43). Third, we do not have data on comedication influencing bone metabolism and thereby altering vitamin D levels in serum; however, because blood was drawn at baseline in initially healthy subjects, this might only be a minor issue. Lastly, we do not have data on other metabolites of bone metabolism (*e.g.* PTH or calcium) available, which might have provided further insights.

Our study has also several strengths, which include the population-based prospective design conducted in initially healthy subjects, the simultaneous measurement of several markers of inflammation, a long follow-up period of more than10 yr, the minimization of the likelihood of a survival bias because fatal and nonfatal coronary events were included in our study, and the careful adjustment for the conventional and several emerging risk factors in multivariable analyses.

Conclusion

In this large prospective case-cohort study, vitamin D (measured as 25-hydroxyvitamin D) predicted future coronary events in apparently healthy, middle-aged women independently of the conventional lipid profile, other traditional cardiovascular risk factors, and sensitive markers of inflammation. In unadjusted analyses, an inverse association was also seen in men, which, however, became nonsignificant after adjustment for traditional risk factors. The current evidence may imply screening for vitamin D deficiency in those who are at high risk for CHD. Nevertheless, a randomized, placebo-controlled trial, the Vitamin D and Omega-3 Trial (www.vitalstudy.org), funded by the National Institutes of Health, is currently investigating whether daily vitamin D supplementation reduces incident heart disease, stroke, or cancer, and physicians should certainly await those results before endorsing widespread screening and treatment programs for vitamin D deficiency. Further clinical and experimental studies are needed to fully understand sex differences and whether supplementation with vitamin D might contribute to the prevention of cardiovascular disease.

Acknowledgments

We thank Gerlinde Trischler (University of Ulm) and Gabi Gornitzka (German Diabetes Center, Düsseldorf) for excellent technical assistance and Lloyd Chambless (School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC) for statistical assistance with the analysis of the case-cohort data set.

Address all correspondence and requests for reprints to: Wolfgang Koenig, M.D., F.R.C.P., F.E.S.C., F.A.C.C., F.A.H.A., Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, Albert-Einstein-Allee 23, D-89081 Ulm, Germany. E-mail: wolfgang.koenig@uniklinik-ulm.de.

This work was supported by research grants from the German Research Foundation (TH-784/2-1 and TH-784/2-2) and additional funds provided by the University of Ulm; the German Diabetes Center, Düsseldorf; the Federal Ministry of Health; the Ministry of Innovation, Science, Research, and Technology of

the state North Rhine Westphalia; and the Helmholtz Zentrum München. Furthermore, the study was supported in part by a grant from the German Federal Ministry of Education and Research to the German Center for Diabetes Research (DZD e.V.).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Judd SE, Tangpricha V** 2009 Vitamin D deficiency and risk for cardiovascular disease. Am J Med Sci 338:40–44
2. **Nibbelink KA, Tishkoff DX, Hershey SD, Rahman A, Simpson RU** 2007 1,25(OH)2-vitamin D3 actions on cell proliferation, size, gene expression, and receptor localization, in the HL-1 cardiac myocyte. J Steroid Biochem Mol Biol 103:533–537
3. **Wu-Wong JR, Nakane M, Ma J, Ruan X, Kroeger PE** 2006 Effects of vitamin D analogs on gene expression profiling in human coronary artery smooth muscle cells. Atherosclerosis 186:20–28
4. **Merke J, Milde P, Lewicka S, Hügel U, Klaus G, Mangelsdorf DJ, Haussler MR, Rauterberg EW, Ritz E** 1989 Identification and regulation of 1,25-dihydroxyvitamin D3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D3. Studies in cultured bovine aortic endothelial cells and human dermal capillaries. J Clin Invest 83:1903–1915
5. **Brewer LC, Michos ED, Reis JP** 2011 Vitamin D in atherosclerosis, vascular disease, and endothelial function. Curr Drug Targets 12:54–60
6. **Meems LM, van der Harst P, van Gilst WH, de Boer RA** 2011 Vitamin D biology in heart failure: molecular mechanisms and systematic review. Curr Drug Targets 12:29–41
7. **Kendrick J, Targher G, Smits G, Chonchol M** 2009 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. Atherosclerosis 205:255–260
8. **Kim DH, Sabour S, Sagar UN, Adams S, Whellan DJ** 2008 Prevalence of hypovitaminosis D in cardiovascular diseases (from the National Health and Nutrition Examination Survey 2001 to 2004). Am J Cardiol 102:1540–1544
9. **Hintzpeter B, Mensink GB, Thierfelder W, Müller MJ, Scheidt-Nave C** 2008 Vitamin D status and health correlates among German adults. Eur J Clin Nutr 62:1079–1089
10. **Snijder MB, Lips P, Seidell JC, Visser M, Deeg DJ, Dekker JM, van Dam RM** 2007 Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. J Intern Med 261:558–565
11. **Melamed ML, Michos ED, Post W, Astor B** 2008 25-hydroxyvitamin D levels and the risk of mortality in the general population. Arch Intern Med 168:1629–1637
12. **Pilz S, März W, Wellnitz B, Seelhorst U, Fahrleitner-Pammer A, Dimai HP, Boehm BO, Dobnig H** 2008 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
13. **Hense HW, Filipiak B, Döring A, Stieber J, Liese A, Keil U** 1998 Ten-year trends of cardiovascular risk factors in the MONICA Augsburg Region in Southern Germany. Results from the 1984/85, 1989/90 and 1994/1995 surveys. CVD Prevention 1:318–327
14. **Karakas M, Koenig W, Zierer A, Herder C, Rottbauer W, Baumert J, Meisinger C, Thorand B** 2012 Myeloperoxidase is associated with incident coronary heart disease independently of traditional risk factors: results from the MONICA/KORA Augsburg study. J Intern Med 271:43–50
15. **Meisinger C, Thorand B, Schneider A, Stieber J, Döring A, Löwel H** 2002 Sex differences in risk factors for incident type 2 diabetes mellitus: the MONICA Augsburg cohort study. Arch Intern Med 162:82–89
16. **Herder C, Baumert J, Zierer A, Roden M, Meisinger C, Karakas M, Chambless L, Rathmann W, Peters A, Koenig W, Thorand B** 2011 Immunological and cardiometabolic risk factors in the prediction of type 2 diabetes and coronary events: MONICA/KORA Augsburg case-cohort study. PLoS One 6:e19852
17. **Karakas M, Zierer A, Herder C, Baumert J, Meisinger C, Koenig W, Thorand B** 2010 Leptin, adiponectin, their ratio and risk of coronary heart disease: results from the MONICA/KORA Augsburg Study 1984–2002. Atherosclerosis 209:220–225
18. **Barlow WE** 1994 Robust variance estimation for the case-cohort design. Biometrics 50:1064–1072
19. **Chambless LE, Diao G** 2006 Estimation of time-dependent area under the ROC curve for long-term risk prediction. Stat Med 25:3474–3486
20. **Chambless LE, Cummiskey CP, Cui G** 2011 Several methods to assess improvement in risk prediction models: extension to survival analysis. Stat Med 30:22–38
21. **Cook NR** 2007 Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 115:928–935
22. **Holick MF** 2007 Vitamin D deficiency. N Engl J Med 357:266–281
23. **Grimes DS** 2006 Are statins analogues of vitamin D? Lancet 368:83–86
24. **Pilz S, Tomaschitz A, Drechsler C, Dekker JM, März W** 2010 Vitamin D deficiency and myocardial disease. Mol Nutr Food Res 54:1103–1113
25. **O'Connell TD, Simpson RU** 1995 1,25-Dihydroxyvitamin D3 regulation of myocardial growth and c-myc levels in the rat heart. Biochem Biophys Res Commun 213:59–65
26. **Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP** 2002 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 110:229–238
27. **Chen S, Cui J, Nakamura K, Ribeiro RC, West BL, Gardner DG** 2000 Coactivator-vitamin D receptor interactions mediate inhibition of the atrial natriuretic peptide promoter. J Biol Chem 275:15039–15048
28. **Rahman A, Hershey S, Ahmed S, Nibbelink K, Simpson RU** 2007 Heart extracellular matrix gene expression profile in the vitamin D receptor knockout mice. J Steroid Biochem Mol Bi 103:416–419
29. **Yiu YF, Chan YH, Yiu KH, Siu CW, Li SW, Wong LY, Lee SW, Tam S, Wong EW, Cheung BM, Tse HF** 2011 Vitamin D deficiency is associated with depletion of circulating endothelial progenitor cells and endothelial dysfunction in patients with type 2 diabetes. J Clin Endocrinol Metab 96:E830–E835
30. **Al Mheid I, Patel R, Murrow J, Morris A, Rahman A, Fike L, Kavtaradze N, Uphoff I, Hooper C, Tangpricha V, Alexander RW, Brigham K, Quyyumi AA** 2011 Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol 58:186–192
31. **Fitzpatrick LA, Bilezikian JP, Silverberg SJ** 2008 Parathyroid hormone and the cardiovascular system. Curr Osteoporos Rep 6:77–83
32. **Takeda M, Yamashita T, Sasaki N, Nakajima K, Kita T, Shinohara M, Ishida T, Hirata K** 2010 Oral administration of an active form of vitamin D3 (calcitriol) decreases atherosclerosis in mice by inducing regulatory T cells and immature dendritic cells with tolerogenic functions. Arterioscler Thromb Vasc Biol 30:2495–2503
33. **Bobryshev YV** 2010 Vitamin D3 suppresses immune reactions in atherosclerosis, affecting regulatory T cells and dendritic cell function. Arterioscler Thromb Vasc Biol 30:2317–2319
34. **Duque G, El Abdaimi K, Macoritto M, Miller MM, Kremer R** 2002 Estrogens (E2) regulate expression and response of 1,25-dihydroxyvitamin D3 receptors in bone cells: changes with aging and hormone deprivation. Biochem Biophys Res Commun 299:446–454
35. **Liel Y, Shany S, Smirnoff P, Schwartz B** 1999 Estrogens increases 1,25-dihydroxyvitamin D receptors and bioresponse in the rat duodenal mucosa. Endocrinology 140:280–285
36. **Correale J, Ysrraelit MC, Gaitán MI** 2010 Gender differences in

1,25 dihydroxyvitamin D3 immunomodulatory effects in multiple sclerosis patients and healthy subjects. J Immunol 185:4948–4958
37. **Thorand B, Zierer A, Huth C, Linseisen J, Meisinger C, Roden M, Peters A, Koenig W, Herder C** 2011 Effect of serum 25-hydroxyvitamin D on risk for type 2 diabetes may be partially mediated by subclinical inflammation: results from the MONICA/KORA Augsburg study. Diabetes Care 34:2320–2322
38. **Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS** 2008 Vitamin D deficiency and risk of cardiovascular disease. Circulation 117:503–511
39. **Kilkkinen A, Knekt P, Aro A, Rissanen H, Marniemi J, Heliövaara M, Impivaara O, Reunanen A** 2009 Vitamin D status and the risk of cardiovascular disease death. Am J Epidemiol 170:1032–1039
40. **Kestenbaum B, Katz R, de Boer I, Hoofnagle A, Sarnak MJ, Shlipak MG, Jenny NS, Siscovick DS** 2011 Vitamin D, parathyroid hormone, and cardiovascular events among older adults. J Am Coll Cardiol 58:1433–1441
41. **Michos E, Reis JP, Post WS, Lutsey PL, Gottesmann RF, Mosley TH, Sharrett AR, Melamed ML** 2010 Vitamin D deficiency is associated with increased risk of fatal stroke among whites but not blacks: the NHANES III linked mortality files. Circulation 122:A9478
42. **Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM; Endocrine Society** 2011 Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96:1911–1930
43. **Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E** 2004 Plasma 1,25-dihydroxy-and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 15:255–265