Association of Differing Qatari Genotypes with Vitamin D Metabolites

Youssra Dakroury

In partial fulfilment of the

requirements for the degree of

M.Sc. by Thesis

Submitted to The University of Hull and The University of York

Hull York Medical School

November 2020

Abstract

This work aimed to determine the possible association between Qatari genotypes and vitamin-D deficiency and diabetes complications. Through next-generation exome sequencing three major genetic Qatari genotypes were determined, Q1 - Bedouin, Q2 - Persian-South Asian and Q3 - African. The hypothesis was that Qatari genotypes would affect vitamin D and metabolite levels independent of cultural factors, perhaps exacerbated by diabetes.

Materials and Methods. Affymetrix 500k SNP arrays determined 398 Qataris genotype (mean age 49.8 years, 56.8% male; type 2 diabetes (T2DM) 220; control 178). LC-MS/MS analysis measured 1,25-dihydroxyvitamin-D ($1,25(OH)_2D$), 25-hydroxyvitamin-D2 ($25(OH)D_2$), 25-hydroxyvitamin-D3 ($25(OH)D_3$), 24,25-dihydroxyvitamin-D ($24,25(OH)_2D$) and 25-hydroxy-3epi-Vitamin-D (3epi25(OH)D). The same study population was used to investigate the association of diabetes and its complications with various genotypes.

Results. There was no difference in 25(OH)D levels between genotype groups; however, $1,25(OH)_2D$ was higher for Q2 and $24,25(OH)_2D$ was higher in Q1 compared to the 'admixed' group. Additionally, the genotype-based ancestry and type 2 diabetes (T2DM) prevalence: 164 (41.2%) with Q1, 60.4% with T2DM; 149 (37.4%) with Q2, 49.7% with T2DM; 31 (7.8%) with Q3, 61.3% with T2DM; and 54 (13.6%) with "admixed", 51.9% with T2DM. In patients with diabetes, hypertension ($p<0.035$) and retinopathy ($p<0.016$) were greater in Q3.

Conclusion. Overall, the study population was vitamin-D deficient, total 25(OH)D was higher in patients with concomitant T2DM, $1,25(OH)_2D$, $24,25(OH)_2D$ and 3epi25(OH)D were lower in diabetes. Vitamin D levels were not associated with a specific genotype. Q3 was found to have a higher frequency of diabetic retinopathy and hypertension.

Contents

List of Figures

List of Tables

Acknowledgements

I would like to first thank both my thesis advisors: - **Prof Thozhukat Sathyapalan** Chair in diabetes, endocrinology and metabolism and an Honorary Consultant Endocrinologist at **Hull University** and **Prof Stephen Atkin** Head of School of Postgraduate Studies & Research of the **RCSI Bahrain** for their enormous encouragement and support since the first day of my MSc journey until the submission day. The door to Prof. Atkin office in WCMQ was always open whenever I ran into a trouble spot or had a question about my research or writing. As well as Dr. Sathya, I was able to reach him through video calls and communicate with him since starting my MSc. studies. They both consistently allowed this paper to be my own work but steered me in the right direction whenever they thought I needed it. I was motivated by my supervisors to apply for the masters, and it would have never been completed without their continuous positivity and support.

I would also like to thank Professor David Hepburn BMSc MBChB MML MD FRCP(Lond) FHEA, Consultant Physician (Honorary Professor), Clinical Dean for Hull University Teaching Hospitals NHS Trust, Director of Undergraduate Medical Education, who was the perfect guiding director all through the TAP meetings. I still remember every single advice that he gave me about writing and organizing the collected information.

Many thanks to Dr. Aziz Asghar Lecturer and neuroscientist at Hull University and Hull York Medical School Postgraduate Research Tutor who was involved in the initiation of this MSc project.

Without all supervisor's passionate participation and input, the MSc research project and thesis writing could have never been successfully conducted.

I would like to acknowledge **ELAINE BROOKES** Postgraduate Research Administrator at University of Hull, as the coordinator of this MSc, and I am gratefully indebted to her for her very valuable efforts and patience through the entire project.

Finally, I must express my very profound gratitude to my parents and to my spouse for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would have not been possible without them. Thank you.

Author

Youssra Dakroury

Publications that have resulted from this work

Dakroury Y, Butler AE, Dargham SR, Latif A, Robay A, Crystal RG, et al. Association of Differing Qatari Genotypes with Vitamin D Metabolites. Int J Endocrinol. 2020;2020:7831590. [1]

Dakroury Y, Atkin SL, Dargham SR, Robay A, Rodriguez-Flores J, Crystal RG, et al. Qatari Genotype May Contribute to Complications in T2DM. J Diabetes Res. 2020;2020:6356973. [2]

Authors Declaration

'*I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this work is my own and does not contain any unacknowledged work from any other sources*'.

Chapter 1: Introduction

Type 2 diabetes (T2DM)

T2DM is a chronic metabolic disease characterized by increased blood glucose levels due to heightened peripheral tissue insulin resistance with possible concomitant insulin deficiency. T2DM represents a major public health challenge in the twenty-first century for both developed and developing countries, accounting for the seventh leading cause of death in the United States. [3, 4] It has been estimated that there will be approximately 629 million people with diabetes by 2045. [5] T2DM has additional economic considerations as it increases strain on healthcare spending, particularly for low- and middle-income countries. [5] The total cost of diabetes in the United States in 2017 was $327 billion. [4]

Diabetes is a chronic metabolic disease with the hallmark characteristic of hyperglycemia through insulin insufficiency, resistance, or both. Accordingly, various mechanisms that lead to the disruption of glucose metabolism result in diabetes. [6] Based on these mechanisms, the three most common types are type 1 Diabetes Mellitus(T1DM), T2DM, and gestational diabetes mellitus (GDM). [7-9] Hyperglycemia leads to further metabolic stress and oxidation leading to related complications in the vascular system which are frequently grouped into microvascular and macrovascular that further add to associated morbidity and costs. [10] A variety of risk factors have been established to increase the risk of diabetes, notably both genetic and environmental. Studies dedicated to US adults have determined that common modifiable risk factors are related to obesity and inactivity. Thus, interventions aimed at decreasing these are employed to prevent diabetes. [4]

Therapeutic approaches for diabetes.

Therapeutic approaches vary according to the type of diabetes but eventually present with convergence. T1DM is characterized by an absolute insulin deficiency most frequently from autoimmune destruction of pancreatic β-cells. α-cell autoantibodies against glutamic acid decarboxylase (GAD65), islet antigen-2, ZnT8 transporter or insulin have all been implicated. [5,

10] T1DM is frequently diagnosed during childhood but current estimates signal that ~85% of T1DM patients are adults. Age of diagnosis may not only be related to the age-onset of symptoms, but also other determinants such as access to healthcare. T1DM is typically diagnosed during childhood, with the mean age of diagnosis ranging across regions from 5 to 11 years. Overall, however, it can occur in adults and 84% of patients with T1DM are adults. Of 1286 individuals with type 1 diabetes, 537 (42%, 95% CI 39–45) were diagnosed when aged 31–60 years and 749 (58%, 55–61) were diagnosed when aged 30 years or younger ($p<0{\cdot}0001$). Type 1 diabetes accounted for 537 (4%, 4–5) of the total 12 233 diabetes cases diagnosed between ages 31 and 60 years and 749 (74%, 71–76) of the 1017 diabetes cases diagnosed aged 30 years or younger. [11] The mainstay treatment for T1DM considering an absolute insulin deficiency is insulin replacement in the form of subcutaneous insulin or insulin pumps. Future therapeutic prospects involve cell transplant and stem cell therapies. [12]

T2DM, as previously mentioned, is characterized by hallmark peripheral tissue insulin resistance with possible concomitant insulin resistance. Exercise and lifestyle interventions can favorably modify insulin resistance. Various treatments are available that aim to modify through various mechanisms peripheral insulin resistance, glucose uptake as well as excretion. Lastly, insulin supplementation is also available for patients with T2DM who are refractory to other therapies. Surgical interventions such as bariatric surgery have also been suggested as possible treatments. [13]

GDM is a hyperglycemic state occurring during pregnancy and resolves after birth. GDM is the most common medical complication in pregnant women. [14] The risk factors of GDM include later age at childbearing, maternal overweight/obesity, and a family history of T2DM. Physical activity and dietary modification are primary treatments for GDM; however, when normoglycemia is not achieved, then insulin treatment is often implemented, though in many countries oral therapy such as metformin is considered safe. [15] GDM carries a risk of long-term complications such as obesity, cardiovascular disease, and impaired glucose metabolism in both the mother and the infant. [14]

Vitamin D metabolism

Vitamin D3 is a liposoluble vitamin which is synthesized by human skin through ultraviolet (UV) light exposure from 7-dehydrocholesterol in two steps. The B ring is broken by UV light that results in the formation of pre-D_3, which subsequently is isomerized to D_3 in a thermo-sensitive reaction. This step is regulated and influenced by both skin pigmentation and light exposure. Both inadequate exposure as well darker pigmentation have been associated with decreased production. [16, 17] Vitamin D can be consumed in ergocalciferol/VD2 form through diet, especially through foods such as fatty fish like tuna, salmon, fortified dairy products, cheese and beef liver. However, it's been established that due to structural differences, Vitamin D3 has a longer half-life than D2. This shorter half-life leads to decreased conversion to 25 hydroxyvitamin D 25OHD. [16]

The next step in the Vitamin D pathways includes 25-hydroxyvitamin D-25(OH)D, which is converted to 25-hydroxylase (25-OHase) in the liver, followed by another hydroxylation step by 25-hydroxyvitamin D-1αhydroxylase (1αOHase) to the biologically active form of VD – $1,25(OH)_2D$ (calcitriol) that occurs in the kidneys. This step is under the regulation and influence of a variety of hormones such as parathormone (PTH), estrogen, thyroxin, cortisol, insulin as well as serum electrolytes such as magnesium and calcium. [16]

Vitamin D is a fat-soluble vitamin with two main forms, Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol). Its main role lies within the regulation of the absorption of calcium magnesium and phosphate in the intestine. [17] Vitamin D3 (25OHD3) is activated through hydroxylation in the liver and kidney to 1,25 dihydroxyvitaminD3 (1,25OHD3calcitriol)". [16, 18]

This process, however, can also be catalyzed at extrarenal sites. An example associated with disease processes such as sarcoidosis sees macrophages with 1 alpha-hydroxylase activity through type 2 interferon response, creating 1,25(OH)d which has binding capacity for Vitamin D receptors. [17, 19]

Vitamin D levels can also be affected by intrinsic metabolic processes such as obesity. Increased adipose tissue favors Vitamin D accumulation within adipose tissue, reducing the bioavailability of Vitamin D. [20] Given the plethora of associated conditions leading to deficiency such as decreased exposure, poor diet, lifestyle and such, vitamin supplementation is commonly available in most countries. Vitamin D deficiency is a worldwide concern. [21] As previously mentioned, adequate sunlight exposure is paramount for adequate synthesis, and thus countries with naturally low levels of sunlight, as well as regions due to cultural and religious customs such as full body coverage have increased prevalence of deficiency. [22, 23]

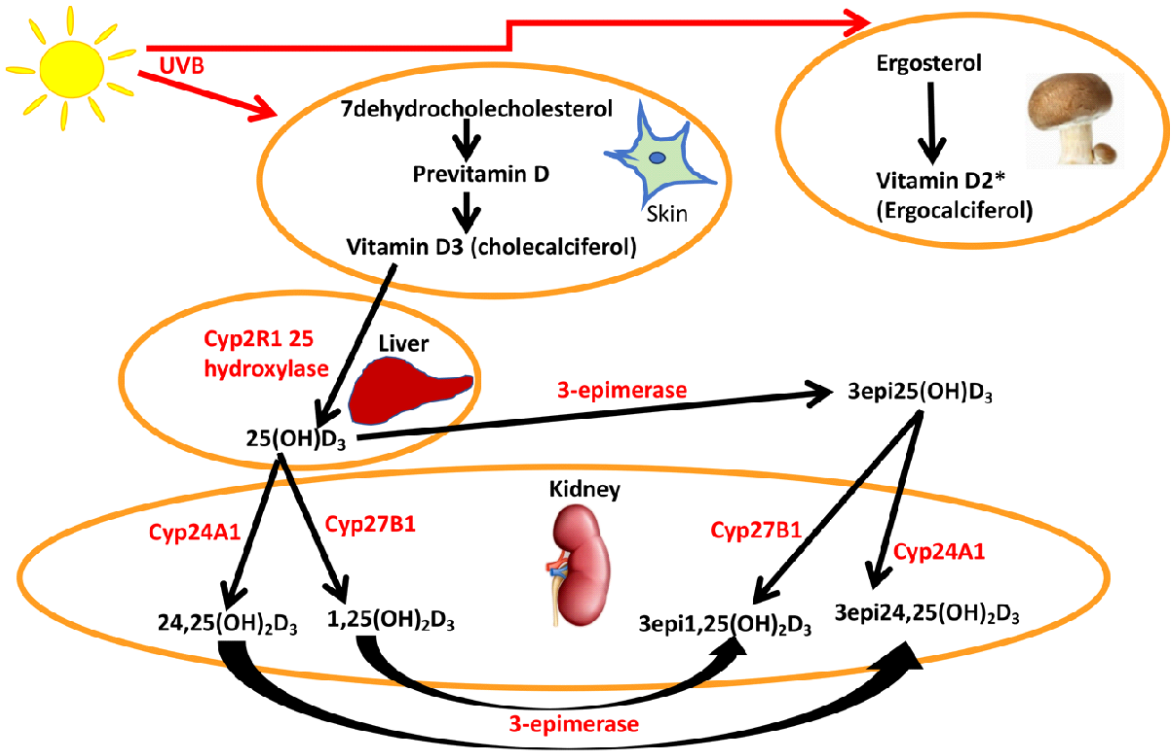

Figure 1. Vitamin D is a fat-soluble vitamin with 2 main forms, Vitamin D3(cholecalciferol) and Vitamin D2(ergocalciferol). Its main role lies within the regulation of the absorption of calcium magnesium and phosphate in the intestine. Vitamin D3 (25OHD3) is activated through hydroxylation in the liver and kidney to 1,25 dihydroxyvitaminD3 (1,25OHD3calcitriol)" [16]

Prevalence of vitamin D deficiency

Vitamin D deficiency does not have a clear or strict definition; however, it is estimated to be highly prevalent worldwide and a significant cause of morbidity. Of the nearly 1 billion estimated worldwide with vitamin D deficiency, half of those who suffer from deficiency are thought to be elderly and/or obese. [23] A study in the adult population from the United States concluded that while 35% of the general population is Vitamin D deficiency, 61% of the elderly population is deficient. [24]

A consensus on the optimal cutoff value for diagnosing Vitamin D deficiency is yet to be determined. A range below 75 nmol/L (or 30 ng/ml) of serum/plasma 25(OH)D concentration is considered vitamin D deficiency by many laboratories in Europe, while the clinical practice guidelines of the Endocrine Society Task Force on Vitamin D used a cutoff level of 50 nmol/L for the United States of America. [25-27] These variations are related to ongoing efforts to determine the optimal cutoff point at which the risk of certain outcomes is significantly increased. Thus, optimal levels may vary between gender, race, and other demographic data.

A recent systematic review and meta-analysis that aimed to determine the prevalence of Vitamin D deficiency in Africa concluded that 20% of people living in Africa were deficient when employing a 30 nmol/L as a cutoff point, while 30% when using 50 nmol/l as a cutoff. A staggering 60% were considered deficient when the cutoff was raised to 75 nmol/l. Authors identified populations with higher prevalence in women, newborns, urban populations, Northern African Countries and South Africa. [28]

In Europe the prevalence of Vitamin D Deficiency is estimated to be 40% using a 50 nmol/L cutoff. Meanwhile, in the United States the reported prevalence of deficiency is estimated to be 20-30%, with variations in certain racial and ethnic subgroups, as African Americans are estimated to have 83% while Hispanics ~70%. [28]

Regarding the Middle East region, a recent study found that among young Saudi women, the prevalence of deficiency was ~80% when using a 25 nmol/L cutoff while adult males had a reported 87.8% prevalence. These findings are like a study from the United Arab Emirates which found an estimated 85.4% prevalence of deficiency. [29]

Available literature from Qatar, suggests the prevalence of vitamin D deficiency is not dissimilar to that of other Northern African and Middle Eastern regions. A recent study showed that almost 70% of the population had 25(OH)D levels below the optimal levels. [30-32] This prevalence increases to 71.4% if a serum level <20 ng/mL was used, and up to 92.7% using broader criteria for its definition. [32] These studies have shown that the prevalence is twice as high in females than males. However, after accounting for Vitamin D insufficiency status, the study concluded there was no difference between genders. [32] This study further analyzed variations in Vitamin D levels through the seasons and concluded springtime had the least deficiency due to the weather allowing people to spend more time outside and by extension, increasing adequate sun exposure. [32]

Vitamin D and diabetes

A large, 20-year longitudinal follow-up study established an inverse relationship between Vitamin D levels and the onset of diabetes. [33, 34] Thus, Vitamin D deficiency has been suggested to increase the risk of T2DM potentially through the mechanism of insulin resistance and beta cell dysfunction. [35-37] Studies on animal models featuring rats determined that Vitamin D Deficiency led to pancreatic beta cell dysfunction. [38] Vitamin D deficiency is reported to exaggerate the features of metabolic syndrome and T2DM. Vitamin D appears to have an antidiabetic effect through activation of calcium and the adenosine monophosphate–activated protein kinase (AMPK) pathway that affects both hepatic glucose and lipid metabolism via. [39]

Recent meta-analysis as well as randomized controlled trials have established the presence of Vitamin D receptors within pancreatic beta cells. These cells had additional capabilities of expressing the enzyme 1-hydroxylase which is encoded by the CUP27B1 gene. Additional links

between the metabolisms and interactions of Vitamin D and glucose metabolism have been established. The promoter region in the gene responsible for insulin contains a Vitamin D related response element that may have additional modulatory effects on T-Cell response to pancreatic beta cells. [31] Thus, certain alterations in these interplaying regions of insulin and Vitamin D could result in heightened immune targeting of pancreatic cells. This notion is supported by a recent study that demonstrated that inflammation through beta cell cytokine production as well as extrapancreatic increases in cytokines led to improper beta cell function and apoptosis. Some further studies have demonstrated that calcitriol has a modulatory effect on IL-1 leading to the inhibition of its activity on beta cell function. [40-44]

Lips et al., studied the implication of Vitamin D deficiency in T2DM, concluding that Vitamin D has stimulatory functions on beta cells and insulin. Insufficient insulin secretion may lead to peripheral resistance and thus establish a possible link between the two conditions. [44] Further indirect relations stem from Vitamin D's impact on lipoprotein and lipid metabolism, leading to increased circulating levels of cholesterol and high-density lipoproteins which contribute to insulin resistance and T2DM. These findings are supported by a case-control study of 2659 Chinese patients. [43]

Due to the immunoregulatory properties of Vitamin D, prior authors have attempted to establish the relationship between Vitamin D deficiency and T1DM. Infante et al., found a high prevalence of deficiency in children with T1DM. [31]

Vitamin D and diabetes related complications

The interaction of Vitamin D and T2DM and its impact of complications have also been studied. Some studies have ascertained that there is a link between Vitamin D deficiency and T2DM with microvascular complications. [41, 42] There is still some debate, vitamin D concentrations have been reported to be lower in those patients with T2DM with microvascular complications. [42] The role of Vitamin D supplementation as a preventive strategy in T2DM and T1DM for complications is yet to be defined, with no current guidelines endorsing its use.

Genotype of the Qatari population

The estimated prevalence of diabetes in Qatar is 20%. This is 2-3x the world average and thus the country faces an increased burden of disease and morbidity through complications. [40] Such is the case of renal failure, as 43% of the current patients undergoing dialysis suffer from diabetic nephropathy. [45] Additionally, over half of the patients with cardiovascular diseases and acute heart failure also have diabetes. [40]

Qatar features a diverse population built through the integration of ancient migration patterns from various regions. The majority of the population originated from the Arabian Peninsula, Oman and Persia, with the minority of the population stemming from Africa and Asia. Arabs are considered descendants of the Arabian Peninsula as well as Bedouins. While Ajam, are descendants who migrated from Persia, while the population from Africa are considered Abd.

There is a high prevalence of incest within the Qatari population. Current estimates suggest that 50% of marriages occur between first- or second-degree cousins. In a study involving 559 Qatari families, there were almost 68% of consanguineous marriages found; this is important to explain the high prevalence of the autosomal recessive disease in Qatar. This complex interplay of marriage customs with a diverse population invites the research into its genetic makeup. Hunter-Zinck et al., had previously established 3 distinct Qatari genotypes, Bedouin/Arab (Q1), Persian/South Asian (Q2) and African (Q3). [46, 47]

Aim of this MSc

This study aims to determine whether variations in Vitamin D levels as well as prevalence of T2DM complications independent of cultural factors existed between three genotype-based Qatari ancestral groups

Chapter 2: Association of differing Qatari genotypes with vitamin D metabolites and prevalence of T2DM Complications

Summary of research

Objective. Genotyping has offered insights into the genetic composition and variation of members of specific geographic regions. Due to patterns of human immigration, prior studies have identified 3 main genotype groups in the Qatar population. These are Q1 Arab, Bedouin; Q2 Asian/Persian, Q3 African and a 4th group termed admixed. Variations in genetic composition have been shown to impact disease predisposition and risk of progression. Additionally, these genotypes lead to different phenotypes, notably variations in skin pigmentation. Vitamin D deficiency is a worldwide health issue that is augmented in the Middle East by cultural and religious factors that decrease skin exposure to sunlight. Both Vitamin D deficiency and T2DM are highly prevalent in the Middle East. This study aims to determine whether variations in Vitamin D levels and prevalence of T2DM complications exist between Qatari genotypes.

Methods: The genotype of 398 Qatari patients was determined through Affymetrix 500k SNP arrays. 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), 25-hydroxyvitamin D2 ($25(OH)D_2$), 25-hydroxyvitamin D3 ($25(OH)D_3$), 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) and 25-hydroxy-3epi-Vitamin D (3epi25(OH)D) concentrations were measured by LC-MS/MS analysis. Participants were classified as either having T2DM or not and queried for presence of related complications.

Results: The study included 398 patients. Mean patient age was 49.8 years and 222 (56.8%) were men. T2DM was present in 220 (55.0%) of participants. Genotype distribution was as follows; 164 (41.2%) genotyped Q1, 149 (37.4%) genotyped Q2, 31 (7.8%) genotyped Q3, and 54 (13.6%) genotyped "admixed". Median levels of 25(OH)D2, 25(OH)D3 and 3epi25(OH)D did not differ across Q1, Q2, Q3, and 'admixed' genotypes, respectively. 1,25(OH)2D levels significantly differed between Q2 and the admixed groups, and 24,25(OH)2D significantly differed between Q1 and the admixed groups. T2DM prevalence by genotype was as follows; Q1 - 60.4% Q2 -

49.7%, Q3 - 61.3%, and “admixed” - 51.9% with T2DM. For patients with diabetes, hypertension ($p<0.035$) and retinopathy ($p<0.016$) were greater in the Q3 ancestry. A total of 220 patients had T2DM.

Conclusion: Findings of this study suggest genotype may play a role in certain steps of Vitamin D regulation although overall 25(OH)D levels were not different between the 4 Qatari genotypes. $1,25(OH)_2D$ was found to be significantly higher in Q2 genotype, and $24,25(OH)_2D$ was higher in Q1 compared to the ‘admixed’ group. These findings suggest that genotypes may play a role in differences in Vitamin D metabolism. When analyzing differences between diabetics and non-diabetics, total 25(OH)D was higher in diabetes while $1,25(OH)_2D$, $_{24},25(OH)_2D$ and 3epi25(OH)D were lower. While prevalence of diabetes was similar between groups, certain genotypes appear to be prone to related complications.

Introduction

The nation of Qatar, residing in a peninsula on the northeast coast of the Arabian Peninsula, sits at the crossroads of human migration out of Africa with human habitation dating over 50,000 years. The current Qatari population comprises approximately 300,000 nationals within a resident population of 1.8 million). The Qataris are descendants of nomadic tribes with European, Persian, and Southern African influences that reflect the complex migration history of the region.

Prior studies aiming to determine the genetic makeup of the diverse populations within Qatar based on ancestry determined the existence of 3 main genotypes: Q1 Bedouin, Q2 Persian-South Asian, Q3 African. [47] Differences in region of origin for these subtypes may account for variations in skin pigmentation in Qatari genotypes. These variations in skin pigmentation related to genotype may play a role in susceptibility to Vitamin D variations and deficiency. [48]

Vitamin-D is a fat-soluble steroid hormone that has an important role in calcium homeostasis and bone metabolism. It has various forms and a complex metabolism. One of the main forms, Vitamin D3 (cholecalciferol) is synthesized by humans while Vitamin D2 (ergocalciferol) synthesized by fungi and consumed in food. Regardless of the form, metabolic pathways converge on the kidney where it's catalyzed through hydroxylation reactions into active forms 1,25-dihydroxyvitamin D by 1 alpha hydroxylase or to 24,25-dihydroxyvitamin D. [16]

These conversion reactions have also been identified in extrarenal tissue. (18) 1,25(OH)2D can bind to vitamin D receptor (VDR) where through heterodimerization with retinoid X results in downstream activation. [16]

Vitamin D Deficiency is regarded as the most common nutritional deficiency worldwide. [49] While multiple factors contribute to this phenomenon, certain regions such as the Middle East are compounded by cultural factors such as customs to wear full body coverage, and decreasing sunlight exposure. [50] Vitamin D is most frequently associated with calcium-phosphate metabolism and the skeletal system; however, it has been furthermore associated with cardiovascular health, implicated in cancer and shown to be involved in immune regulation. [51]

These associations have been furtherer related and proposed as treatment to a spectrum of autoimmune diseases such as type 1 diabetes mellitus, multiple sclerosis, SLE, or rheumatoid arthritis as detailed in Chapter 1. [49, 52] Further links to immune regulation have implicated Vitamin D deficiency in several inflammatory pathways and diseases such as metabolic syndrome, obesity, and certain cardiovascular diseases. 1,25(OH)2D levels have been shown to be inversely correlated with inflammatory cytokines IL-6 and IL-1 through modifying gene expression in the monocytes. Additional clinical studies have established the risk of Vitamin D deficiency in risk of infections and hospitalization [52].

T2DM is a disease resulting from complex alterations to multiple pathways related to glucose metabolism either to dysregulated peripheral resistance or through insufficient production. [53] Both environmental, lifestyle and genetic factors have been implicated in these dysregulations leading to T2DM. It's a major worldwide health issue, with an estimated worldwide prevalence of 20%. In the Middle East the prevalence is estimated to be 2-3x higher. [40] Prior studies have established genetic susceptibility in certain populations, such as findings from one study that determined SNP rs4506565 to be present in European populations and in admixed Qatari genotypes. However, unlike the European cohort, this SNP was not concluded to be responsible for the increased prevalence in Qatar. [54, 55] The genetic diversity of the Qatari population as well as the possible impact of Vitamin D in inflammatory pathways may play a role in Vitamin D deficiency, prevalence of T2DM and risk of complications in the Qatari population.

Research Question

Do Qatari genotypes relate to significant differences in Vitamin D levels, prevalence of T2DM and T2DM-related complication rates?

Methods

Study population

This study recruited patients who were at least 30 years old, regardless of gender and were at least Qatari for 3 generations. Patients were voluntarily recruited from clinics affiliated to the Hamad Hospital in Doha, Qatar from routine diabetes screening attendees and participation was offered to their companions. Patients with T1DM, GDM or those on steroid treatment were excluded from participating.

Study Definitions

T2DM diagnosis was considered as satisfying at least one of the following: fasting plasma glucose >7 mmol/l, HbA1c > 6.5, or a diagnostic glucose tolerance test. Dyslipidemia was defined as any of the following: total cholesterol greater than 190mg/dl (>4.9mmol/l) and/or fasting triglycerides >150mg/dl (>1.7mmol/l) untreated, or current treatment for dyslipidemia.

Microvascular diseases considered diabetic retinopathy as diagnosed through fundoscopy, and diabetic neuropathy as diagnosed through vibration perception threshold using a Neurothsiometer NU-1 (Horwell, UK) of the first lower extremity digit >25V.

Vitamin D deficiency was defined as proposed by the Endocrine Society: deficiency, insufficiency, and repletion as ≤ 20ng/mL, 20-30 ng/mL and ≥30 ng/mL, respectively.

Sample Processing

As part of a standardized protocol, all patients providing blood glucose samples had an overnight fast and additionally had weight and blood pressure measured at the baseline visit. Blood samples were collected into serum gel tubes with fluoride. Sample processing involved 15-minute centrifugation at 2000g at 4°C and storing at -80°C within 1 hour of collection.

Fasting plasma glucose (FPG) was measured using a Synchron LX 20 analyzer (Beckman-Coulter) according to the manufacturer's recommended protocol. HbA1c was measured on a COBAS analyzer according to the manufacturer's recommended protocol (Roche Diagnostics).

Blood pressure was obtained using a NPB-3900 automated device (Nellcor Puritan Bennet, Pleasanton, CA). This measurement was obtained after subjects had been sitting in a quiet environment for at least 5 minutes with their right arm supported at heart level. Three measurements were obtained and averaged.

Quantification of serum Vitamin D levels was performed with isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). 25 µL of internal standards (d6-1calcitriol (1.5 ng/mL), d6-25OHD3 (50 ng/mL) and d6-25OHD2 (20 ng/mL) were added into each micro centrifuge tube containing 250 L of calibration standards, Quality Control or serum samples and kept for 30 minutes to reach binding equilibrium. The samples were diluted with 250 L of pretreatment solution (isopropanol and water; 50:50 v/v) and left to stand for at least 15 min to displace binding protein.

300 µL of pre-treated samples were then loaded onto ISOLUTE® supported liquid extraction (SLE+) columns (Biotage), followed by elution with 1.8 mL of n-heptane (2 x 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 L of reconstituted solution consisting of methanol and deionized water, 70:30 v/v, and 0.006% methylamine were added into all tubes. The derivatized extracts were transferred into LC insert vials and 10 L from each was injected into the LC-MS/MS system.

Genotype Determination

DNA was extracted from blood using the QIAamp DNA Blood Maxi Kit (Qiagen Sciences Inc, Germantown, MD). Patients were assigned to one of the three genotype-based ancestries described in the Qatari population (47, 61, 62) using a TaqMan SNP Genotyping Assay (Life Technologies, Carlsbad, CA) for a previously described panel of 48 informative SNPs (47, 48, 61, 62). Average

genotype call rate was 96% and was analyzed in STRUCTURE with K = 3. The Q1, Q2 or Q3 population was assigned if the highest proportion was >65%; otherwise, subjects were classed as "admixed." The SLMAP allele was noted specifically given its association with retinopathy in the Qatari diabetic population.

Institutional Oversight

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.

Statistical analyses

Continuous data were tested for normality of distribution using the Kolmogorov-Smirnov Test. Parametric testing was used for normally distributed data using t-Student and ANOVA with Tukey's post-hoc where applicable, otherwise Mann-Whitney-U and Kruskall Wallis were used. Normally distributed variables are reported as mean and standard deviation(sd) otherwise they are reported as median and interquartile range (IQR). Multiple iterations were adjusted using Bonferroni correction. Statistical analysis was performed in SPSS v 24. Categorical and ordinal variables were tested using Chi-square or Fisher's exact were applicable. Single logistic regression analysis was performed and is expressed as Odds Ratio (OR) with 95% Confidence Intervals (CI). P values of under 0.05 were considered significant.

Results

Baseline Characteristics

A total of 398 patients were included in this study. Mean patient age was 49.8 years and 222 (56.8%) were male. Distribution of analyzed genotypes was as follows; 164 (41.2%) - Q1, 149 (37.4%) - Q2, 31 (7.8%) - Q3, and 54 (13.6%) - "admixed". 220(55%) were diabetic. Baseline characteristics of the entire cohort are presented in (Table 1).

Study 1: Vitamin D

Vitamin D

Analysis aimed at comparing Vitamin D levels between the different genotypes revealed median levels of 25(OH)D2, 25(OH)D3 and 3epi25(OH)D were non-different between any of the genotypes. However, 1,25(OH)2D levels significantly differed between Q2 and the admixed groups, and 24,25(OH)2D significantly differed between Q1 and the admixed groups. These findings are summarized in (Table 2). The overall cohort was Vitamin D deficient.

Vitamin D and T2DM

Comparisons of Vitamin D levels across T2DM+ and T2DM- patients per genotype revealed significant differences between total 25(OH)D, which was higher in the T2DM while 1,25(OH)2D, 24,25(OH)2D and 3epi25(OH)D were lower in T2DM. These findings are summarized in (Table 3).

Study 2: T2DM

Genotype and T2DM

Of the 4 analyzed genotypes, the prevalence of T2DM was non-different and as follows: Q1 - 99 (60.4%), Q2 - (49.7%), Q3 - (61.3%), and Admixed - 28. These findings are displayed in (Table 4).

Genotype and T2DM-related complications

Analysis of prevalence of complications by genotype revealed hypertension was greater in Q3 (71%) ancestry as compared to Q1(47%), Q2(42.3%) and admixed (44.4%), p=0.035. Diabetic retinopathy was more frequent in Q3 ancestry (35.5%) as opposed to Q1(15.2%), Q2(12.8%) or admixed (20.4%), p=0.016. Differences in rates of dyslipidemia and neuropathy were not found to be significant. These findings are displayed in Table 5.

Risk factors for T2DM-related complications

Regression analysis determined age, HbA1c, glucose levels, hypertension, and dyslipidemia to significantly increase odds of both diabetic retinopathy and diabetic neuropathy. Full model is summarized in (Table 6).

SLMAP Variants and T2DM-related complications

The association of diabetic retinopathy with Q3 ancestry led to further analysis of the SLMAP genetic variants which had been previously established to play a role in susceptibility. The allele frequency was determined for all genotypes but was not found to be different across any of the ancestries. These findings are displayed in Table 7.

Summary Study

Findings presented by this study conclude that the Qatari population has a high prevalence of Vitamin D deficiency and genotypes are associated with slight variations between Vitamin D levels. However, overall, 25(OH) levels were not significantly different suggesting skin color may not play a role in deficiency in this population. The prevalence of T2DM is similar between groups, although overall T2DM did induce differences in Vitamin D levels and group Q3 was more prone to diabetic retinopathy even in the absence of significant differences in the SLMAP allele.

	Population n=398
Age, mean (sd)	49.80 (10.56)
Gender, N (%)	
Male	226 (56.8)
Female	172 (43.2)
HBA1C, median (IQR)	6.9 (2.7)
Glucose, median (IQR)	6.1 (3.9)
Diabetes-Yes, N (%)	220 (55.3)
Hypertension-Yes, N (%)	186 (46.7)
Dyslipidemia-Yes, N (%)	224 (56.3)

Table 1. Demographics and baseline characteristics of the 398 patients.

	Q1	Q2	Q3	Admixed	Significance
Gender, N (%)					
Male	85 (51.8)	98 (65.8)	12 (38.7)	31 (57.4)	
Female	79 (48.2)	51 (34.2)	19 (61.3)	23 (42.6)	
Age, mean (SD)	49.54 (11.05)	49.72 (10.01)	51.80 (9.57)	49.63 (11.23)	0.744
HBA1C, median (IQR)	7.1 (2.6)	6.6 (2.9)	7.7 (2.6)	6.7 (3.2)	0.503
Glucose, median (IQR)	6.85 (4.03)	6.05 (3.98)	6.05 (4.40)	5.50 (3.70)	0.100
Diabetes, N (%)					
No	65 (39.6)	75 (50.3)	12 (38.7)	26 (48.1)	0.228
Yes	99 (60.4)	74 (49.7)	19 (61.3)	28 (51.9)	0.112
Total 25(OH)D, median (IQR)	22.76 (18.16)	20.77 (16.44)	22.27 (17.13)	22.98 (20.58)	0.389
Toal $1,25(OH)_2D$, median (IQR)	0.034 (0.036)	0.042 (0.043)	0.038 (0.043)	0.025 (0.033)	0.035
Total 3epi-25(OH)D, median (IQR)	0.33 (0.60)	0.55 (0.68)	0.40 (1.02)	0.36 (0.34)	0.099
Total $24,25(OH)_2D$, median (IQR)	0.36 (0.40)	0.34 (0.45)	0.30 (0.24)	0.25 (0.25)	0.047

Table 2. Demographic data, genotype (Q1, Q2, Q3, admixed) and vitamin D metabolites in 398 Qatari subjects:

1,25-dihydroxyvitamin D3, ($1,25(OH)_2D_3$); 25-hydroxyvitamin D2 ($25(OH)D_2$); 25-hydroxyvitamin D3 ($25(OH)D_3$); 24,25-dihydroxyvitamin D3 ($24,25(OH)_2D_3$); 25-hydroxy-3epi-Vitamin D3 ($3epi25(OH)D_3$).

	Control	Diabetes	
	Median (Range)	**Median (Range)**	**P-value**
Age (years)	46.1 (10.8)	55.2 (9.9)	<0.001
BMI (kg/m^2)	30.1 (34.8)	32.4 (44.0)	<0.001
HbA1c (%)	5.6 (4.6)	7.9 (11.2)	<0.001
Glucose (mmol/L)	5.2 (14.6)	8.6 (26.7)	<0.001
Total 1,25(OH)$_2$D (ng/dl)	0.044 (2.087)	0.02 (0.189)	<0.001
Total 25(OH)D (ng/dl)	19.58 (59.32)	26.46 (17.84)	<0.001
Total 24,25(OH)D (ng/dl)	0.387 (4.486)	0.290 (7.772)	<0.001
Total 3epi25(OH)D (ng/dl)	0.387 (4.486)	0.290 (7.772)	0.005

Table 3. Vitamin D levels between diabetes (n=220) and controls (n=178).

	Q1	Q2	Q3	Admixed	Significance
Gender, N (%)					
Male	85 (51.8)	98 (65.8)	12 (38.7)	31 (57.4)	
Female	79 (48.2)	51 (34.2)	19 (61.3)	23 (42.6)	
Age (years), mean (SD)	49.54 (11.05)	49.72 (10.01)	51.80 (9.57)	49.63 (11.23)	0.744
BMI (kg/m^2), mean (SD)	32.80 (6.37)	31.87 (5.61)	33.40 (7.62)	32.62 (6.24)	0.462
HbA1c (%), median (IQR)	7.1 (2.6)	6.6 (2.9)	7.7 (2.6)	6.7 (3.2)	0.503
Fasting glucose (mmol/l), median (IQR)	6.85 (4.03)	6.05 (3.98)	6.05 (4.40)	5.50 (3.70)	0.100
Diabetes, N (%)					
No	65 (39.6)	75 (50.3)	12 (38.7)	26 (48.1)	0.228
Yes	99 (60.4)	74 (49.7)	19 (61.3)	28 (51.9)	0.112

BMI= body mass index; HbA1c= glycated hemoglobin; SD= standard deviation; IQR= interquartile range

Table 4. Baseline demographic data for the entire cohort of Qatari subjects (n=398), categorized according to genotype ((Q1 Bedouin, Q2 Persian-South Asian, Q3 African, Admixed).

	Q1	Q2	Q3	Admixed	
	N=164	N=149	N=31	N=54	
T2DM, N (%)	**99 (60.4)**	**74 (49.7)**	**19 (61.3)**	**28 (51.9)**	**p value**
Gender, N (%)					
Male	85 (51.8)	98 (65.8)	12 (38.7)	31 (57.4)	
Female	79 (48.2)	51 (34.2)	19 (61.3)	23 (42.6)	0.014
Diabetes, N (%)					
No	65 (39.6)	75 (50.3)	12 (38.7)	26 (48.1)	
Yes	99 (60.4)	74 (49.7)	19 (61.3)	28 (51.9)	0.228
Hypertension, N (%)					
No	87 (53.0)	86 (57.7)	9 (29.0)	30 (55.6)	
Yes	77 (47.0)	63 (42.3)	22 (71.0)	24 (44.4)	0.035
Dyslipidemia, N (%)					
No	70 (42.7)	66 (44.3)	13 (41.9)	25 (46.3)	
Yes	94 (57.3)	83 (55.7)	18 (58.1)	29 (53.7)	0.964
Diab Retinopathy, N (%)					
No	139 (84.8)	130 (87.2)	20 (64.5)	43 (79.6)	
Yes	25 (15.2)	19 (12.8)	11 (35.5)	11 (20.4)	0.016
Diab Neuropathy, N (%)					
No	148 (90.2)	134 (89.9)	26 (83.9)	49 (90.7)	
Yes	16 (9.8)	15 (10.1)	5 (16.1)	5 (9.3)	0.724

Table 5. The relationship of the Qatari genotypes (Q1 Bedouin, Q2 Persian-South Asian, Q3 African) to diabetes and diabetes complications (microvascular disease) and cardiovascular complications.

	Diabetic Retinopathy		Diabetic Neuropathy	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age	1.07 (1.04-1.09)	<0.001	1.06 (1.03-1.09)	<0.001
Gender-Female	1.32 (0.83-2.12)	0.246	0.97 (0.55-1.72)	0.924
BMI (kg/m^2)	1.04 (0.99-1.07)	0.057	1.00 (0.96-1.05)	0.867
HbA1c (%)	1.55 (1.36-1.77)	<0.001	1.31 (1.14-1.50)	<0.001
Fasting glucose (mmol/l)	1.14 (1.07-1.21)	<0.001	1.15 (1.07-1.23)	<0.001
Hypertension	4.00 (2.38-6.72)	<0.001	4.80 (2.46-9.39)	<0.001
Dyslipidemia	3.11 (1.82-5.32)	<0.001	3.98 (1.95-8.13)	<0.001

Table 6. Odds ratio for the risk factors of T2DM complications.

Population	**Chromosome**	**DbSNP**	**Reference**	**Alternate**	**Alternate allele frequency**	**Depth of coverage**
Q1	3	rs17058639	C	T	0.343	222150
Q1	3	rs1057719	A	G	0.357	207986
Q1	3	rs1043045	T	C	0.361	222911
Q2	3	rs17058639	C	T	0.343	222150
Q2	3	rs1057719	A	G	0.357	207986
Q2	3	rs1043045	T	C	0.361	222911
Q3	3	rs17058639	C	T	0.343	222150
Q3	3	rs1057719	A	G	0.357	207986
Q3	3	rs1043045	T	C	0.361	222911

Table 7. The genetic variation of sarcolemma-associated protein (SLMAP; OMIM ID 602701) in the Q1, Q2 and Q3 ancestries of the Qatari population.

Discussion and conclusions

This study compared the levels of Vitamin D in various forms and stages of its metabolism across the four main Qatari genotypes. Prior studies have established that due to the convergence of various migration patterns of distinct regional cultures in Qatar, these genotypes carry significant variations in phenotypes such as skin color. The three genotypes include immigrants from the Bedouin region in Q1, immigrants of Persia and South Asia in Q2 and, immigrants from African regions in Q3. [47, 48, 50] Given its genetic heritage, this last genotype has been associated to darker skin pigmentation. Prior studies on the relation betweeen skin pigmentation and Vitamin D metabolism derangements had previously established that darker pigmentation had lower levels of Vitamin D and was more prone to deficiency. [48, 50] Under the premise that darker skin pigmentation leads to lower Vitamin D levels, this study in part hypothesized that patients belonging to genotypes with associated darker skin color would have lower levels of measured Vitamin D. This study however, found differences in the levels of measurable 1,25(OH)D but not 25(OH)D between the genotypes and additionally found similar prevalence of Vitamin D deficiency.

While skin pigmentation has already been validated as a significant factor in Vitamin D synthesis rate and deficiency, overall Vitamin D regulation is subject to the intersection of multiple pathways of genetic and environmental factors. [48, 56] Prior studies on the impact of genetic variations of certain components of the Vitamin D metabolism such as polymorphisms in the Vitamin D receptor could explain the variation in the observed differences in 1,25(OH)D levels found in this cohort. However, another important and possible limitation is that while pigmentation has been found to vary significantly between genotypes, these variations are not accounted for in this study. Additional integration of objective measurements of skin pigmentation per genotype could further shed light and quantify the impact of variations in pigmentation within groups and their impact in the measured outcome. Genetic variations such as VDR polymorphism leading to slight differences in 1,25(OH)D variations seen in groups could be overall blunted by cultural factors such as whole-body covering limit sunlight exposure. [16, 57] This would result in the overall non-different levels of the clinically relevant 25(OH)D as determined in this study. However, this premise could also benefit from further integration of objective measurements of sunlight exposure

time and body surface area exposed, as this study in the present form did not account for the included participant's adherence to full-body clothing and thus these factors could induce further confounding in the performed analysis.

Nonetheless, the impact of genetic composition on Vitamin D levels within this studied population should be further explored. Results from twin studies have suggested that up to 80% of variations in Vitamin D levels are attributable to the genetic composition of the included patients. [58-62] Prior literature has established the key role epigenetics and evolutionary genetics have played in the development of skin pigmentation as a regulator of Vitamin D synthesis, as populations who emigrated north towards regions with decreased sunlight needed to compensate and adapted through evolution to increase Vitamin D synthesis from decreased exposure. [56]

A recent study summarizing results form a systematic search found 29 studies performing analysis of GWAS and determining key genes along the Vitamin D pathway involved in regulation. [56] Among them, these studies have found and stratified genes into the following groups: skin-related, lipid-related, binding protein-related, enzyme related, inactivation and excretion-related, obesity and muscle-related and enzyme related. Over 20 genes have been identified and future studies would benefit from analyzing the presence of these genes in the Qatari population as well as across the previously established genotypes as well as comparing the genetic composition related to these genes in Qataris and other populations. [56]

Additionally, there is ample room in the literature to determine the composition of total variance of Vitamin D within this population through elucidation of the ratio of genetic variance and environmental variance within this population. As previously mentioned, while some twin studies have claimed a genetic heritability of ~80% in Vitamin D Deficiency, other studies including other races such as Hispanics and African Americans have reported much lower levels at 28% and 41% and thus inherent genetic factors related to race could influence the percent of genetic heritability for susceptibility to variations in a population. [63, 64]

Prior studies have also attempted to determine whether genetics play a role in therapeutic success in patients with Vitamin deficiency. These have resulted in genetic risk scores that suggest optimal

treatment approach and dosage can be tailored using genetic insights. [65] However, it is important to note that while genetic risk scores may be useful in determining potential treatment approaches, they should not be used as a sole determinant and should be used in conjunction with clinical assessment and other factors such as patient preferences and comorbidities. Additionally, more research is needed to fully understand the utility and implications of genetic risk scores in the treatment of Vitamin D deficiency.

T2DM is a complex disease, and much like Vitamin D, insulin and glucose metabolisms are the complex intersection of various genetic and environmental factors. [53, 56] Given the diverse ancestry of each group, representing heritage from very distinct geographic regions it's somewhat surprising to find that in this study all four cohorts had similar prevalence of T2DM.

Similarly, to Vitamin D as previously mentioned, T2DM has also been benefitted from intense research employing GWAS focused on determining and stratifying genetic risk and susceptibility to not only T2DM but related complications. While both genetic and environmental factors contribute to the onset of T2DM, it is currently thought that T2DM is developed once the summation of the effects of this both factors reach a certain threshold. Twin studies have estimated that T2DM and susceptibility to complications to have a ~40% of heritability. [66] Studies on diverse populations have identified specific loci mutations for specific populations. For example, while the *TCF7L2* loci has been found to affect both European and East Asian populations, *DGKD and ZNF257* were found to only have a significant effect modifier on East Asian populations. Authors speculated that changes in adipose tissue distribution may play a more significant role in causing T2DM in East Asian populations compared to others. [67]

The interplay of genetic makeup and relation to predisposition and environmental factors is yet to be fully defined in this cohort. While all the analyzed genotypes had similar prevalence of T2DM, future considerations should incorporate external genotypes. Certain genes may be thoroughly conserved across the four genotypes and thus susceptibility obfuscated by a common genetic makeup. These could be fully elucidated by comparing against other genotypes in future studies. The findings of this present study are also limited by the relatively low number of events and non-events per group.

T2DM and its impact or relation with Vitamin D deficiency was also explored. In this study, the T2DM population had a higher total 25(OH)D but lower levels of 1,25(OH)D, 24,25(OH)D and 3epi25(OH)D. There is a possibility that these findings could be confounded by the concurrent use of Vitamin D supplements in the analyzed patient cohort. Prior studies have explored the relation of Vitamin D and T2DM. Some authors have proposed that Vitamin D deficiency is related to T2DM severity, as they found patients with lower Vitamin D levels to relate to higher concentrations of HbA1C, fasting plasma glucose and various interleukins. [68] However, authors have also hypothesized that increased adiposity in patients with T2DM allows for higher sequestration of Vitamin D in adipose tissue. [44, 69, 70]

Authors have attempted to better understand the association and relation of T2DM and Vitamin D in a relatively similar patient cohort in Saudi Arabia. These authors found that Vitamin D levels were inversely correlated with HbA1C, triglycerides and low-density lipoprotein cholesterol (LDL). Contrasting to previously established findings, this study also found Vitamin D to be lower in patients with higher BMI. [69] Further studies in a similar Saudi cohort determined that patients with lower Vitamin D levels through polymorphisms in the VDR genes *BSML* and *TAQ1* and concurrent T2DM were at increased risk of complications of coronary artery disease. [71]

This study additionally sought to explore whether the different genotypes presented with varying susceptibility to different T2DM-related complications. Whether through direct diversity in the genetic composition of each genotype or through downstream effects of variations in genes regulating Vitamin D, this study concluded certain significant findings. As previously demonstrated, the Q3 genotype made up by Sub-Saharan ancestry had an increased frequency of diabetic retinopathy and hypertension when compared to the Q1, Q2 and admixed ancestries.

Diabetic retinopathy is a complex degeneration of optical pathways due to oxidative damage and accumulation of glucose-related metabolites that frequently leads to blindness in patients with T2DM. [9, 57, 72] A GWAS study on a Mexican population found that a locus related to the protein kinase *CAMK4* was found to increase the risk of T2DM patients of developing diabetic

retinopathy. [73] Further loci have been implicated in genes related to angiogenesis, growth factor receptors, oxidase, and redox enzymes as well as capillary permeability. [74]

Studies specific to Qatari populations determined the role of SLMAP genetic variants as a factor to susceptibility to diabetes and retinopathy. [54] This study featured analysis of SLAMP genetic variants within the analyzed genotypes. While SLMAP has been previously established as a risk variant, it was not found to be more frequent in the Q3 genotype which was also found to have increased risk. Further studies are needed to fully determine the genetic culprits or confounders of the findings suggesting the increased risk of complications in the Q3 genotype. While these findings must be confirmed by replication in larger datasets, if proven true this could be the foundation for further personalized care integrating genetics. These findings would be limited however, as studies have shown that Qatari ancestry has more than 30,000 years of divergence with European, and thus the validity of these, even within Qatari populations with European ancestry remains unclear. [75]

Cardiovascular diseases such as hypertension may be related to or independently coexistent with T2DM. Hypertension has also benefited from an array of multiple population and GWAS studies attempting to determine the genetic culprits, however while various genes at various levels of hemodynamic regulation have been proposed, findings remain inconclusive. [76-81] This suggests that genetic susceptibility is a complex interplay of polygenic factors and environmental factors and the proportion of contribution of both of these factors to risk is yet to be defined. [81]

In conclusion, this study found that genotypes are not associated with variations in total 25(OH) D levels and these could be influenced by cultural factors. Variations in 1,25(OH)D suggest that genotypes may play a role in Vitamin D regulation. Additionally, T2DM prevalence was similar between genotypes, with Q3 being more prone to complications such as hypertension and diabetic retinopathy.

This study is limited by the relatively low number of participants and its impact on group distribution, leading to the application of non-parametric tests. Certain of the non-different variables may prove to be significantly different in a larger cohort size. Homozygosity may signal

substantial consanguinity. In this cohort homozygosity is exceptionally high, although identity-by-descent sharing generally appears to be lower than expected for a population in which nearly half of marriages are between first cousins. [82]

Future research

Future studies can corroborate our findings and improve their strength by employing a larger sample size to reduce the risk of a type 2 error. Future studies employing data from the Qatar Biobank with currently 15,000 enrolled participants could be performed. [83, 84]

Differences in 1,25(OH)D levels between Q1 and admixed could lead to further research exploring more specific genetic implications of genes within Vitamin D metabolism. This may be of importance to differing populations and how vitamin D may interact with genetic disposition to the development of diabetes complications. As previously mentioned, further studies should attempt to integrate objective measures of established variables such as skin pigmentation and sunlight exposure to better quantify the strength of association if any. Additional genetic-focused studies should also be performed to define the culprit genes for increased risk in Q3 genotype. Additional comparisons to outside populations both with relative similar genetic composition such as other gulf populations and other further populations is encouraged.

A prospective study is necessary to validate our findings. Furthermore, our findings here may not be applicable to other ethnic groups or countries, since Middle Easterners have low vitamin D status, in part, because of their primarily vegetable-based diet, near total skin coverage and tendency to stay indoors to avoid the hot summer sun, although these variables could be adjusted for in a future study.

References

1. Dakroury Y, Butler AE, Dargham SR, Latif A, Robay A, Crystal RG, et al. Association of Differing Qatari Genotypes with Vitamin D Metabolites. International Journal of Endocrinology. 2020; 2020:7831590.
2. Dakroury Y, Atkin SL, Dargham SR, Robay A, Rodriguez-Flores J, Crystal RG, et al. Qatari Genotype May Contribute to Complications in Type 2 Diabetes. Journal of Diabetes Research. 2020; 2020:6356973.
3. Zimmet P, Alberti KG, Magliano DJ, Bennett PH. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. Nature Reviews Endocrinology. 2016;12(10):616-622.
4. National Diabetes Statistics Report 2020. Centers for Disease Control and Prevention Atlanta, GA: Centers for Disease Control and Prevention, US Department of Health and Human Services. 2020.
5. Organization WH. Classification of diabetes mellitus. 2019.
6. Hurtado MD, Vella A. What is type 2 diabetes? Medicine. 2019;47(1):10-15.
7. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, et al. Type 1 diabetes mellitus. Nature Reviews Disease Primers. 2017;3(1):17016.
8. Plows J, Stanley J, Baker P, Reynolds C, Vickers M. The Pathophysiology of Gestational Diabetes Mellitus. International Journal of Molecular Sciences. 2018;19(11):3342.
9. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Type 2 diabetes mellitus. Nature Reviews Disease Primers. 2015;1(1):15019.
10. Goyal R, Jialal I. Diabetes Mellitus Type 2. StatPearls. Treasure Island (FL): StatPearls Publishing; 2022.
11. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. The Lancet Diabetes & Endocrinology. 2018;6(2):122-129.
12. DiMeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. The Lancet. 2018;391(10138):2449-2462.

13. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, et al. 16. Diabetes Care in the Hospital: Standards of Care in Diabetes—2023. Diabetes Care. 2023;46(Supplement_1):S267-S278.
14. Bianco ME, Josefson JL. Hyperglycemia During Pregnancy and Long-Term Offspring Outcomes. Current Diabetes Reports. 2019;19(12):143.
15. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nature Reviews Disease Primers. 2019;5(1):47.
16. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. Physiological Reviews. 2016;96(1):365-408.
17. Bikle Daniel D. Vitamin D Metabolism, Mechanism of Action, and Clinical Applications. Chemistry & Biology. 2014;21(3):319-329.
18. Adams JS, Rafison B, Witzel S, Reyes RE, Shieh A, Chun R, et al. Regulation of the extrarenal CYP27B1-hydroxylase. The Journal of Steroid Biochemistry and Molecular Biology. 2014;144:22-27.
19. Lösel R, Wehling M. Nongenomic actions of steroid hormones. Nature Reviews Molecular Cell Biology. 2003;4(1):46-55.
20. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. The American Journal of Clinical Nutrition. 2000;72(3):690-693.
21. Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. The American Journal of Clinical Nutrition. 1999;69(5):842-856.
22. Chakhtoura M, Rahme M, Chamoun N, El-Hajj Fuleihan G. Vitamin D in the Middle East and North Africa. Bone Reports. 2018;8:135-146.
23. Holick MF. High Prevalence of Vitamin D Inadequacy and Implications for Health. Mayo Clinic Proceedings. 2006;81(3):353-373.
24. Cashman KD. Vitamin D Deficiency: Defining, Prevalence, Causes, and Strategies of Addressing. Calcified Tissue International. 2020;106(1):14-29.
25. Giustina A, Adler RA, Binkley N, Bollerslev J, Bouillon R, Dawson-Hughes B, et al. Consensus statement from 2nd International Conference on Controversies in Vitamin D. Reviews in Endocrine and Metabolic Disorders. 2020;21(1):89-116.

26. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 Dietary Reference Intakes for Calcium and Vitamin D: What Dietetics Practitioners Need to Know. Journal of the American Dietetic Association. 2011;111(4):524-527.
27. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. The American Journal of Clinical Nutrition. 2004;80(6):1678S-1688S.
28. Mogire RM, Mutua A, Kimita W, Kamau A, Bejon P, Pettifor JM, et al. Prevalence of vitamin D deficiency in Africa: a systematic review and meta-analysis. The Lancet Global Health. 2020;8(1):e134-e142.
29. AlFaris NA, AlKehayez NM, AlMushawah FI, AlNaeem AN, AlAmri ND, AlMudawah ES. Vitamin D Deficiency and Associated Risk Factors in Women from Riyadh, Saudi Arabia. Scientific Reports. 2019;9(1):20371.
30. Zainel A-JAL, Qotba H, Al Nuaimi A, Syed M. Vitamin D status among adults (18–65 years old) attending primary healthcare centres in Qatar: a cross-sectional analysis of the Electronic Medical Records for the year 2017. BMJ Open. 2019;9(8):e029334.
31. Al-Dabhani K, Tsilidis KK, Murphy N, Ward HA, Elliott P, Riboli E, et al. Prevalence of vitamin D deficiency and association with metabolic syndrome in a Qatari population. Nutrition & Diabetes. 2017;7(4):e263-e263.
32. Al Saleh Y, Beshyah SA, Hussein W, Almadani A, Hassoun A, Al Mamari A, et al. Diagnosis and management of vitamin D deficiency in the Gulf Cooperative Council (GCC) countries: an expert consensus summary statement from the GCC vitamin D advisory board. Archives of Osteoporosis. 2020;15(1):35.
33. Nakashima A, Yokoyama K, Yokoo T, Urashima M. Role of vitamin D in diabetes mellitus and chronic kidney disease. World Journal of Diabetes. 2016;7(5):89.
34. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, et al. Vitamin D and Calcium Intake in Relation to Type 2 Diabetes in Women. Diabetes Care. 2006;29(3):650-656.
35. Kadowaki S, Norman AW. Dietary vitamin D is essential for normal insulin secretion from the perfused rat pancreas. Journal of Clinical Investigation. 1984;73(3):759-766.

36. Chiu KC, Chu A, Go VLW, Saad MF. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. The American Journal of Clinical Nutrition. 2004;79(5):820-825.
37. Husemoen LLN, Thuesen BH, Fenger M, Jørgensen T, Glümer C, Svensson J, et al. Serum 25(OH)D and Type 2 Diabetes Association in a General Population. Diabetes Care. 2012;35(8):1695-1700.
38. Leung P. The Potential Protective Action of Vitamin D in Hepatic Insulin Resistance and Pancreatic Islet Dysfunction in Type 2 Diabetes Mellitus. Nutrients. 2016;8(3):147.
39. Bajaj S, Singh R, Dwivedi N, Singh K, Gupta A, Mathur M. Vitamin D levels and microvascular complications in type 2 diabetes. Indian Journal of Endocrinology and Metabolism. 2014;18(4):537.
40. Alhyas L, McKay A, Balasanthiran A, Majeed A. Prevalences of overweight, obesity, hyperglycaemia, hypertension and dyslipidaemia in the Gulf: systematic review. JRSM Short Reports. 2011;2(7):1-16.
41. Mutlu U, Ikram MA, Hofman A, de Jong PTVM, Uitterlinden AG, Klaver CCW, et al. Vitamin D and retinal microvascular damage: The Rotterdam Study. Medicine. 2016;95(49):e5477.
42. Joergensen C, Hovind P, Schmedes A, Parving H-H, Rossing P. Vitamin D Levels, Microvascular Complications, and Mortality in Type 1 Diabetes. Diabetes Care. 2011;34(5):1081-1085.
43. Wang L, Liu X, Hou J, Wei D, Liu P, Fan K, et al. Serum Vitamin D Affected Type 2 Diabetes though Altering Lipid Profile and Modified the Effects of Testosterone on Diabetes Status. Nutrients. 2020;13(1):90.
44. Lips P, Eekhoff M, van Schoor N, Oosterwerff M, de Jongh R, Krul-Poel Y, et al. Vitamin D and type 2 diabetes. The Journal of Steroid Biochemistry and Molecular Biology. 2017;173:280-285.
45. Shigidi MMT, Fituri OM, Chandy SK, Asim M, Al Malki HA, Rashed AH. Peritoneal dialysis, an expanding mode of renal replacement therapy in Qatar. Saudi Journal of Kidney Diseases and Transplantation: An Official Publication of the Saudi Center for Organ Transplantation, Saudi Arabia. 2011;22(3):587-593.

46. Rodriguez-Flores JL, Fakhro K, Hackett NR, Salit J, Fuller J, Agosto-Perez F, et al. Exome Sequencing Identifies Potential Risk Variants for Mendelian Disorders at High Prevalence in Qatar. Human Mutation. 2014;35(1):105-116.
47. Hunter-Zinck H, Musharoff S, Salit J, Al-Ali KA, Chouchane L, Gohar A, et al. Population Genetic Structure of the People of Qatar. The American Journal of Human Genetics. 2010;87(1):17-25.
48. Webb A, Kazantzidis A, Kift R, Farrar M, Wilkinson J, Rhodes L. Colour Counts: Sunlight and Skin Type as Drivers of Vitamin D Deficiency at UK Latitudes. Nutrients. 2018;10(4):457.
49. Holick MF. Vitamin D Deficiency. New England Journal of Medicine. 2007;357(3):266-281.
50. Bassil D, Rahme M, Hoteit M, Fuleihan GE-H. Hypovitaminosis D in the Middle East and North Africa: Prevalence, risk factors and impact on outcomes. Dermato-Endocrinology. 2013;5(2):274-298.
51. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nature Clinical Practice Endocrinology & Metabolism. 2008;4(2):80-90.
52. Ginde AA, Mansbach JM, Camargo CA. Association Between Serum 25-Hydroxyvitamin D Level and Upper Respiratory Tract Infection in the Third National Health and Nutrition Examination Survey. Archives of Internal Medicine. 2009;169(4):384.
53. Gloyn AL, Drucker DJ. Precision medicine in the management of type 2 diabetes. The Lancet Diabetes & Endocrinology. 2018;6(11):891-900.
54. Upadhyay R, Robay A, Fakhro K, Abi Khadil C, Zirie M, Jayyousi A, et al. Role of SLMAP genetic variants in susceptibility of diabetes and diabetic retinopathy in Qatari population. Journal of Translational Medicine. 2015;13(1):61.
55. O'Beirne SL, Salit J, Rodriguez-Flores JL, Staudt MR, Abi Khalil C, Fakhro KA, et al. Exome sequencing-based identification of novel type 2 diabetes risk allele loci in the Qatari population. Public Library of Science ONE. 2018;13(9):e0199837.
56. Hyppönen E, Vimaleswaran KS, Zhou A. Genetic Determinants of 25-Hydroxyvitamin D Concentrations and Their Relevance to Public Health. Nutrients. 2022;14(20):4408.

57. Zhang Y, Xia W, Lu P, Yuan H. The Association between VDR Gene Polymorphisms and Diabetic Retinopathy Susceptibility: A Systematic Review and Meta-Analysis. BioMed Research International. 2016;2016:1-10.
58. Jablonski NG, Chaplin G. The roles of vitamin D and cutaneous vitamin D production in human evolution and health. International Journal of Paleopathology. 2018;23:54-59.
59. Berry D, Hyppönen E. Determinants of vitamin D status: focus on genetic variations. Current Opinion in Nephrology and Hypertension. 2011;20(4):331-336.
60. Wjst M, Altmüller J, Braig C, Bahnweg M, André E. A genome-wide linkage scan for 25-OH-D3 and 1,25-(OH)2-D3 serum levels in asthma families. The Journal of Steroid Biochemistry and Molecular Biology. 2007;103(3-5):799-802.
61. Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino RB, et al. Genetic and non-genetic correlates of vitamins K and D. European Journal of Clinical Nutrition. 2009;63(4):458-464.
62. Orton S-M, Morris AP, Herrera BM, Ramagopalan SV, Lincoln MR, Chao MJ, et al. Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. The American Journal of Clinical Nutrition. 2008;88(2):441-447.
63. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, Wagenknecht LE, et al. Genetic and Environmental Determinants of 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Levels in Hispanic and African Americans. The Journal of Clinical Endocrinology & Metabolism. 2008;93(9):3381-3388.
64. Sutherland JP, Zhou A, Leach MJ, Hyppönen E. Differences and determinants of vitamin D deficiency among UK biobank participants: A cross-ethnic and socioeconomic study. Clinical Nutrition. 2021;40(5):3436-3447.
65. Sallinen RJ, Dethlefsen O, Ruotsalainen S, Mills RD, Miettinen TA, Jääskeläinen TE, et al. Genetic Risk Score for Serum 25-Hydroxyvitamin D Concentration Helps to Guide Personalized Vitamin D Supplementation in Healthy Finnish Adults. The Journal of Nutrition. 2021;151(2):281-292.
66. Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. Nature Reviews Nephrology. 2020;16(7):377-390.
67. Spracklen CN, Horikoshi M, Kim YJ, Lin K, Bragg F, Moon S, et al. Identification of type 2 diabetes loci in 433,540 East Asian individuals. Nature. 2020;582(7811):240-245.

68. Sun H-m, Yu Y, Gao X-r, Wei Y-d, Qi C-z, Ma M-d, et al. Potential role of 25(OH)D insufficiency in the dysfunction of glycolipid metabolism and cognitive impairment in patients with T2DM. Frontiers in Endocrinology. 2022;13:1068199.
69. Gharib A, Askary A, Almehmadi M, Allam H, Elsayyad L, Althobaiti B, et al. Association of Vitamin D Deficiency, Dyslipidemia, and Obesity with the Incidence of Coronary Artery Diseases in Type 2 Diabetic Saudi Patients. Clinical laboratory, 68(10)
70. Liu E, Meigs JB, Pittas AG, Economos CD, McKeown NM, Booth SL, et al. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. The American Journal of Clinical Nutrition. 2010;91(6):1627-1633.
71. Shafie A, Askary AE, Almehmadi M, Allam HH, Elsayyad LK, Hassan AF, et al. Association of Vitamin D Deficiency and Vitamin D Receptor Genetic Variants With Coronary Artery Disease in Type 2 Diabetic Saudi Patients. In Vivo. 2022;36(3):1444-1452.
72. Teo ZL, Tham Y-C, Yu M, Chee ML, Rim TH, Cheung N, et al. Global Prevalence of Diabetic Retinopathy and Projection of Burden through 2045. Ophthalmology. 2021;128(11):1580-1591.
73. Fu Y-P, Hallman DM, Gonzalez VH, Klein BEK, Klein R, Hayes MG, et al. Identification of Diabetic Retinopathy Genes through a Genome-Wide Association Study among Mexican-Americans from Starr County, Texas. Journal of Ophthalmology. 2010;2010:1-9.
74. Grassi MA, Tikhomirov A, Ramalingam S, Below JE, Cox NJ, Nicolae DL. Genome-wide meta-analysis for severe diabetic retinopathy. Human Molecular Genetics. 2011;20(12):2472-2481.
75. Rodriguez-Flores JL, Fakhro K, Agosto-Perez F, Ramstetter MD, Arbiza L, Vincent TL, et al. Indigenous Arabs are descendants of the earliest split from ancient Eurasian populations. Genome Research. 2016;26(2):151-162.
76. Fahiminiya S, Almuriekhi M, Nawaz Z, Staffa A, Lepage P, Ali R, et al. Whole exome sequencing unravels disease-causing genes in consanguineous families in Qatar. Clinical Genetics. 2014;86(2):134-141.
77. Cowley AW. The genetic dissection of essential hypertension. Nature Reviews Genetics. 2006;7(11):829-840.

78. Levy D, Larson MG, Benjamin EJ, Newton-Cheh C, Wang TJ, Hwang S-J, et al. Framingham Heart Study 100K Project: genome-wide associations for blood pressure and arterial stiffness. BMC Medical Genetics. 2007;8(Suppl 1):S3.
79. Ehret GB, Morrison AC, O'Connor AA, Grove ML, Baird L, Schwander K, et al. Replication of the Wellcome Trust genome-wide association study of essential hypertension: the Family Blood Pressure Program. European Journal of Human Genetics. 2008;16(12):1507-1511.
80. Sõber S, Org E, Kepp K, Juhanson P, Eyheramendy S, Gieger C, et al. Targeting 160 Candidate Genes for Blood Pressure Regulation with a Genome-Wide Genotyping Array. PLOS ONE. 2009;4(6):e6034.
81. Harrap SB. Hypertension: genes versus environment. The Lancet. 1994;344(8916):169-171.
82. Ben-Omran T, Al Ghanim K, Yavarna T, El Akoum M, Samara M, Chandra P, et al. Effects of consanguinity in a cohort of subjects with certain genetic disorders in Qatar. Molecular Genetics & Genomic Medicine. 2020;8(1),: e1051
83. Zayed H. The Qatar genome project: translation of whole-genome sequencing into clinical practice. International Journal of Clinical Practice. 2016;70(10):832-834.
84. Sivadas A, Scaria V. Pharmacogenomic survey of Qatari populations using whole-genome and exome sequences. The Pharmacogenomics Journal. 2018;18(4):590-600.

Appendix: Measurement of serum Vitamin D and metabolite measurements: -

Serum vitamin D concentrations were quantified using isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS). 25µL of internal standards (d6-1calcitriol (1.5ng/mL), d6-25OHD_3 (50ng/mL) and d6-epi-25(OH)D_3 (20 ng/mL)) were added into each microcentrifuge tube containing 250L of calibration standards, Quality Control or serum samples, and kept for 30 minutes to reach binding equilibrium. The samples were diluted with 250L of pretreatment solution (isopropanol and water; 50:50 v/v) and left to stand for at least 15 min to displace binding protein.300µL of pre-treated samples were loaded onto ISOLUTE® supported liquid extraction (SLE+) columns (Biotage), followed by elution with 1.8mL of n-heptane (2 x 900µL) into a collection tube already containing 200µL 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 38C. Once dried, 50L 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 L from each was injected into the LC-MS/MS system. Data for the 25(OH)D_3 and metabolite validation is shown below.

Vit. D Metabolites	LOQ	Linearity Range	Accuracy	Reproducibility CV%
25OHD3	0.5 ng/mL	0.5-100 ng/mL	102-118%	6.80
25OHD2	0.25 ng/mL	0.25-50 ng/mL	97-112%	13.26
24-R-25(OH)2D2	0.05 ng/mL	0.05-10 ng/mL	92-101%	7.47
24-R-25(OH)2D3	0.05 ng/mL	0.05-10 ng/mL	94-102%	5.68
3-epi-25(OH)D2	0.05 ng/mL	0.05-10 ng/mL	97-104%	10.25
1,25(OH)2D3	0.01 ng/mL	0.01-0.20 ng/mL	89-105%	12.26
1,25(OH)2D2	0.01 ng/mL	0.01-0.20 ng/mL	90-100%	13.90
3-epi-25(OH)D3	0.05 ng/mL	0.05-10 ng/mL	67-106%	13.72

Analyses for all 8 Vitamin D metabolites were validated for LOQ, linearity, accuracy, repeatability, reproducibility and robustness. All analytes showed good recoveries with acceptable accuracy and within ±15% reproducibility.