

# Serum 25-Hydroxyvitamin D3 Concentrations and Prevalence of Cardiovascular Disease Among Type 2 Diabetic Patients

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Accumulating research suggests that low 25-hydroxyvitamin D3 [25(OH)D] concentrations may be inversely associated with type 2 diabetes (1–3), metabolic syndrome (4,5), insulin resistance (6), and cardiovascular disease (CVD) (7).

Much remains to be learned, however, about the relationships between vitamin D status, metabolic syndrome, and CVD. Furthermore, the published data in humans arguing that hypovitaminosis D is a CVD risk factor remain conflicting (8,9).

Because this topic has received scant attention and the available information on associations between vitamin D status and CVD among type 2 diabetic adults was lacking, we examined the relationships between serum 25(OH)D concentrations and prevalent CVD in type 2 diabetic adults.

## RESEARCH DESIGN AND METHODS

We studied 459 consecutive type 2 diabetic outpatients attending our clinic after exclusion of those with recent acute illness or advanced chronic liver or renal disease and those who were taking medications known to alter vitamin D metabolism. The control group consisted of 459 (64% men, age  $61 \pm 6$  years) age- and sex-matched nondiabetic volunteers.

Biochemical blood measurements were determined by standard laboratory procedures. Serum 25(OH)D concentra-

tions were measured during winter months using an automated chemiluminescence immunoassay (DiaSorin Liaison). Metabolic syndrome was defined according to the Adult Treatment Panel III criteria (10). Presence of coronary (myocardial infarction, angina, or revascularization procedures), cerebrovascular (ischemic stroke, recurrent transient ischemic attacks, or carotid endarterectomy), and peripheral (claudication, lower-extremity amputation, or revascularization procedures) vascular disease was confirmed by chart review, medical history and examination, and vascular laboratory studies.

Data are means  $\pm$  SD or frequencies. Skewed variables were logarithmically transformed to improve normality before analysis. Statistical analyses included unpaired *t* test,  $\chi^2$  test, and logistic regression analysis. In this latter analysis, CVD was considered as an aggregate end point inclusive of patients with at least one atherosclerotic manifestation. In fully adjusted logistic regression models, sex, age, BMI, smoking, diabetes duration, HbA<sub>1c</sub> (A1C), LDL cholesterol, calcium, creatinine, albumin excretion rate, use of medications, metabolic syndrome, and inflammatory markers (fibrinogen or C-reactive protein [CRP]) were also included as covariates. Hypovitaminosis D was defined as a serum 25(OH)D concentration  $<20$  ng/ml (6,11,12).

**RESULTS**— The mean ( $\pm$ SD) 25(OH)D concentration was  $24.1 \pm 9.1$  ng/ml (median 22.3, range 4.9–91.0) among control subjects and  $19.7 \pm 10$  ng/ml (17, 3–76) among diabetic patients. The age- and sex-adjusted prevalence of hypovitaminosis D was higher in diabetic patients than in control subjects (60.8 vs. 42.8%,  $P < 0.001$ ).

As shown in Table 1, diabetic patients with hypovitaminosis D were more likely to be women and had increased prevalence of higher values of A1C, triglycerides, CRP, and fibrinogen than their vitamin D-sufficient counterparts. The proportion using insulin, lipid-lowering, or antiplatelet drugs was higher among those with hypovitaminosis D, whereas the proportion using hypoglycemic drugs was similar in both groups. Age, BMI, waist circumference, diabetes duration, smoking, LDL cholesterol, creatinine, calcium, albuminuria, and metabolic syndrome components did not differ between the groups.

Overall, 143 (31.1%) of 459 patients were coded positive for CVD. Of these, 81 patients had coronary heart disease, 51 had cerebrovascular disease, and 41 had peripheral vascular disease; many subjects had CVD in multiple sites. As shown in Table 1, the prevalence of CVD was greater among those with hypovitaminosis D. Similarly, 25(OH)D was lower ( $P < 0.01$ ) among those with CVD ( $17.9 \pm 9$  vs.  $20.6 \pm 10$  ng/ml), coronary disease ( $17 \pm 9$  vs.  $20.3 \pm 10$  ng/ml), and cerebrovascular disease ( $16.9 \pm 7$  vs.  $20 \pm 10$  ng/ml) than among those without CVD.

In logistic regression analysis, the association between hypovitaminosis D and prevalent CVD (odds ratio 1.70 [95% CI 1.1–2.6],  $P < 0.01$ ) remained statistically significant after adjustment for classical risk factors, A1C, metabolic syndrome, renal function tests, calcium, and use of medications (1.77 [1.1–2.9],  $P = 0.023$ ); additional adjustment for fibrinogen (or CRP) levels abolished this association (1.43 [0.9–2.3],  $P = \text{NS}$ ). Almost identical results were obtained in models that included the individual components of

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**Abbreviations:** 25(OH)D, 25-hydroxyvitamin D3; CRP, C-reactive protein; CVD, cardiovascular disease. A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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**Table 1—Baseline characteristics of the study participants, grouped according to vitamin D status**

Variables	Without hypovitaminosis D [25(OH)D: 29.0 ± 9 ng/ml]	With hypovitaminosis D [25(OH)D: 13.6 ± 3.3 ng/ml]	P
n	180	279	—
Sex (% men)	74	57	0.001
Age (years)	61 ± 7	62 ± 7	NS
BMI (kg/m <sup>2</sup> )	29.2 ± 4.5	29.7 ± 5.1	NS
Waist circumference (cm)	106.8 ± 12	107 ± 12	NS
Diabetes duration (years)	11 ± 8	12 ± 8	NS
Diet only (%)	17	14	NS
Oral hypoglycemic agents only (%)	64	57	NS
Insulin treatment (%)	19	29	0.01
Statin users (%)	20.6	31.2	0.01
Aspirin users (%)	20.6	33.7	0.01
Current smokers (%)	16.7	19.7	NS
Systolic blood pressure (mmHg)	144 ± 18	145 ± 17	NS
Diastolic blood pressure (mmHg)	80 ± 7	80 ± 8	NS
A1C (%)	7.0 ± 1.3	7.4 ± 1.3	0.01
(ln)triglycerides (mmol/l)	1.74 ± 0.8	2.02 ± 1.3	0.01
HDL cholesterol (mmol/l)	1.33 ± 0.3	1.36 ± 0.4	NS
LDL cholesterol (mmol/l)	3.49 ± 0.9	3.40 ± 0.9	NS
Creatinine (μmol/l)	74 ± 15	76 ± 18	NS
Calcium (mmol/l)	2.35 ± 0.1	2.38 ± 0.2	NS
Fibrinogen (g/l)	4.02 ± 0.9	4.56 ± 0.9	0.001
(ln)hs-CRP (mg/l)	3.85 ± 5.4	5.10 ± 6.7	0.001
Microalbuminuria (%)	18	18.4	NS
Macroalbuminuria (%)	5.8	6.5	NS
Adult Treatment Panel III: metabolic syndrome (%)	78.7	84.2	NS
Cardiovascular disease (%)			
“Aggregate” end point	24.4	35.5	0.01
Coronary	9.5	22.9	0.001
Cerebrovascular	7.8	13.5	0.06
Peripheral	9.9	8.0	NS

Data are the means ± SD, unless otherwise indicated. Differences were assessed by the unpaired *t* test (for normally distributed variables) and by the  $\chi^2$  test (for categorical variables).

metabolic syndrome and in models in which a more restrictive threshold to define hypovitaminosis D ( $\leq 15$  ng/ml) was used (13).

**CONCLUSIONS**— We found a high prevalence of hypovitaminosis D and a strong inverse association between 25(OH)D concentrations and prevalent CVD among type 2 diabetic outpatients. Interestingly, our data suggest that the putative elevated CVD risk associated with hypovitaminosis D is probably mediated by correlated elevations in plasma inflammatory markers. Moreover, since elevations of CRP and fibrinogen levels increase the risk for CVD (14), these findings could help to explain the CVD excess typically observed during winter months,

a period in which vitamin D status tends to be poor (15), and suggest a rationale for vitamin D supplementation in prevention of CVD, especially in the elderly.

Our findings are supported by few available data in humans showing that 25(OH)D levels are inversely related to coronary artery calcifications (16,17) and are lower in patients with myocardial infarction (7) and by experimental studies (18–22) suggesting that low 25(OH)D influences the activity/expression of macrophages and lymphocytes in atherosclerotic plaques, thus promoting chronic inflammation in the artery wall. Interestingly, in two recent clinical trials (23,24), vitamin D supplementation markedly reduced serum levels of CRP, interleukin-6, and tissue matrix metalloproteinases. Additionally, low vi-

tamin D3 concentrations result in elevations of parathyroid hormone, which has been linked to insulin resistance and significant increases in the serum levels of many acute-phase proteins (25).

Evidently, these findings are all consistent with the proposition that hypovitaminosis D and subsequent secondary hyperparathyroidism may promote the acute phase response and may help to explain how hypovitaminosis D might act as a risk factor for CVD.

This study has some limitations. Because our study was a cross-sectional one, the causative nature of the associations cannot be established. Additionally, parathyroid hormone and  $1\alpha,25(\text{OH})_2\text{D}$  were not measured in this study. Further investigation is necessary to evaluate whether hypovitaminosis D is associated with incident CVD among type 2 diabetic adults and to determine possible mechanisms of any preventive effect from vitamin D supplementation against CVD.

## References

1. Boucher BJ, Mannan N, Noonan K, Hales CN, Evans SJ: Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in East London Asians. *Diabetologia* 38:1239–1245, 1995
2. Isaia G, Giorgino R, Adami S: High prevalence of hypovitaminosis D in female type 2 diabetic population (Letter). *Diabetes Care* 24:1496, 2001
3. Scragg R, Sowers M, Bell C: Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 27:2813–2818, 2004
4. Boucher BJ: Inadequate vitamin D status: does it contribute to the disorders comprising syndrome X? *Br J Nutr* 79:315–327, 1998
5. Ford ES, Ajani UA, McGuire LC, Liu S: Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care* 28:1228–1230, 2005
6. Chiu KC, Chu A, Go VL, Saad MF: Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 79:820–825, 2004
7. Scragg R, Jackson R, Holdaway I, Lim T, Beaglehole R: Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. *Int J Epidemiol* 19: 559–563, 1990
8. Holick MF: Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 79:362–371, 2004

9. Norman PE, Powell JT: Vitamin D, shedding light on the development of disease in peripheral arteries. *Arterioscler Thromb Vasc Biol* 25:39–46, 2005
10. National Cholesterol Education Program: Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 285:2486–2497, 2001
11. Fuleihan GE, Deeb M: Hypovitaminosis D in a sunny country. *N Engl J Med* 340:1840–1841, 1999
12. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR: Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* 30:771–777, 2002
13. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, Vamvakas EC, Dick IM, Prince RL, Finkelstein JS: Hypovitaminosis D in medical inpatients. *N Engl J Med* 338:777–783, 1998
14. Willerson JT, Ridker PM: Inflammation as a cardiovascular risk factor. *Circulation* 109 (Suppl. 1):2–10, 2004
15. Pell JP, Cobbe SM: Seasonal variations in coronary heart disease. *QJM* 92:689–696, 1999
16. Doherty T, Tang W, Dascolas S, Watson KE, Demer LL, Shavelle R, Detrano R: Ethnic origin and serum levels of 1,25-dihydroxyvitamin D3 are independent predictors of coronary calcium mass measured by electron-beam computed tomography. *Circulation* 96:1477–1481, 1997
17. Watson KE, Abrolat ML, Malone LL, Hoeg JM, Doherty T, Detrano R, Demer LL: Active serum vitamin D levels are inversely correlated with coronary calcification. *Circulation* 96:1755–1760, 1997
18. Brown A, Dusso A, Slatopolsky E: Vitamin D. *Am J Physiol* 277:F157–F175, 1999
19. Barsony J, Prufer K: Vitamin D receptors and retinoid X receptors interactions in motion. *Vitam Horm* 65:345–376, 2002
20. Pierce R, Kolodzie M, Parks W: 1,25-dihydroxyvitamin D3 repress tropoelastin expression by a posttranscriptional mechanism. *J Biol Chem* 267:11593–11599, 1992
21. Veldman C, Cantorna M, DeLuca H: Expression of 1,25 dihydroxyvitamin D3 receptor in the immune system. *Arch Biochem Biophys* 374:334–338, 2000
22. Willheim M, Thien R, Schratlbauer K, Bajna E, Holub M, Gruber R, Baier K, Pietschmann P, Reinisch W, Scheiner O, Peterlik M: Regulatory effects of 1 $\alpha$ , 25 dihydroxyvitamin D3 on the cytokine production of human peripheral blood lymphocytes. *J Clin Endocrinol Metab* 84:3739–3744, 1999
23. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R: Bone turnover in prolonged critical illness: effect of vitamin D. *J Clin Endocrinol Metab* 88:4623–4632, 2003
24. Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercombe-Court D, Aganna E, Price CP, Boucher BJ: Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *QJM* 95:787–796, 2002
25. McCarty MF: Secondary hyperparathyroidism promotes the acute phase response: a rationale for supplemental vitamin D in prevention of vascular events in the elderly. *Med Hypotheses* 64:1022–1026, 2005