Evaluation of vitamin D relationship with type 2 diabetes and systolic blood pressure

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ABSTRACT
Objective: To investigate whether relationships exist among vitamin D, type 2 diabetes mellitus (T2DM), and blood pressure in Trinidadian subjects with T2DM.

Research design and methods: This was a case–controlled study to determine if vitamin D levels were lower in patients with T2DM. After data analysis, an exploratory hypothesis of vitamin D relationship to systolic blood pressure (SBP) was developed. Plasma calcifediol (25(OH)D) concentrations were used as a measurement for vitamin D levels and were determined by ELISA. Cholesterol levels were measured by an automated dry chemistry analyzer and blood pressure was measured using an automatic blood pressure monitor.

Results: There was no significant difference (p=0.139, n=76) in 25(OH)D levels between patients with T2DM and controls. Subjects with SBP above 130 mm Hg were 8 times more likely to have a 25(OH)D plasma concentration above 25 ng/mL (OR 7.9 (2.2 to 28.7)), and were 5 times (OR 4.7 (1.7 to 15.1)) more likely to have a 25(OH)D plasma concentration above 30 ng/mL (OR 7.5 (2.3–24.2)). Vitamin D levels moderately and positively correlated with SBP (r=0.38, p=0.001).

Conclusions: There was no significant difference in the 25(OH)D levels between patients with T2DM and controls (p=0.139). Patients with SBP under 130 mm Hg were 8 times more likely to have a vitamin D level above 25 ng/mL (OR 7.9 (2.2 to 28.7)). Further investigations are required to examine the relationship between vitamin D and SBP.

INTRODUCTION
Vitamin D is well known for its role in calcium and bone metabolism; however, its deficiency may play a role in type 2 diabetes mellitus (T2DM). The exact pathogenesis of T2DM remains unknown, but the condition is a result of different environmental and biochemical factors. It is important then to look at different biochemical components to determine their role in T2DM. The biochemical component that is of particular interest in this study is vitamin D.

Cholecalciferol (vitamin D3) is photosynthesized from 7-dehydroxycholecalciferol within the epidermal layer of the skin. When ultraviolet B (UVB) radiation from a source such as the sun strikes the skin, 7-dehydroxycholecalciferol transforms into vitamin D3. Vitamin D3 undergoes hydroxylation in the liver to form calcifediol (25-hydroxyvitamin D). Calcifediol (25(OH)D) is further hydroxylated in the kidneys to form calcitriol (active form of vitamin D). Calcitriol (1,25-dihydroxyvitamin D3) mediates its metabolic effect by binding to the Vitamin D Receptor (VDR) found inside the cell.

Calcitriol (1,25(OH)2D) has a half-life of ~4 hours, so it is not effective in reflecting the overall vitamin D status of humans. 25(OH)D has a minimal circulating half-life of 2 months since it can be stored and released from adipose and muscle tissue. For the purposes of this study, 25(OH)D will be used to reflect the subjects’ vitamin D levels.

Significance of this study
What is already known about this subject?
▪ An unclear relationship between type 2 diabetes mellitus (T2DM) and the vitamin D axis.
▪ Vitamin D levels are lower in hypertensive individuals as compared with normotensive individuals.

What are the new findings?
▪ Vitamin D levels are higher in patients with systolic blood pressure (SBP) above 130 mm Hg as compared with patients with SBP lower than 130 mm Hg.

How might these results change the focus of research or clinical practice?
▪ Future studies of vitamin D relationship to blood pressure and T2DM need to be conducted in tropical regions since vitamin D is regarded as a ‘sunshine vitamin’.

All study participants are from the Caribbean, in the country of Trinidad (10.6667° N; 61.5167° W), which generally has a warm and sunny climate throughout the year. The study participants generally have skin type V (brown) according to the Fitzpatrick classification of skin type. It is expected that most study participants...
experience sufficient sunlight which can result in participants having sufficient levels of 25(OH)D.\textsuperscript{10} \textsuperscript{11} If a patient normally remains indoors, synthesis of vitamin D\textsubscript{3} from sunlight will be low but vitamin D can be obtained from fish, eggs, and vitamin D fortified milk.\textsuperscript{10} Vitamin D deficiency and insufficiency are characterised as 25(OH)D <20 and 21–29 ng/mL, respectively.\textsuperscript{5}

Studies have shown that T2DM and hypertension are related;\textsuperscript{12} \textsuperscript{14} however, 25(OH)D’s relation to blood pressure (BP) is unclear and the literature surveyed for 25(OH)D and BP gave conflicting reports of this relationship.\textsuperscript{10} \textsuperscript{11} \textsuperscript{15} In this study, it was hypothesized that 25(OH)D levels were significantly lower in patients with T2DM and systolic BP (SBP) over 130 mm Hg.

**RESEARCH DESIGN AND METHODS**

Ethical approval to conduct the study was obtained from the University of the West Indies (UWI), St. Augustine, the South West Regional Health Authority (SWRHA), and the North Central Regional Health Authority (NCRHA) in Trinidad and Tobago. Subjects were randomly chosen at the Eric Williams Medical Sciences Complex (EWMSC) and San Fernando General Hospital (SFGH) in Trinidad. The sample size chosen for the study was 80 because of limitations in resources for assays. Both effect size and sample size with 80% power were estimated for future studies.

Hospital records were used to select at random patients who were diagnosed with T2DM. From the hospital records, patients with T2DM had a history of glucose (FBG) values of ≥6.5% and ≥120 mg/dL, respectively. Patients with T1DM; having any form of liver disease; having thyroid or parathyroid problems; being pregnant; and being under the age of 18. Additionally, exclusion criteria for controls only were HbA1c or FBG values of ≥6.5% or ≥120 mg/dL, respectively.

Subjects fasted and did not take any medication 8–10 hours before venous blood samples were drawn. On the morning of the blood draw, before venous samples were taken, the subjects’ height and mass were measured. SBP and diastolic BP (DBP) were measured using a digital BP monitor. Venous blood samples drawn into blood collection tubes were centrifuged at 2000 g and separated into serum and plasma fractions. All blood fractions and two whole blood samples were stored at −70°C subsequent to analysis. Plasma 25(OH)D was determined by ELISA (ADL-900-215, Enzo Life Sciences, USA). Serum cholesterol was assayed using an automated dry chemistry analyzer (Cobas 6000, Roche Diagnostics, USA). Four subjects were removed from the study because of blood sample hemolysis. The final sample size for the study was 76 subjects (24 males and 52 females).

Software packages used for statistical analyses were IBM SPSS Statistics V.21, Minitab 16, and G*Power 3.1.7. Statistical analyses performed were Anderson-Darling test, independent t-test, Mann-Whitney U-test, Fisher’s exact test, Spearman’s correlation (r), logistic regression, and general linear model (GLM) univariate

| Parameter | All subjects, n=76 (%) | Controls, n=35 (%) | Patients with T2DM, n=41 (%) | 25(OH)D>25 ng/mL, n=58 (%) | 25(OH)D≤25 ng/mL, n=18 (%) |
|-----------|-----------------------|-------------------|-----------------------------|---------------------------|---------------------------|
| Age, years |                       |                   |                             |                           |                           |
| 40–50     | 15 (20)               | 12 (34)           | 4 (10)                      | 13 (22)                   | 7 (39)                    |
| 51–70     | 51 (67)               | 18 (52)           | 32 (78)                     | 34 (59)                   | 10 (55)                   |
| 70–80     | 10 (13)               | 5 (14)            | 5 (12)                      | 11 (19)                   | 1 (6)                     |
| Ethnicity |                       |                   |                             |                           |                           |
| East Indian| 43 (56)               | 16 (46)           | 27 (66)                     | 31 (53)                   | 12 (67)                   |
| African   | 22 (29)               | 14 (40)           | 8 (19)                      | 20 (35)                   | 2 (11)                    |
| Mixed     | 11 (15)               | 5 (14)            | 6 (15)                      | 7 (12)                    | 4 (22)                    |
| BMI, kg/m\textsuperscript{2} |                      |                   |                             |                           |                           |
| <25       | 15 (20)               | 10 (29)           | 5 (12)                      | 11 (19)                   | 4 (22)                    |
| 25–30     | 26 (34)               | 11 (31)           | 15 (37)                     | 19 (33)                   | 7 (39)                    |
| >30       | 35 (46)               | 14 (40)           | 21 (51)                     | 28 (48)                   | 7 (39)                    |
| Subjects on antihypertensive medication | 40 (53) | 15 (43) | 25 (61) | 33 (57) | 7 (39) |
| Kidney disease | 7 (9)    | 5 (14)   | 2 (5)   | 7 (12)  | 0 (0)  |

BMI, body mass index; T2DM, type 2 diabetes mellitus.
analysis. A p value <0.05 meant a statistically significant result. SBP was transformed via natural logarithm (ln) in order for the requirements of the GLM to be met.

RESULTS
Overall, no relationship was found among 25(OH)D, T2DM and use of specific anti-hypertensive agents. A significant relationship existed between 25(OH)D and SBP.

Table 2 Characteristics of the variables in study in relation to T2DM

| Variable                  | Total (n=76) | Controls (n=35) | Patients with T2DM (n=41) | Distribution | Test statistic | p Value | Effect sized | Sample size estimate, 80% power |
|---------------------------|--------------|-----------------|---------------------------|--------------|----------------|---------|--------------|-------------------------------|
| Age (years)               | 58.9±9.6     | 57.7±10.8       | 59.8±8.6                  | Normal       | t              | 0.375   | 0.2          | 792                           |
| BMI (kg/m²)               | 30.2±6.8     | 29.1±6.5        | 31.1±7.0                  | 3P-Weibull   | U              | 0.293   | 0.3          | 410                           |
| SBP (mm Hg)               | 145.6±22.7   | 141±20          | 149.8±24.2                | Normal       | t              | 0.095   | 0.4          | 200                           |
| DBP (mm Hg)               | 87.7±10.8    | 87±11           | 88.3±10.9                 | Normal       | t              | 0.598   | 0.1          | 3162                          |
| 25(OH)D (ng/mL)           | 38.3±17.8    | 41.3±18.6       | 35.7±16.9                 | 3P-Weibull   | U              | 0.139   | 0.3          | 410                           |
| Chol (mg/dL)              | 191.0±49.8   | 195.1±49.0      | 187.6±50.8                | Normal       | t              | 0.459   | 0.2          | 792                           |

25(OH)D, vitamin D; BMI, body mass index; Chol, cholesterol; DBP, diastolic blood pressure; SBP, systolic blood pressure; t, independent t-test; T2DM, type 2 diabetes mellitus; U, Mann-Whitney U-test.

Table 3 Means of 25(OH)D, adjusted for gender and age

| Subjects                  | Gender      | Adjusted mean 25(OH)D, ng/mL | 95% CI | p Value |
|---------------------------|-------------|------------------------------|--------|---------|
| Controls, n=35            | Male, n=12  | 40.4                         | 30.3   | 0.472   |
|                           | Female, n=23| 43.6                         | 35.6   |         |
| Patients with T2DM, n=41  | Male, n=12  | 36.3                         | 24.8   |         |
|                           | Female, n=29| 33.5                         | 26.7   |         |

T2DM, type 2 diabetes mellitus.

Table 4 GLM output for ln (SBP) as a dependent variable

| Source                  | Significance | Effect size (d) | Observed power (%) |
|-------------------------|--------------|-----------------|--------------------|
| Corrected model         | 0.006        | 0.4             | 86                 |
| T2DM/controls           | 0.046        | 0.2             | 52                 |
| Gender                  | 0.558        | 0.1             | 9                  |
| 25(OH)D>25 ng/mL        | 0.002*       | 0.4             | 89                 |

*p<0.05=statistically significant.
GLM, general linear model; ln, natural logarithm; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

Table 5 displayed the OR, adjusted for age, gender, and T2DM diagnosis, when separating 25(OH)D and BP into categorical variables. The 25(OH)D levels were divided into two categories for SBP >130 and >140 mm Hg, respectively (p<0.001, 25(OH)D data were log-normal transformed to follow normal distribution). A Mann-Whitney U-test was also applied for the 25(OH)D between the SBP >130 and >130 mm Hg categories, which gave a significant difference (p<0.001). There was a moderately positive correlation between SBP and 25(OH)D (r=0.38, p=0.001).

Table 4 displayed the results of a GLM univariate analysis applied to ln (SBP) as a dependent variable with categorical variables T2DM/controls, gender, and 25(OH)D >25 and ≤25 ng/mL as fixed factors. There was a significant difference between 25(OH)D levels >25 and ≤25 ng/mL for the dependent variable ln (SBP).

Table 5 displayed the OR, adjusted for age, gender, and T2DM diagnosis, when separating 25(OH)D and BP into categorical variables. The 25(OH)D levels were divided into two categories for concentrations >30 and >25 ng/mL. SBP data were divided into two categories for SBP values >130 and >140 mm Hg. SBP/DBP data were divided into five categories for SBP/DBP >130/90, >130/100, >135/100, >140/80, and >140/90 mm Hg.
Table 5  Adjusted ORs for blood pressure and 25(OH)D

| Blood pressure, mm Hg | Adjusted OR (95% CI) | 25(OH)D>25 ng/mL |
|-----------------------|----------------------|------------------|
| SBP>130               | 4.7 (1.5 to 15.1)    | p=0.009*         |
| SBP>140               | 2.0 (0.8 to 5.4)     | p=0.157          |
| SBP/DBP>130/90       | 6.8 (1.8 to 25.4)    | p=0.005*         |
| SBP/DBP>130/100      | 5.5 (1.7 to 18.3)    | p=0.005*         |
| SBP/DBP>135/100      | 4.0 (1.4 to 11.5)    | p=0.010*         |
| SBP/DBP>140/80       | 2.3 (0.9 to 6.4)     | p=0.096          |
| SBP/DBP>140/90       | 2.3 (0.9 to 6.4)     | p=0.096          |

n=76; OR adjusted for Age, gender, T2DM diagnosis.
*p<0.05=statistically significant.
DBP, diastolic blood pressure; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

Table 6  Contingency table for 25(OH)D categories and antihypertensive therapy

| Category                        | 25(OH)D>25 ng/mL | 25(OH)D≤25 ng/mL | p-Value |
|---------------------------------|------------------|-----------------|---------|
| Subjects not on antihypertensive drug therapy | 25               | 11              | 0.181*  |
| Subjects on antihypertensive drug therapy | 33               | 7               |         |
| Antihypertensive drug used      |                  |                 |         |
| None                            | 25               | 11              | 0.386†  |
| ACE inhibitors                  | 14               | 4               |         |
| β-Blockers                      | 8                | 0               |         |
| Diuretics                       | 1                | 1               |         |
| Calcium channel blockers        | 4                | 0               |         |
| α-Blockers                      | 3                | 1               |         |
| Angiotensin receptor blockers   | 1                | 1               |         |
| Other drugs                     | 2                | 0               |         |

*p<0.05=statistically significant.
†Fisher’s exact test.

DISCUSSION

In table 2, the results of the Mann-Whitney U-test for T2DM and controls indicated no significant difference in 25(OH)D between patients with T2DM and controls (p=0.139). After adjustments for gender and age were made, table 2 also indicated no significant differences in 25(OH)D levels between patients with T2DM and controls (p=0.472). The tropical island of Trinidad, in which the subjects reside, generally has a sunny climate which may account for the production of vitamin D being similar in both groups. Vitamin D is synthesized when sunlight strikes the skin, resulting in the conversion of 7-dehydroxycholesterol to vitamin D₃. Studies have demonstrated that low 25(OH)D levels are related to T2DM susceptibility; thus, for the Trinidadian population, the problem with the vitamin D axis is that there may be a problem with the VDR. The VDR gene polymorphism may cause subtle changes in the three-dimensional conformation of VDRs. These subtle conformational changes may result in individuals having VDRs with different affinities toward 1,25(OH)₂D₃. The differences in affinities may account for an individual's susceptibility toward T2DM. It can be hypothesized then that someone with VDR receptors of low 1,25(OH)₂D₃ affinity is susceptible to T2DM. Thus, further investigations are required to elucidate the VDR polymorphisms, which may cause variance in VDRs, in relation to T2DM.

The study undertaken did not meet the requirements of the estimated sample size, so it would be noteworthy...
to expand the sample size in order to effectively draw a better conclusion on the correlation between 25(OH)D and SBP in the Trinidadian population. The GLM univariate analysis displayed in table 4 compensated for the weak sample size when considering the 25(OH)D categories >25 and ≤25 ng/mL in relation to ln SBP (p=0.002, 89% power). The 25 ng/mL 25(OH)D categories were examined in relation to the use of a specific antihypertensive; however, table 6 displayed that there was no significant relationship. This may indicate that antihypertensive medication did not influence a change in vitamin D levels.

The study is a pilot study, which would enable researchers to better determine future sample sizes with sufficient power in relation to a particular outcome variable of interest. There is some obvious complexity in relating T2DM to the vitamin D axis. Based on current studies and the pilot study conducted, it seems that 25(OH)D cannot be used as a biochemical marker or predictor for T2DM, but there is some role of vitamin D in the pathogenesis of T2DM. Further elucidation of the binding interaction of vitamin D to VDR as well as VDR polymorphisms is required to obtain clarity on a possible relationship between T2DM and the vitamin D axis.

Acknowledgements The authors would like to thank the staff at the Eric Williams Medical Sciences Complex and San Fernando General Hospital, Trinidad and Tobago.

Contributors TGR contributed to the acquisition of data, analysis, manuscript writing, revisions and accuracy of work. SBN contributed to the conception, drafting, integrity of work, sourcing of funding and correspondence. Both the authors contributed to the final version of the article to be published.

Funding This work was supported by the University of the West Indies, St. Augustine Campus (grant number 26600457573).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Campus Ethics Committee of the University of the West Indies.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All data collected have been published in the article. Additional genetic data will be produced for a future study; however, DNA extraction has not started. The future genetic analysis, which focuses on the vitamin D receptor gene polymorphism, will be available to the research team and will be published in a future study.

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