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Association of vitamin D_3 and its metabolites in patients with and without type 2 diabetes and their relationship to diabetes complications

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Abstract

Background: Epidemiological studies have suggested that vitamin D deficiency is associated with the development of type 2 diabetes (T2DM) and is related to diabetes complications. This study was undertaken to determine the relationship between diabetes complications and cardiovascular risk factors with vitamin D_3 and its metabolites: 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), 25-hydroxyvitamin D_3 ($25(OH)D_3$), 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$); and 25-hydroxy-3epi-vitamin D_3 ($3epi25(OH)D_3$).

Methods: 750 Qatari subjects, 460 (61.3%) with and 290 (38.7%) without T2DM, who were not taking vitamin D_3 supplements, participated in this cross-sectional, observational study. Plasma concentrations of vitamin D_3 and its metabolites were measured by liquid chromatography tandem mass spectrometry analysis.

Results: T2DM subjects had lower concentrations of all vitamin D_3 metabolites ($p < 0.001$) except $3epi25(OH)D_3$ ($p < 0.071$). Males had higher concentrations of all vitamin D_3 metabolites ($p < 0.001$). In the T2DM subjects, lower $25(OH)D_3$ was associated with retinopathy ($p < 0.03$) and dyslipidemia ($p < 0.04$), but not neuropathy or vascular complications; lower $1,25(OH)_2D_3$ was associated with hypertension ($p < 0.009$), dyslipidemia ($p < 0.003$) and retinopathy ($p < 0.006$), and coronary artery disease ($p < 0.012$), but not neuropathy; lower $24,25(OH)_2D_3$ concentrations were associated with dyslipidemia alone ($p < 0.019$); $3epi25(OH)D_3$ associated with diabetic neuropathy alone ($p < 0.029$). In nondiabetics, $25(OH)D_3$, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ were associated with dyslipidemia ($p < 0.001$, $p < 0.001$, $p < 0.015$, respectively) and lower $1,25(OH)_2D_3$ was associated with hypertension ($p < 0.001$). Spearman's correlation showed $1,25(OH)_2D_3$ to be negatively correlated to age and diabetes duration.

Conclusions: Different diabetes complications were associated with differing vitamin D parameters, with diabetic retinopathy related to lower $25(OH)D_3$ and $1,25(OH)_2D_3$ levels, hypertension significantly associated with lower $1,25(OH)_2D_3$, while dyslipidemia was associated with lower $25(OH)D_3$, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$. While 25(OH)D metabolites were lower in females, there was not an exaggeration in complications.

Keywords: diabetes complications, type 2 diabetes, vitamin D_3, vitamin D_3 epimers, vitamin D_3 metabolites

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Background

Accumulating evidence suggests that vitamin D deficiency increases the risk of type 2 diabetes (T2DM).[1,2] Deficiency of vitamin D is associated with both insulin resistance and beta cell dysfunction.[3] As long ago as 1980, impaired insulin secretion was demonstrated in isolated perfused rat pancreas in the setting of vitamin D deficiency.[4] Later reports suggested that vitamin D deficiency could contribute to metabolic syndrome and T2DM.[5,6] Vitamin D appears to exert its antidiabetic effect through modulation of hepatic glucose and lipid metabolism *versus* activation of calcium and the adenosine monophosphate–activated protein kinase (AMPK) pathway, and through promotion of beta cell function and survival.[7]

A large prospective study of women, with 20 years of follow up, reported an inverse relationship between vitamin D concentrations and the onset of diabetes.[8] Vitamin D deficiency in T2DM has also been correlated with microvascular complications of retinopathy, neuropathy and nephropathy,[9,10] though there is still some debate.

Vitamin D_3 (cholecalciferol) is endogenously produced by UV-B irradiation of 7-dehydrocholesterol, while ergosterol is derived from the diet (primarily from mushrooms and fungi) and converted to previtamin D_2 (ergocalciferol) by UV-B light, though both are hydroxylated to 25(OH)D_3 and 25(OH)D_2, respectively, by multiple 25-hydroxylases in the liver (Figure 1).[11] 25(OH)D is transported to the kidney and converted to either the active 1,25$(OH)_2$D by 1 alpha hydroxylase, or to 24,25$(OH)_2$D, which is also active, by the 24 alpha hydroxylase present in the renal tubular cells (Figure 1).[12] It has recently been reported that extrarenal tissues may also convert 25(OH)D to 1,25$(OH)_2$D, although, notably, activation in renal and non-kidney tissues is regulated differently with macrophage production of 1,25$(OH)_2$D through the type 2 interferon response.[13] 1,25$(OH)_2$D binds to the vitamin D receptor (VDR) but, to exert its effect (which may take several hours), it heterodimerizes with the retinoid X receptor;[12] however, reports suggest alternative, more rapid mechanisms of action through binding to membrane VDR or through the 1,25D-membrane-associated, rapid response steroid-binding protein receptor with activation of protein kinases A and C.[14] Obesity can exacerbate vitamin D deficiency through decreased bioavailability due to deposition of vitamin D in the body fat compartments.[15] In many countries, vitamin D_2 is available as a pharmaceutical and a supplement to counter vitamin D deficiency.[16]

While vitamin D deficiency is a global issue,[17] cultural norms dictating full-body coverage in parts of the world such as the Middle East magnify the issue of vitamin D deficiency in these regions.[18]

In Qatar, T2DM is a particularly serious health issue affecting approximately 20% of the population, a figure 2–3 times higher than the world average.[19] As a consequence, the burden associated with diabetes complications in Qatari patients is tremendous. A total of 43% of patients referred to dialysis have diabetic nephropathy.[20] A significant proportion of the total patient population presenting with cardiac pathologies in Qatar have T2DM: 57% of patients presenting with acute heart failure, and 30% of atrial fibrillation patients.[21]

The aim of this study was specifically to answer the question as to whether vitamin D deficiency was associated with diabetes retinopathy. In addition, given the pervasively low vitamin D status in the Middle East, we hypothesized that full-body coverage for women would lead to lower vitamin D parameter concentrations compared to men and lead to increased diabetes complications.

Methods

Study population

A total of 750 Qatari patients, 460 (61.3%) with T2DM, were recruited from the Hamad outpatient clinic, along with 290 (38.7%) nondiabetic participants, who were relatives accompanying the T2DM patients to their clinic visits from July 2013 to July 2015, as part of a study investigating gene expression and genomics in individuals with diabetes (Table 1). None of the diabetic patients were taking vitamin D_3 supplements, though all had been prescribed vitamin D_2 supplements.

Inclusion criteria were male or female Qataris, aged 30 years or older. The diagnosis of T2DM was made according to the WHO guidelines;[22] for

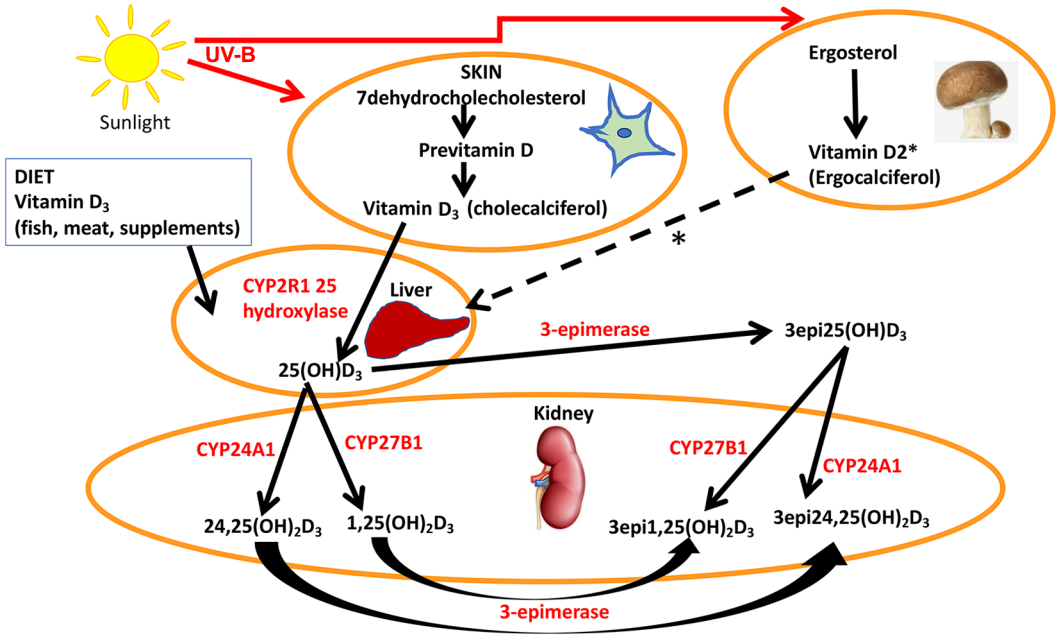

Figure 1. A simplified schematic representation of the synthesis and metabolism of vitamin D.
7-dehydrocholesterol in the skin is converted to previtamin D_3 by UV-B and then is thermally isomerized to vitamin D_3. Transport of vitamin D_3 from the skin to the liver is mediated by vitamin D binding protein (DBP) where vitamin D_3 is hydroxylated at position 25 to 25(OH)D_3, DBP then transports 25(OH)D_3 to the kidney. 25(OH)D_3/DBP is filtered by the glomeruli and 25(OH)D_3 is taken up into the tubular cells, following DBP binding to megalin, a transmembrane protein. 25(OH)D_3 undergoes a second hydroxylation step by the 1-alpha-hydroxylase Cyp27B1, converting to the active 1α,25 $(OH)_2D_3$, while 24 hydroxylase Cyp27A1 converts to 24,25$(OH)_2D_3$. Keratinocytes contribute to the 3-epimerase activity, but the exact sites of activity remain unknown. 3epi25(OH)D_3 is converted by Cyp27A1 to 24,25(OH)D_3 and Cyp27B1 to 1,25(OH)D_3, in equal measure. Ergosterol (provitamin D_2) is plant-based and is converted to ergocalciferol by UV-B light that then follows the same pathway as vitamin D_3 that is depicted with the dotted line * to the liver to form 25(OH)D_2 and then transported to the kidney and epimerized to the D_2 metabolites.

inclusion in the T2DM group, at least one of the following was required: fasting plasma glucose >7 mmol/L, HbA1c >6.5%, or a diagnostic glucose tolerance test. Inclusion in the nondiabetic control group required a normal glucose tolerance test. Patients were excluded from the study if they had a diagnosis of type 1 diabetes or any active form of diabetes, such as gestational diabetes or diabetes secondary to steroid treatment. All diabetes patients had retinal photography, a clinical foot examination, blood pressure measurement and urine analyzed for the albumin:creatinine ratio collected at their outpatient visit. Dyslipidemia was defined as a total cholesterol greater than 190 mg/dl (>4.9 mmol/L) and/or fasting triglycerides greater than 150 mg/dl (>1.7 mmol/L) untreated, or if patients were under treatment.

The study was approved by Weill Cornell IRB (IRB# 13-00063) and all participants provided written informed consent. The conduct of the trial was in accordance with ICH GCP and the Declaration of Helsinki.

Study design

Following an overnight fast, blood samples were collected, and weight and blood pressure were measured at the baseline visit. Fasting venous blood was collected into fluoride oxalate and serum gel tubes. Samples were separated by centrifugation at 2000 g for 15 min at 4°C, and the blood, serum and plasma aliquots were stored at –80°C within 1 h of collection. Overnight urine samples were collected, and aliquots were stored at –80°C until batch analysis. Blood pressure was measured using an automated device (NPB-3900; Nellcor Puritan Bennett, Pleasanton, CA, USA) during each study visit. Blood pressure measurements were performed after the participants had been seated quietly for at least 5 min and with the right arm supported at heart level. Three readings

Table 1. Demographic data and vitamin D_3 levels in the control ($n = 290$) and type 2 diabetic ($n = 460$) groups.

	Control	Diabetes	*p* value
	Median (IQR)	Median (IQR)	
Age (years)	44.5 (38.0–53.0)	56.0 (48.0–62.0)	<0.001
BMI (kg/m^2)	30.1 (26.7–35.1)	32.4 (28.6–37.2)	<0.001
Hypertensive – *n* (%)	71 (24.5)	320 (69.6)	<0.001
HbA1c (%)	5.6 (5.3–5.9)	7.9 (6.7–9.5)	<0.001
Glucose (mmol/L)	5.2 (4.7–5.7)	8.6 (6.4–12.2)	<0.001
1,25(OH)$_2$D$_3$ (ng/ml)	0.043 (0.025–0.066)	0.025 (0.013–0.044)	<0.001
25(OH)D$_3$ (ng/ml)	8.82 (4.88–14.47)	6.49 (3.40–13.57)	<0.001
24,25(OH)$_2$D$_3$ (ng/ml)	0.38 (0.21–0.66)	0.29 (0.18–0.56)	0.001
3epi25(OH)D$_3$ (ng/ml)	0.47 (0.23–0.93)	0.39 (0.22–0.75)	0.071

BMI, body mass index; HbA1c, glycosylated hemoglobin A1c; 1,25(OH)$_2$D$_3$, 1,25-dihydroxyvitamin D3; 25(OH)D$_3$, 25-hydroxyvitamin D$_3$; 24,25(OH)$_2$D$_3$, 24,25-dihydroxyvitamin D3; 3epi25(OH)D$_3$, 25-hydroxy-3epi-vitamin D$_3$; IQR, interquartile range.

were taken, each at least 2 min apart, and then the average of the readings was calculated.

Serum vitamin D and metabolite measurements

Serum vitamin D concentrations were quantified using isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). A total of 25µl of internal standards (d6-1calcitriol (1.5 ng/ml), d6-25OHD$_3$ (50 ng/ml) and d6-epi-25(OH)D$_3$ (20 ng/ml)) was added into each microcentrifuge tube containing 250 µl of calibration standards, quality control or serum samples, and kept for 30 min to reach binding equilibrium. The samples were diluted with 250 µl of pretreatment solution (isopropanol and water; 50:50 v/v) and left to stand for at least 15 min to displace binding protein.

Pretreated samples of 300 µl were loaded onto ISOLUTE® supported liquid extraction (SLE+) columns (Biotage), followed by elution with 1.8 ml of n-heptane (2 × 900 µl) into a collection tube already containing 200 µl of 0.25 mg/ml PTAD solution in ethyl acetate and heptane (8:92 v/v). The eluate was evaporated to dryness using turbovap under nitrogen gas heated at 38°C. Once dried, 50 µl of reconstituted solution consisting of methanol and deionized water, 70:30 v/v, and 0.006% methylalamine were added into all tubes. The derivatized extracts were transferred into LC insert vials and 10 µl from each was injected into the LC-MS/MS system. Data for the 25(OH)D$_3$ and metabolite validation are shown in Supplemental Table 1.

Diabetes-related complications

Coronary artery disease (CAD) was defined as a history of myocardial infarction or angina, confirmed by coronary angiography. Peripheral arterial disease (PAD) was defined as a history of claudication or rest pain with evidence of artery stenosis on ultrasound or lower limb angiography. Stroke was defined as a sudden onset neurological deficit lasting more than 24 h.

Diabetic retinopathy was diagnosed by fundoscopy. Diabetic neuropathy (DN) was diagnosed based on the vibration perception threshold (Neurothesiometer NU-1, Horwell, UK) of the great toe being >25 V.

Study outcomes

Statistical analyses. A pilot study of 270 diabetes patients was previously undertaken in this

population based on previous literature[9] that suggested that 460 total patients, of whom 40% had retinopathy, would be required at $\alpha = 0.05$ to detect a standard deviation difference of 0.27 in mean vitamin D levels with 80% power, considered a small to moderate-sized effect.

Data trends were visually and statistically evaluated for normality. Non-parametric tests (Mann–Whitney U) were applied to data that violated the assumptions of normality when tested using the Kolmogorov–Smirnov test. Bonferroni correction was applied to account for multiple testing. Statistical analysis was performed using SPSS for Windows, version 24.0. All values are given as mean ± SD or as mean with 95% confidence interval (CI) unless specified. Correlations between vitamin D and its metabolites were undertaken with Spearman's correlation.

Results

Baseline characteristics

Diabetes patients were older than controls (median age (range) 55.2 (9.9) and 46.1 (10.8) years, respectively, $p < 0.001$)) and diabetes patients had a greater body mass index (BMI) than controls (median age (range) 32.4 (44) and 30.1 (34.8) years, respectively, $p < 0.001$)), and concentrations of $25(OH)D_3$, $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ were significantly different to controls ($p < 0.001$), though $3epi25(OH)D_3$ did not differ ($p < 0.071$) (Table 1). The diabetes complications for the T2DM cohort are shown in Table 2.

Vitamin D metabolite measurements

Vitamin D_3 concentrations are lower in T2DM. Concentrations of $25(OH)D_3$, and its active form $1,25(OH)_2D_3$, and its metabolite $24,25(OH)_2D_3$ were lower in the T2DM patients (median (ng/dl) 6.45, 0.02 and 0.29, respectively) compared with nondiabetic controls (median (ng/dl) 8.82, 0.04 and 0.38, respectively: $p < 0.001$), but $3epi25(OH)D_3$ did not differ ($p = 0.07$) (Table 1).

Gender differences in vitamin D_3 concentrations. In T2DM, concentrations of $25(OH)D_3$, $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ and $3epi25(OH)D_3$ were all lower in females *versus* males ($p < 0.01$) (Table 2).

Concentrations of $25(OH)D_3$, $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ were also lower in nondiabetic females *versus* nondiabetic males ($p < 0.01$) (Table 3). Despite the lower vitamin D parameters in females, there was no difference in diabetes complications between males and females (data not shown).

Relationship of vitamin D_3 metabolite concentrations with diabetic complications. In this T2DM Qatari cohort, lower concentrations of $1,25(OH)_2D_3$ ($p < 0.006$) and lower levels of $25(OH)D_3$ were weakly associated with diabetic retinopathy ($p < 0.031$), as shown in Table 2.

Spearman's correlation showed $1,25(OH)_2D_3$ to be negatively correlated to age and diabetes duration (R^2 –0.301, $p < 0.01$; –0.16, $p < 0.01$, respectively); $25(OH)D_3$ was negatively correlated to age (R^2 –0.08, $p < 0.024$), with no correlation evident for $24,25(OH)_2D_3$ or $3epi25(OH)D_3$.

There was no difference in the frequency of diabetic complications between males and females.

Relationship of vitamin D_3 metabolite concentrations with cardiovascular complications. In this T2DM Qatari cohort, lower concentrations of $1,25(OH)_2D_3$ were associated with hypertension ($p < 0.009$), dyslipidemia ($p < 0.003$) and coronary heart disease ($p < 0.01$). Lower levels of $25(OH)D_3$ were weakly associated with dyslipidemia ($p < 0.041$) and low concentrations of $24,25(OH)_2D_3$ were associated with dyslipidemia ($p < 0.02$), as shown in Table 2.

Relationship of vitamin D_3 metabolite concentrations in nondiabetic participants. In nondiabetic participants, concentrations of $25(OH)D_3$, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$, but not $3epi25(OH)D_3$, were lower in females *versus* males ($p < 0.01$) (Table 3).

Lower concentrations of $1,25(OH)_2D_3$ were associated with hypertension ($p < 0.001$) and dyslipidemia ($p < 0.001$). Lower levels of $25(OH)D_3$ were associated with dyslipidemia ($p < 0.001$), as were low concentrations of $24,25(OH)_2D_3$, as shown in Table 3.

eGFR and vitamin D_3 concentrations

We considered the possibility that $25(OH)D_3$ metabolite concentrations were merely reflecting the eGFR in these patients. However, the lack of correlation between eGFR and 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), 25-hydroxyvitamin D_3

Table 2. The relationship of vitamin D_3 and its epimers to diabetes complications in the group of participants ($n = 460$) with type 2 diabetes.

	$1,25(OH)_2D_3$ (ng/dl) median (IQR)	*p* value	$25(OH)D_3$ (ng/dl) median (IQR)	*p* value	$24,25(OH)_2D_3$ (ng/dl) median (IQR)	*p* value	3-epi-$25(OH)D_3$ (ng/dl) median (range)	*p* value
Gender								
Male ($n = 227$)	0.030 (0.014–0.050)	<0.001	8.3 (4.4–15.3)	<0.001	0.35 (0.20–0.65)	0.002	0.511 (0.284–0.839)	0.003
Female ($n = 233$)	0.020 (0.011–0.037)		4.7 (2.9–10.3)		0.26 (0.16–0.44)		0.304 (0.190–0.579)	
Hypertension								
No ($n = 140$)	0.033 (0.016–0.049)	0.009	7.0 (3.5–14.5)	0.465	0.31 (0.20–0.65)	0.059	0.330 (0.208–0.746)	0.390
Yes ($n = 320$)	0.023 (0.012–0.041)		6.4 (3.4–13.4)		0.27 (0.18–0.53)		0.415 (0.228–0.744)	
Dyslipidemia								
No ($n = 109$)	0.031 (0.015–0.052)	0.003	7.9 (4.0–16.1)	0.041	0.34 (0.22–0.65)	0.019	0.340 (0.203–0.732)	0.719
Yes ($n = 351$)	0.023 (0.012–0.041)		6.2 (3.3–12.5)		0.27 (0.18–0.53)		0.402 (0.223–0.752)	
Diabetic retinopathy								
No ($n = 300$)	0.029 (0.014–0.046)	0.006	6.3 (3.3–13.9)	0.031	0.29 (0.19–0.62)	0.207	0.350 (0.208–0.691)	0.369
Yes ($n = 160$)	0.021 (0.012–0.035)		7.1 (3.6–13.5)		0.29 (0.18–0.47)		0.513 (0.262–0.853)	
Diabetic Neuropathy								
No ($n = 439$)	0.028 (0.014–0.046)	0.084	6.4 (3.4–13.9)	0.518	0.29 (0.19–0.53)	0.948	0.349 (0.208–0.708)	0.029
Yes ($n = 21$)	0.023 (0.012–0.035)		7.1 (3.5–13.5)		0.30 (0.17–0.62)		0.471 (0.265–0.757)	
PAD								
No ($n = 378$)	0.024 (0.013–0.043)	0.334	6.4 (3.4–13.5)	0.355	0.29 (0.19–0.55)	0.705	0.381 (0.219–0.746)	0.812
Yes ($n = 82$)	0.030 (0.017–0.054)		8.8 (3.6–15.3)		0.32 (0.14–0.65)		0.352 (0.251–0.794)	
CAD								
No ($n = 378$)	0.027 (0.014–0.046)	0.012	6.5 (3.4–13.9)	0.978	0.29 (0.19–0.56)	0.347	0.364 (0.209–0.740)	0.196
Yes ($n = 82$)	0.020 (0.012–0.033)		6.4 (4.0–12.0)		0.26 (0.16–0.59)		0.429 (0.287–0.757)	
Stroke								
No ($n = 439$)	0.026 (0.014–0.044)	0.052	6.5 (3.4–14.0)	0.264	0.29 (0.19–0.58)	0.085	0.385 (0.223–0.746)	0.716
Yes ($n = 21$)	0.015 (0.010–0.023)		5.5 (3.4–8.4)		0.22 (0.14–0.46)		0.346 (0.211–0.744)	

$1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; CAD, coronary artery disease; IQR, interquartile range; PAD, peripheral arterial disease.

Table 3. The relationship of vitamin D_3 and its epimers to complications in the group of nondiabetic control participants ($n = 290$).

	$1,25(OH)_2D_3$ (ng/dl) median (range)	*p* value	$25(OH)D_3$ (ng/dl) median (range)	*p* value	$24,25(OH)_2D_3$ (ng/dl) median (IQR)	*p* value	$3epi25(OH)D_3$ (ng/dl) median (IQR)	*p* value
Gender								
Male	0.048 (0.031–0.069)	0.001	10.68 (6.96–15.78)	<0.001	0.44 (0.26–0.69)	0.007	0.24 (5.17)	0.081
Female	0.036 (0.017–0.059)		6.21 (3.38–10.82)		0.30 (0.17–0.58)		0.12 (7.15)	
Hypertension								
No	0.047 (0.030–0.070)	0.001	9.11 (5.14–15.18)	0.350	0.39 (0.20–0.68)	0.557	0.17 (5.17)	0.868
Yes	0.032 (0.021–0.047)		7.96 (4.37–13.12)		0.37 (0.23–0.55)		0.19 (7.15)	
Dyslipidemia								
No	0.047 (0.029–0.068)	0.001	9.40 (5.92–15.74)	<0.001	0.41 (0.20–0.69)	0.015	0.55 (0.26–1.02)	0.148
Yes	0.035 (0.020–0.054)		7.49 (3.62–12.63)		0.33 (0.21–0.54)		0.36 (0.21–0.84)	

$1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; IQR, interquartile range.

($25(OH)D_3$) and 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) demonstrated that this was not the case (Table 4).

Discussion

This study was specifically powered on retinopathy, and both $25(OH)D_3$ and the active form $1,25(OH)_2D_3$ were highly associated with diabetic retinopathy. The association of vitamin D with diabetes complications is controversial and unclear, with some studies suggesting that it is associated with microvascular complications[9,10] and others that it is not.[23] An association specifically with diabetic retinopathy was reported,[9,24] and low concentrations of vitamin D have been associated with development of microvascular complications in T2DM,[25] which is in accord with the results reported here, and may, in fact, be predictive of diabetic complication risks.[26] This discrepancy between studies may be due to measuring total vitamin D rather than the D_3 form specifically, and perhaps contributed to by having less precise measurement of vitamin D levels than the gold standard measurement employed here. Of note, vitamin D deficiency is not related to seasonal variability in the Middle East, likely due to the population remaining covered throughout the year.[27]

Only low $3epi25(OH)D_3$ levels were found with DN and otherwise $3epi25(OH)D_3$ did not relate to any other complication. Little is known about the epimers of vitamin D, and the assumption has been that they are biologically less potent.[28,29] It is therefore unclear if this is a random chance observation or of specific significance that needs further clarification on the role of vitamin D epimers in disease, and specifically in diabetes.

The metabolite $24,25(OH)_2D_3$ was highly associated with dyslipidemia, and may just reflect $25(OH)D_3$ concentrations that were also highly associated with dyslipidemia; however, $24,25(OH)_2D_3$ may not be an inactive metabolite, as it has been shown to induce non-genomic signaling pathways and to suppress Apo A-1 in Hep G cells,[30] and it may have a physiological role in growth plate formation;[11] therefore, a direct effect on lipid metabolism cannot be excluded.

Hypertension specifically was associated with lower $1,25(OH)_2D_3$ levels for those with and without diabetes. No relationship of 25(OH)D with hypertension was found in a meta-analysis,[31] in accordance with the findings here for $25(OH)D_3$, $24,25(OH)_2D_3$ or the $3epi25(OH)D_3$. This suggests that only low $1,25(OH)_2D_3$ is related to hypertension and has therefore been missed in

Table 4. Correlation of eGFR with levels of vitamin D and its epimers in patients with diabetes.

Diabetic + control participants		
	eGFR	
	R	***p* value**
$1,25(OH)_2D_3$	0.141	0.001
$25(OH)D_3$	−0.028	0.461
$24,25(OH)_2D_3$	0.096	0.012
Diabetic patients		
	eGFR	
	R	***p* value**
$1,25(OH)_2D_3$	0.067	0.242
$25(OH)D_3$	−0.032	0.507
$24,25(OH)_2D_3$	0.148	0.002
Controls		
	eGFR	
	R	***p* value**
$1,25(OH)_2D_3$	0.108	0.119
$25(OH)D_3$	−0.112	0.076
$24,25(OH)_2D_3$	−0.063	0.324

eGFR, estimated glomerular filtration rate; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3.

the past; however, as $1,25(OH)_2D_2$ was not measured, we do not know if this is specifically related to the $1,25(OH)_2D_3$ metabolite.

Dyslipidemia was significantly associated with lower $25(OH)_2D_3$, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ for both participants with and without diabetes in accord with what others have reported.[32]

Lower $1,25(OH)_2D_3$ levels were found with CAD, though this was not seen for $25(OH)D_3$, $24,25(OH)_2D_3$ or $3epi25(OH)D_3$. Vitamin D has been associated epidemiologically with CAD, though a systematic review concluded that there was no effect on vascular function.[33] This study was not powered for this outcome and, therefore, future studies are needed to determine if this is a true association, although vitamin D_3 supplements seem to be of limited therapeutic impact.[33] It is of note that no vitamin D_3 metabolite was associated with either PAD or stroke; however, vitamin D has been associated with increased risk of PAD[34] and stroke in some studies.[35]

The lower $25(OH)D_3$ concentrations in the Qatari T2DM females *versus* males are also in accord with other studies,[36] though this is not a universal finding.[9] However, despite the lower vitamin D levels in women, they did not have a higher rate of diabetes.

The strength of this study was the number of participants, all of whom were vitamin D_3 supplement naive, and the use of state-of-the-art measurement of vitamin D_3 metabolites in this homogeneous population. However, this was a cross-sectional observational study that could not address the underlying causality or mechanisms, and interventional vitamin D_3 studies are needed. All of the participants were Qatari, but the controls without T2DM were recruited from the relatives that accompanied the patients to clinic, which may have resulted in a source of bias. Vitamin D deficiency is not related to seasonal variability in the Middle East, likely due to the population remaining covered throughout the year,[27] and therefore would not have been a confounder.

Conclusion

These data showed that both $25(OH)D_3$ and the active form $1,25(OH)_2D_3$ were highly associated with diabetic retinopathy for which the study was powered, but in addition a relationship between $1,25(OH)_2D_3$ concentrations and hypertension and CAD was found: $25(OH)D_3$ and $24,25(OH)_2D_3$ levels were associated with dyslipidemia, giving further support to the evidence that vitamin D metabolites may have a role in the development of diabetes as well as cardiovascular complications.

Author contributions

AEB researched the data and wrote the manuscript. SRD performed the statistical analysis. AL and HRM performed the vitamin D measurements. AR, OMC, AJ and JAS researched the data. RGC, SLA and CAK designed the study and contributed to the discussion. SLA is the guarantor of this work.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

Ethical approval

The study was approved by Weill Cornell IRB (IRB# 13-00063) and all participants provided written informed consent. The conduct of the trial was in accordance with ICH GCP and the Declaration of Helsinki.

Consent to publish

All authors agree to publish this work.

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Availability of data and materials

All data are available upon reasonable request by contacting the corresponding author.

Supplemental material

Supplemental material for this article is available online.

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