



Vitamin D receptor methylation attenuates the association between physical activity and type 2 diabetes mellitus: A case-control study

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Abstract

Background: Physical activity and vitamin D receptor (VDR) have been associated with type 2 diabetes mellitus (T2DM). However, the associations of VDR methylation with T2DM and physical activity remained unknown. We aimed to investigate whether VDR methylation was a link between physical activity and T2DM.

Methods: A 1:1 matching case-control study was designed based on the Henan Rural Cohort Study, including 272 pairs of T2DM patients and non-patients. Physical activity level was assessed using the International Physical Activity Questionnaire. The high-resolution melt method was applied to determine the methylation level of the promoter region of VDR. The association between physical activity and T2DM was analyzed with a conditional logistic regression model. The effect modification of VDR methylation levels on the association between physical activity and T2DM was conducted. A multivariate correlation analysis model was applied to investigate correlations of VDR methylation with insulin sensitivity.

Results: Physical activity level was associated with T2DM risk (crude model: odds ratio [OR] 0.611; 95% CI, 0.416-0.897; adjusted model: OR 0.619; 95% CI, 0.418-0.917). In effect modification analysis, the effects of physical activity on T2DM were stronger for low VDR methylation levels than for high ($P = .025$). Moreover, VDR methylation levels were associated with insulin ($r = -0.089$, $P = .039$) as well as homeostatic model assessment of insulin resistance ($r = -0.098$, $P = .022$).

Conclusions: The methylation status of the VDR promoter is associated with the secretion and sensitivity of insulin. VDR methylation attenuates the association between physical activity and T2DM, indicating that proactively physical activity may reduce the risk of T2DM, especially in people with low VDR methylation level.

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KEYWORDS

DNA methylation, insulin resistance, physical activity, type 2 diabetes mellitus, vitamin D receptor

Highlights

- Vitamin D receptor (VDR) methylation attenuates the association between physical activity and type 2 diabetes mellitus (T2DM).
- The methylation status of the VDR promoter is associated with the secretion and sensitivity of insulin.
- A high physical activity level is associated with a decreased risk of T2DM compared to a low physical activity level.

1 | INTRODUCTION

Physical activity plays a significant role in prevention of type 2 diabetes mellitus (T2DM).^{1,2} Many studies have indicated an inverse association between physical activity and the risk of T2DM.^{1–3} Increased physical activity could reduce the risk caused by sedentary behavior.^{2,4} Besides increasing glycolipid uptake and utilization, another beneficial effect of physical activity on T2DM is to regulate expressions of metabolic genes by modulating DNA methylation.⁵ Variations of DNA methylation in human islets, skeletal muscle, adipose tissue, and liver were found to be associated with T2DM.⁶ Therefore, a better insight into the relationship between physical activity and T2DM could be obtained by investigating DNA methylation caused by physical activity.

The widely known function of vitamin D is to maintain calcium homeostasis and bone metabolism.⁷ In the past two decades, accumulative evidence suggested that vitamin D played a role in other biological activities, for example, proliferation, differentiation, apoptosis, and immune response.⁸ Thus, vitamin D is associated with many diseases including T2DM.^{9–11} More and more studies showed that vitamin D deficiency may be a risk factor of T2DM.^{12,13} Vitamin D supplementation would benefit prevention and control of T2DM.^{14,15}

The diverse biological functions of vitamin D are accomplished by precise changing of gene expression through the vitamin D receptor (VDR). As the key protein to mediate vitamin D function, VDR may play an important role in the development of T2DM. Meta-analysis suggested that polymorphism in the VDR gene was associated with T2DM.^{16,17} Recently, Wei et al reported that VDR as a key modulator of inflammation and β -cell survival was a potential therapeutic target for T2DM.¹⁸ Thus, aberrant expression of VDR may contribute to the development of T2DM.

DNA methylation as an important epigenetic regulator plays a key role in gene expression.¹⁹ Studies suggested

that increased methylation of the VDR promoter was associated with decreased mRNA and VDR protein.^{20,21} Methylation variation of the VDR promoter had been reported to be associated with cancer.^{20,22} However, the association between methylation status of the VDR promoter and T2DM remained unknown. Furthermore, whether physical activity is associated with the methylation status of VDR was also unclear.

Therefore, we hypothesized that physical activity could change VDR methylation status and further affect the development of T2DM. This study aimed to examine the relationship between physical activity and methylation variation of the VDR promoter and its effect on T2DM in a case-control study. The results would shed more light on the role of physical activity in the prevention and control of T2DM.

2 | METHODS

2.1 | Study subjects

A total of 272 people with T2DM aged 18 to 79 years were from Zhengzhou, Henan province, China. Then 272 individuals without T2DM from the same residential area were selected according to the rule of same gender, same exposure of smoking and drinking, and similar age (less than 3 years difference) to carry out a 1:1 matching case-control study. The guidelines of the American Diabetes Association (2002) and the criteria of the World Health Organization (1999) were applied to diagnose patients with T2DM. And all the non-T2DM participants were determined by an oral glucose tolerance test with 75 g glucose.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Life Science Ethics Review Committee of Zhengzhou



University. Written informed consent was obtained from all subjects.

2.2 | Data collection

The 544 subjects' information was collected by a standardized questionnaire and physical examination. The questionnaires were completed in face-to-face interviews by well-trained investigators. All the subjects' weight and height were measured three times, and the averages were used to calculate their body mass index (BMI). All the participants were asked to complete the International Physical Activity Questionnaire-Short Form (IPAQ-SF). The IPAQ recorded the different intensity level of physical activity in the last 7 days, including running, brisk walking, hoeing, mountain climbing, jogging, walking, sedentary behavior, etc. The information collected through the IPAQ can be transformed into continuous variables, indicating metabolic equivalents of tasks (MET). According to IPAQ's algorithm, combined with MET and the time and intensity of daily activities, physical activities were divided into three levels: low, moderate, and high. For a detailed scoring protocol, please refer to the website: <https://sites.google.com/site/theipaq/scoring-protocol>.

2.3 | Measurements of glucose and insulin

A kit with the glucose oxidase method was applied for measurement of fasting blood glucose (Kehua Bio-Engineering Co Ltd, Shanghai, China). The levels of fasting insulin were determined with a radioimmunoassay kit (North Institute of Biotechnology, Beijing, China).

2.3.1 | Methylation levels of the VDR promoter region

Peripheral blood DNA was extracted using a whole blood genome DNA isolation kit (Bioteke Co Ltd, Beijing, China) according to the manufacturer's guidelines. The high-resolution melt (HRM) method was applied to determine the methylation levels of the promoter region of VDR. The target sequence contains 27 CpGs within 300 bp (position from 47 905 396 to 47 905 695, chromosome 12, GRCh38.p12 assembly, National Center for Biotechnology Information [NCBI]). HRM assay was performed on the MeltDoctor HRM platform in the 7500 Fast System (Applied Biosystems, California). The results were processed with the HRM Software in Applied Biosystems.

2.4 | Statistical analysis

Categorical variables are described as frequency and percentage and compared by the chi-square test. Continuous variables with normal distribution are presented as means \pm SD and compared with Student's *t* test. Continuous variables with skew distribution are presented as median (interquartile range) and compared with the Wilcoxon rank sum test.

We analyzed the data with the following strategy. First, we investigated the association between physical activity and T2DM with a conditional logistic regression model in the case-control study. Second, to identify the combined associations of VDR methylation levels and physical activity with T2DM, VDR methylation levels were grouped according to corresponding medians values, and physical activity was divided into low physical activity and moderate to vigorous physical activity levels according to the criteria of IPAQ. The effect modification on a multiplicative scale was assessed by including the main effect of VDR methylation levels and physical activity and product term in analysis.²³ The *P* values of the product term were used to identify the effect modification on the multiplicative scale. The effect modification of physical activity on the association between VDR methylation levels and T2DM risk was computed based on the following equation:

$$(\hat{Q}_1 - \hat{Q}_2) \pm 1.96 \left(\sqrt{SE_1^2 + SE_2^2} \right),$$

where \hat{Q}_1 and \hat{Q}_2 are the estimates for the two subgroups, and SE_1 and SE_2 are their respective standard errors. Finally, a multivariate correlation analysis model was applied to investigate correlations of VDR methylation levels with insulin and homeostatic model assessment of insulin resistance (HOMA-IR).

All the statistical analyses, except the interaction investigation, were completed with SPSS 21.0 (IBM SPSS, New York). A two-tailed *P* value less than .05 was considered as statistically significant.

3 | RESULTS

3.1 | Subject characteristics

The demographic and biochemical characteristics of the study participants are shown in Table 1. Gender, smoking, and drinking were exactly matched between case and control. There was no significant difference in age between case and control (*P* = .973). BMI in the case group was higher than in controls (*P* = .002). Both the level of glucose and insulin in the case group were higher

TABLE 1 Demographic and biochemical characteristics of the study participants

Variables	Control (n = 272)	Case (n = 272)	P
Gender			-
Male (%)	101 (37.1)	101 (37.1)	
Female (%)	171 (62.9)	171 (62.9)	
Age (y)	59.4 ± 12.7	59.4 ± 12.6	.973
Smoking			-
Never (%)	206 (75.7)	206 (75.7)	
Ever (%)	19 (7.0)	19 (7.0)	
Current (%)	47 (17.3)	47 (17.3)	
Drinking			-
Never (%)	241 (88.6)	241 (88.6)	
Ever (%)	11 (4.0)	11 (4.0)	
Current (%)	20 (7.4)	20 (7.4)	
BMI (kg/m ²)	25.5 ± 3.2	26.5 ± 3.8	.002*
Glucose (mmol/L)	4.7 ± 0.8	9.4 ± 3.5	<.001*
Insulin (mIU/L)	11.7 ± 6.5	14.8 ± 8.1	<.001*
VDR methylation (%)	1.24 (1.15, 1.56)	1.34 (1.15, 1.45)	.673

Note: Categorical variables are described as frequency and percentage and compared by a chi-square test. Continuous variables with normal distribution are presented as means ± SD and compared with Student's *t* test. Continuous variables with skew distribution are presented as median (interquartile range) and compared with the Wilcoxon rank sum test. "-" shows exact case-control matching.

Abbreviations: BMI, body mass index; VDR, vitamin D receptor.

**P* < .05.

TABLE 2 Associations of physical activity and T2DM

Physical activity	Control (n = 272)	Case (n = 272)	Crude model		Adjusted model ^a	
			OR (95% CI)	P	OR (95% CI)	P
Low	104 (38.2)	133 (48.9)	Reference		Reference	
Medium	54 (19.9)	49 (18.0)	0.695 (0.434, 1.113)	.130	0.645 (0.398, 1.047)	.076
High	114 (41.9)	90 (33.1)	0.611 (0.416, 0.897)	.012*	0.619 (0.418, 0.917)	.017*

Note: The conditional logistic regression model was applied to investigate the association between physical activity and T2DM.

Abbreviations: OR, odds ratio; T2DM, type 2 diabetes mellitus.

^aAdjusted for age and body mass index.

**P* value <.05.

than those in the control (*P* < .001). There was no significant difference in methylation level of VDR between case and control (*P* = .673).

3.2 | Association between physical activity and T2DM

The conditional logistic regression model was applied to investigate the association between physical activity and T2DM (Table 2). The result suggests that a high physical activity level is associated with a decreased risk of T2DM

compared to a low physical activity level (odds ratio [OR] 0.611; 95% CI, 0.416-0.897; *P* = .012). These associations persisted after adjusting for BMI and age (OR 0.619; 95% CI, 0.418-0.917; *P* = .017).

3.3 | Effect modification of VDR methylation levels on T2DM by physical activity

The VDR methylation levels were divided into low and high, and according to the criteria of IPAQ, physical

**TABLE 3** Modification of the effect of physical activity on T2DM by VDR methylation levels

	LPA		MVPA		
	T2DM/non-T2DM	OR (95% CI)	T2DM/non-T2DM	OR (95% CI)	OR (95% CI)
VDR methylation levels					
Low	67/44	1	66/60	0.759 (0.447-1.289)	0.464 (0.227-0.948)
High	64/98	0.422 (0.253-0.705)	75/70	0.713 (0.428-1.185)	1.174 (0.764-1.802)
<i>P</i>			.025		

Note: Conditional logistics regression analyses were used to assess the effect modification of VDR methylation on T2DM by physical activity. Models were adjusted for body mass index and age. The measure of effect modification on the multiplicative scale: $P = .025$.

Abbreviations: LPA, low physical activity; MVPA, moderate to vigorous physical activity; OR, odds ratio; T2DM, type 2 diabetes mellitus; VDR, vitamin D receptor.

TABLE 4 Correlations of VDR methylation levels with insulin and HOMA-IR

Variables	VDR methylation levels	
	Correlation coefficient	<i>P</i>
Insulin	−0.089	.039*
HOMA-IR	−0.098	.022*

Note: A multivariate correlation analysis model was applied to investigate correlations of VDR methylation levels with insulin and HOMA-IR.

Adjusted for physical activity, age, and body mass index.

Abbreviations: HOMA-IR, homeostatic model assessment of insulin resistance; VDR, vitamin D receptor.

* P value $< .05$.

activity was divided into low physical activity and moderate to vigorous physical activity level. Effect modification analysis was used to test the association of VDR methylation levels and physical activity with T2DM. The results of effect modification analysis are shown in Table 3. VDR methylation status modified the correlation between physical activity and T2DM. The measure of effect modification on the multiplicative scale is represented by P values ($P = .025$). In the stratified analysis, the effects of physical activity on T2DM were stronger for low VDR methylation levels than for high (OR 0.464; 95% CI, 0.227-0.948), which suggests that for people with low VDR methylation level, low physical activity increased the risk of T2DM.

3.4 | Associations of VDR methylation levels with insulin and HOMA-IR

A multivariate correlation analysis model was applied to investigate correlations of VDR methylation levels with insulin and HOMA-IR (Table 4). The results suggest that increased methylation levels of VDR are associated with decreased levels of insulin ($r = -0.089$, $P = .039$) as well as decreased HOMA-IR ($r = -0.098$, $P = .022$), adjusted for covariates including physical activity, age, and BMI.

4 | DISCUSSION

Associations of physical activity with T2DM and VDR methylation were investigated in this study. The results suggest that a high physical activity level is associated with a decreased risk of T2DM compared to a low physical activity level. On the other hand, there was effect modification between VDR methylation status with physical activity and T2DM, which indicated that VDR methylation attenuated the association between physical activity and T2DM. In addition, increased methylation levels of VDR was associated with decreased levels of serum insulin as well as decreased HOMA-IR.

The islets had a significant expression of the VDR gene.²⁴ VDR may play a role in maintaining the maturity of β -cells.²⁵ Recently, Wei reported that VDR as a key modulator of inflammation and β -cell survival was a potential therapeutic target for T2DM.¹⁸ Thus, VDR is associated with insulin secretion. Pilon and colleagues reported that VDR gene promoter methylation may play a role in reduced expression of the VDR gene.²⁰ Meyer found that rs11568820 (AA vs AG/GG) in the VDR gene was associated with both higher VDR promoter methylation and lower VDR mRNA induction, which suggests that there may be an association between VDR promoter methylation and the levels of VDR mRNA.²¹ Therefore, an increased methylation level of the VDR promoter may lead to decreased VDR expression and then less secretion of insulin.

Maestro reported that there was a vitamin D response element in the promoter of human insulin receptors, and VDR could specifically recognize the region to regulate the expression of the insulin receptors.²⁶ In summary, increased methylation levels of the VDR promoter may lead to decreased VDR expression. Decreased VDR levels may cause fewer insulin receptors, which would result in decreased insulin sensitivity. Due to the associations of VDR with insulin secretion

and insulin sensitivity, VDR may play a role in development of T2DM. A meta-analysis conducted by Han indicated that variants of ApaI, BsmI, and FokI in the VDR gene were associated with T2DM.¹⁶ Another meta-analysis we conducted also suggested that the FokI polymorphism in the VDR gene may be a risk factor of T2DM.¹⁷ All these results demonstrate that VDR may play a role in development of T2DM.

It is worth noting that some studies reported a correlation between VDR methylation status and physical activity, which may be partly responsible for the effect modification, although this association has not been found in this study. Voisin et al reviewed the connection between exercise training and DNA methylation in humans. They found that both acute and chronic exercise could impact DNA methylation in a tissue- and gene-specific manner.²⁷ Denham and colleagues reported that the sperm methylome could be reprogrammed by 3 months of exercise training.²⁸ Thus, physical activity may decrease the methylation level of the VDR promoter and increase VDR expression. Physical activity may be beneficial for T2DM prevention by improving the biological functions of vitamin D through maintaining low methylation of the VDR promoter.

However, limitations must be acknowledged in the current study. First, the threshold of physical activity or VDR methylation status for T2DM risk could not be determined in the current analysis, which has significant implications for prevention strategies in susceptible populations. Future work will need to focus on the quantitative analysis of this association. Second, the current study could not establish causal associations because of the cross-sectional design. Prospective, larger sample size research is needed in the future.

In conclusion, the methylation status of the VDR promoter is associated with the secretion and sensitivity of insulin. VDR methylation attenuates the association between physical activity and T2DM, indicating that physical activity should be proactively increased to reduce the risk of T2DM, especially in people with low VDR methylation levels.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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