Low Vitamin D Levels Predict Stroke in Patients Referred to Coronary Angiography

Stefan Pilz, MD; Harald Dobnig, MD; Joachim E. Fischer, MD; Britta Wellnitz, LLD; Ursula Seelhorst, MA; Bernhard O. Boehm, MD; Winfried März, MD

Background and Purpose—Vitamin D deficiency is common among the elderly and may contribute to cerebrovascular diseases. We aimed to elucidate whether low vitamin D levels are predictive for fatal stroke.

Methods—The LUdwigshafen RIsk and Cardiovascular Health (LURIC) study includes 3316 patients who were referred to coronary angiography at baseline between 1997 and 2000. 25-Hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D] were measured in 3299 and 3315 study participants, respectively. To account for the seasonal variation of vitamin D metabolites, we calculated z values for the 25(OH)D and 1,25(OH)2D concentrations within each month of blood draw.

Results—During a median follow-up time of 7.75 years, 769 patients died, including 42 fatal (ischemic and hemorrhagic) strokes. When compared with survivors in binary logistic-regression analyses, the odds ratios (with 95% CIs) for fatal stroke were 0.58 (0.43 to 0.78; $P<0.001$) per z value of 25(OH)D and 0.62 (0.47 to 0.81; $P<0.001$) per z value of 1,25(OH)2D. After adjustment for several possible confounders, these odds ratios remained significant for 25(OH)D at 0.67 (0.46 to 0.97; $P=0.032$) and for 1,25(OH)2D at 0.72 (0.52 to 0.99; $P=0.047$). Z values of 25(OH)D and 1,25(OH)2D were also reduced in the 274 patients who had a history of previous cerebrovascular disease events at baseline.

Conclusions—Low levels of 25(OH)D and 1,25(OH)2D are independently predictive for fatal strokes, suggesting that vitamin D supplementation is a promising approach in the prevention of strokes. **(*Stroke*. 2008;39:2611-2613.)**

Key Words: stroke ■ vitamins ■ epidemiology

Insufficient vitamin D is found in at least half of the elderly population.[1] The classic role of vitamin D as a regulator of calcium and bone homeostasis has recently been extended by reports that show that vitamin D deficiency might be involved in the development of several diseases, including arterial hypertension, diabetes mellitus, and heart failure.[2] The importance of vitamin D for overall health is further supported by a meta-analysis that found a significant reduction of total mortality in patients who received vitamin D.[3] In line with this concept, we have previously shown that low levels of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)2D] are independent predictors of total mortality in patients scheduled for coronary angiography who were participating in the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study.[4] In cross-sectional analyses, hemiplegic patients with acute stroke showed significantly reduced 25(OH)D concentrations compared with healthy controls.[5] Data from a population-based study showed that elderly persons with a low intake of vitamin D and low serum concentrations of 1,25(OH)2D were at increased risk for future strokes even after adjustment for age, sex, smoking, and functional capacity.[6] We aimed to extend the currently rare knowledge about vitamin D and stroke by addressing the question whether low levels of 25(OH)D and 1,25(OH)2D are predictive for fatal stroke in patients from the LURIC study.

Subjects and Methods

A detailed description of the baseline examinations of the LURIC study has been published elsewhere.[7] In brief, the LURIC study is a prospective cohort study of 3316 white patients who were routinely referred to coronary angiography at a single tertiary center in southwestern Germany. All study participants gave their informed consent, and the ethics committee at the Ärztekammer Rheinland-Pfalz approved the study. Previous cerebrovascular disease (CVD) events were defined as a documented history of a foregoing transient ischemic attack, prolonged ischemic deficit, or cerebral infarction with or without a remaining neurologic deficit.[7] 25(OH)D was determined in serum samples by radioimmunoassay (RIA) (DiaSorin Antony, France; Stillwater, Minn, USA) with an intra-assay and interassay coefficient of variation of 8.6% and 9.2%, respectively. In 100 randomly chosen samples, we determined 25(OH)D by liquid

Received January 3, 2008; final revision received January 28, 2008; accepted February 12, 2008.

From the Department of Public Health, Social and Preventive Medicine (S.P., J.E.F., W.M.), Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany; the Department of Internal Medicine (S.P., H.D.), Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Graz, Austria; LURIC Database LLC (B.W., U.S.), Freiburg, Germany; the Department of Internal Medicine I (B.O.B.), Division of Endocrinology and Diabetes, Ulm University, Ulm, Germany; and the Synlab Center of Laboratory Diagnostics (W.M.), Heidelberg, Germany.

Correspondence to Winfried März, Synlab Center of Laboratory Diagnostics, Heidelberg, PO Box 10470, D-69037 Heidelberg, Germany. E-mail maerz@synlab.de

Stroke **is available at http://stroke.ahajournals.org** **DOI: 10.1161/STROKEAHA.107.513655**

chromatography–tandem mass spectrometry with an isotope-labeled internal standard and 2 fragments of *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 those obtained by liquid chromatography–tandem mass spectrometry ($r=0.875$; $P<0.001$). Serum concentrations of 1,25(OH)2D were measured by RIA (Nichols Institute Diagnostika GmbH, Bad Nauheim, Germany). Fatal stroke (ischemic and hemorrhagic) was classified by reviewing death certificates. To account for the seasonal variation in vitamin D metabolites with usually higher concentrations in the summer and lower concentrations in the winter, we calculated *z* values for 25(OH)D and 1,25(OH)2D. Because of their skewed distributions, 25(OH)D and 1,25(OH)2D levels were logarithmically transformed, and *z* values for these parameters were calculated for each month of blood draw from the following formula: $x-\text{mean}/\text{SD}$. Binary logistic-regression analyses of survivors and patients with fatal stroke were performed with *z* values of 25(OH)D and 1,25(OH)2D as explanatory variables and inclusion of several possible confounders. Similarly, we also calculated logistic-regression analyses with a dichotomous outcome variable of patients with a history of previous CVD events at baseline and all other study subjects, who served as controls.

Results

Serum concentrations of 25(OH)D and 1,25(OH)2D were available for 3299 (99.5%) and 3315 (99.9%) LURIC subjects, respectively. After a median follow-up time of 7.75 years, 769 patients died, including 42 fatal strokes (27 ischemic, 8 hemorrhagic, and 7 of unknown etiology). Baseline characteristics of patients with fatal stroke and controls (survivors) are presented in Table 1. Patients who died of causes other than stroke were excluded, as we have previously shown that these subjects had lower levels of 25(OH)D and 1,25(OH)2D and hence, were at increased risk of fatal stroke by considering the competing risks of stroke and other causes of death in these patients.[4] In binary logistic-regression analyses, the odds ratios (with 95% CIs) for fatal stroke per *z* value were 0.58 (0.43 to 0.78, $P<0.001$) for 25(OH)D and 0.62 (0.47 to 0.81; $P<0.001$) for 1,25(OH)2D (Tables 2 and 3). These odds ratios remained significant after adjustments for cardiovascular risk factors, physical activity level, and calcium and parathyroid hormone levels (Tables 2 and 3). At baseline, 274 patients had a history of previous CVD events. In age- and sex-adjusted binary logistic-regression analyses including the entire LURIC cohort (survivors and all deceased patients), the odds ratios per *z* value were 0.76 (0.67 to 0.86; $P<0.001$) for 25(OH)D and 0.79 (0.70 to 0.88; $P<0.001$) for 1,25(OH)2D. After multivariate adjustments (according to model 5 in Tables 2 and 3), these odds ratios remained significant for 25(OH)D with 0.82 (0.71 to 0.94, $P=0.006$) but not for 1,25(OH)2D with 0.92 (0.80 to 1.50; $P=0.209$).

Discussion

In a cohort of >3000 patients referred to coronary angiography, low levels of 25(OH)D and 1,25(OH)2D were independent predictors for fatal stroke and were reduced in patients with a history of previous CVD events at baseline. In particular, patients after acute stroke are at increased risk for vitamin D insufficiency due to reduced sun exposure and low dietary intake.[8] Vitamin D supplementation in stroke patients has already been shown to reduce osteopenia, fractures, and falls while improving muscle strength.[9,10] Apart from these beneficial effects, our results suggest that vitamin D might also directly protect against stroke. This hypothesis is supported by data indicating that vitamin D may protect against hypertension, diabetes mellitus, and atherosclerosis.[2] In addition, vitamin D exerts antithrombotic and neuroprotective

Table 1. Baseline Characteristics for Survivors and Patients With Fatal Stroke

	Survivors	Fatal Stroke
Numbers	2547	42
Female, %	31.6	38.1
Previous CVD event, %	6.1%	31%
Age, y	61.7 (54.8–61.7)	69.3 (64.0–76.0)
BMI, kg/m^2	27.2 (24.9–29.8)	26.0 (23.5–29.0)
Triglycerides, mmol/L	1.65 (1.23–2.27)	1.57 (1.07–2.18)
LDL cholesterol, mmol/L	2.98 (2.46–3.57)	2.90 (2.18–3.44)
HDL cholesterol, mmol/L	0.98 (0.83–1.17)	0.93 (0.75–1.09)
Diabetes mellitus, %	27.1	50.0
Arterial hypertension, %	71.1	83.3
Active smokers, %	20.6	9.5
GFR, mL/min per 1.73 m^2	81.9 (71.2–92.7)	78.7 (59.1–90.4)
NT-pro-BNP, ng/mL	225 (89–580)	608 (289–1820)
C-reactive protein, mg/L	2.9 (1.2–7.5)	4.5 (1.5–14.9)
Physical activity category, %		
Below average	21.6	39.0
Average	55.7	56.1
Above average	22.7	4.9
Serum calcium, mmol/L	2.33 (2.27–2.39)	2.33 (2.24–2.43)
Parathyroid hormone, ng/L	28 (21–38)	30 (21–48)
25(OH)D, nmol/L	41.7 (28.0–59.7)	27.5 (20.7–44.9)
Z values for 25(OH)D	0.19 (−0.49–0.78)	−0.50 (−0.84–0.29)
1,25(OH)2D, pmol/L	88.9 (69.2–114.4)	69.2 (62.9–95.9)
Z values for 1,25(OH)2D	0.15 (−0.45–0.73)	−0.23 (−0.85–0.37)

Continuous data are shown as medians with interquartile range. BMI indicates body mass index; GFR, glomerular filtration rate; and NT-pro-BNP, *N*-terminal pro-B-type natriuretic peptide.

Table 2. Risk for Fatal Stroke per 25(OH)D *Z* Value

Model	Odds Ratio (95% CI)	*P* Value
Unadjusted	0.58 (0.43–0.78)	<0.001
Model 1*	0.62 (0.45–0.85)	0.003
Model 2†	0.63 (0.46–0.87)	0.005
Model 3‡	0.65 (0.47–0.92)	0.013
Model 4§	0.67 (0.47–0.94)	0.022
Model 5‖	0.67 (0.46–0.97)	0.032

Binary logistic-regression analyses for risk of fatal stroke with odds ratios (with 95% CI) per 25-hydroxyvitamin D *z* value.

*Adjusted for age and sex.

†Additionally adjusted for LDL and HDL cholesterol, active smokers, body mass index, C-reactive protein, and glomerular filtration rate.

‡Additionally adjusted for arterial hypertension, diabetes mellitus, and *N*-terminal pro-B-type natriuretic peptide.

§Additionally adjusted for physical activity level.

‖Additionally adjusted for calcium and parathyroid hormone values.

Table 3. Risk for Fatal Stroke per 1,25(OH)2D *Z* Value

Model	Odds Ratio (95% CI)	*P* Value
Unadjusted	0.62 (0.47–0.81)	<0.001
Model 1*	0.66 (0.50–0.87)	0.003
Model 2†	0.70 (0.52–0.87)	0.022
Model 3‡	0.69 (0.51–0.94)	0.020
Model 4§	0.71 (0.52–0.97)	0.031
Model 5‖	0.72 (0.52–0.99)	0.047

Binary logistic-regression analyses for risk of fatal stroke with odds ratios (with 95% CIs) per 1,25-dihydroxyvitamin D *z* value.

*Adjusted for age and sex.

†Additionally adjusted for LDL and HDL cholesterol, active smokers, body mass index, C-reactive protein, and glomerular filtration rate.

‡Additionally adjusted for arterial hypertension, diabetes mellitus, and *N*-terminal pro-B-type natriuretic peptide.

§Additionally adjusted for physical activity level.

‖Additionally adjusted for calcium and parathyroid hormone values.

effects and was shown to attenuate ischemic cortical injury in rats.[11,12] We acknowledge that our results may be limited because the *z* values for vitamin D metabolites were based on data from patients referred to coronary angiography and not from a "healthy" control group.

With reference to our work and the meta-analysis that found an increased survival in persons treated with vitamin D, we are of the opinion that it is a promising and safe preventive/therapeutic approach to supplement vitamin D in patients after stroke and at high risk for stroke to maintain 25(OH)D concentrations of at least 75 nmol/L (30 ng/mL), which have been shown to be most effective in producing favorable health outcomes.[13,14]

Acknowledgments

We thank the LURIC study team either temporarily or permanently involved in patient recruitment and sample and data handling and the laboratory staff at the Ludwigshafen General Hospital and the Universities of Freiburg, Ulm, and Graz and the German registration offices and local public health departments for their assistance.

Sources of Funding

The LURIC study was funded by grants from the Deutsche Forschungsgemeinschaft (GRK 1041 and SFB 518). B.O.B. is supported by the State Baden-Württemberg, Centre of Excellence "Metabolic Disorders".

Disclosures

None.

References

1. Norman AW, Bouillon R, Whiting SJ, Vieth R, Lips P. 13th workshop consensus for vitamin D nutritional guidelines. *J Steroid Biochem Mol Biol*. 2007;103:204–205.
2. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266–281.
3. Autier P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. *Arch Intern Med*. 2007; 167:1730–1737.
4. Dobnig H, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, Riedmüller G, Brück C, Tiran B, Boehm BO, Maerz W. Serum concentrations of vitamin D are significant and independent predictors of mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Exp Clin Endocrinol Diabetes* 2007;115. Abstract.
5. Poole KE, Loveridge N, Barker PJ, Halsall DJ, Rose C, Reeve J, Warburton EA. Reduced vitamin D in acute stroke. *Stroke*. 2006;37:243–245.
6. Marniemi J, Alanen E, Impivaara O, Seppänen R, Hakala P, Rajala T, Rönnemaa T. Dietary and serum vitamins and minerals as predictors of myocardial infarction and stroke in elderly subjects. *Nutr Metab Cardiovasc Dis*. 2005;15:188–197.
7. Winkelmann BR, März W, Boehm BO, Zotz R, Hager J, Hellstern P, Senges J. LURIC Study Group (LUdwigshafen RIsk and Cardiovascular Health). Rationale and design of the LURIC study: a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics*. 2001;2:1–73.
8. Poole KE, Reeve J, Warburton EA. Falls, fractures, and osteoporosis after stroke: time to think about protection? *Stroke*. 2002;33:1432–1436.
9. Sato Y, Maruoka H, Oizumi K. Amelioration of hemiplegia-associated osteopenia more than 4 years after stroke by 1α-hydroxyvitamin D and calcium supplementation. *Stroke*. 1997;28:736–739.
10. Sato Y, Iwamoto J, Kanoko T, Satoh K. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis*. 2005;20:187–192.
11. Aihara K, Azuma H, Akaike M, Ikeda Y, Yamashita M, Sudo T, Hayashi H, Yamada Y, Endoh F, Fujimura M, Yoshida T, Yamaguchi H, Hashizume S, Kato M, Yoshimura K, Yamamoto Y, Kato S, Matsumoto T. Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice. *J Biol Chem*. 2004;279:35798–35802.
12. Kiraly SJ, Kiraly MA, Hawe RD, Makhani N. Vitamin D as a neuroactive substance: review. *Sci World J*. 2006;6:125–139.
13. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *Am J Clin Nutr*. 2007;85:6–18.
14. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr*. 2006;84:18–28.

Stroke
Journal of the American Heart Association

Low Vitamin D Levels Predict Stroke in Patients Referred to Coronary Angiography

Stefan Pilz, Harald Dobnig, Joachim E. Fischer, Britta Wellnitz, Ursula Seelhorst, Bernhard O. Boehm and Winfried März

Stroke. 2008;39:2611-2613; originally published online July 17, 2008;
doi: 10.1161/STROKEAHA.107.513655
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628