

Vitamin D Deficiency and Coronary Artery Calcification in Subjects With Type 1 Diabetes

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OBJECTIVE—The objective of this study is to examine the relationship among serum levels of 25-hydroxyvitamin D (25[OH]D), polymorphisms in vitamin D-associated genes, and the presence and progression of coronary artery calcification (CAC) in adults with type 1 diabetes.

RESEARCH DESIGN AND METHODS—This prospective study included 374 non-Hispanic white individuals with type 1 diabetes (mean age 40 ± 9 years; 46% were male). CAC was measured at the baseline and 3- and 6-year follow-up visits were determined by electron beam computed tomography. Serum 25[OH]D levels were measured by liquid chromatography tandem mass spectrometry at the 3-year visit.

RESULTS—Normal (>30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL) 25[OH]D levels were present in 65%, 25%, and 10% of the individuals with type 1 diabetes, respectively. 25[OH]D deficiency was associated with the presence of CAC at the 3-year visit, odds ratio (OR) = 3.3 (95% CI 1.6–7.0), adjusting for age, sex, and hours of daylight. In subjects free of CAC at the 3-year visit, 25[OH]D deficiency predicted the development of CAC over the next 3 years in those with the vitamin D receptor M1T CC genotype (OR = 6.5 [1.1–40.2], *P* = 0.04) than in those with the CT or TT genotype (OR = 1.6 [0.3–8.6], *P* = 0.57).

CONCLUSIONS—Vitamin D deficiency independently predicts prevalence and development of CAC, a marker of coronary artery plaque burden, in individuals with type 1 diabetes.

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Coronary artery disease (CAD) is the major cause of morbidity and mortality in patients with type 1 diabetes (1,2). Coronary artery calcification (CAC), a marker of coronary artery plaque burden, predicts coronary events in persons with type 1 diabetes (3). The presence and progression of CAC have become useful surrogate CAD end points in evaluating novel CAD risk factors (3,4).

Vitamin D deficiency has been associated with increased risk of CAD (5–7) in people without diabetes, as well as cardiovascular mortality (8). Studies have also shown that vitamin D levels are lower in individuals with type 1 diabetes (9,10), although whether this relationship holds

for different geographic populations is unclear (11). Vitamin D regulates calcium blood levels and affects vascular smooth muscle cell proliferation (12), inflammation (13), and the renin-angiotensin system (14), all of which have cardiovascular effects and could lead to coronary calcification. Serum level of 25-hydroxyvitamin D (25[OH]D) assesses vitamin D status best. 25[OH]D is the major circulating form of vitamin D and the precursor of the active form 1,25[OH]D. This active form binds in the circulation to vitamin D-binding protein (VDBP), to be transported to target organs where it mediates its biological effects by binding to the vitamin D receptor (VDR).

Cross-sectional studies of the relationship between 25[OH]D levels and CAC have been inconclusive. A study of 650 Amish subjects found no association (15); lower 25[OH]D levels were associated with carotid and aortal, but not with coronary artery, calcified plaques in 340 African Americans with type 2 diabetes (16). The Multi-Ethnic Study of Atherosclerosis (MESA) study (17) reported that lower 25[OH]D levels predicted development of CAC, but not progression of CAC. Our study examined the cross-sectional and longitudinal relationship between 25[OH]D levels and CAC in adults with type 1 diabetes. We also explored whether the potential relationship between 25[OH]D levels and CAC differed by polymorphisms in the VDBP and VDR genes.

RESEARCH DESIGN AND METHODS

The Coronary Artery Calcification in Type 1 Diabetes (CACTI) study population has been described previously (4). In brief, 652 men and women with type 1 diabetes and 764 nondiabetic control subjects aged 19–56 years, with no history of CAD, were enrolled in a prospective follow-up of the development and progression of CAC. Patients with type 1 diabetes had long-standing diabetes (mean duration 23 years, range 4–52 years) on enrollment. Control subjects had not been diagnosed with diabetes of any type, had fasting blood glucose in the normal range, and were generally spouses, friends, or neighbors of the patients. All subjects provided informed consent, and the study was approved by the Colorado Multiple Institutional Review Board.

Participants were examined at a baseline visit in 2000–2002. Data used in these analyses are from the second (3-year) and third (6-year) follow-up visits, both conducted approximately 2.5 ± 0.4 years after each prior visit. For these analyses, all subjects with type 1 diabetes who had complete data at the 3-year visit were included. A total of 374 non-Hispanic white subjects with type 1 diabetes were analyzed. Anthropometric measurements were obtained, including height, weight,

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minimal waist circumference, and hip circumference. BMI was calculated in kilograms/meters squared. Resting systolic blood pressure and fifth-phase diastolic blood pressure were measured three times while the patients were seated after a 5-min rest, and the second and third measurements were averaged. Hypertension was defined as a blood pressure $\geq 140/90$ mmHg or participant receiving current antihypertensive treatment. Current medication, tobacco use, and family medical history were obtained by standardized questionnaire. Vitamin D and calcium intake were obtained by a validated food frequency questionnaire (Harvard 1988) and included the use of supplements.

Laboratory measures

Total, HDL-cholesterol, and triglycerides were measured by standard enzymatic methods, and LDL-cholesterol was calculated by the Friedewald formula. Albumin excretion rate (AER) was calculated from urinary albumin measured in two timed overnight urine samples, and the results from the two nights were averaged. 25 [OH]D levels were measured by liquid chromatography tandem mass spectrometry by Quest Diagnostics on samples collected at the 3-year visit. Season of blood draw was classified as fall/winter for blood drawn during the months of October to March and spring/summer as April to September, and the number of hours of average daylight over the 2 weeks before the study visit was calculated on the basis of study date and latitude of the study visit (Denver, CO, latitude 39° 45' N). Individuals were categorized as vitamin D sufficient (25[OH]D >30 ng/mL), insufficient (20–30 ng/mL), or deficient (<20 ng/mL), according to current clinical guidelines (18).

Genomic DNA was extracted from leukocytes by the salting out method. Vitamin D-binding protein (VDP) gene polymorphisms 420 (C/A) and 416 (T/G) and vitamin D receptor (VDR) gene polymorphism M1T (T/C) and Bsm1 (G/A) were genotyped in a multiplex dot-blot sequence-specific oligonucleotide polymerase chain reaction (SSO-PCR) assay (Roche Molecular Systems, Pleasanton, CA).

Coronary artery calcification

Two sets of high-resolution noncontrast contiguous 3-mm tomographic images were acquired at 0.1-s exposure on an Imatron C-150XLP electron-beam CT scanner (Imatron, San Francisco, CA) as

Table 1—Characteristics of study participants with type 1 diabetes at the 3-year visit

	Subjects with type 1 diabetes (N = 374)
25[OH]D level (ng/mL): mean \pm SD (median)	35.4 \pm 13.0 (34.0)
Vitamin D status	
Normal (>30 ng/mL)	244 (65.2%)
Insufficient (20–30 ng/mL)	92 (24.6%)
Deficient (<20 ng/mL)	38 (10.2%)
Age at 3-year visit: mean \pm SD (median)	39.7 \pm 8.9 (39.7)
Female	202 (54.0%)
Male	172 (46.0%)
BMI (kg/m ²): mean \pm SD (median)	26.2 \pm 4.3 (25.5)
Current smoker†	
Yes	29 (8.3%)
No	321 (91.7%)
Total cholesterol (mg/dL)	175.7 \pm 32.2 (172.3)
HDL-cholesterol (mg/dL)	62.3 \pm 19.5 (59.0)
LDL-cholesterol (mg/dL)	99.3 \pm 26.6 (98.2)
Triglycerides (mg/dL)	70.2 \pm 47.4 (59.0)
Systolic blood pressure (mmHg)	111.6 \pm 12.6 (110.0)
Diastolic blood pressure (mmHg)	74.2 \pm 8.7 (74.0)
Hypertensive‡	
Yes	175 (46.9%)
No	198 (53.1%)
Coronary artery calcification	
Yes	170 (45.5%)
No	204 (54.5%)
VDR 420 CC	196 (59.2%)
CA	111 (33.5%)
AA	24 (7.3%)
VDR 416 TT	132 (41.1%)
TG	126 (39.3%)
CC	63 (19.6%)
VDR M1T TT	64 (17.1%)
CT	178 (47.6%)
CC	132 (35.3%)
VDR Bsm1 GG	123 (32.9%)
GA	178 (47.6%)
AA	73 (19.5%)

†Smoking status is missing for 25 participants. ‡Hypertensive is defined as blood pressure $\geq 140/90$ mmHg or current hypertensive treatment. Hypertensive is missing for two participants.

described previously (4). The two sets of scans were acquired within 5 min, and CAC scores were averaged. Calcium volume scores (CVS) were square root transformed, and the difference in square root transformed CVS between the 3- and 6-year examinations was calculated. Progression of CAC was defined as a change in square root transformed CVS of ≥ 2.5 (19).

Statistical analyses

All statistical analyses were conducted in the SAS 9.2 system (SAS Institute, Cary, NC). Statistical significance was defined as $P < 0.05$. The association of 25[OH]D level with genotype was determined after adjustment for age, sex, hours of

daylight, AER, vitamin D intake, and calcium intake. The association of vitamin D deficiency with the presence of CAC at the 3-year follow-up visit was examined in a multiple logistic regression model initially adjusted for age, sex, and hours of daylight. Additional models were run adjusting for BMI, HDL-cholesterol, LDL-cholesterol, and triglycerides. Models adjusted for factors that may affect vitamin D levels (AER, total vitamin D intake, and total calcium intake) were also run to examine the precision of our estimates. Genotypic data were added to these models.

The effect of vitamin D deficiency on progression of CAC from the 3- to 6-year visit was examined in a multiple logistic regression model adjusted for age, sex,

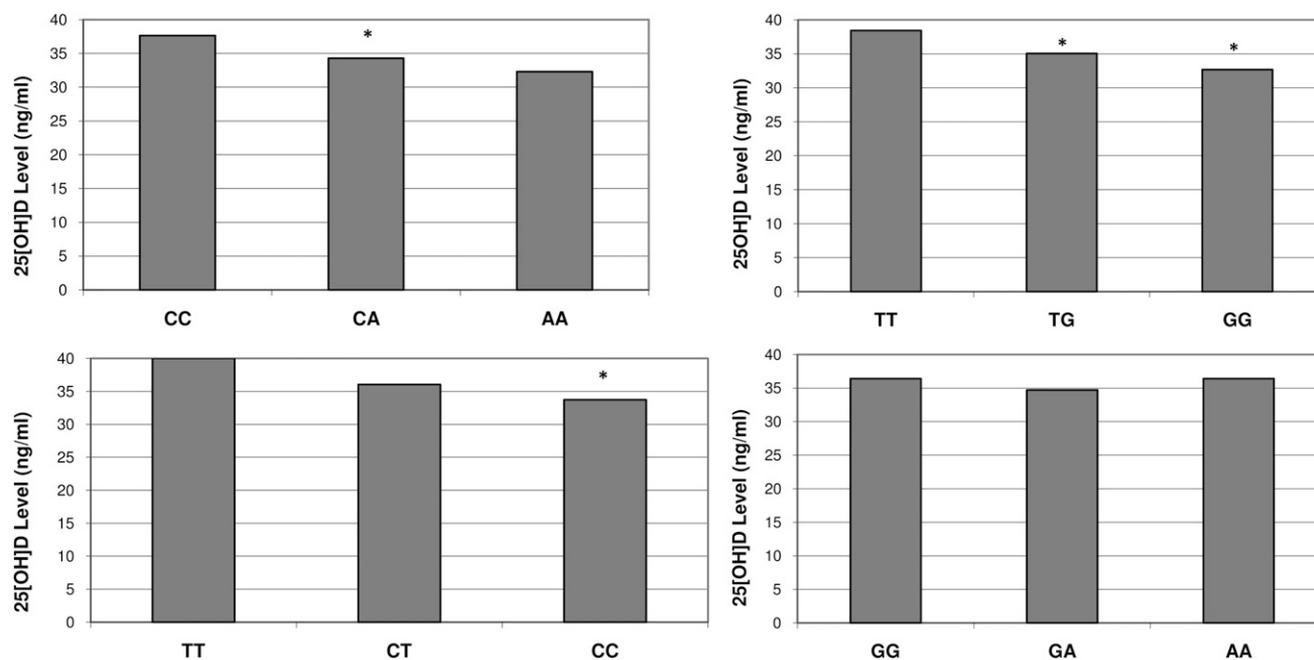


Figure 1—Vitamin D levels by genotype. Least square means adjusted for age, sex, diabetes status, hours of daylight, AER, vitamin D intake, and calcium intake. Upper left, VDP 420; upper right, VDP 416; lower left, VDR M1T; lower right, VDR Bsm1. **P* < 0.05 compared with major genotype.

hours of daylight, presence of CAC at the 3-year visit, AER, total vitamin D intake, and total calcium intake. An interaction term for vitamin D deficiency and presence of CAC at the 3-year visit was included to examine incident compared with prevalent CAC. In addition, an interaction term for vitamin D deficiency and the VDR genotype M1T was included to explore a possibility of gene–environment interaction on CAC progression.

RESULTS—Characteristics of study participants at the 3-year visit are summarized in Table 1. Age- and sex-adjusted mean 25[OH]D levels for type 1 diabetes subjects were 35.4 ± 0.7 ng/mL. Normal (>30 ng/mL), insufficient (20–30 ng/mL), and deficient (<20 ng/mL) 25-[OH]D levels were present in 65%, 25%, and 10% of the patients with diabetes, respectively.

Polymorphisms in the VDP and VDR genes were associated with levels of 25 [OH]D after adjusting for age, sex, hours of daylight, AER, total vitamin D intake, and total calcium intake (Fig. 1). Levels of 25[OH]D were higher among individuals with the CC (37.6 ± 1.0 ng/mL) genotype at VDP 420 compared with those with the CA (34.3 ± 1.2 ng/mL, *P* = 0.03) and AA (32.3 ± 2.8 ng/mL, *P* = 0.08) genotypes. At VDP 416, levels of 25[OH]D were significantly higher among individuals with the TT (38.4 ± 1.0 ng/mL) genotype

compared with those with the TG (35.1 ± 1.2 ng/mL, *P* = 0.04) and GG ($32.7 \pm 1.1 \pm 1.7$ ng/mL, *P* = 0.007) genotypes. Of the polymorphisms studied in the VDR gene, only those at VDR M1T showed significant associations with level of 25[OH]D. Levels of 25[OH]D were significantly higher among individuals with the TT (40.0 ± 1.7 ng/mL) genotype at VDR M1T compared with those with the CT (36.0 ± 1.1 ng/mL, *P* = 0.05) and CC (33.7 ± 1.0 ng/mL, *P* = 0.002) genotypes.

Lower levels of vitamin D as a continuous measure were significantly associated with presence of CAC at the 3-year visit, adjusting for age, sex, and hours of daylight (odds ratio [OR] = 0.98 [95% CI 0.96–1.00]; *P* = 0.02). Additional adjustment for BMI, HDL-cholesterol, LDL-cholesterol, and triglycerides

attenuated these results (OR = 0.99 [0.97–1.00]; *P* = 0.21).

Vitamin D deficiency (25[OH]D <20 ng/mL) was associated with the presence of CAC at the 3-year visit (OR = 3.3 [95% CI 1.6–7.0; *P* = 0.002]), adjusting for age, gender, and hours of daylight (Table 2). Similar results were found when examining the same models with vitamin D insufficiency (<30 ng/mL) (OR = 1.8 [1.1–3.0]). Additional adjustment for BMI, HDL-cholesterol, LDL-cholesterol, and triglycerides did not significantly affect the association with vitamin D deficiency and CAC (OR = 2.4 [1.1–5.3]). The addition of factors that could affect vitamin D levels (AER, vitamin D intake, and calcium intake) attenuated the results only slightly (2.2 [0.8–5.8]). Adjustment for VDP 420, VDP 416, VDR M1T, and VDR Bsm1 did not change these findings.

Table 2—Association of vitamin D deficiency and presence of CAC at 3-year visit

Model	Vitamin D deficiency (yes vs. no) OR (95% CI)
Model 1: Adjusted for age, sex, and hours of daylight	3.3 (1.6–7.0)
Model 1 + AER, vitamin D intake, and total calcium intake	2.8 (1.1–7.0)
Model 1 + BMI, HDL-cholesterol, LDL-cholesterol, and triglycerides	2.4 (1.1–5.3)
Model 1 + BMI, HDL-cholesterol, LDL-cholesterol, triglycerides, AER, vitamin D intake, and total calcium intake	2.2 (0.8–5.8)

No polymorphisms were associated with the presence of CAC at the 3-year visit.

In the longitudinal analyses of the relationship between vitamin D deficiency and progression of CAC, we first explored if the effect of vitamin D deficiency differed between persons free of CAC and those who already had detectable CAC at the 3-year visit. The interaction term was borderline significant ($P = 0.06$), indicating that vitamin D-deficient subjects without preexisting CAC at the 3-year visit were more likely to develop CAC than those with sufficient vitamin D levels, whereas vitamin D-deficient subjects with CAC already present did not experience more CAC progression than those with sufficient vitamin D levels.

The multivariate model that takes into account the interactions among vitamin D deficiency, presence/absence of CAC at 3-year visit, and VDR M1T genotype is shown in Table 3. Vitamin D deficiency predicted development of CAC among subjects free of CAC at the 3-year visit. Among those, vitamin D deficiency was a stronger risk factor for CAC in the presence of the VDR M1T CC genotype (OR = 6.5 [1.1–40.2], $P = 0.04$) than in the presence of the CT or TT genotype (OR = 1.6 [0.3–8.6], $P = 0.57$). Vitamin D deficiency did not predict progression of CAC among subjects with preexisting CAC at the 3-year visit.

CONCLUSIONS—The results of this study are consistent with the growing body of evidence for the role of vitamin D deficiency in development of coronary atherosclerosis. They are also internally consistent with regard to cross-sectional and longitudinal analyses. The novel findings include demonstration of a potential gene–environment interaction effect between the VDR M1T CC genotype and vitamin D deficiency on CAC development. Furthermore, these results indicate that vitamin D deficiency may be of particular importance during the early events leading to development of coronary calcification.

The only other prospective study (The Multi-Ethnic Study of Atherosclerosis [MESA]) has also found that lower 25 [OH]D levels predicted incident but not prevalent CAC (17). Both studies used similar methods to measure CAC. 25 [OH]D was measured by radioimmunoassay in MESA and by high-performance liquid chromatography in our study; however, both studies found similar prevalence of vitamin D deficiency (10% with

Table 3—Association of vitamin D with progression of CAC at the 6-year visit in subjects free of CAC at the 3-year visit and in subjects with CAC present at the 3-year visit

	Subjects free of CAC at 3-year visit	P value	Subjects with CAC present at 3-year visit	P value
Vitamin D deficiency				
In VDR M1T (CC)	6.5 (1.1–40.2)	0.04	1.5 (0.2–9.4)	0.67
In VDR M1T (CT or TT)	1.6 (0.3–8.6)	0.57	0.4 (0.1–1.5)	0.16

Odds ratios and 95% CI are presented for vitamin D deficiency in those with the VDR M1T CC genotype and in those with the VDR M1T CT or TT genotype. Models are adjusted for age, sex, hours of daylight, total vitamin D intake, total calcium intake, and AER.

<20 ng/mL in our study and 10% with <15 ng/mL in MESA). Dietary vitamin D intake was available in CACTI and included in our analyses, but not in the report from MESA. In contrast with the MESA study population, the CACTI participants analyzed all had type 1 diabetes. Furthermore, the age of vitamin D-deficient CACTI participants was lower (40.1 ± 8.9 years) than that of those in the MESA study (62.1 ± 10.3 years). Despite these differences in CACTI and MESA study populations, results were remarkably similar, indicating a robust role of vitamin D deficiency in CAC development.

Our study population was limited to non-Hispanic white subjects, whereas the MESA study (17) had a more diverse study population. African Americans and Hispanics have an increased prevalence of vitamin D deficiency; however, the MESA study did not find any differences by race (17). Results of our study demonstrate that the effects of vitamin D deficiency on CAC may be modified by genetic factors, especially the VDR M1T polymorphism. In our population, the CC genotype, associated with the lowest levels of serum 25[OH]D, conferred increased risk of CAC development indicating a possible pathway. This finding also is consistent with a previously reported association between the CC genotype and acute coronary syndrome (20). Future studies should expand our understanding of the genetic architecture of vitamin D metabolism and its implications to atherosclerosis.

Our study has some limitations that should be noted. Because we measured 25 [OH]D levels only at the 3-year visit, we do not know whether this measure is a stable indicator of overall vitamin D status. We only studied subjects with type 1 diabetes, so we are uncertain if these findings differed between subjects with

and without type 1 diabetes. Our study was also underpowered to test gene–environment interactions of modest magnitude, so it is possible that such interactions exist but were not detected in this study.

In the CACTI study, the association of vitamin D deficiency with prevalent CAC was independent of known CAD risk factors, including confounders such as BMI and mediators such as lipids. This suggests that vitamin D may not be related to CAD through common pathways with these traditional CAD risk factors but rather through a unique biologic mechanism. Vitamin D regulates the renin-angiotensin system (14) and may lower cardiovascular risk through this mechanism. Cardioprotective effects of 1,25 [OH]₂D treatment have been demonstrated in animal models (21–23). Our results suggest that vitamin D deficiency may be involved in the initiation of coronary calcification process. Vitamin D has an effect on antigen-presenting cells, such as dendritic cells and macrophages, and treatment with 1,25[OH]₂D can reduce foamy macrophages and suppress cholesterol uptake (24).

In conclusion, this study has strengthened the evidence for the role of vitamin D deficiency in the development of coronary atherosclerosis, especially during the early events leading to development of coronary calcification. The novel findings include demonstration of this effect in patients with type 1 diabetes and evidence for a gene–environment interaction between the VDR M1T CC genotype and vitamin D deficiency on CAC development.

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