

## Association between FokI Vitamin D Receptor Gene Polymorphism and Visceral Obesity in Patients with Type 2 Diabetes: A Single-Blinded Randomised Clinical Trial

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### ABSTRACT

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**Background:** Role of genetic variants on the effect of vitamin D on adiposity measures is still unclear. This study aimed to investigate the effect of vitamin D on visceral adiposity using intake of vitamin D fortified doogh.

**Methods:** This was a single-blinded randomised clinical trial which seventy type 2 diabetic Iranian subjects were randomly allocated to two groups of receiving plain Doogh (PD; n 32, containing 170 mg Ca and no vitamin D/250 ml) or vitamin D3-fortified Doogh (FD; n 38, containing 170 mg Ca and 12.5 µg/250 ml) twice a day for 3 months. Serum 25-hydroxyvitamin D (25(OH)D), anthropometric measures, body composition, glycaemic status and visceral obesity indicators assessed twice before and after the intervention. Genotyping was conducted for FokI single nucleotide polymorphisms (SNPs) of the VDR gene by polymerase chain reaction.

**Results:** Serum 25(OH)D ( $P < 0.001$ ) was increased in FD compared to PD and waist circumference ( $P = 0.022$ ), fat mass ( $P < 0.001$ ), visceral adipose tissue ( $P < 0.001$ ), and trunk fat ( $P < 0.001$ ) were decreased after 3-month intervention. Analysis of FOK-1 genotyping was showed significant change for 25(OH)D ( $P = 0.03$ ), FSG ( $P = 0.036$ ), HbA1c ( $P = 0.003$ ), waist circumference ( $P = 0.045$ ), waist: hip ratio ( $P = 0.04$ ), fat mass ( $P = 0.016$ ), visceral adipose tissue ( $P = 0.024$ ), and Truncal fat ( $P = 0.004$ ) after Tukey's post hoc analysis.

**Conclusion:** In conclusion, FD compared to PD could increase serum 25(OH)D and improve glycaemic, anthropometric, and visceral obesity indicators. Moreover, significant differences was seen between FOK-1 (FF, Ff, and ff) genotypic groups for 25(OH)D, FSG, HbA1c, waist circumference, waist: hip ratio, fat mass, visceral adipose tissue, and Truncal fat.

#### Introduction

Type 2 diabetes (T2D) is a chronic diseases and characterized by hyperglycemia, insulin resistance, and other serious disorders like neuropathy, nephropathy and retinopathy which imposes a large socioeconomic burdens on health system [1]. Several risk factors associated with diabetes have been identified, including physical inactivity [2], alcohol consumption [3], smoking [4], and obesity [5]. Among these factors, obesity is one of the major metabolic disorder and a pathological condition defined by defecting in metabolism of carbohydrate, fat, and protein [6]. An increase in obesity and diabetes is seen

together, which indicates a direct linkage between these two disorders. Central obesity, where visceral adipose tissue (VAT) stored, is more predictive indexes for diabetes incidents and to measure it, waist circumference (WC) and waist to hip ratio (WHR) have been used. However, body mass index (BMI) is a measure of general obesity [6-7]. Although, the etiology of obesity was not fully examined, it is an interaction between genetics and environmental factors. Studies have been shown that genetics is responsible for 50-70 % of the variation in BMI [8]. Moreover, up to about 80% of the differences in WC are explained by inheritance [9]. Due to

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the decisive role of the gene in obesity and as a consequence in diabetes, many genome wide association studies (GWAS) focusing on the genes involved in obesity, have been designed.

Numerous studies have demonstrated a link between vitamin D and obesity [10-11]. There is a hypothesis that justifies this relationship by increasing the lipogenesis following an increase in serum parathyroid hormone in vitamin D deficiency [12]. So that, the hypothesis of vitamin D role in obesity is taken into account. Active form of vitamin D (1,25(OH)<sub>2</sub>D<sub>3</sub>), a fat-soluble hormone, is a ligand for vitamin D receptor (VDR) which is a member of the nuclear receptor super family [13]. Actually, about 3% of the human genomes expression are regulated by VDR [14]. VDR is expressed in many tissues such as pancreatic β-cells which are related to glucose metabolism and also diabetes [15]. Moreover, studies have been shown that vitamin D deficiency reduced glucose tolerance and inhibit insulin secretion [16-17]. There are several single nucleotide polymorphisms (SNPs) for VDR gene which the most important of them including TaqI (rs731236), BsmI (rs1544410), ApaI (rs7975232), and FokI (rs2228570). FokI is located in exon 2 of the DNA gene segment and near the promoter at 5' end of the gene [18-19].

Some studies confirm the relationship between FokI polymorphisms and obesity [20]. There are controversial studies though [18]. However, the relation between FokI VDR gene polymorphism and obesity and diabetes are conflicting and needs more randomized clinical trials. The current study was conducted to investigate the effect of vitamin D-fortified Doogh on obesity considering the FokI VDR gene polymorphism.

## Subjects and methods

### *Study protocol*

This was a 12-week randomized controlled clinical trial (RCT) which was a part of a larger trial that has previously been described and conducted during mid-October 2010 to late March 2011. The trial was done in compliance with the Declaration of Helsinki and received ethical approval from the ethics committees of the National Nutrition and Food Technology Research Institute (NNFTRI), Shahid Beheshti University of Medical Sciences (SBUMS), and Tehran University of Medical Sciences (TUMS). The trial protocol has been published in more details, previously [21]. The registration number of the RCT at ClinicalTrials.gov is NCT01236846.

### *Participants recruitment*

Patients with T2D were recruited from Iranian Diabetes Society and Gabric Diabetes Society in Tehran, Iran. The inclusion criteria were comes as follow: (1) Having type 2 diabetes, (2) Willingness to participate, (3) BMI between 25 to 35 kg/m<sup>2</sup>, (4) 30-60 years old, (5) No use of any vitamin, dietary, herbal or omega-3 supplements since at least 3 months to and during the intervention period. Participants were excluded if they had history of cardiovascular, gastrointestinal, renal, and other endocrinological diseases or receive vitamin D, omega-3 or other types of dietary supplements. Volunteers were asked to attend Laboratory of Nutrition Research at NNFTRI while they were fasting for 12–14 h. Data gathering, bleeding, and all the measurement are done during 07.30 and 10.00 am. For more details find our previous paper [22].

### *Intervention, compliance and quality control*

In this was a single-blinded RCT after 2-week run-in period, all study subjects were randomly assigned to one of the two groups of either fortified yogurt drink (doogh) (FD, n =38) or plain yogurt drink (PD, n =32) using permuted blocks of random numbers allocation method. The subjects were consumed FD containing 170 mg calcium and 500 IU vitamin D<sub>3</sub>/250 mL or PD containing 170 mg calcium and no vitamin D/250 mL twice a day. Participants were completely instructed and biweekly visited to provide the next 2-week doogh and evaluate their consumption and compliance. Every two weeks, participants were given the same yogurt drink in colour, size, taste and packaging. They instructed to mark the yogurt drink consumption table prepared for them after drinking a yogurt drink with each meal. We asked them to return the yogurt drink bottles every two-week. Vitamin D<sub>3</sub> composition of yogurt drink were measured at first, the end and in the middle of storage by Maad Laboratory of Foods, Drinks and Cosmetics, accredited by the Deputy of Food and Drug of the Iranian Ministry of Health to ensure the stability of vitamin D<sub>3</sub>.

### *Outcome Measurements*

#### *Dietary assessment*

A three-day dietary 24-recall questionnaire was used to evaluate dietary intakes. It is included two work day and one weekend. Then, the data from questionnaire was analyzed using N4 software (NUTRITIONIST 4, First Data Bank, San Bruno, CA, USA).

*Dietary assessment*

Weight was measured in light clothing without shoes using a digital scale (Seca808; Seca, Hamburg, Germany) with a sensitivity of 0.1 kg and height by a stadiometer (Seca) with a sensitivity of 0.1 cm. BMI was calculated as weight in kilograms divided by the height square in meters. Waist circumference was measured by strip tape between the lowest gear and the iliac crest and in expiratory state. Then the waist to hip ratio (WHR) will be calculated for each person.

*Body composition analysis*

Body fat mass percentage was assessed using bioelectrical impedance analysis (Quadscan 4000 system; Bodystat). The percentage of trunk fat (TF) and the degree of VAT were evaluated by the Tanita AB-140 ViScan (Tanita Corporation). The study subjects were asked to avoid eating and exercising for at least 4 h before assessment. Other measurement conditions are available in more details [23].

*Laboratory investigations*

After 12-14 hours fasting, 20 mL of venous blood taken. Then divided into two tubes with or without an anticoagulant, EDTA. The anticoagulated tube was employed for extracting DNA genom and HbA1c, and the one without EDTA was used for biochemical analysis. Fasting bloodsugar (FBS) was determined by commercial kits (Pars Azmoon) using enzymatic method and an auto-analyzer system (Selectra E; Vitalab). Glycated Hb (HbA1c) was measured using colorimetric method after an initial chromatographic separation (BioSystems). Fasting serum insulin was assayed by immunoradiometric assay (Biosource) and a gamma-countersystem (Gamma I; Genesys). Insulin sensitivity was evaluated by Quantitative Insulin Check Index (QUICKI) calculated as [28]: QUICKI index:  $1/(\log(\text{insulin}) (\mu\text{U/ml}) + \log(\text{glucose}) (\text{mg/dl}))$ .

*DND extraction and genotyping*

The extraction of DNA was done in whole blood samples using Genet Bio DNA Isolation kit (Prime Prep) according to the procedures in the kit protocol. The VDR genotypes at FokI SNP sites are determined using polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. PCR was performed for thirty cycles and at 68°C annealing temperature. DNA was digested with FOKI enzyme (Fermentas; Thermo Scientific).

DNA was digested with Bpu10I (Fermentas; Thermo Scientific), and the products were analyzed by electrophoresis on a 1.5 % agarose gel containing ethidium bromide and were visualized in a gel documentation system (UVIdoc; UVIttec).

*Statistical analysis*

All statistical analyses were conducted using the SPSS (version 21). A P value of <0.05 was considered significant. Data were reported as means and standard deviations. To check the normality of data distribution Kolmogorov-Smirnov was used.

To compare the variable between two groups of PD and FD, an independent sample t tests (for normally distributed variables) was used. Repeated-measures ANOVA was used to evaluate time×group interactions, with time and group as factors. In case of significant time–group interaction, between-group comparison of changes at week 12 was carried out using ANOVA followed by Tukey's post hoc analysis with polynomial contrast analysis for trend when indicated. When time effect was significant, the within-group comparison of values was performed by paired sample t tests. The differences in frequency of the variable among polymorphic groups (FF, Ff, and ff) are compared by analysis of variance (ANOVA) and, if the differences were significant, Tukey's post hoc analysis was done.

**Results**

Participants recruitment is shown in flow diagram of (Figure 1). Thirty-seven women and thirty-three men with mean age of FD:  $51.53 \pm 7.19$  and PD:  $53.66 \pm 7.69$  and no significant differences about age ( $P=0.236$ ) were included in the study. There were no significant differences between sex distributions ( $P=0.57$ ), diabetes duration ( $P=0.625$ ), physical activity ( $P=0.75$ ), and sun exposure ( $P=0.407$ ) between groups. Moreover, sex ( $P=0.75$ ), diabetes duration ( $P=0.522$ ), physical activity ( $P=0.41$ ), and sun exposure ( $P=0.37$ ) distributions were not different between groups of FOK-1 genotypes. Age distributions differences between genotype groups of FF ( $53.73 \pm 6.12$ ), Ff ( $52.15 \pm 8.04$ ), and ff ( $44.67 \pm 10.42$ ) were significant ( $P=0.017$ ) (Table 1).

Anthropometric measures, body composition, 25(OH)D concentration and other serum biomarkers were not significantly different between two groups at baselines. Significant time effect was observed for 25(OH)D ( $P<0.001$ ), HbA1c ( $P<0.001$ ), and FM

(P=0.036). Serum 25(OH)D concentration in FD group showed a significant increase compared to baseline (P<0.001). WC (P<0.001), and VAT (P=0.036) were decreased significantly after fortified Doogh consumption in FD group compared to PD group. A marginally significant reduction was also observed for FM (P=0.052). Analysis of within group changes revealed a significant Time × group interaction for 25(OH)D (P<0.001), QUICKI (P<0.001), WC (P= 0.022), FM (P<0.001), VAT (P<0.001), and Truncal fat (P<0.001)in which WC, FM, VAT, and truncal fat decreased (Table2).

There were no significant differences among variables among three genotypic groups at baseline. We observed a significant time effect for FSG (P=0.021), QUICKI (P=0.004), weight (P=0.009), BMI (P=0.009), WC (P=0.004), WHR (P<0.001), and truncal fat (P=0.019) while the same significant effect was not seen for 25(OH)D, HbA1c, FM, and VAT after 12 weeks

of intervention. Significant difference was observed for serum 25(OH)D (P=0.008) between 3 genotypic groups and the increase of serum 25(OH)D in FF genotypic group was significant (P<0.001). In contrast, no significant increase was reported for two other groups of Ff (P=0.431) and ff (P=0.315). HbA1c was also different between groups (P=0.012) while this difference was significant for FF genotypic group (P=0.001) after 12 weeks intervention. Between groups changes for weight and BMI were also showed a significant decrease (p=0.037 and p=0.016, respectively). The changes was related to ff genotype for weight (P=0.001) and BMI (P=0.001). The analysis of Time×group showed significant effects for 25(OH)D (P=0.03), FSG (P=0.036), HbA1c (P=0.003), WC (P=0.045), WHR (P=0.04), FM (P=0.016), VAT (P=0.024), and Truncal fat (P=0.004) (Table3).

**Table 1. characteristics of the subjects enrolled in the randomised clinical trial (RCT) study and FOK-1 genotypic groups**

Variables	Treatment groups (n 70)				FOK-1 genotypic groups (n 70)							
	FD (n 38)		PD (n 32)		P	FF (n 44)		Ff (n 20)		ff (n 6)		P
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	
Age (years)	51.53	7.19	53.66	7.69	0.236	53.73	6.12	52.15	8.04	44.67	10.42	0.017
Sex (male/female)	15.17		18.20		0.57	22.22		8.12		3.3		0.75
Diabetes duration (years)	7.47	5.58	8.13	5.46	0.625	7.77	5.77	8.45	5.53	5.50	2.51	0.522
Physical activity (%)	30.4		29.0		0.75	40.6		15.9		2.9		0.41
Sun exposure (min/d)	54.03	62.22	66.59	37.71	0.407	60.68	46.24	47.14	27.64	86.00	120.7	0.37

PD, plain doogh; FD, vitamin D-fortified doogh.

\* Very low physical activity.

**Table 2. Comparison of the initial and final values of the variables**

	Plain doogh (n 32)						Fortified doogh (n 38)						Time Group Time×group		
	Before		After		Change		Before		After		Change		P1	P2	P3
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
25(OH)D (nmol/l)	16.30	7.99	14.24	9.1	-5.14	16.13	16.41	6.19	30.43	7.45	35.03	18.19	0.000	0.000	0.000
FSG (mmol/l)	160.18	50.53	170.00	52.88	9.81	46.51	167.32	42.96	156.32	42.41	-11.00	49.08	0.919	0.754	0.076
HbA1c (proportional)	9.04	1.69	8.28	1.62	-0.75	1.89	8.87	2.35	7.58	1.83	-1.2	2.00	0.000	0.279	0.263
QUICKI	0.28	0.01	0.27	0.01	-0.009	0.01	0.27	0.02	0.28	0.02	0.01	0.02	0.801	0.884	0.000
Weight (kg)	78.83	13.75	79.74	13.80	0.91	2.68	73.78	11.71	73.53	11.47	-0.25	2.63	0.32	0.077	0.083
BMI (kg/m2)	29.58	4.22	29.92	4.14	0.33	1.07	27.75	4.56	27.67	4.62	-0.07	0.98	0.303	0.066	0.112
WC (cm)	101.13	9.81	102.76	10.08	1.63	4.20	96.82	11.42	95.81	8.85	-1.01	4.76	0.584	0.02	0.022
WHR	0.94	0.05	0.95	0.05	0.01	0.03	0.93	0.04	0.92	0.04	-0.002	0.03	0.166	0.083	0.051
FM%	38.89	9.63	40.30	9.42	1.41	3.41	36.68	10.62	33.33	9.58	-3.35	4.01	0.036	0.052	0.000
VAT (AU)	14.37	3.77	14.92	3.42	0.55	1.7	13.26	4.3	11.92	4.58	-1.34	1.47	0.46	0.036	0.000
Truncal fat (%)	38.84	8.59	39.66	7.81	0.81	1.7	35.85	9.60	34.69	9.51	-1.16	1.63	0.406	0.68	0.000

P1, probability level by repeated-measures ANOVA for difference in time course; P2, probability level by repeated-measures ANOVA for difference between types of yogurt drink; P3, probability level by repeated-measures ANOVA for interaction between time course and type of yogurt drink; 25(OH)D, 25-hydroxyvitamin D; FSG, fasting serum glucose; HbA1c, glycated Hb; QUICKI, Quantitative Insulin Check Index; WC, waist circumference; WHR, waist:hip ratio; FM, fat mass; VAT, visceral adipose tissue.

**Table 3. Comparison of the initial and final values across FokI polymorphism and type of intervention**

	FF (n 42)									Ff (n 14)									Ff (n 5)									Time × group
	Before			After			Change	Before			After			Change	Before			After			Change	TimeGroup						
	Mean	SD	p	Mean	SD	p		Mean	SD	p	Mean	SD	p		Mean	SD	p	Mean	SD	p		P1	P2	P3				
<b>25(OH)D (nmol/l)</b>	40.29	18.81	58.88	28.87	18.58	25.54	0.000	43.88	14.71	50.34	30.48	6.45	29.73	0.431	40.9	17.69	49.81	29.31	12.16	23.71	0.315	0.1	0.008	0.03				
<b>FSG (mmol/l)</b>	158.69	47.78	160.39	51.64	1.69	48.06	0.818	168.85	45.17	169.9	45.97	1.05	45.66	0.919	186.03	7.96	154.83	45.5	-31.16	61.26	0.26	0.021	0.014	0.036				
<b>HbA1c (propotional)</b>	8.91	1.9	7.82	1.74	-1.08	1.93	0.001	9.12	2.09	8.25	1.94	-0.86	2.11	0.091	8.68	3.21	7.48	1.31	-1.2	1.92	0.188	0.13	0.012	0.003				
<b>QUICKI</b>	0.28	0.01	0.282	0.02	0.002	0.02	0.513	0.27	0.01	0.27	0.01	-0.001	0.01	0.699	0.28	0.02	0.28	0.02	0.005	0.02	0.677	0.004	0.04	0.009				
<b>Weight (kg)*</b>	75.54	12.81	75.89	12.69	0.34	2.72	0.41	79.01	13.83	80.1	13.57	1.08	2.52	0.095	71.88	10.04	69.46	10.41	-2.41	0.89	0.001	0.009	0.037	0.118				
<b>BMI (kg/m2)</b>	28.56	4.74	28.70	4.71	0.13	1.02	0.381	29.03	4.39	29.46	4.42	0.42	1.03	0.107	27.56	2.81	26.63	3.06	-0.9	0.35	0.001	0.009	0.016	0.119				
<b>WC (cm)</b>	98.35	10.26	99.28	10.34	0.92	4.1	0.153	101.94	13.21	100.73	9.90	-1.2	5.97	0.417	93.16	3.06	92.33	4.88	-0.8	3.6	0.601	0.004	0.05	0.045				
<b>WHR</b>	0.93	0.05	0.94	0.05	0.01	0.04	0.127	0.93	0.04	0.93	0.03	0.002	0.027	0.727	0.93	0.04	0.91	0.02	-0.01	0.02	0.222	0.000	0.04	0.04				
<b>FM%</b>	37.09	10.49	36.18	10.24	-0.9	4.46	0.184	40.24	10.21	39.03	9.41	-1.21	4.33	0.228	33.6	5.89	30.64	9.61	-2.95	4.85	0.196	0.075	0.04	0.016				
<b>VAT (AU)</b>	13.05	3.82	12.79	3.81	-0.25	1.96	0.387	15.94	4.18	15.12	4.79	-0.81	1.6	0.034	11.80	3.02	10.83	4.91	-0.96	2.08	0.309	0.068	0.108	0.024				
<b>Truncal fat (%)</b>	35.47	9.77	35.28	9.43	-0.19	2.08	0.546	42.25	5.39	41.97	5.32	-0.28	1.5	0.040	23.25	9.58	32.61	10.67	-0.63	2.72	0.594	0.019	0.129	0.004				

**Discussion**

The findings of this study showed that the response of participants to vitamin D intake was different according to FokI genotypes. Significant difference in 25(OH)D, WC, WHR, FM, VAT, and trunk fat was seen after 12 weeks interventions between two groups, these differences remained significant for 25(OH)D, FSG, HbA1c, WC, WHR, FM, VAT, and trunk when considering genotypes of FokI polymorphism.

Recently, many studies, in line with our study, have identified significant relationships between vitamin D deficiency, obesity and diabetes progression. It seems that these three factors can strongly affect each other, as many interventions have shown that vitamin D deficiency can lead to obesity and chronic diseases such as type 2 diabetes, cardiovascular disease, and etc. On the other hand, supplementation and fortification with vitamin D has been shown that can reduce obesity and its complications [24]. The probable mechanisms vitamin D by which could affect BMI and obesity is alteration in expression of genes related to vitamin D metabolism, because more than 3% of human genome is regulated by vitamin D [25]. Moreover, vitamin D suppress tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) which both of them increase in inflammatory situation like obesity and deteriorate the metabolic response [26]. Furthermore, some studies have been shown that hypovitaminosis D is associated with higher C-reactive proteins (CRP) [27].

In contrast with the effect of vitamin D on obesity, researchers have shown that obesity can mutually lower vitamin D levels. In fact, due to the high fat content of the obese person and the fat-soluble nature of vitamin D, a small amount of this vitamin is bioavailable in circulation. On

the other hand, it has been seen that the amount of two hormones of 25-Hydroxylase and 1-hydroxylase which are responsible for converting this vitamin to its active form is lower in obese people [28-29].

In our study, although FD had no significant effect on weight and BMI, it lowered WC, FM, and VAT which are better indicators than BMI for central obesity and diabetes. Previously, this effect of vitamin D plus ca on FM, regardless of the effect on weight and BMI has been observed in other studies [30]. Moreover, another study has also shown that a 1 cm increase in WC is associated with 0.17 ng/mL decrease in vitamin D [31]. Interestingly, when in Framingham Heart Study, the relationships between vitamin D and adiposity tested categorically for VAT and SAT (subcutaneous adipose tissue), revealed that the differences in VAT is more clear [32]. VAT is more associated with impaired fasting glucose and diabetes [33].

Although the geographic area of our country indicates that it is exposed to sunlight for most of the year, vitamin D deficiency is one of the main problems [34]. This problem can be largely explained by genetics, social and cultural status [35]. The current study showed significant associations between FOK-1 genotypes and diabetes, anthropometric and obesity biomarkers after 3-month study. The association between genetics, VDR polymorphisms, and visceral obesity and T2D is controversial. In line with the relationships seen in this study, some studies have supported the relation between other vitamin D polymorphisms including BsmI and TaqI and visceral obesity [36-37]. However, Walsh et al. in their cross-sectional study on Northern-European adults, reported no connection between FOK-1 polymorphisms and visceral obesity biomarkers including FM [38]. Moreover, Ochs-Balcom et al. in their study on

healthy women living in western US found no significant association between FOK-1 and BMI [39]. Differences between our study and these studies can be explained by different population, lower sample size and cross-sectional study design of these studies versus our study.

This study is not without limitation. Taking FOK-1 VDR polymorphism along with other VDR polymorphisms gives a better view of the relationships between vitamin D genetic variants and visceral obesity. In contrast, the nature of the single-blinded randomised clinical trial which is used in this study is much stronger to find a cause and affect relationships than cross-sectional studies conducted in this regard.

### Conclusion

The present study suggests that vitamin D fortified Doogh as a good way to increase serum vitamin D levels and reduce visceral adiposity biomarkers and FOK-1 VDR SNP plays a dominant role in obesity-related phenotypes. More studies with larger sample sizes and longer duration should be conducted to see more reliable relationships between VDR polymorphisms and obesity.

### Conflict of interest

None of authors have conflict of interests.

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None.

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