Association between 25-hydroxyvitamin D deficiency and cardiovascular disease in type 2 diabetic patients with mild kidney dysfunction

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Abstract

Background. A potentially modifiable and underesti-mated risk factor for cardiovascular disease (CVD) in subjects with kidney dysfunction is 25-hydroxyvitamin D deficiency, although the relationship between inadequate vitamin D status and manifest CVD in type 2 diabetic subjects with mild kidney impairment has not been extensively examined.

Methods. We evaluated the relationship between serum 25-hydroxyvitamin D concentrations, baseline kidney function (estimated using the modification of diet in renal disease equation) and manifest CVD (myocardial infarction, angina, ischaemic stroke, coronary revascularization or carotid endarterectomy) among 462 consecutive patients with type 2 diabetes.

Results. In the whole population, the mean age was 62 ± 7 years, 64% were men, 76.3% had hypertension and the mean estimated glomerular filtration rate (GFR) was 94 ± 33 ml/min/1.73 m². Kidney function was strongly and inversely associated with CVD. In multivariate logistic regression analysis, there was an inverse association between serum 25-hydroxyvitamin D concentrations and prevalent CVD [odds ratio 0.95 (95% CI 0.92–0.98; P = 0.001)] in the whole population independent of baseline kidney function and other known risk factors. Additionally, the association between serum 25-hydroxyvitamin concentrations and CVD [odds ratio 0.97 (95% CI 0.94–0.99; P = 0.045)] remained statistically significant in participants in the lowest estimated GFR tertile after adjustment for potential confounders.

Conclusions. Decreased 25-hydroxyvitamin D concentrations are independently associated with prevalent CVD in type 2 diabetic patients with mild kidney dysfunction.

Keywords: 25-hydroxyvitamin D; cardiovascular disease; kidney dysfunction; myocardial infarction; vitamin D deficiency

Introduction

A substantial amount of data has accumulated in recent years suggesting that even mild kidney dysfunction, as reflected by an increase in urinary albumin excretion and/or a decrease in glomerular filtration rate (GFR), is an important and independent cardiovascular risk factor [1–9]. The relationship of kidney dysfunction with the risk for adverse cardiac outcomes appears to be mainly related to coexisting cardiovascular risk factors [10].

Given that a number of investigators [11–15] have shown that low levels of 25-hydroxyvitamin D may be associated with hypertension, impaired glucose metabolism, diabetes risk and cardiovascular disease (CVD), including congestive heart failure, in subjects with normal kidney function, it seems reasonable to propose that the presence of CVD among participants with mild kidney dysfunction may also be associated with reduced 25-hydroxyvitamin D concentrations.

Currently, the available data on this specific topic are lacking, especially among type 2 diabetic individuals, a patient population in whom the prevalence and incidence rates of CVD and kidney dysfunction are extremely high.

Thus, the purpose of this study was to examine the association between serum 25-hydroxyvitamin D concentrations and manifest CVD in persons with mild kidney dysfunction and type 2 diabetes. Clarification of this relationship may help to suggest possible underlying mechanisms, and might be of clinical importance in planning preventive and therapeutic strategies.
Subjects and methods

We enrolled 462 outpatients with type 2 diabetes who consecutively attended the diabetes clinic at ‘Sacro Cuore’ Hospital of Negrar (Verona, Italy) during the winter months (November–March) after exclusion of those with recent history of acute illness or with any clinical evidence of cancer, advanced kidney disease and cirrhosis, and those who were taking any drugs known to affect 25-hydroxyvitamin D metabolism, including vitamin/mineral supplements. The protocol was approved by the local Ethical Committee. Written informed consent was obtained from all participants.

A detailed medical history was collected by questionnaire, administered by a trained physician, in order to record previous or current coronary heart disease (i.e. myocardial infarction, angina pectoris or revascularization procedures) and cerebrovascular disease (ischemic stroke or carotid endarterectomy). Presence of vascular disease was confirmed by reviewing medical records of the hospital of all patients and by a careful physical examination by one of the investigators that also included vascular laboratory studies (e.g. electrocardiogram and echo-Doppler scanning of carotid arteries, which were performed in all participants). No participants had a CVD-induced immobility (with consequent diminished exposure to sunshine) or significant physical disability.

Body mass index (BMI) was calculated by dividing weight in kilograms by height in metres squared. Waist circumference was measured in a standing position at the level of the umbilicus. Blood pressure was measured in triplicate with a standard mercury manometer. Information on smoking status and other lifestyle characteristics was obtained from all participants by questionnaire.

Blood samples were drawn in the morning after an overnight fast. Serum creatinine was measured using the modified kinetic Jaffe method. Lipids and other biochemical blood measurements were determined by standard laboratory procedures (DAX 96, Bayer Diagnostics). Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald’s equation, except when triglycerides exceeded 399 mg/dl (10 subjects). Glycosilated haemoglobin (HbA1c) was measured by an automated high-performance liquid chromatography analyser (HA-S140, Menarini, Florence, Italy); the upper limit of normal for our laboratory was 5.9%.

High sensitivity C-reactive protein (hs-CRP) was determined with a highly sensitive immuno-turbidimetric assay (Roche Diagnostics, Milan, Italy). Serum 25-hydroxyvitamin D concentrations, as a reliable measure of overall vitamin D status [16], were measured using an automated chemiluminescence assay (DiaSorin Liaison, Stillwater, MN); intra- and inter-assay coefficients of variation were below 4% and 9%, respectively. To avoid seasonal variations, all 25-hydroxyvitamin D samples were collected during the winter months in all participants. Reference ranges for serum 25-hydroxyvitamin D concentrations in our laboratory during the winter months were 10–64 ng/ml. Urinary albumin excretion rate was measured as the albumin-to-creatinine ratio (ACR) by an immuno-nephelometric method; micro- and macro-albuminuria were defined as an ACR ≥2.5 and ≥25 mg/mmol, respectively.

Because a number of factors such as age, ethnicity and gender can influence serum creatinine concentrations, the level of kidney function was defined by estimated GFR using the formula developed and validated in the Modification of Diet in Renal disease (MDRD) study [17]. The MDRD formula is as follows:

\[
\text{GFR} = 186.3 \times (\text{Serum creatinine}^{\text{−}\left(\frac{1.154}{\text{Age}}\right)}) \times (\text{Age}^{\text{−}\left(\frac{0.203}{1.212}\right)})
\]

Because serum creatinine values measured in different laboratories may vary, serum creatinine values were indirectly calibrated to results obtained at the Cleveland Clinic Laboratory (where serum creatinine was measured in the MDRD study) by using the calibration method described by Froissart et al. [18,19]. This calibration method was used as it has been validated in large non-US cohorts as the one represented in this analysis.

Data are presented as means ± SD or proportions. Skewed variables were logarithmically transformed to improve normality prior to analysis. Statistical analyses included the one-way analysis of variance, the chi-squared test with Yates’ correction for continuity (for categorical variables) and the multivariate logistic regression analysis. To examine the associations between 25-hydroxyvitamin D and prevalent CVD in participants with mild kidney dysfunction, we have arbitrarily created tertiles of the study population according to estimated GFR values. Subjects were classified as having CVD (considered as composite endpoint) if they had a history of myocardial infarction, angina pectoris, coronary revascularization procedures, ischemic stroke or carotid endarterectomy. Vitamin D deficiency was defined as a serum 25-hydroxyvitamin D concentration ≤15 ng/ml, as previously established [16,20]. For prediction of prevalent CVD events, men and women were combined and first-order interaction terms for sex-by-vitamin D and sex-by-estimated GFR interactions on risk for CVD were examined. Because the interactions were not statistically significant, sex-pooled multivariate logistic regression analyses were used to assess the independence of the association of 25-hydroxyvitamin D with prevalent CVD in the whole population (Table 1), and in those in the lowest tertile of estimated GFR (Table 2).

In the fully adjusted multivariate logistic regression model—in the whole population (Table 1)—together with 25-hydroxyvitamin D (modelled as either a linear variable, without logarithmic transformation, per 1 ng/ml increase or as a categorical measure), age, gender, BMI, smoking, hypertension, hyperlipidaemia, history of diabetes and micro- and macro-albuminuria were included in the model. The associations between 25-hydroxyvitamin D and prevalent CVD were examined only in those in the lowest tertile of estimated GFR (Table 2). The interactions were not statistically significant, sex-pooled GFR interactions on risk for CVD were examined. Because the interactions were not statistically significant, sex-pooled multivariate logistic regression analyses were used to assess the independence of the association of 25-hydroxyvitamin D with prevalent CVD in the whole population (Table 1), and in those in the lowest tertile of estimated GFR (Table 2).

### Table 1. Determinants of cardiovascular disease (as composite endpoint) in the entire diabetic cohort as evaluated by multiple logistic regression analysis (n = 462).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>1.05 (1.02; 1.10)</td>
<td>0.011</td>
</tr>
<tr>
<td>Male gender (male vs. female)</td>
<td>1.66 (1.02; 2.75)</td>
<td>0.047</td>
</tr>
<tr>
<td>Body mass index (per unit)</td>
<td>0.99 (0.94,1.05)</td>
<td>0.838</td>
</tr>
<tr>
<td>Current smoking (yes vs no)</td>
<td>1.03 (0.56; 1.91)</td>
<td>0.877</td>
</tr>
<tr>
<td>Duration of diabetes (per year)</td>
<td>1.02 (0.99; 1.05)</td>
<td>0.156</td>
</tr>
<tr>
<td>Arterial hypertension (yes vs no)</td>
<td>2.31 (1.21; 4.39)</td>
<td>0.0125</td>
</tr>
<tr>
<td>LDL cholesterol (per unit)</td>
<td>0.92 (0.76; 1.11)</td>
<td>0.386</td>
</tr>
<tr>
<td>hs-CRP (per unit)</td>
<td>1.0 (0.993; 1.004)</td>
<td>0.5819</td>
</tr>
<tr>
<td>25(OH)D (per unit)</td>
<td>1.03 (0.99; 1.02)</td>
<td>0.157</td>
</tr>
<tr>
<td>GFR (per unit)</td>
<td>0.95 (0.92; 0.98)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

GFR, estimated glomerular filtration rate; HbA1c, glycosilated haemoglobin; hs-CRP, high sensitivity C-reactive protein; LDL, low density lipoprotein; 25(OH)D, 25-hydroxyvitamin D.
Largest cholesterol, estimated GFR, presence of hypertension (defined as blood pressure values ≥140/90 mmHg or on treatment), diabetes duration, HbA1c, and hs-CRP were also included as covariates. In this model, only those cardiovascular risk factors that had biological plausibility based on a well-accepted association with CVD in the literature were included. Given the lower number of CVD events (n = 44), in the fully adjusted multivariate logistic regression model performed in those in the lowest tertile of estimated GFR (Table 2)—together with 25-hydroxyvitamin D (modelled as either a linear variable, without logarithmic transformation, per 1 ng/ml increase or as a categorical measure)—age, gender, estimated GFR and presence of hypertension were only included as covariates. These variables were also chosen because of biological plausibility with CVD in patients with mild kidney dysfunction. In both models P-values <0.05 were considered statistically significant.

Predictors of cardiovascular disease

Overall, 112 (24.2%) out of 462 patients were coded positive for CVD as composite endpoint. Of these, 82 (17.7%) patients had definite coronary heart disease (myocardial infarction, angina pectoris or coronary revascularization) and 51 (11%) had cerebrovascular disease (ischemic stroke or carotid endarterectomy); many subjects had CVD in multiple sites. As shown in Table 3, participants in the lowest estimated GFR tertile had a markedly greater prevalence of manifest CVD, mainly coronary heart disease.

Concordantly, serum 25-hydroxyvitamin D concentrations and estimated GFR values were significantly lower (P < 0.01–0.001) among those with ‘composite’ CVD [25(OH)D: 17.8 ± 9 vs 20.6 ± 10 ng/ml; estimated GFR: 85.2 ± 23 vs 98 ± 30 ml/min/1.73 m²], coronary (17.2 ± 9 vs 20.3 ± 10 ng/ml and 84.3 ± 24 vs 97 ± 29 ml/min/1.73 m²) and cerebrovascular (16.9 ± 7 vs 20 ± 10 ng/ml and 87.2 ± 23 vs 96 ± 30 ml/min/1.73 m²) disease than among those without CVD.

In multivariate logistic regression analysis (Table 1), there was an inverse and independent association between serum 25-hydroxyvitamin D concentrations and prevalent CVD in the whole population [odds ratio 0.95 (95% CI 0.92–0.98; P = 0.001)], which implies that worsening CVD is more likely to occur in participants with lower vitamin D concentrations. Of note, other independent predictors of prevalent CVD in this cohort included older age, male gender, hypertension and baseline kidney function (inversely).

Almost identical results were observed when both estimated GFR and 25-hydroxyvitamin D were entered as categorical measures (i.e. estimated GFR tertiles and vitamin D deficiency defined as serum 25-hydroxyvitamin D concentrations ≤15 ng/ml tended to be higher (P = 0.071) in participants with lowest estimated GFR tertile. Body mass index, waist circumference, smoking status, prevalence of hypertension, diabetes duration, HbA1c, LDL-cholesterol and hs-CRP concentrations were not significantly different among the groups. Furthermore, medical therapy with lipid-lowering agents was administered to a similar proportion of participants across tertiles of kidney function.

Almost identical results were obtained when the study population was stratified according to the median or the quartiles of distribution of estimated GFR values (not shown).

Table 2. Determinants of cardiovascular disease (CVD) in the lowest estimated glomerular filtration rate tertile as evaluated by multiple logistic regression analysis (n = 148).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per year)</td>
<td>1.07 (1.02; 1.13)</td>
<td>0.049</td>
</tr>
<tr>
<td>Male gender (male vs female)</td>
<td>1.48 (0.70; 3.23)</td>
<td>0.544</td>
</tr>
<tr>
<td>Arterial hypertension (yes vs no)</td>
<td>2.98 (1.20; 12.10)</td>
<td>0.022</td>
</tr>
<tr>
<td>25(OH)D (per unit)</td>
<td>0.97 (0.94; 0.99)</td>
<td>0.045</td>
</tr>
<tr>
<td>GFR (per unit)</td>
<td>0.96 (0.93; 0.99)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

25(OH)D, 25-hydroxyvitamin D; GFR, estimated glomerular filtration rate.
In participants with the lowest estimated GFR tertile, CVD was also independently related to serum 25-hydroxyvitamin D concentrations [odds ratio 0.97 (95% CI 0.94–0.99; P = 0.045)] or to 25-hydroxyvitamin D deficiency [odds ratio 2.19 (95% CI 1.04–4.61; P = 0.040)] when this variable was entered as categorical measure. Similar to the risk model for the whole cohort, older age, hypertension and baseline kidney function were additional predictors of CVD in this subgroup (Table 2). The results did not substantially change after further adjustment for smoking, LDL-cholesterol, hs-CRP, glycaemic control, diabetes duration and use of medications (not shown).

Of note, 25-hydroxyvitamin D as a continuous or categorical measure (25-hydroxyvitamin D concentration <15ng/ml) in univariate logistic regression analysis was also a significant predictor of prevalent CVD for subjects in the 2nd tertile of estimated GFR (i.e. 82–99 ml/min/1.73 m²) but did not reach statistical significance in the highest tertile of kidney function (i.e. >100 ml/min/1.73 m²) (data not shown).

### Discussion

In this analysis we have demonstrated in a large outpatient cohort of type 2 diabetic adults that significant vitamin D deficiency (i.e. 25-hydroxyvitamin D ≤15ng/ml), as defined by the recent K/DOQI Clinical Practice Guidelines for Bone Mineral Metabolism and Disease in Chronic Kidney Disease [20], was associated with higher prevalence of manifest CVD independent of kidney function. In addition, among individuals with mild kidney dysfunction this significant association persisted. Notably, the observed relationship in the general cohort and in participants with lowest estimated GFR tertile remained statistically significant after adjustment for a broad spectrum of traditional and non-traditional CVD risk factors.

Our findings complement recent observations suggesting that vitamin D deficiency is strongly associated with greater carotid intima-media thickness and plaques—a reliable index of early, generalized atherosclerosis—in people with type 2 diabetes [21]. Additionally, type 2 diabetic patients with manifest CVD had lower serum 25-hydroxyvitamin D concentrations than their vitamin D-sufficient diabetic counterparts without CVD [22].

Accumulating evidence [20,23] exists that calcitriol [1,25(OH)2D3] levels may be more dependent on the availability of 25-hydroxyvitamin D in patients with impaired kidney function than in those with normal kidney function. In addition, most tissues and cells in the body including bone, brain, heart, pancreas,
Vitamin D and kidney dysfunction in type 2 diabetes

skin, breast, gonads, activated T and B lymphocytes, and monocytes possess vitamin D receptors (VDRs) [24]. Specifically, it is well established that the smooth muscle cells of the cardiovascular system have VDRs [25]. Thus, in addition to its traditional calcium-related effects on the skeleton, 1,25(OH)\textsubscript{2}D\textsubscript{3} can affect cardiac health by altering the inflammatory response associated with atherosclerosis and modulating cardiac vascular smooth muscle function [16,24]. Other non-skeletal biologic effects of 1,25(OH)\textsubscript{2}D\textsubscript{3} may include: stimulating the secretion/action of insulin, regulation of blood pressure through its modulation of renin production and inhibition of cellular proliferation [16,24].

Recent K/DOQI Clinical Practice Guidelines for Bone Mineral Metabolism and Disease in Chronic Kidney Disease (CKD) have only recommended the measurement of serum 25-hydroxyvitamin D levels in patients with CKD stage 3 and 4; the rationale being that low levels of 25-hydroxyvitamin D are likely to play a role in the development of secondary hyperparathyroidism by limiting the synthesis of 1,25(OH)\textsubscript{2}D\textsubscript{3} [20]. Although our findings should be used as hypothesis generating, they suggest a rationale for the measurement and supplementation of 25-hydroxyvitamin D at all levels of kidney dysfunction with the objective of preventing CVD, particularly in the type 2 diabetic population.

This study has several strengths, including the large number of participants, the complete nature of the dataset and the ability for the adjustment of multiple traditional and non-traditional CVD risk factors. Despite the comprehensive nature of the dataset, there are some possible limitations to our study. First, the measurement of kidney function was based on a single creatinine measure, however, serum creatinine was measured in the same laboratory and using the same technique. In addition, we used estimated GFR rather than more precise measures of kidney function, like iothalamate clearance.

Second, questions remain about the generalizability of the MDRD study equation, because it has not been definitively validated in diabetic kidney disease, in patients with serious comorbid conditions or in persons older than 70 years of age. Third, this cohort of participants consisted of white Caucasians, which limits generalizability. Finally, 1,25(OH)\textsubscript{2}D\textsubscript{3} and parathyroid hormone levels were not measured in this study.

In summary, the present study found that 25-hydroxyvitamin D deficiency is strongly and independently associated with manifest CVD among type 2 diabetic adults with mild kidney impairment. These findings suggest that vitamin D deficiency may play a role in CVD pathogenesis in diabetic subjects with mild kidney dysfunction, and may not be merely a marker for other associated risk factors. Whether therapeutic intervention to replace vitamin D stores may be useful to prevent the development and progression of CVD in this high-risk population remains to be determined in future interventional studies.

Conflict of interest statement. None declared.

References


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