

# 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

BARBARA THORAND, PHD<sup>1</sup>  
 ASTRID ZIERER, DIPL STAT<sup>1</sup>  
 CORNELIA HUTH, PHD<sup>1</sup>  
 JAKOB LINSEISEN, PHD<sup>2</sup>  
 CHRISTA MEISINGER, MD<sup>1</sup>

MICHAEL RODEN, MD<sup>3,4</sup>  
 ANNETTE PETERS, PHD<sup>1</sup>  
 WOLFGANG KOENIG, MD<sup>5</sup>  
 CHRISTIAN HERDER, PHD<sup>3</sup>

inflammation. Therefore, we assessed the association between 25-OHD and incident type 2 diabetes with and without adjustment for markers of inflammation. Furthermore, we examined possible interactions of 25-OHD with sex and age.

**OBJECTIVE**—To assess the association between serum 25-hydroxyvitamin D (25-OHD) and incident type 2 diabetes and to determine whether the association is mediated by subclinical inflammation.

**RESEARCH DESIGN AND METHODS**—Using a case-cohort design, baseline levels of 25-OHD were measured in 416 case subjects with incident type 2 diabetes and 1,267 noncase subjects selected from a source population of 7,936 middle-aged participants in the population-based Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region of Augsburg (KORA) study.

**RESULTS**—A significant inverse association was observed between serum 25-OHD and incident type 2 diabetes after adjustment for diabetes risk factors and season. The hazard ratio (HR) and 95% CI comparing tertile extremes was 0.63 (0.44–0.90) ( $P_{\text{trend}} = 0.010$ ). Further adjustment for C-reactive protein, interleukin-6, soluble intercellular adhesion molecule-1, and interferon- $\gamma$ -inducible protein-10 attenuated this association by 16% (HR 0.73 [0.50–1.05],  $P = 0.090$ ).

**CONCLUSIONS**—Vitamin D status is inversely related to type 2 diabetes risk and our data suggest that this association may be partially mediated by subclinical inflammation.

*Diabetes Care* 34:2320–2322, 2011

Prospective studies demonstrated an inverse relationship between vitamin D status determined by measurement of serum or plasma 25-hydroxyvitamin D (25-OHD) and incident type 2 diabetes (1–5). However, results have not always been consistent, especially regarding associations in women (2,6). Several mechanisms may explain the link between vitamin D and type 2 diabetes. These

include direct and indirect effects of 1,25-OHD, the active vitamin D metabolite, on insulin secretion and action (7,8). Immunomodulatory effects of vitamin D (7) could also mediate the association, as it is well established that subclinical inflammation is an important risk factor for type 2 diabetes (9,10). To date, no prospective epidemiological study extensively addressed the mediating role of subclinical

## RESEARCH DESIGN AND METHODS

Results are based on a prospective case-cohort study within the population-based Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Cooperative Health Research in the Region of Augsburg (KORA) cohort (10). The final study sample comprised 1,683 participants aged 35–74 years (231 male/185 female case subjects; 657 male/610 female noncase subjects). The subcohort for the case-cohort study was selected randomly, stratifying by sex and survey from a source population of 7,936 subjects without diabetes at baseline, with available blood samples and a follow-up time of  $\geq 1$  year, as previously described (10). All participants provided written informed consent.

Incident diabetes was assessed using questionnaires or interviews. Incident cases were validated by contacting the treating physician or medical chart review. The mean duration of follow-up ( $\pm$ SD) was  $11.0 \pm 4.7$  years. Further details regarding study design and assessment of covariables have been described previously (10,11). Serum samples collected at baseline were used to analyze 25-OHD in 2010 using an enzyme immunoassay (IDS, Frankfurt, Germany). The intra- and interassay coefficients of variation were 3.3 and 6.3%, respectively. Thirteen inflammation-related biomarkers were measured (see Table 1) (12). Biomarkers with skewed distributions were log transformed. Geometric means of 25-OHD and antilogs of SEs were compared with  $t$  tests. Cox proportional hazard models were used to assess associations between sex-specific tertiles of 25-OHD and incident type 2 diabetes. To account for the case-cohort design, correction of variance estimation was performed (13). Interactions were examined using likelihood ratio

From the <sup>1</sup>Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; the <sup>2</sup>Institute of Epidemiology I, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; the <sup>3</sup>Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany; the <sup>4</sup>Department of Metabolic Diseases, Heinrich Heine University, Düsseldorf, Germany; and the <sup>5</sup>Department of Internal Medicine II—Cardiology, University of Ulm Medical Center, Ulm, Germany.

Corresponding author: Wolfgang Koenig, wolfgang.koenig@uniklinik-ulm.de.

Received 24 April 2011 and accepted 26 July 2011.

DOI: 10.2337/dc11-0775

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc11-0775/-/DC1>.

© 2011 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

Table 1—HRs for type 2 diabetes risk according to baseline levels of serum 25-OHD

	Tertiles of 25-OHD			P value for trend
	T1	T2	T3	
Men: median [lower–upper] (nmol/L)	27.7 [5.08–36.12]	43.9 [36.13–54.70]	68.0 [54.71–153.92]	
Women: median [lower–upper] (nmol/L)	27.0 [9.87–33.13]	39.9 [33.14–48.24]	58.0 [48.25–127.69]	
Number case/noncase subjects	175/418	145/428	96/421	
HR (95% CI)				
Model 1*	1.0	0.77 (0.59–1.01)	0.52 (0.38–0.70)	<0.001
Model 2†	1.0	0.77 (0.56–1.06)	0.63 (0.44–0.90)	0.010
Model 3‡	1.0	0.85 (0.61–1.17)	0.73 (0.50–1.05)	0.090
Number case/noncase subjects	99/235	104/268	68/293	
HR (95% CI)				
Model 1a**	1.0	0.91 (0.64–1.29)	0.55 (0.38–0.80)	0.001
Model 2a‡	1.0	0.89 (0.58–1.38)	0.61 (0.39–0.96)	0.017
Model 2a + WHR	1.0	0.92 (0.59–1.43)	0.66 (0.42–1.03)	0.036
Model 3a + WHR#	1.0	1.04 (0.66–1.64)	0.78 (0.48–1.27)	0.224

HRs were estimated by Cox proportional hazard models. Correction for SEs was made using the method by Barlow. Weighting was performed using survey- and sex-specific sampling weights. Tertiles of the weighted distributions in the subcohort, stratified by sex, were used. Tests for trend were conducted, assigning the median value within each tertile to the corresponding tertile. WHR, waist-to-hip ratio. \*Model 1, adjusted for age, sex, survey, and season. †Model 2, adjusted for factors in model 1 + BMI, lifestyle factors (i.e., smoking status [never smoker, former smoker, current smoker], alcohol consumption [0, 0.1–39.9,  $\geq 40$  g/day for men; 0, 0.1–19.9,  $\geq 20$  g/day for women]), physical activity [inactive, active]), systolic blood pressure, total cholesterol/HDL cholesterol, and parental history of diabetes (negative, positive, unknown). ‡Model 3, adjusted for factors in model 2 + C-reactive protein, interleukin-6, soluble intercellular adhesion molecule-1, and interferon- $\gamma$ -inducible protein-10/CXCL10 (all coded as tertiles). Markers of inflammation, which were available in addition, include interleukin-18, macrophage-migration inhibitory factor, monocyte chemoattractant protein-1/CCL2, interleukin-8/CXCL8, adiponectin, leptin, RANTES/CCL5, transforming growth factor- $\beta$ 1, and soluble E-selectin. \*\*Model 1a, adjusted for factors in model 1, based on data of surveys 2 and 3 only with available WHR measurements ( $n = 1,067$ ). ‡Model 2a, adjusted for factors in model 2 ( $n = 1,067$ ). ||Model 2a + WHR, adjusted for factors in model 2 + WHR (as polynomial of degree 2) ( $n = 1,067$ ). #Model 3a + WHR, adjusted for factors in model 3 + WHR (as polynomial of degree 2) ( $n = 1,067$ ).

tests.  $P < 0.05$  was considered statistically significant. Evaluations were performed with the SAS software (version 9.2; SAS Institute, Cary, NC).

**RESULTS**—Baseline characteristics for case and noncase subjects are provided in Supplementary Table 1. The geometric mean serum 25-OHD concentration was 37.8 nmol/L in case and 42.0 nmol/L in noncase subjects. Significant inverse correlations with  $r$  less than  $-0.10$  were observed between 25-OHD and soluble intercellular adhesion molecule-1, interleukin-6, interferon- $\gamma$ -inducible protein-10/CXCL10, and C-reactive protein (Supplementary Table 2). Therefore, these four markers of inflammation were considered potential mediating factors.

After adjustment for age, sex, survey, and season, high 25-OHD was strongly associated with a reduced risk for type 2 diabetes with a hazard ratio (HR) (95% CI) of 0.52 (0.38–0.70) comparing tertile extremes ( $P_{\text{trend}} < 0.001$ ) (Table 1). Further adjustment for “classic” diabetes risk factors attenuated the association but it remained statistically significant (HR 0.63 [0.44–0.90];  $P_{\text{trend}} = 0.010$ ). Additional adjustment for C-reactive protein, interleukin-6, soluble intercellular adhesion molecule-1, and interferon- $\gamma$ -inducible protein-10 further attenuated the association by 16%.

To examine the effect of residual confounding by body fat distribution, where available, we added waist-to-hip ratio to the “classical” risk factor model. The addition of markers of inflammation to this model attenuated the HR for the upper tertile of 25-OHD by 18% (Table 1). Linear regression models showed similar results, but effects remained significant for model 3 (Supplementary Table 3). Interaction and stratified analyses revealed no statistically significant sex differences but stronger associations in younger ( $< 52$  years) than in older ( $\geq 52$  years) subjects. Since the third-order interaction term 25-OHD\*sex\*age-group was statistically significant ( $P = 0.010$ ), we performed additional analyses simultaneously stratifying by age-group and sex (Supplementary Table 4). In women, the inverse association was confined to those aged  $< 52$  years ( $P$  for age-group\*25-OHD interaction = 0.016), whereas in men, differences between age-groups were less clear ( $P$  for age-group\*25-OHD interaction = 0.046).

**CONCLUSIONS**—This study demonstrated an independent association between serum 25-OHD and incident type 2 diabetes after adjustment for “classic” diabetes risk factors. Further adjustment for markers of inflammation attenuated the HRs for the upper tertile of 25-OHD by 16–18%, suggesting that the relationship

between 25-OHD and type 2 diabetes risk may be partially mediated by subclinical inflammation. Stratified analyses demonstrated a significant association between 25-OHD and type 2 diabetes in younger (presumably mainly premenopausal) women, but not in older (most likely postmenopausal) women.

Our results are in line with three other prospective studies reporting inverse associations between 25-OHD and incident type 2 diabetes after adjustment for “classic” diabetes risk factors (1,2,4,5). Furthermore, they support results from the Women’s Health Initiative where no association between 25-OHD and type 2 diabetes was seen in postmenopausal women (6). Our study is the first prospective study on 25-OHD and type 2 diabetes 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 type 2 diabetes.

The current study has several strengths, including the population-based prospective design, the large number of incident case subjects, the long follow-up, and the availability of many covariables, including 13 markers of subclinical inflammation. Major limitations are the self-reported diagnoses of diabetes, single-time-point

25-OHD measurements, and missing data on fasting glucose or insulin at baseline. Also, it is conceivable that prior adjustment for more sophisticated indicators of obesity and body fat distribution could have diminished the risk reduction caused by the addition of markers of inflammation.

In conclusion, our results suggest that the relationship between vitamin D status and incident type 2 diabetes may be partially mediated by subclinical inflammation. Furthermore, they suggest a modulating role of age and possibly sex hormones that needs to be clarified in further studies.

**Acknowledgments**—This study was supported by research grants from the German Research Foundation (TH-784/2-1 and TH-784/2-2) and funds provided by the University of Ulm; the German Diabetes Center; the Federal Ministry of Health; the Ministry of Innovation, Science, Research, and Technology of the state North Rhine Westphalia; and the Helmholtz Zentrum München. C.Hu. was supported by a grant from the German Federal Ministry of Education and Research to the German Center for Diabetes Research (DZD e.V.).

No potential conflicts of interest relevant to this article were reported.

B.T. developed the study concept and design, acquired data and funding, and drafted the manuscript. A.Z. performed statistical analysis and critically revised the manuscript. C.Hu. and J.L. critically revised the manuscript. C.M. acquired data and funding and critically revised the manuscript. M.R. and

A.P. critically revised the manuscript. W.K. and C.He. developed the study concept and design, acquired data and funding, and critically revised the manuscript.

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

## References

1. Mattila C, Knekt P, Männistö S, et al. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. *Diabetes Care* 2007;30:2569–2570
2. Knekt P, Laaksonen M, Mattila C, et al. Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology* 2008;19:666–671
3. Grimnes G, Emaus N, Joakimsen RM, et al. Baseline serum 25-hydroxyvitamin D concentrations in the Tromsø Study 1994-95 and risk of developing type 2 diabetes mellitus during 11 years of follow-up. *Diabet Med* 2010;27:1107–1115
4. Pittas AG, Sun Q, Manson JE, Dawson-Hughes B, Hu FB. Plasma 25-hydroxyvitamin D concentration and risk of incident type 2 diabetes in women. *Diabetes Care* 2010;33:2021–2023
5. Gagnon C, Lu ZX, Magliano DJ, et al. Serum 25-hydroxyvitamin D, calcium intake, and risk of type 2 diabetes after 5 years: results from a national, population-based prospective study (the Australian Diabetes, Obesity and Lifestyle study). *Diabetes Care* 2011;34:1133–1138
6. Robinson JG, Manson JE, Larson J, et al. Lack of association between 25(OH)D levels and incident type 2 diabetes in older women. *Diabetes Care* 2011;34:628–634
7. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–281
8. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes* 2008;57:2619–2625
9. Kolb H, Mandrup-Poulsen T. An immune origin of type 2 diabetes? *Diabetologia* 2005;48:1038–1050
10. Thorand B, Baumert J, Kolb H, et al. Sex differences in the prediction of type 2 diabetes by inflammatory markers: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. *Diabetes Care* 2007;30:854–860
11. Thorand B, Kolb H, Baumert J, et al. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. *Diabetes* 2005;54:2932–2938
12. Herder C, Baumert J, Zierer A, et al. Immunological and cardiometabolic risk factors in the prediction of type 2 diabetes and coronary events: MONICA/KORA Augsburg case-cohort study. *PLoS One* 2011;6:e19852
13. Barlow WE. Robust variance estimation for the case-cohort design. *Biometrics* 1994;50:1064–1072