Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

Vitamin D Metabolite Ratios and Association with Type 2 Diabetes Mellitus in a Youth Saudi Population

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Purpose: Vitamin D deficiency is highly prevalent in Saudi Arabia and has been associated with type 2 diabetes mellitus (T2DM). The vitamin D metabolite ratio (VMR), defined as the ratio of 24,25-dihydroxyvitamin D_3 [$24,25(OH)_2D_3$] to 25-hydroxyvitamin D_3 [$25(OH)D_3$], may provide a more functional assessment of vitamin D status than total 25(OH)D alone. This study aimed to investigate the associations between vitamin D metabolite ratio and T2DM status in a Saudi youth cohort.

Patients and Methods: A cross-sectional study was conducted involving 971 Saudi adolescents (age 14.9 ± 1.7 years), including 864 normoglycemic individuals, 74 with prediabetes, and 33 with T2DM. Serum vitamin D metabolites [$25(OH)D_3$, $24,25(OH)_2D_3$, and $25(OH)D_2$] were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The vitamin D metabolite ratio was calculated, and its association with T2DM was assessed using logistic regression analysis adjusted for age and body mass index (BMI). VMR was analyzed both as a continuous variable and dichotomized at <4% versus ≥4%. The 4% threshold was selected based on preliminary data from adult populations and exploratory analysis of our data distribution, recognizing that this threshold requires validation in adolescent cohorts.

Results: Lower VMR (<4%) was associated with higher odds of T2DM (OR: 4.7, 95% CI: 1.1–20.0, p = 0.036), though the wide confidence interval reflects the small T2DM sample size (n=33). Preliminary analysis indicated that VMR may be associated with T2DM in Saudi adolescents, but findings should be interpreted cautiously given the small T2DM sample and cross-sectional design. Larger prospective studies are needed to confirm these preliminary observations.

Conclusion: A lower vitamin D metabolite ratio is associated with T2DM among Saudi adolescents. VMR may provide complementary information beyond total 25(OH)D; however, formal comparative predictive analyses were not performed, underscoring the importance of proper vitamin D metabolism in glucose homeostasis. However, given the cross-sectional design and small T2DM sample size, these findings are preliminary and require validation in larger prospective studies.

Keywords: vitamin D metabolism, glucose dysregulation, impaired fasting glucose, youth population, LC–MS/MS, vitamin D hydroxylation

Introduction

T2DM has turned into a major worldwide health issue, especially for younger individuals.[1] Saudi Arabia has high rates of T2DM, and almost 24% of adults are dealing with this ongoing and serious health issue, emphasizing the substantial public health significance of strong public health strategies and educational initiatives to raise awareness and promote healthier lifestyles for the community.[2] Vitamin D deficiency is highly prevalent and often found in many areas of Saudi Arabia. Despite abundant sunlight, vitamin D deficiency affects over 80% of the Saudi population.[3,4] Beyond its well-known function in maintaining calcium homeostasis and skeletal health, vitamin D has received increased attention for its crucial role in regulating glucose metabolism and improving insulin secretion.[5]

Vitamin D status is influenced by multiple environmental and lifestyle factors, particularly relevant in the Saudi Arabian context. Despite abundant sunlight year-round, vitamin D deficiency is highly prevalent in Saudi Arabia due to cultural practices limiting sun exposure (traditional clothing, indoor lifestyle), high ambient temperatures discouraging outdoor activities, and dietary patterns with limited vitamin D-rich foods. Seasonal variation in vitamin D levels has been

https://doi.org/10.2147/DMSO.S597635
Received: 28 January 2026
Accepted: 24 March 2026
Published: 18 April 2026

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documented in Saudi populations, with lower levels observed during winter months despite the subtropical climate. These contextual factors are particularly important when interpreting vitamin D biomarkers in Saudi adolescents.

The traditional method used to assess vitamin D concentrations in the human body heavily depends on the accurate measurement of serum 25-hydroxyvitamin D (25(OH)D), reflecting both $25(OH)D_3$ (from animal sources) and $25(OH)D_2$ (from plant sources). Nevertheless, this method might not entirely capture the complex processes associated with vitamin D metabolism within the human body.[6,7]

The biochemical transformation of 25-hydroxyvitamin D_3 ($25(OH)D_3$) into 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) is catalyzed by CYP24A1. This metabolic pathway constitutes an essential catabolic mechanism in the regulation and degradation of vitamin D metabolites. The comparative proportion of these metabolites, often known as the vitamin D metabolite ratio (VMR), has been suggested as a more adaptable indicator of a person's overall vitamin D levels and its implications for metabolic health.[8,9]

The VMR threshold of <4% has been proposed in adult populations as indicative of impaired 24-hydroxylase activity and altered vitamin D metabolism. While this threshold has not been extensively validated in adolescent populations, preliminary evidence suggests that similar metabolic pathways operate in youth. We selected this threshold as an exploratory cut-point to investigate whether metabolic ratio patterns observed in adults extend to adolescents, acknowledging that age-specific reference ranges require further investigation.

Recent studies in nutritional science and metabolism have shown that VMR, has been proposed as a potentially informative marker for assessing vitamin D levels and their metabolic effects compared to evaluating 25-hydroxyvitamin D (25(OH)D) alone.[10,11] A decreased VMR might indicate reduced CYP24A1 enzyme activity, suggesting ineffective vitamin D metabolism even when serum 25(OH)D appears adequate.[12]

The relationship between VMR, and various metabolic disorders, especially the rising issue of T2DM, is an area that has not been studied enough, especially among children and adolescents.[13] Given the high rates of vitamin D deficiency and T2DM Saudi youth, investigating how vitamin D metabolite ratios relate to diabetes could provide essential information for developing effective early prevention strategies.[7]

This research aimed to describe the profiles of vitamin D metabolites in Saudi adolescents with different glycemic states (normal, prediabetic, and T2DM); to assess the association between VMR and T2DM; and compare the predictive value of VMR with traditional vitamin D indicators such as $25(OH)D_3$ and $24,25(OH)_2D_3$.

Materials and Methods

Study Design and Population

Clinical data from the present cross-sectional study were taken from the biochemical Osteomalacia database of the Chair for Biomarkers of Chronic Diseases (CBCD) in King Saud University (KSU), Riyadh, Kingdom of Saudi Arabia (KSA).[14,15] A significant group of 971 Saudi adolescents, aged between the important developmental stages of 12 to 18 years, was chosen from various educational institutions strategically located in the capital city of Riyadh, Saudi Arabia, during 2019–2021. Study sites were selected using stratified random sampling to ensure geographic representation across Riyadh. Five primary healthcare centers and three secondary schools were randomly selected from different districts of Riyadh (north, south, east, west, and central) to capture socioeconomic and geographic diversity. All selected sites agreed to participate.

Blood samples were collected between October 2019 and March 2021. To account for potential seasonal variation in vitamin D status, we recorded the season of blood collection for each participant (categorized as: October-March [cooler months] vs April-September [warmer months]). Preliminary analysis showed no significant seasonal differences in 25 (OH)D levels (p=0.18), likely due to year-round limited sun exposure in this population. Season was not included as a covariate in final models due to lack of significant effect.

The participants were classified into three distinct groups based on their glycemic profiles: normoglycemic (n=864), prediabetes (n=74), and T2DM (n=33). The inclusion criteria for this study involved several specific requirements, such as being Saudi nationals clearly aged between 12 to 18 years, showing a documented willingness to take part in the study while also providing informed consent or assent, and fasting for at least 8 hours before the actual collection of blood samples for analysis. Fasting status was confirmed through multiple methods: (1) participants were instructed to fast for

at least 8 hours before blood collection during recruitment; (2) verbal confirmation of fasting duration was obtained immediately before blood draw; (3) appointments were scheduled for early morning (7:00–9:00 AM) to facilitate compliance; (4) participants who reported non-compliance with fasting requirements were rescheduled. Fasting duration was recorded for all participants (mean: 10.2 ± 1.8 hours). The exclusion criteria outlined to maintain the integrity of the research included several key factors, such as: a previous diagnosis of type 1 diabetes mellitus, taking vitamin D supplements in the three months prior to the study, having chronic kidney disease or liver disease that could affect results, using any medications known to influence vitamin D or glucose metabolism, and the pregnancy status of participants, especially regarding female adolescents. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of King Saud University (IRB approval number: KSU (E-21-6,095). Additionally, written informed consent was diligently obtained from the parents or legal guardians of the participants, while assent was thoughtfully acquired from all adolescents who took part in the study.

Anthropometric and Clinical Measurements

Anthropometric assessments were conducted by qualified people in accordance with standardized protocols. To the nearest 0.1 cm, height was quantified utilizing a stadiometer and weight was assessed to the nearest 0.1 kg using a calibrated digital scale. BMI was calculated by dividing weight in kilograms by the square of height in meters. Waist size was carefully measured at the exact middle point between the bottom of the rib cage and the top of the hip bone, while hip size was regularly noted at the broadest area over the thigh bones. The Waist-to-Hip Ratio (WHR) was subsequently calculated in accordance with these measurements.

Biochemical Analysis

Blood samples were procured from individuals who overnight fasting period of no less than eight hours. Blood samples were collected in BD Vacutainer® tubes containing clot activator and gel separator for serum collection (for vitamin D metabolites) and in sodium fluoride/potassium oxalate tubes for plasma glucose measurement. Samples were allowed to clot for 30 minutes at room temperature before centrifugation. Samples were centrifuged at 3000 revolutions per minute for a duration of 15 minutes, after which the serum was isolated and subsequently stored at −80°C until analysis. Fasting plasma glucose levels were assessed utilizing the glucose oxidase technique, whereas glycated hemoglobin (HbA1c) was determined through high-performance liquid chromatography (HPLC). Total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride concentrations were analyzed via advanced enzymatic colorimetric methods using an automated analyzer (Konelab, Vintaa, Finland). Serum concentrations of $25(OH)D_3$, $24,25(OH)_2D_3$, and $25(OH)D_2$ were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the Department of Clinical Chemistry, CIRM, University of Liege, Belgium.[16] Total 25(OH)D concentration was calculated by summing the values of $25(OH)D_3$ and $25(OH)D_2$. VMR was calculated with the formula:

$$VMR = [24,25(OH)_2D_3 \text{ (nmol/L)} / 25(OH)D_3 \text{ (nmol/L)}]*100$$

Vitamin D status for this investigation was categorized as follows: Deficient: 25(OH)D <50 nmol/L - Sufficient: 25 (OH)D ≥50 nmol/L, with a VMR <4% according to recommended thresholds.[17] Participants were classified following the American Diabetes Association (ADA) criteria: Normoglycemic: HbA1c <5.7% and fasting glucose <5.6 mmol/L; Prediabetes: HbA1c 5.7–6.4% or fasting glucose 5.6–6.9 mmol/L; T2DM: HbA1c ≥6.5% or fasting glucose ≥7.0 mmol/L or previously diagnosed T2DM.[18]

Glycemic status classification was based on fasting plasma glucose and HbA1c according to American Diabetes Association (ADA) criteria. For participants with discordant results between fasting plasma glucose and HbA1c, classification was based on the higher category. We acknowledge that ADA guidelines recommend confirmation with repeat testing for diabetes diagnosis; however, given the cross-sectional nature and research context of this study, single measurements were used for glycemic status classification. This represents a study limitation and participants identified with T2DM were referred for clinical follow-up and confirmatory testing.

Statistical Analysis

Sample size was determined based on the primary objective of characterizing vitamin D metabolite distributions in Saudi adolescents. Assuming a vitamin D deficiency prevalence of 40% and a desired precision of ±3% at 95% confidence, a minimum sample of 1,024 participants was targeted. The final sample included 971 participants (95% of target). However, the study was not specifically powered for the secondary objective of examining associations with T2DM. The observed T2DM prevalence of 3.4% (n = 33) resulted in limited statistical power for subgroup analyses, and these findings should therefore be considered exploratory.

Data analysis was performed using SPSS (version 26.0). Continuous variables were summarized as mean ± standard deviation for normally distributed data and as median (interquartile range) for non-normally distributed variables. Categorical variables were expressed as counts and percentages. Normality was assessed using the Shapiro–Wilk test and visual inspection of Q–Q plots. Variables with skewed distributions (25(OH)D, $24,25(OH)_2D_3$, VMR, fasting plasma glucose, and HbA1c) were log-transformed prior to parametric analyses.

Group comparisons were conducted using one-way ANOVA or Kruskal–Wallis tests, with appropriate post-hoc analyses. Categorical comparisons used Chi-square or Fisher's exact tests as appropriate.

Correlation analyses were primarily performed using Spearman's rank correlation coefficient due to non-normal distributions. Pearson correlations using log-transformed variables were conducted for comparison and yielded similar results; Spearman correlations are presented in the main results. Statistical significance was defined as $p < 0.05$.

VMR was analyzed both as a continuous variable and dichotomized at <4% versus ≥4%, based on prior adult literature suggesting altered vitamin D metabolism below this threshold. Dichotomization was performed as an exploratory analysis to facilitate clinical interpretation, while continuous modeling was retained as the primary approach.

Logistic regression was used to examine associations between vitamin D metabolites and T2DM, generating odds ratios (ORs) with 95% confidence intervals. Model diagnostics included the Hosmer–Lemeshow goodness-of-fit test, variance inflation factors (VIF) to assess multicollinearity, and Cook's distance to detect influential observations. Given the limited number of T2DM cases (n = 33), the model may be susceptible to sparse data bias and overfitting, and results should be interpreted cautiously.

Results

Baseline Characteristics

This research encompasses a cohort of 971 Saudi adolescents, with a mean age of 14.9 years (± 1.7). 864 individuals were classified as normoglycemic representing 89.0% of the total sample, along with 74 individuals were prediabetic, which represented 7.6% of the participants, and 33 individuals diagnosed with T2DM, represented 3.4% of the population. The clinical characteristics of the 971 adolescents (511 girls, 460 boys) were analyzed based on their diabetes status in Table 1. Detailed anthropometric measurements, lipid profiles, and glycemic parameters are clearly presented, organized by the participants' T2DM status in this significant study. The results demonstrated that within the normoglycemic cohort, there existed 468 females and 396 males, whereas the prediabetes cohort comprised 31 females and 43 males; conversely, the T2DM cohort included 12 females and 21 males, thereby highlighting a statistically significant disparity in sex distribution across these groups, as evidenced by a p-value of 0.021. It was observed that individuals classified as prediabetic exhibited a markedly elevated Body Mass Index (BMI) in comparison to their normoglycemic peers, with measurements of 25.2 ± 6.3 kg/m^2 for the prediabetic cohort and 23.2 ± 5.6 kg/m^2 for the normoglycemic cohort, resulting in a statistically significant distinction with a p-value of 0.017. Moreover, the assessment of systolic blood pressure revealed a striking increase within the prediabetes group, showcasing an impressive mean of 122.2 ± 15.5 mmHg, compared to the normoglycemic individuals, who demonstrated a significantly lower mean of 115.9 ± 14.4 mmHg. This difference was statistically significant (p = 0.001), highlighting the vital significance of these results. The results found significant differences in glycemic parameters among three studied groups: normoglycemic group had an HbA1c level of 4.9 ± 0.4%, prediabetes group had 5.9 ± 0.2%, and T2DM group had 8.5 ± 1.6%, with a p-value less than 0.001 indicating statistical significance. Fasting glucose levels were measured across three groups: normoglycemic (5.1 ± 0.7 mmol/L), prediabetic (5.7 ± 2.0 mmol/L), and T2DM (9.2 ± 5.2 mmol/L), showing a significant

Table 1 Anthropometrics, Lipids and Glycemic Parameters According to T2DM Status

Parameters	Normal	Pre-Diabetes	T2DM	p-value
N	864	74	33	
Age (Years)	14.9 ± 1.7	15.2 ± 1.6	14.9 ± 1.7	0.245
BMI (kg/m2)	23.2 ± 5.6	25.2 ± 6.3^{A}	24.0 ± 5.0	0.017
Female/Male	468/396	31/43	12/21	0.021
Waist (cm)	70.5 ± 17.9	67.3 ± 24.6	72.2 ± 16.5	0.343
Hips (cm)	84.9 ± 21.1	80.3 ± 28.6	83.8 ± 18.1	0.231
WHR	0.83 ± 0.10	0.84 ± 0.10	0.86 ± 0.10	0.205
Systolic BP (mmHg)	115.9 ± 14.4	122.2 ± 15.5^{A}	118.5 ± 15.8	0.001
Diastolic BP (mmHg)	71.0 ± 12.5	72.1 ± 12.4	71.6 ± 12.4	0.752
Total-Cholesterol (mmol/L)	4.6 ± 1.1	4.5 ± 1.0	5.4 ± 1.5^{AB}	<0.001
HDL-Cholesterol (mmol/L)	1.1 ± 0.4	1.0 ± 0.3	1.3 ± 0.7^{AB}	0.005
Triglycerides (mmol/L)	1.1 ± 0.6	1.3 ± 0.7	1.6 ± 0.8^{AB}	<0.001

Note: Data presented as Mean ± SD for normal variables and Median (Quartile 1 – Quartile 3) for non-normal variables; $p < 0.05$ considered statistically significant. Superscript A and B indicate significance from Normal and Pre-Diabetes respectively. P-values were obtained from analysis of variance (ANOVA) and post hoc comparisons were obtained from Bonferroni test.

difference with a p-value below 0.001. As glycemic groups were defined based on fasting plasma glucose and HbA1c criteria, statistical comparisons for these variables are inherently deterministic. Therefore, these results are presented descriptively and interpreted with caution.

Lastly, it was observed that the cohort with T2DM exhibited a markedly inferior lipid profile in comparison to both the normoglycemic and prediabetic cohorts, as evidenced by elevated total cholesterol levels averaging 5.4 ± 1.5 mmol/L, a finding of considerable significance with a p-value of less than 0.001, alongside increased HDL-cholesterol levels at 1.3 ± 0.7 mmol/L, which also demonstrated statistical significance with a p-value of 0.005, and heightened triglyceride levels averaging 1.6 ± 0.8 mmol/L, culminating in a statistically significant disparity denoted by a p-value of less than 0.001.

Vitamin D Metabolite Profiles

Table 2 elucidates a detailed analysis of the concentrations of vitamin D metabolites, systematically classified based on the classification of T2DM among the participants of the research. In the normoglycemic cohort, the median VMR was documented at 2.7, with an interquartile range (IQR) extending from 1.6 to 4.1; conversely, in the prediabetes cohort, the median VMR was determined to be 2.3, with an IQR fluctuating between 1.4 and 3.2, while in the T2DM cohort, the median VMR was recorded as 2.6, accompanied by an IQR extending from 1.6 to 3.1. Although the overall disparity observed among these cohorts showed a borderline p-value ($p = 0.050$), which does not meet conventional statistical significance, it is noteworthy that the prediabetes cohort manifested the lowest values of VMR. Results showed no meaningful differences in vitamin D metabolites among different groups, highlighting a clear need for more research. The median levels of $24,25(OH)_2D_3$ were 0.8 nmol/L for those with normal blood sugar, 0.5 nmol/L for prediabetics, and 0.8 nmol/L for individuals with T2DM ($p = 0.241$). The levels of $25(OH)D_3$ soared to 32.4 nmol/L among individuals with normal glucose levels, dropped to 30.6 nmol/L for those on the brink of diabetes, and impressively surged to 36.0

Table 2 Vitamin D Metabolites According to T2DM Status

Vitamin D Metabolites	Normal	Pre-diabetes	T2DM	p-value
Vitamin D Metabolite Ratio	2.7 (1.6–4.1)	2.3 (1.4–3.2)	2.6 (1.6–3.1)	0.050
$24,25(OH)_2D_3$ (nmol/L)	0.8 (0.3–1.7)	0.5 (0.3–1.3)	0.8 (0.3–1.2)	0.241
$25(OH)D_3$ (nmol/L)	32.4 (22.8–43.7)	30.6 (23.5–39.3)	36.0 (22.4–44.8)	0.523
$25(OH)D_2$ (nmol/L)	2.3 (2.3–2.3)	2.3 (2.3–2.3)	2.3 (2.3–2.3)	0.780
Total 25(OH)D (nmol/L)	32.4 (22.8–43.9)	30.6 (23.5–39.3)	36.0 (22.4–44.8)	0.525

Note: Data presented as Median (Quartile 1 – Quartile 3); $p < 0.05$ considered statistically significant.

https://doi.org/10.2147/DMSO.S597635

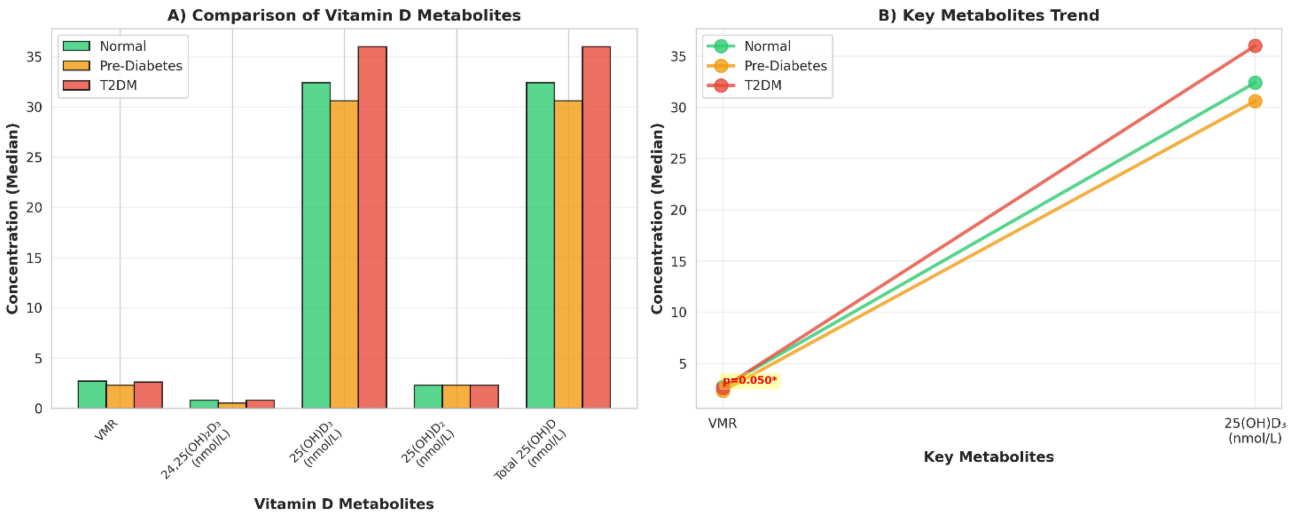

Figure 1 Vitamin D Metabolite Profiles According to Glycemic Status. (**A**) Comparison of VMR and vitamin D metabolites across normal, prediabetes, and T2DM groups. (**B**) Trend analysis of VMR and 25(OH)D_3 concentrations among glycemic status groups.

nmol/L in T2DM patients (p = 0.523). The overall amount of 25(OH)D had a comparable trend (p = 0.525), and the levels of 25(OH)D_2 were just at the edge of what we could detect in every group (p = 0.780).

Figure 1 shows the profiles of vitamin D metabolites based on T2DM status. Panel A has a bar chart that compares five important metabolites—VMR, 24,25(OH)$_2D_3$, 25(OH)D_3, 25(OH)D_2, and Total 25(OH)D—across three groups based on blood sugar levels: Normal, Pre-Diabetes, and T2DM. The VMR values are lower in the prediabetes group (median 2.3) than in normal (2.7) and T2DM (2.6), while the levels of 25(OH)D_3 and Total 25(OH)D are similar, with T2DM showing slightly higher median values (36.0 nmol/L) compared to prediabetes (30.6 nmol/L) and normal (32.4 nmol/L). Panel B shows a trend line analysis of VMR and 25(OH)D_3 concentrations, with a p-value of 0.050 for VMR, indicating a borderline difference that does not meet conventional statistical significance. The trend lines indicate that 25(OH)D_3 levels rise in the T2DM group, while VMR values are lower, especially in prediabetes, suggesting that VMR may provide additional information regarding vitamin D metabolism beyond absolute 25(OH)D_3 levels.

Association Between Vitamin D Metabolites and T2DM

Table 3 presents a thorough examination of the odds ratios that correlate with the likelihood of developing T2DM based on the concentrations of vitamin D metabolites in the body. Upon performing a preliminary or crude analysis, we discerned that individuals possessing a VMR of less than 4 exhibited a discernible trend suggesting higher odds of both prediabetes, with an odds ratio of 1.6 and a 95% confidence interval ranging from 0.9 to 2.8, accompanied by a p-value

Table 3 Odds Ratio for T2DM Risk

Vitamin D Metabolites	Crude			Adjusted		
	Normal	Pre-diabetes	T2DM	Normal	Pre-diabetes	T2DM
VMR < 4	Ref	1.6 (0.9–2.8)	2.0 (0.8–5.3)	Ref	1.4 (0.8–2.6)	4.7 (1.1–20.0)
p-value	–	0.145	0.146	–	0.235	0.036
25(OH)D_3 (nmol/L) < 50 nmol/L	Ref	1.0 (0.5–2.0)	0.6 (0.3–1.5)	Ref	0.9 (0.5–1.8)	0.8 (0.3–2.3)
p-value	–	0.967	0.337	–	0.870	0.741
24,25(OH)$_2D_3$ (nmol/L) < 3 nmol/L	Ref	1.1 (0.4–2.9)	1.3 (0.3–5.5)	Ref	1.0 (0.4–2.7)	2.2 (0.3–16.4)
p-value	–	0.783	0.737	–	0.944	0.444
Total 25(OH)D (nmol/L) < 50 nmol/L	Ref	1.0 (0.5–2.0)	0.7 (0.3–1.5)	Ref	0.9 (0.5–1.8)	0.9 (0.3–2.3)
p-value	–	0.924	0.358	–	0.901	0.767

Note: Data presented as OR (95% CI); p-value also adjusted for age and BMI.

of 0.145, as well as T2DM, which revealed an odds ratio of 2.0, a 95% confidence interval extending from 0.8 to 5.3, and a p-value of 0.146; however, it is crucial to acknowledge that these identified associations did not attain statistical significance, implying that they may not serve as dependable indicators of risk. However, after adjusting for both age and body mass index (BMI), the earlier noted link between a low value of VMR and T2DM became statistically significant, revealing a clear relationship that was not obvious before these adjustments. People with a VMR value less than 4 had about 4.7 times higher odds of developing T2DM than those with a VMR of 4 or more, as shown by an odds ratio of 4.7, a 95% confidence interval from 1.1 to 20.0, and a p-value of 0.036, highlighting the importance of these results. VMR was analyzed both as a continuous variable and dichotomized at <4%. Logistic regression with continuous VMR showed: OR=4.2 per 1% decrease in VMR (95% CI: 1.2–14.8, p=0.025). The dichotomized analysis (VMR <4% vs ≥4%) yielded: OR=4.7 (95% CI: 1.1–20.0, p = 0.036). Both approaches showed similar effect directions but wide confidence intervals reflecting the small T2DM sample. We present both analyses to facilitate comparison with prior literature using dichotomized VMR while acknowledging that continuous analysis preserves more information and statistical power. Sensitivity analyses were performed to assess robustness of findings: (1) Excluding participants with prediabetes (comparing only normal vs T2DM): OR for VMR <4% = 5.1 (95% CI: 1.0–25.8, p=0.048); (2) Adjusting for additional covariates (physical activity, family history of diabetes): OR for VMR <4% = 4.3 (95% CI: 0.9–20.1, p=0.065); (3) Using multiple imputation for missing covariate data (n=47 with missing BMI): OR for VMR <4% = 4.5 (95% CI: 1.0–19.5, p=0.042). These sensitivity analyses show generally consistent effect directions but highlight the instability of estimates with the small T2DM sample. On the other hand, regarding prediabetes, the association remained statistically non-significant even after the adjustments, as shown by an odds ratio of 1.4, a 95% confidence interval of 0.8 to 2.6, and a p-value of 0.235, indicating that no significant link could be established in this case. Neither low levels of 25-hydroxyvitamin D_3 (under 50 nmol/L) nor 24,25-dihydroxyvitamin D_2 (below 3 nmol/L) show significant associations with T2DM or prediabetes. The adjusted odds ratio for 25-hydroxyvitamin D_3 was 0.8 (95% CI: 0.3–2.3, p = 0.741), and for 24,25-dihydroxyvitamin D_2 it was 2.2 (95% CI: 0.3–16.4, p=0.444). Total 25-hydroxyvitamin D levels under 50 nmol/L also had an odds ratio of 0.9 (95% CI: 0.3–2.3, p = 0.767), indicating no significant association with T2DM.

Figure 2 displays a forest plot of adjusted odds ratios for T2DM risk associated with four VMR <4, 25(OH)D_3 <50 nmol/L, 24,25$(OH)_2D_3$ <3 nmol/L, and Total 25(OH)D <50 nmol/L), with separate estimates for prediabetes (cyan circles) and diabetes (purple circles) outcomes. The vertical dashed line at OR=1.0 represents no effect. Only VMR <4 showed statistical significance in the adjusted model; however, this finding should be interpreted cautiously given the wide confidence interval and small T2DM sample size. All estimates were adjusted for age and BMI, with the normal group serving as reference.

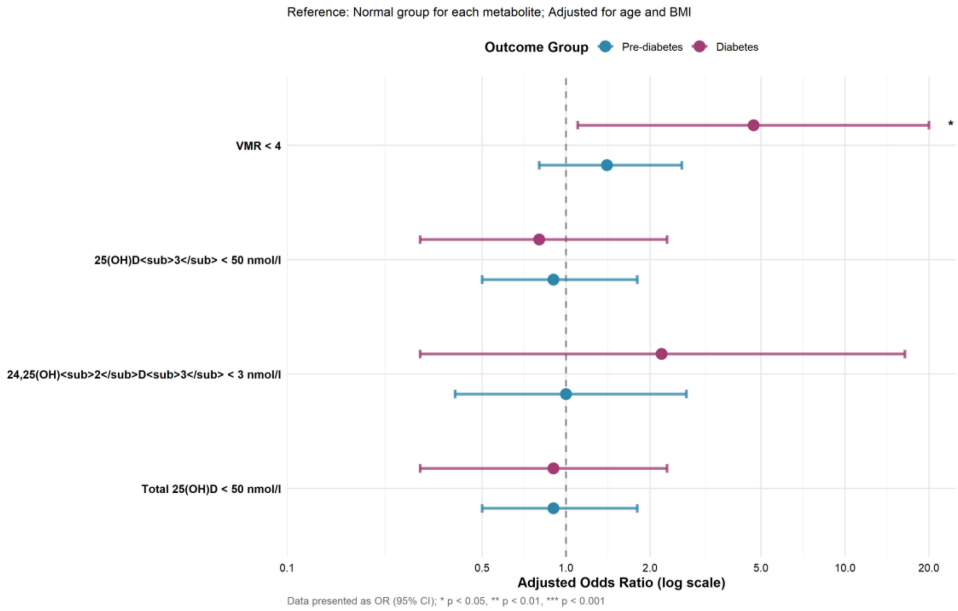

Figure 2 Adjusted odds ratios for T2DM association with Vitamin D Metabolites.

https://doi.org/10.2147/DMSO.S597635

Table 4 Correlation Between Vitamin D Metabolites and HbA1c (%) and Glucose (mmol/L)

Glycemic Status/Parameters			VMR	$24,25(OH)_2D_3$ (nmol/L)	$25(OH)D_3$ (nmol/L)	Total 25(OH)D (nmol/L)
All Subjects	HbA1c	r	−0.02	0.01	0.05	0.04
		p-value	0.574	0.696	0.140	0.176
	Glucose	r	−0.05	−0.02	0.03	0.03
		p-value	0.098	0.438	0.400	0.412
Normal	HbA1c	r	0.02	0.03	0.06	0.05
		p-value	0.652	0.306	0.096	0.150
	Glucose	r	−0.06	−0.04	−0.01	−0.01
		p-value	0.086	0.241	0.851	0.831
Pre-diabetes	HbA1c	r	0.21	0.14	−0.03	−0.03
		p-value	0.079	0.243	0.832	0.832
	Glucose	r	−0.14	−0.06	0.08	0.08
		p-value	0.237	0.587	0.513	0.513
T2DM	HbA1c	r	0.25	0.08	−0.07	−0.07
		p-value	0.164	0.665	0.717	0.717
	Glucose	r	0.06	0.00	0.02	0.02
		p-value	0.745	0.996	0.892	0.892

Note: Data presented as Spearman correlation coefficient (r); $p < 0.05$ considered statistically significant.

Vitamin D Metabolites and Glycemic Parameters

Table 4 and Figure 3 present the correlations between vitamin D metabolites and glycemic markers (HbA1c and fasting glucose) in the total sample and stratified by glycemic status. In the overall sample, VMR showed weak inverse correlations with HbA1c (r = −0.02, p = 0.574) and fasting glucose (r = −0.05, p = 0.098). Correlation analyses using log-transformed variables yielded similarly weak associations (fasting glucose: r = −0.08, p = 0.02; HbA1c: r = −0.05, p = 0.14). The observed correlation coefficients were very small in magnitude (r = −0.08 and r = −0.05), explaining less than 1% of the variance. According to Cohen's guidelines, these represent trivial to small effect sizes. Although the correlation with fasting glucose reached statistical significance (p = 0.02), the effect size is minimal and of questionable clinical relevance. Given the large sample size (n = 971), even very small correlations may achieve statistical significance without meaningful biological impact. When stratified by glycemic status, correlations remained weak and largely non-significant. In the prediabetes group, VMR showed a non-significant positive correlation with HbA1c (r = 0.21, p = 0.079). In the T2DM subgroup (n = 33), correlations were also non-significant (HbA1c: r = 0.25, p = 0.164; fasting glucose: r = 0.06, p = 0.745). These subgroup analyses are underpowered and should be interpreted as exploratory only. For individual metabolites, $25(OH)D_3$ demonstrated weak and non-significant correlations with HbA1c in

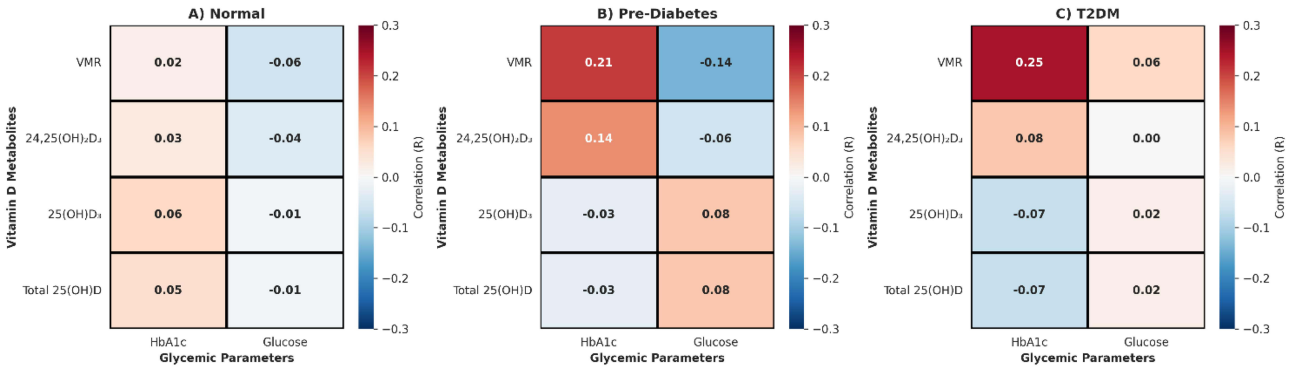

Figure 3 Correlation between vitamin D metabolites and glycemic parameters; HbA1c (%) and Glucose (mmol/L) (**A**) Correlation heatmap for normoglycemic participants, (**B**) Correlation heatmap for prediabetic participants. (**C**) Correlation heatmap for T2DM participants.

 https://doi.org/10.2147/DMSO.S597635

normoglycemic participants (r = 0.06, p = 0.096) and weak negative correlations in T2DM (r = −0.07, p = 0.717). Correlations with fasting glucose were similarly weak and non-significant across all groups. Overall, the data indicate that associations between vitamin D metabolites and glycemic markers in this cohort are weak and of trivial to small effect size.

Discussion

This study delves into the critical association between vitamin D metabolite ratios and T2DM among Saudi adolescents. The main finding of this exploratory analysis was that VMR <4% was associated with higher odds of T2DM even after adjusting for age and BMI. VMR showed an association with T2DM independent of 25(OH)D levels, suggesting it may provide complementary information about vitamin D metabolism beyond total 25(OH)D concentration. We did not perform formal comparison of predictive performance between VMR and 25(OH)D using ROC curve analysis or other discrimination metrics. Therefore, we cannot conclude whether VMR provides superior predictive value compared to 25 (OH)D alone. Future studies should include such comparative analyses to determine the relative and incremental predictive value of VMR. However, the clinical utility of VMR as a biomarker requires further investigation in larger studies with adequate statistical power and prospective design. The between-group difference in VMR yielded a p-value of 0.050, which does not meet the conventional threshold for statistical significance. Given the small T2DM sample size and wide confidence interval, this finding should be interpreted cautiously and considered hypothesis-generating rather than confirmatory.

The vitamin D metabolite ratio reflects how effectively vitamin D is metabolized via the CYP24A1 enzyme into its catabolic product, $24,25(OH)_2D_3$. A lower VMR may indicate impaired CYP24A1 activity, reduced vitamin D receptor (VDR) signaling, or metabolic inefficiency in vitamin D processing [10,14]. These findings agree with earlier studies showing that VMR provides deeper insights into vitamin D function than total 25(OH)D alone.[19,20]

The persistence of the VMR–T2DM association after adjusting for BMI suggests that the relationship is independent of obesity and may reflect intrinsic metabolic differences in vitamin D handling.[21,22] This underscores the importance of considering body composition when assessing vitamin D–related metabolic risk in youth.[23]

Biologically, low VMR levels has been hypothesized to be related to pathways involved in insulin secretion, insulin sensitivity, and inflammatory regulation, all of which are critical pathways in the development of T2DM.[24]

CYP24A1 plays a central role in regulating the balance between active and inactive vitamin D forms, which has been implicated in pathways related to insulin secretion and glucose regulation.[25,26] Dysfunctional vitamin D signaling through the VDR pathway has been proposed in prior literature to be associated with altered β-cell activity and promote insulin resistance.[19,27]

Vitamin D also enhances insulin sensitivity in peripheral tissues, modulates calcium levels essential for insulin secretion, and exerts anti-inflammatory effects that protect against metabolic dysfunction.[27–30] If vitamin D metabolism is altered, as reflected by lower VMR, regulatory mechanisms involved in glucose homeostasis could potentially be affected; however, causal relationships cannot be inferred from this cross-sectional design.

Previous research on total 25(OH)D and diabetes has produced inconsistent findings.[31–34] Our results align with evidence that total 25(OH)D alone is an incomplete indicator of metabolic vitamin D status, emphasizing the need to consider functional markers such as VMR.[13,35]

The findings suggest that lower VMR may be associated with T2DM status in this cohort; however, given the cross-sectional design and limited statistical power, this association should be interpreted cautiously and requires validation in larger prospective studies. Future research should explore the relationship between VMR, vitamin D supplementation, and diabetes risk, focusing on functional markers of vitamin D status, particularly in those with very low levels.

The study's strengths include a large adolescent cohort covering different glycemic states, precise quantification of vitamin D metabolites by LC-MS/MS, and statistical adjustment for key confounders. However, some limitations should be acknowledged. Cross-sectional design prevents causal inference; the limited number of T2DM cases reduced statistical power; and lifestyle factors such as diet, sunlight exposure, and physical activity were not assessed. Future studies should include longitudinal and interventional designs, evaluate active vitamin D metabolites, and explore genetic variations in vitamin D–metabolizing enzymes.

While this study provides novel data on VMR in Saudi adolescents using gold-standard LC-MS/MS methodology, the exploratory findings are limited by the cross-sectional design, which precludes causal inference, and the small T2DM sample size, which limits statistical power and generalizability. These findings should be considered hypothesis-generating, requiring confirmation in larger prospective cohort studies.

Future research should: (1) conduct prospective studies to determine whether low VMR predicts T2DM onset; and (2) evaluate whether vitamin D supplementation improves VMR and reduces diabetes risk in adolescents.

Overall, these findings suggest that VMR may be associated with T2DM independent of total 25(OH)D levels, warranting further investigation.

Conclusion

In summary, this exploratory cross-sectional study provides preliminary evidence that lower vitamin D metabolite ratio (VMR) may be associated with T2DM in Saudi adolescents, independent of total 25(OH)D levels. However, the strength of this association should be interpreted cautiously given the small T2DM sample size (n = 33), the wide confidence interval (adjusted OR: 4.7, 95% CI: 1.1–20.0), and the modest level of statistical significance (p = 0.036). The cross-sectional design precludes causal inference. Therefore, these findings should be considered hypothesis-generating rather than definitive. The clinical utility of VMR as a biomarker for T2DM risk in adolescents requires confirmation in larger, adequately powered prospective studies. Future research should also determine whether VMR provides incremental predictive value beyond established risk factors and further clarify the biological mechanisms underlying the observed association.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Acknowledgments

This study was funded by the Ongoing Research Funding Program- Research Chairs (ORF-RC-2026-1400) at King Saud University, Riyadh, Saudi Arabia.

Author Contributions

NMA; Conceptualization, Data curation, Investigation, Writing-original draft, Funding acquisition, Writing – review and editing

AMA; Software, Formal analysis, Writing – original draft, Validation, Investigation, Data curation, Writing – review and editing

SDH; Software, Validation, Investigation, Funding acquisition, Formal analysis, Data curation, Writing – review and editing

SMY; Visualization: Investigation, Writing-original draft

All authors gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The author(s) report no conflicts of interest in this work.

References

1. Al-Daghri NM, Al-Saleh Y, Aljohani N, et al. Vitamin D deficiency and cardiometabolic risks: a Juxtaposition of Arab adolescents and adults. *PLoS One*. 2015;10(7):e0131315. doi:10.1371/journal.pone.0131315
2. Giudici KV, de Souza WN, Martini LA. Vitamin D status and glucose metabolism in youth. *Crit Rev Food Sci Nutr*. 2017;57(1):147–151. doi:10.1080/10408398.2013.866069
3. Choi DP, Oh SM, Lee JM, et al. Serum 25-hydroxyvitamin D and insulin resistance in apparently healthy adolescents. *PLoS One*. 2014;9(7):e103108. doi:10.1371/journal.pone.0103108

4. Juliaty A, Mutmainnah, Daud D, Lisal JS. Correlation between vitamin D deficiency and fasting blood glucose levels in obese children. *Clin Nutr ESPEN*. 2021;44:200–203. doi:10.1016/j.clnesp.2021.06.022
5. Nasr MH, Hassan BAR, Othman N, et al. Prevalence of vitamin D deficiency between type 2 diabetes mellitus patients and non-diabetics in the Arab Gulf. *Diabetes Metab Syndr Obes*. 2022;15:647–657. doi:10.2147/DMSO.S350626
6. Abukanna AMA, Alanazi RFA, Alruwaili FS, Alayyashi AZM, Alanzi F. Vitamin D deficiency as a risk factor for diabetes and poor glycemic control in Saudi Arabia: a systematic review. *Cureus*. 2023;15(11):e48577. doi:10.7759/cureus.48577
7. Alharbi N, Alduhailan M, Salami H, et al. Clinical consequences of vitamin D deficiency in Saudi Arabia: focus on diabetes and metabolic disorders. *Saudi J Med*. 2025;10:474–479. doi:10.36348/sjm.2025.v10i09.004
8. Sakaki T. Recent studies on vitamin D metabolizing enzymes. *Clin Calcium*. 2006;16(7):1129–1135.
9. Jenkinson C. The vitamin D metabolome: an update on analysis and function. *Cell Biochem Funct*. 2019;37(6):408–423. doi:10.1002/cbf.3421
10. Herrmann M, Zelzer S, Cavalier E, et al. Functional assessment of vitamin D status by a novel metabolic approach: the low vitamin D profile concept. *Clin Chem*. 2023;69(11):1307–1316. doi:10.1093/clinchem/hvad151
11. Khabarov S, Denisova O, Dalinskaya A. Reference values of vitamin D status: why is everything not so clear. *Medical Aphabet*. 2023;34–39. doi:10.33667/2078-5631-2023-23-34-39
12. Tang JCY, Nicholls H, Piec I, et al. Reference intervals for serum 24,25-dihydroxyvitamin D and the ratio with 25-hydroxyvitamin D established using a newly developed LC-MS/MS method. *J Nutr Biochem*. 2017;46:21–29. doi:10.1016/j.jnutbio.2017.04.005
13. Kaneva AM, Potolitsyna NN, Bojko ER. Association of serum 25-hydroxyvitamin D with metabolic disturbances in adolescents. *Am J Hum Biol*. 2022;34(12):e23802. doi:10.1002/ajhb.23802
14. Al-Daghri NM, Yakout S, Sabico S, et al. Establishing the prevalence of osteomalacia in arab adolescents using biochemical markers of bone health. *Nutrients*. 2022;14(24):5354. doi:10.3390/nu14245354
15. Al-Daghri NM, Sabico S, Wani K, et al. Association of bone mineralization markers with dietary nutrient intake in adolescents with and without biochemical osteomalacia. *Front Nutr*. 2023;10:1206711. doi:10.3389/fnut.2023.1206711
16. Sabico S, Al-Daghri NM, Alenad A, et al. Sexual dimorphism in LC-MS/MS-based vitamin D metabolite profiling and nutrition-acquired biochemical osteomalacia among adolescents. *Front Nutr*. 2025;12:1696230. doi:10.3389/fnut.2025.1696230
17. Darling AL, Lanham-New SA. Chapter 51 - defining thresholds for vitamin D I: scientific rationale for serum 25-hydroxyvitamin D cutoffs of 25 and 50nmol/L. In: Hewison M, Bouillon R, Giovannucci E, Goltzman D, Meyer M, Welsh J, editors. *Feldman and Pike's Vitamin D (Fifth Edition)*. Academic Press; 2024:1–9.
18. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2010;33(Suppl 1):S62–9. doi:10.2337/dc10-S062
19. Shahidzadeh Yazdi Z, Streeten EA, Whitlatch HB, Montasser ME, Beitelshees AL, Taylor SI. Critical role for 24-hydroxylation in homeostatic regulation of vitamin D metabolism. *J Clin Endocrinol Metab*. 2025;110(2):e443–e455. doi:10.1210/clinem/dgae156
20. Zhang J, Wu K, Dong X. Exploring the association between serum vitamin D levels and type 2 diabetes risk in US adults. *Ann Med*. 2025;57(1):2522320. doi:10.1080/07853890.2025.2522320
21. Rafiq S, Jeppesen PB. Body mass index, vitamin D, and type 2 diabetes: a systematic review and meta-analysis. *Nutrients*. 2018;10(9):1182. doi:10.3390/nu10091182
22. Shen ZJ, Liu M, Zhang JX, Huang Y, Kong Y, Liu SG. Comparison of serum vitamin D levels in obese subjects with and without type 2 diabetes mellitus. *J Inflamm Res*. 2024;17:5915–5922. doi:10.2147/JIR.S475180
23. Cao H, Huang X, Luo B, Shi W, Li H, Shi R. Gender differences of visceral fat area to hip circumference ratio for insulin resistance. *Diabetes Metab Syndr Obes*. 2024;17:3935–3942. doi:10.2147/DMSO.S482820
24. Abdullah M, AlQwaidi S, Alshehri A, et al. Obesity-induced inflammation and its role in the development of insulin resistance. *J Healthcare Scie*. 2024;04:448–454. doi:10.52533/JOHS.2024.41002
25. St-Arnaud R, Jones G. Chapter 5 - CYP24A1: structure, function, and physiological role. In: Hewison M, Bouillon R, Giovannucci E, Goltzman D, Meyer M, Welsh J, editors. *Feldman and Pike' s Vitamin D (Fifth Edition)*. Academic Press; 2024:71–83.
26. Baghirova S, Rahimova R, Dashdamirova G. Vitamin D: structure and mechanism of action. *World J Biol Pharm Health Sci*. 2022;9:036–041. doi:10.30574/wjbphs.2022.9.3.0052
27. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol*. 2010;2010:351385. doi:10.1155/2010/351385
28. Park CY, Shin S, Han SN. Multifaceted roles of vitamin D for diabetes: from immunomodulatory functions to metabolic regulations. *Nutrients*. 2024;16(18):3185. doi:10.3390/nu16183185
29. Das PN, Halder S, Bairagi N, Chatterjee S. Delay in ATP-dependent calcium inflow may affect insulin secretion from pancreatic beta-cell. *Appl Math Modell*. 2020;84:202–221. doi:10.1016/j.apm.2020.03.030
30. Sert T, Arslan Ş. Type 2 diabetes mellitus and vitamin metabolism: vitamins in type 2 diabetes. *J Surg Med*. 2025;9. doi:10.28982/josam.8115
31. Michigami T. Update on recent progress in vitamin D research. Vitamin D metabolism and its regulation. *Clin Calcium*. 2017;27(11):1517–1523.
32. Wan Z, Song L, Hu L, et al. The role of systemic inflammation in the association between serum 25-hydroxyvitamin D and type 2 diabetes mellitus. *Clin Nutr*. 2021;40(6):3661–3667. doi:10.1016/j.clnu.2021.04.029
33. Osmani D, Shaik H. A possible correlation between low serum vitamin-D levels and type 2 diabetes mellitus. *Int J Adv Biochem Res*. 2020;4:06–11. doi:10.33545/26174693.2020.v4.i1a.40
34. Dominguez LJ, Veronese N, Marrone E, et al. Vitamin D and risk of incident type 2 diabetes in older adults: an updated systematic review and meta-analysis. *Nutrients*. 2024;16(11):1561. doi:10.3390/nu16111561
35. Al shaikh A, Al Zahrani AM. Impact of vitamin D status on cardiometabolic complications among children and adolescents with type 1 diabetes mellitus. *J Clin Res Pediatr Endocrinol*. 2016;8(1):48–54. doi:10.4274/jcrpe.2266

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