Contents available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Does high-dose vitamin D supplementation impact insulin resistance and risk of development of diabetes in patients with pre-diabetes? A double-blind randomized clinical trial

Mahtab Niroomand [a,*], *Akbar Fotouhi* [b], *Navid Irannejad* [c], *Farhad Hosseinpanah* [d]

[a] *Endocrinology Division, Department of Internal Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[b] *Department of Epidemiology and Biostatistics, Tehran University of Medical Sciences, Tehran, Iran*
[c] *Department of Internal Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[d] *Obesity Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Article history:
Received 5 September 2018
Received in revised form
18 November 2018
Accepted 17 December 2018
Available online 21 December 2018

Keywords:
Insulin resistance
Prevention
Diabetes
Vitamin D

ABSTRACT

Aims: The aim of this study is to evaluate the effect of high-dose vitamin D on insulin sensitivity and the risk of progression to diabetes.

Methods: In this double-blind, placebo-controlled randomized clinical trial adults with pre-diabetes and vitamin D deficiency were randomly assigned to either vitamin D_3 or placebo. Fasting plasma glucose (FPG), 2-h oral glucose tolerance test plasma glucose (OGTT PG), Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), and the rate of progression of glucose tolerance was compared.

Results: A total of 162 patients were randomized, from which 83 finished the 6-month follow-up (44 in intervention group and 39 in control group). In 6 months, serum 25-hydroxyvitamin D levels were significantly higher in the intervention group (36 ng/ml vs 16 ng/ml, P value < 0.001). There was no significant difference between FPG or 2H-OGTT PG in two groups. HOMA-IR score was significantly lower in the vitamin D group (2.6 vs. 3.1; P value = 0.04). The rate of progression toward diabetes was significantly lower in the intervention group (28% vs. 3%; P value = 0.002).

Conclusions: In patients with pre-diabetes and hypovitaminosis D, high dose vitamin D improves insulin sensitivity and decreases risk of progression toward diabetes.

1. Introduction

The rate of diabetes is soaring globally, with about 451 million people being affected throughout the world [1]. According to the World Health Organization, in 2030, diabetes will be the seventh leading cause of death [2]. In the early phases of the disease, a pre-diabetic stage develops with two distinct phases: impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) [3]. Preventive efforts while the patient is still in the pre-diabetes stage have been shown to be

* Corresponding author at: Arabi Ave, Daneshjoo Blvd, Velenjak, 7th Floor, Bldg. No. 2, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Work address). No. 12, Sangar St., Moqadas Ardebili St., Tehran, Iran (Home address).
E-mail address: m.niroomand@sbmu.ac.ir (M. Niroomand).
https://doi.org/10.1016/j.diabres.2018.12.008
0168-8227/

effective in preventing or delaying the onset of diabetes [3]. While lifestyle modifications may deter progression to diabetes, in practice, they are difficult to implement [4]. Medications may also have a role in slowing the progression of diabetes, but they may be costly and give rise to untoward side effects [5].

Vitamin D, a steroid hormone with its receptors present in diverse cell types, is presumed to be involved in several cellular processes, including insulin secretion by pancreatic beta cells and tissue response to insulin. Changes in insulin secretion can be accounted for by the presence of vitamin D receptors as well as vitamin D-dependent calcium-binding proteins on pancreatic islet cells. Expression of vitamin D receptor in adipocytes and possibly on myocytes may give rise to the modulation in tissue response to insulin. Based on these findings, it has been suggested that supplementation with vitamin D may be an alternative approach to improve glucose tolerance and prevent or delay progression of diabetes [6,7].

Some studies have reported a positive association [8–12] between low serum 25-hydroxyviamin D [25(OH)D] levels and diabetes, but, after multivariable adjustment for confounders [13–15] this association has been attenuated. On the other hand, cross-sectional studies have shown a lower serum level of 25(OH)D in patients with diabetes and pre-diabetes than in those with normal glucose tolerance [15,16]. However, after adjustment for other confounders, such as obesity, fasting glucose, hypertension, and body mass index (BMI), the results were not uniform. Furthermore, a few prospective studies have shown an association between baseline 25(OH)D and insulin resistance and fasting insulin concentration [17–20]. A 12-year cohort study revealed that higher 25(OH) D levels were associated with lower risk of diabetes with hazard ratio of 0.64 for each 10 ng/mL increase in serum 25(OH) D concentration [21]. These studies at best reveal associations and do not prove causation. Several prospective interventional studies have been conducted to assess the effect of vitamin D supplementation on insulin resistance [22–25] and all, but one [26], have yielded negative results. The effect of vitamin D on the risk of diabetes and whether vitamin D supplementation can successfully prevent or delay the progression of pre-diabetes has been the subject of three randomized trials [27–29]. No benefit has been shown except a subgroup analysis on patients with pre-diabetes in one study [27]. Since methodological limitations including mixed study populations, small sample sizes, or failure to prove that supplementation has resulted in adequate serum 25(OH)D concentrations exist in these studies, the results are subject to skepticism.

The aim of this double blind randomized clinical trial was to evaluate the effect of high-dose vitamin D supplementation on insulin sensitivity, as well as the risk of progression along the spectrum of glucose tolerance.

2. Subjects, materials and methods

2.1. Study design

This is a parallel design, double blind, randomized, placebo-controlled clinical trial. The study protocol was approved by the University Institutional Review Board. Since fasting plasma glucose (FPG) screening test for diabetes for patients with at least one risk factor for diabetes is considered standard of care [30], the Board decided that providing a description of the study and obtaining informed consent was necessary only before the oral glucose tolerance test (OGTT) [Approval No. 308-11270]. The trial has been registered at IRCT.ir [registration number IRCT2013050413223N1].

2.2. Study population

The study population consisted of adult (18 years $\leq$ age $\leq$ 80 - years) patients with pre-diabetes and vitamin D deficiency presenting to one of the 3 university-affiliated endocrinology clinics located in the metropolitan city of Tehran, Iran. All adult individuals presenting to any of the three clinics were considered as potentially eligible. Any prospective subject with at least one risk factor for diabetes [30] received an FPG testing after at least 8 h of fasting. Patients with normal FPG (<100 mg/dl) or with overt diabetes (FPG $\geq$ 126 mg/dl) were not included in the study. Patients suffering from medical conditions or taking medications that interfere with glucose metabolism were excluded from the study. Subjects with FPG levels of 100 through 125 mg/dl without exclusion criteria were approached and invited to participate in the study. A sample size of 80 patients in each group was calculated for an effect size of 0.5 considering primary outcome of Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) score standard deviation of 1.1, type I error of 0.05, power of 0.80, and attrition rate of 5%.

2.3. Study protocol

When consented, patients were formally enrolled in the study and their weight (using digital scales to the nearest 100 g while the patient minimally clothed), height (using a tape measure in the standing position without shoes to the nearest 1 cm), waist circumference (WC; using unstretched measuring tape measure at the narrowest level over light clothing to the nearest 1 cm), and blood pressure (BP; using an automatic monitoring system Cardioset FX7, SaIRAN Medical Industry, Isfahan, Iran) were measured. BMI was calculated as weight in kilograms divided by height in meter square. In addition, after an 8-h overnight fast, a blood sample was obtained to assess baseline FPG, as well as serum levels of 25(OH)D; Radioimmunoassay method BioSource Europes SA, Nivelles, Belgium), insulin (Electrochemiluminescence immunoassay method, Rosche, Berlin, Germany) and calcium (Cresolphthalein Complexon Metod; Pars Azmoon Kit, Pars Azmoon Inc., Tehran, Iran). Subsequently, the patients underwent a 75-gram OGTT according to the following protocol: 75 g anhydrous glucose made up to 250 ml of solution with plain water was administered orally within 5 min and blood samples were obtained after 120 min [31]. All glucose measurements were conducted by enzymatic colorimetric method using glucose oxidase. IGT was diagnosed if the 2-h post challenge test was between 140 and 200 mg/dl. Values less than 140 mg/dl were considered diagnostic of IFG, and concentrations equal or above 200 mg/dl were considered overt diabetes [30].

All individuals with pre-diabetes (IFG or IFG+ IGT) and serum 25(OH)D levels less than 30 ng/mL were, then, randomly assigned to either intervention or control group. Balanced block randomization was performed using computer generated random sequence of blocks of four by one of the investigators (MN). For randomization concealment, enrollment was decided by another one of the investigators (NI). A pack of sequentially numbered sealed envelopes containing identical appearing pearls (either placebo or 50000 IU vitamin D) were available to the investigator. Once enrollment was confirmed, he opened the next sealed envelope in the sequence and handed the content to the patient, thereby assigning the participant to either intervention or control group. Participants, care providers, and those assessing baseline data and outcomes were all blinded to the study.

The intervention group received oral weekly doses of 50,000 IU Vitamin D_3 pearls (Zahravi Pharmaceutical Company, Tabriz, Iran) for 3 months, followed by 1 pearl per month for an additional period of 3 months. The control group received placebo pearls (prepared by Zahravi Pharmaceutical Company using paraffin with the size, shape, and color similar to the original pearls) with a similar schedule to vitamin D. Subjects were instructed to return their remaining medications in the follow up visits to ensure compliance. Lifestyle modification recommendations were made to both groups. They were allowed to continue their current supplements, if taking one. Participants were followed up for 6 months. Each participant was seen three times during the study period: at baseline and subsequently two follow-up visits every three months. Participants' weight, height, and WC, BP, as well as FPG, serum levels of 25OHD, insulin, and calcium were measured and 2-h OGTT was repeated during each follow-up visit.

2.4. *Outcome variables*

The primary outcome of interest was insulin resistance, as measured by HOMA-IR index. Progression (or regression) of glucose tolerance in the continuum of diabetes (from to normal to IFG, IGT, and overt diabetes) was another outcome that we sought for.

2.5. *Statistical analysis*

Baseline data were compared between two groups to check the comparability of treatment groups and randomization process. Intention to treat approach was used in analyses of outcomes. Considering the loss to follow up which could potentially lead to differences in the two treatment groups considering some prognostic variables and repeated measurement of outcomes over follow up times, we used generalized estimation equation (GEE) analyses for comparing groups over time. This analysis model adjusted differences in treatment groups in terms of prognostic variables and accounted for correlation of repeated measurements of outcomes over time. We used Chi Square test [Chi-Square Test Calculator (Social Science Statistics, 2018)] to compare the proportion of patients in different stages of glucose tolerance and also for proportion of patients whose glucose tolerance status remained unchanged, progressed, or regressed. We did not perform subgroup analyses.

3. Results

A total of 1018 individuals with one or more of the risk factors for diabetes presenting to the three recruitment sites between July 2015 and November 2017 were screened for pre-diabetes using the FPG criteria of at least 100 and less than 126. 162 subjects with pre-diabetes and hypovitaminosis D were randomized to receive either vitamin D (n = 81) or placebo (n = 81). Fig. 1 illustrates the flow of subjects through the study. According to the number of the packs returned at follow-up visits, the compliance rate was perfect.

The baseline demographic characteristics (age, sex, education level), physical characteristics (body weight, BMI, BP, WC) and the glycemic indices (i.e. FPG, 2-h OGTT, serum insulin, and HOMA-IR), and 25OHD levels were comparable in the two groups. Table 1 shows the characteristics of the participants in the intervention and control groups at the beginning of the study.

Fig. 2 illustrates the changes in the serum 25OHD levels of the subjects over the study period in the two groups. There was a rapid increase in serum 25OHD levels in the intervention group, while the levels in the control group expectedly remained almost constant.

Table 2 shows the changes in the glycemic indices and BMIs of the subjects in the two groups, at baseline and after 3 and 6 months. Only patients who had completed both follow-ups (n = 88) were analyzed for these changes. As can be noted, 6 months after randomization the serum 25OHD levels were significantly higher in the intervention group (36 ng/ml vs 16 ng/ml, P value <0.001). There were no differences between the two groups during the 6-month follow-up in terms of FPG, 2-h OGTT plasma glucose, waist circumference or BMI. Fasting serum insulin levels, 2 h OGTT plasma glucose, and HOMA-IR score (Fig. 3) were slightly but significantly lower in the vitamin D group compared to the placebo group [10 μU/ml vs. 12 μU/ml (P value = 0.05), 129 mg/dl vs. 139 mg/dl (P value = 0.06), and 2.6 vs. 3.1 (P value = 0.04), respectively].

Table 3 shows the distribution of participants in the four groups according to the glucose tolerance [i.e. normal, IFG, IGT with or without IFG (IGT ± IFG), and overt diabetes]. In order to evaluate the effect of vitamin D supplementation on the progression or regression of glucose tolerance, we combined changes from IFG or IGT to normal or from IGT to IFG as "regression". Moving from IFG to IGT or from IFG or IGT to diabetes was considered "progression". Other states were considered as no change ("constant"). As can be shown in Table 4, there was a significant difference between the two groups at 6 months.

4. Discussion

This double-blind randomized clinical trial sought to determine the effect of vitamin D supplementation on insulin resistance and incidence of diabetes. The result obtained revealed that in patients with prediabetes and hypovita-

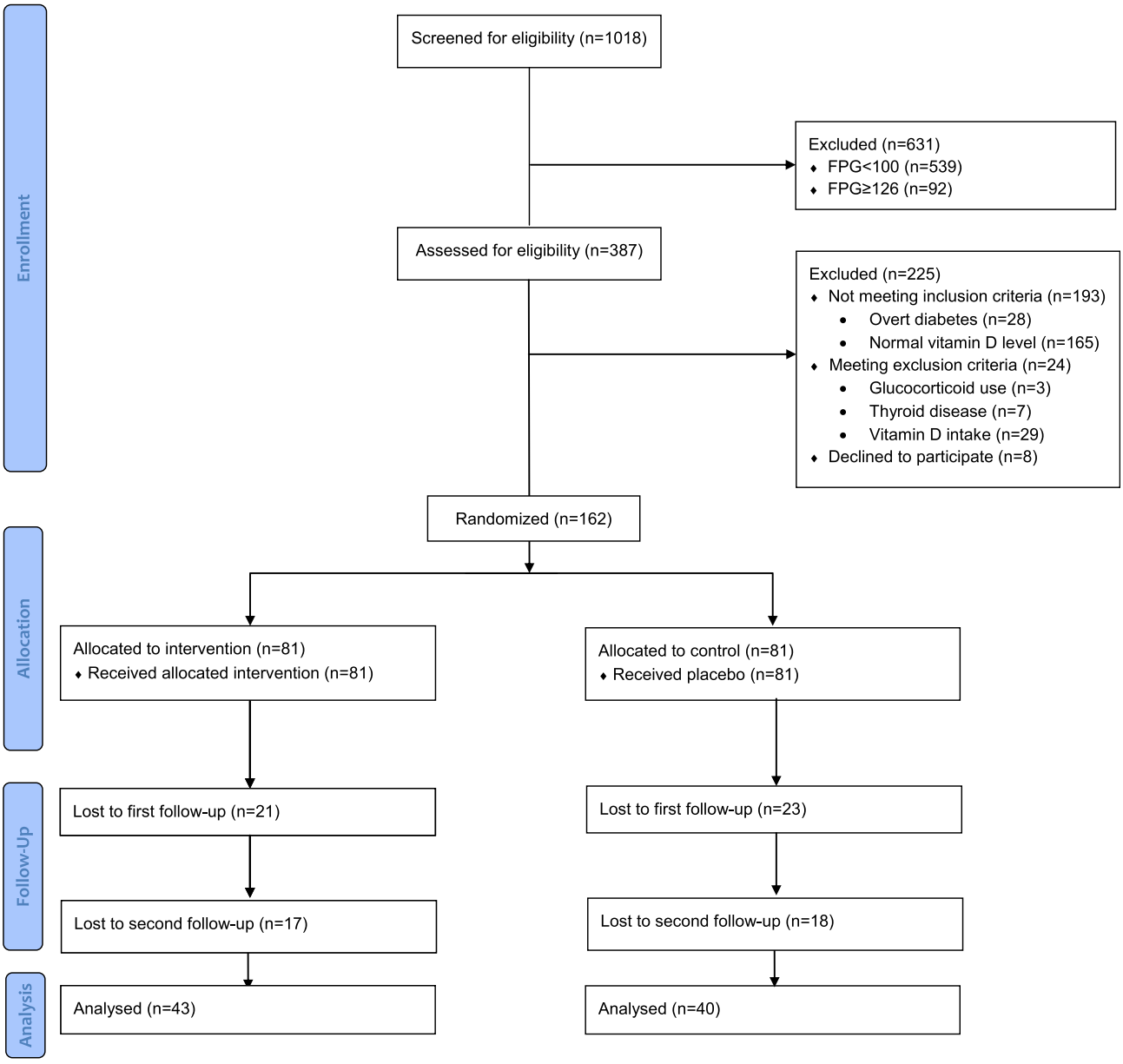

Fig. 1 – Participants' flow in the study.

minosis D high dose vitamin D supplementation results in improved insulin sensitivity and decreased risk of progression toward diabetes.

Although the change in BMI, WC and serum calcium level of the patients receiving vitamin D was similar to those who did not, there was a small (although not statistically significant) decrease in fasting serum insulin and 2H OGTT PG after 6 months of supplementation with high dose vitamin D. More notably, insulin resistance, as measured by HOMA-IR, decreased significantly in patients on vitamin D. In their clinical trial on patients with prediabetes and hypovitaminosis D, Davidson et al (2013) found no significant change in HOMA-IR, FPG 2H OGTT PG levels after 6 months of vitamin D administration. HOMA-IR remained unchanged at 2.1 in intervention group and increased from 2.2 to 2.4 in control group. They concluded that vitamin D supplementation does not alter insulin resistance in this population. Continuing the intervention for 12 months failed to change their conclusion. They, however, noticed that A1C levels were slightly but significantly lower in the intervention group at 12 months (25). Orwoll et al (1994) [32], Sugden et al (2008) [33], Jorde et al (2009) [34] and Witham et al (2010) [35], in their studies on patients with type 2 diabetes, failed to show any benefit for administering vitamin D in terms of improvement in glycemic control. Unlike our trial on patients with IFG, these studies were performed on small sample sizes of patients with known diabetes. In two of the studies [32,33] all participants had documented low serum levels of 25(OH)D. In one study [32] 1, 25 $(OH)_2$ vitamin D_3 was used, while another study [33] used single dose of vitamin D_2 and the other two trials [34,35] used high dose vitamin D_3. Vitamin D_2 has been shown to have a lower potency than D_3 [36]. In 2007, Pitas et al conducted a post hoc analysis on the data of 92 patients with IFG from another randomized trial to evaluate the effect of low

Table 1 – Baseline characteristics of the study participants upon recruitment in the intervention and control groups (N = 162).

	Vitamin D group (N = 81)	Placebo group (N = 81)
Age (years); Mean ± SD	45 ± 14	48 ± 11
Sex; Female/Male ratio	63/18	61/20
Education Level; N (%)		
Elementary	14 (53.8)	12 (46.2)
High school	48 (53.3)	42 (46.7)
College or higher	19 (42.2)	26 (57.8)
Hypertension; N (%)	19 (23.5)	18 (22.5)
Dyslipidemia; N (%)	41 (50.6)	44 (55)
Smoking; N (%)	5 (6.2)	5 (6.3)
Family History of Diabetes in first-degree relatives; N (%)	48 (60)	47 (58.8)
Ischemic Heart Disease; N (%)	4 (4.9)	5 (6.3)
History of GDM; N (%)	8 (13.1)	5 (8.3)
History of Macrosomia; N (%)	2 (3.3)	6 (10)
Body Weight (kg); Mean ± SD	82 ± 16	85 ± 17
BMI (kg/m^2); Mean ± SD	31 ± 6	32 ± 6
Waist Circumference (cm); Mean ± SD	101 ± 3	104 ± 3
Systolic BP (mmHg); Mean ± SD	121 ± 14	123 ± 15
Diastolic BP (mmHg); Mean ± SD	77 ± 9	76 ± 8
Fasting plasma glucose (mg/dl); Mean ± SD	107 ± 5	110 ± 8
2 h-OGTT PG (mg/dl); Mean ± SD	139 ± 29	139 ± 35
Serum Calcium (mg/dl); Mean ± SD	9.4 ± 0.6	9.4 ± 0.4
Serum 25OHD (ng/ml); Mean ± SD	12.3 ± 6.6	12.7 ± 6.3
Fasting Insulin (μU/ml); Mean ± SD	12.9 ± 5.6	14.3 ± 8.9
HOMA-IR; Mean ± SD	3.4 ± 1.6	3.9 ± 2.5

N = number; SD = standard deviation; GDM = gestational diabetes mellitus; BMI = body mass index; BP = blood pressure; 2 h-OGTT PG = 2 h pral glucose tolerance test plasma glucose; 25OHD = 25 hydroxyvitamin D; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.

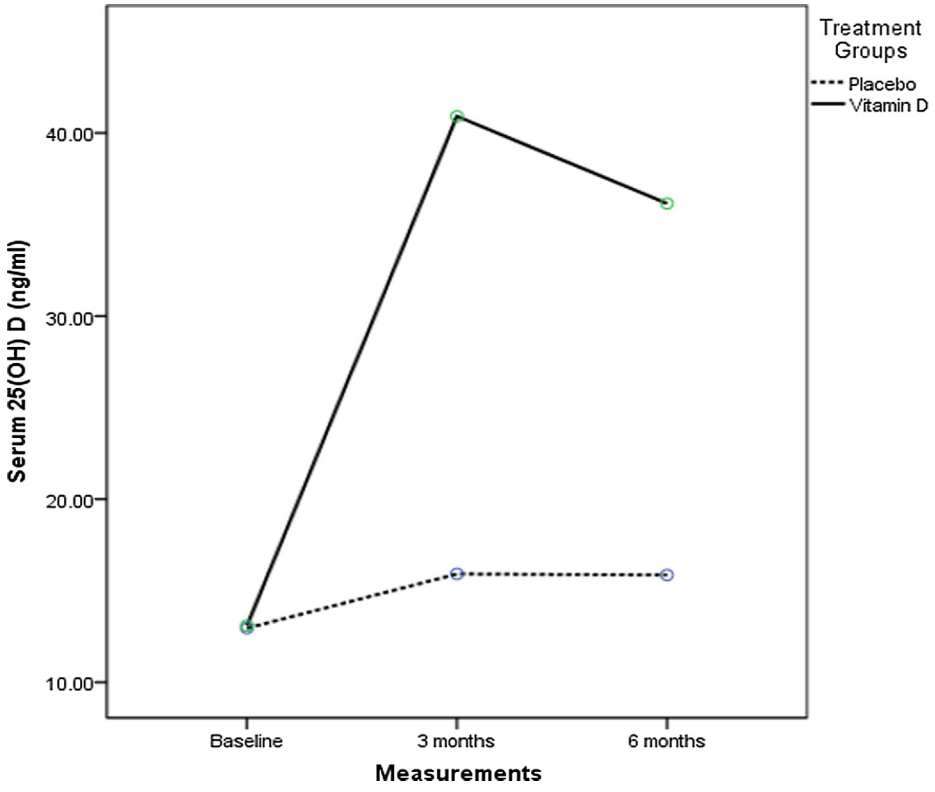

Fig. 2 – Serum 25(OH)D levels in the intervention and control groups over time.

dose vitamin D and calcium supplementation and showed a small but significant effect on FPG and insulin resistance (HOMA-IR) at 3 years [27]. They found that patients who had received vitamin D and calcium showed a lower rise in FPG (0.4 mg/dl vs. 6.1 mg/dl, p = 0.042; 0.05 vs. 0.91, p = 0.031, respectively) and HOMA-IR as compared to those on placebo.

Table 2 – Demographics, glycemic indices, 25(OH)D levels and insulin resistance of the participants in the intervention and control groups who completed the 6-month follow-up at baseline and after 3 and 6 months (N = 83).

	Vitamin D Group			Placebo Group			P value
	Baseline	3 months	6 months	Baseline	3 months	6 months	
BW; kg (mean, SD)	80 ± 16	78 ± 15	77 ± 14	83 ± 14	82 ± 15	82 ± 15	0.24
BMI; kg/m^2 (mean, SD)	31 ± 6	30 ± 6	29 ± 5	32 ± 5	31 ± 5	31 ± 5	0.26
WC; cm (mean, SD)	100 ± 14	98 ± 12	96 ± 11	102 ± 12	100 ± 12	98 ± 13	0.81
Serum Ca level; mg/dl (mean, SD)	9.4 ± 0.5	9.5 ± 0.5	9.3 ± 0.4	9.4 ± 0.4	9.4 ± 0.4	9.3 ± 0.4	0.13
Serum 25OHD level; ng/ml (mean, SD)	13 ± 7	41 ± 13	36 ± 11	13 ± 7	16 ± 9	16 ± 10	<0.001
SBP; mmHg (mean, SD)	121 ± 15	119 ± 14	117 ± 13	123 ± 16	120 ± 13	117 ± 12	0.32
DBP; mmHg (mean, SD)	76 ± 9	76 ± 8	76 ± 8	75 ± 10	76 ± 11	76 ± 9	0.98
FPG level; mg/dl (mean, SD)	107 ± 6	102 ± 8	100 ± 8	108 ± 8	102 ± 8	105 ± 14	0.15
2 h-OGTT PG level; mg/dl (mean, SD)	141 ± 27	138 ± 30	129 ± 27	140 ± 33	140 ± 45	139 ± 38	0.07
Fasting serum Insulin level; μU/ml (mean, SD)	13 ± 6	12 ± 5	10 ± 5	13 ± 8	13 ± 8	12 ± 7	0.05
HOMA-IR score (mean, SD)	3.4 ± 1.6	2.9 ± 1.5	2.6 ± 1.3	3.4 ± 2.0	3.1 ± 2.0	3.1 ± 2.0	0.04

N = number; BW = body weight; SD = standard deviation; BMI = body mass index; WC = waist circumference; 25OHD = 25 hydroxyvitamin D; SBP = systolic blood pressure; DBP = diastolic blood pressure; 2 h-OGTT PG = 2 h pral glucose tolerance test plasma glucose; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance.

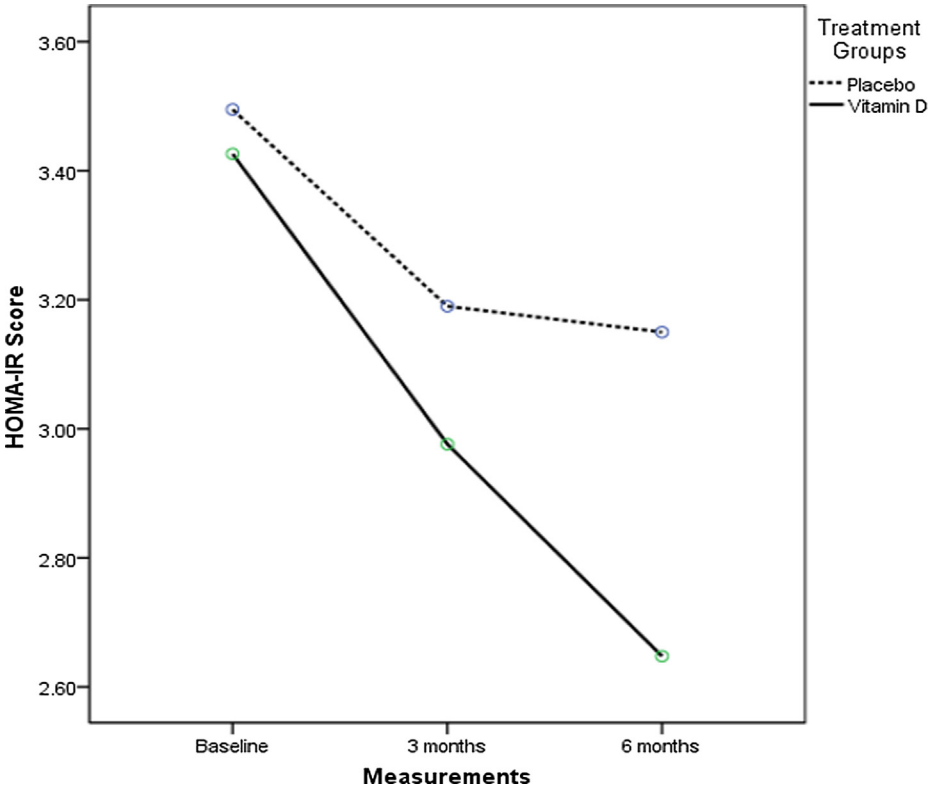

Fig. 3 – HOMA-IR indices in the intervention and control groups over time.

Their study, however, was different from ours and that of Davidson et al in that they used low doses of vitamin D. The dose taken and on the type of vitamin (D_2 or D_3) determines changes in serum 25(OH) D level resulting from vitamin D supplementation [36]. It has been shown that ingesting 100 IU/d (2.5 μg) of vitamin D increases serum 25(OH)D approximately by about 1 ng/ml [37]. Therefore, the dose of 50,000 IU per week used in our study is expected to cause a rise of about 70 ng/mL in serum 25(OH)D levels. Considering the fact that the average serum 25(OH)D levels of the participants was around 12, this dose ensured that a normal to high-normal level is achieved.

Furthermore, the average age of the patients in their study was 71 years. Moreover, it was a post hoc analysis and was not specifically designed to assess this outcome.

Our study also showed that vitamin D supplementation in patients with any level of glucose intolerance and concomitant hypovitaminosis D decreases the rate of progression

Table 3 – Frequency distribution of all study participants in the four categories of glucose tolerance at baseline and after 3 and 6 months.

	Baseline		3 months		6 months	
	Intervention (N = 81)	Control (N = 81)	Intervention (N = 60)	Control (N = 56)	Intervention (N = 43)	Control (N = 40)
Normal N (%)	0 (0)	0 (0)	20 (33)	18 (32)	17 (40)	11 (28)
Only IFG N (%)	42 (52)	43 (53)	20 (33)	11 (20)	14 (33)	10 (25)
IGT ± IFG N (%)	39 (48)	38 (47)	17 (28)	25 (44)	11 (26)	15 (38)
Diabetes N (%)	0 (0)	0 (0)	3 (5)	2 (4)	1 (2)	4 (10)
P Value			0.22		0.23	

N = number; IFG = impaired fasting glucose; IGT ± IFG = impaired glucose tolerance with or without impaired fasting glucose.

Table 4 – Rate of progression, regression, or staying constant along the continuum of glucose tolerance in intervention and control groups, 3 and 6 months after enrollment.

		0–3 months (N = 83)		0–6 months (N = 83)	
		Vitamin D N (%)	Placebo N (%)	Vitamin D N (%)	Placebo N (%)
Regression	IFG only to Nml	9 (21)	11(28)	13 (30)	5 (13)
	IGT ± IFG to Nml	7 (16)	4 (10)	5 (12)	6 (15)
	IGT ± IFG to IFG	3 (7)	5 (13)	6 (14)	2 (5)
	Total	*19 (43)*	*20 (50)*	*24 (56)*	*13 (32)*
Constant	IGT ± IFG to IGT	11 (25)	10 (25)	11 (26)	8 (20)
	IFG only to IFG	8 (19)	4 (10)	7 (16)	8 (20)
	Total	*19 (43)*	*14 (35)*	*18 (41)*	*16 (40)*
Progression	IFG only to IGT	3 (7)	5 (13)	0 (0)	7 (18)
	IFG only to DM	0 (0)	0 (0)	0 (0)	0 (0)
	IGT ± IFG to DM	2 (5)	1 (3)	1 (3)	4 (10)
	Total	*5 (14)*	*6 (15)*	*1 (3)*	*11 (28)*
P Value*		0.68		0.002	

N = number; IFG = impaired fasting glucose; IGT = impaired glucose tolerance; DM = diabetes mellitus; IGT ± IFG = impaired glucose tolerance with or without impaired fasting glucose.

* P values relate to the Chi-Square test of the total numbers for progression, regression, and constant categories between vitamin D and Placebo groups at 3 and 6 months.

toward overt diabetes. We considered any upgrading in the spectrum from IFG to IGT and from IGT to diabetes as progression. Davidson et al in their randomized trial found that 1 year administration of high doses of vitamin D to patients with prediabetes and hypovitaminosis D did not affect the proportion of subjects who developed diabetes as compared to placebo. After 6 months of supplementation, in the placebo group 36% were normal, 57% had prediabetes, and 7% had diabetes. In vitamin D group, these figures were 48%, 43% and 9%, respectively. Their participants, however, were mainly Latino women and hence the generalizability of their results to Caucasian populations is questionable [25]. In a trial in 2009, Avenell et al showed that administration of vitamin D_3 and calcium to the elderly (>70 years) non-diabetic patients with low serum 25(OH)D levels did not decrease the incidence of diabetes [29]. Most (85%) participants in this study were women, and diabetes was diagnosed based on the patients' self-report. Another study by de Boer et al in the US conducted on non-diabetic postmenopausal women with hypovitaminosis D also had similar results [28].

Our study has several strengths. It is a double blind randomized clinical trial with a sample size large enough to detect reasonable effect sizes with a good power. We also designed and analyzed our study well so that we were able to assess several confounders and adjust the results to precisely evaluate the effect of our intervention. Furthermore, we not only administered supplemental vitamin D but also proved that the serum levels have reached normal values. Our study, however, suffers from several limitations. The most important limitation is the relatively high number of loss to follow-ups. This could have biased the baseline characteristics of the participants in the two groups. We tried to overcome this issue by using GEE analysis, which adjusted for the differences in prognostic variables among the two groups and provided us better power by considering all information in repeated follow-ups together. Women outnumbered men in our study, which can be accounted for by the higher prevalence of hypovitaminosis D among women compared to men. We also diagnosed diabetes based on OGTT, which has a limited reproducibility. We did not measure HbA1C, which is an important index for overall glycemia, and relied on other parameters of glycemic control, namely FPG, 2-h OGTT, serum insulin and HOMA-IR. Another shortcoming of our study is the rather short duration of follow-up (6 months). As a suggestion, another trial with smaller losses, and longer duration of follow-up may be warranted.

We administered supplemental vitamin D in a dose which is considered safe. We also monitored serum 25(OH) D and calcium levels. However, we did not measure liver or kidney function tests or complete blood count.

In conclusion, our study showed that supplementation with high-dose vitamin D_3 in patients with prediabetes and hypovitaminosis D can improve insulin sensitivity and reduce the rate of progression toward diabetes.

Acknowledgements

The authors thank Mohammad Jalili, Tehran University of Medical Sciences, for help with preparing the manuscript.

REFERENCES

[1] Cho NH, Shaw JE, Karuranga S, et al. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 2018;138:271–81.
[2] World Health Organization. Diabetes fact sheet no. 312 [article online]; 2013. Available from <http://www.who.int/mediacentre/factsheets/fs312/en/> [accessed 31 March 2014].
[3] Knowler WC, Barrett-Connor E, Fowler SE, et al. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403.
[4] Wing RR, Look AHEAD Research Group. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. Arch Intern Med 2010;170:1566–75.
[5] DeFronzo RA, Abdul-Ghani M. Type 2 diabetes can be prevented with early pharmacological intervention. Diabetes Care 2011;34(Suppl. 2):S202–9.
[6] Rosen CJ, Adams JS, Bikle DD, et al. The non-skeletal effects of vitamin D: an Endocrine Society scientific statement. Endocr Rev 2012;33(3):456–92.
[7] Wallace IR, Wallace HJ, McKinley MC, Bell PM, Hunter SJ. Vitamin D and insulin resistance. Clin Endocrinol (Oxf) 2015;2. https://doi.org/10.1111/cen.12760 [Epub ahead of print] PubMed PMID: 25727561.
[8] Scragg R, Sowers M, Bell C. Third National Health and Nutrition Examination Survey. Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care 2004;27(12):2813–8.
[9] Liu E, Meigs JB, Pittas AG, et al. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. Am J Clin Nutr 2010;91(6):1627–33.
[10] Mattila C, Knekt P, Männistö S, et al. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. Diabetes Care 2007;30(10):2569–70.
[11] Shankar A, Sabanayagam C, Kalidindi S. Serum 25-hydroxyvitamin d levels and prediabetes among subjects free of diabetes. Diabetes Care 2011;34(5):1114–9.
[12] Lim S, Kim MJ, Choi SH, et al. Association of vitamin D deficiency with incidence of type 2 diabetes in high-risk Asian subjects. Am J Clin Nutr 2013;97(3):524–30.
[13] Pittas AG, Dawson-Hughes B, Li T, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 2006;29(3):650–6.
[14] Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. Diabetes Care 2005;28(12):2926–32.
[15] Gao Y, Zheng T, Ran X, et al. Vitamin D and incidence of prediabetes or Type 2 diabetes: a four-year follow-up community-based study. Dis Markers 2018;18:1926308. https://doi.org/10.1155/2018/1926308. eCollection 2018. PubMed PMID: 29743959; PubMed Central PMCID: PMC5878872.
[16] Fondjo LA, Sakyi SA, Owiredu WKBA, et al. Evaluating vitamin D status in pre- and postmenopausal type 2 diabetics and its association with glucose homeostasis. Biomed Res Int 2018;2:9369282. https://doi.org/10.1155/2018/9369282. eCollection 2018. PubMed PMID: 29808168; PubMed Central PMCID: PMC5902073.
[17] Issa CM. Vitamin D and type 2 diabetes mellitus. Adv Exp Med Biol 2017;996:193–205.
[18] Kayaniyil S, Retnakaran R, Harris SB, et al. Prospective associations of vitamin D with β- cell function and glycemia: the PROspective Metabolism and ISlet cell Evaluation(PROMISE) cohort study. Diabetes 2011;60(11):2947–53.
[19] Napoli N, Schafer AL, Lui LY, et al. Serum 25-hydroxyvitamin D level and incident type 2 diabetes in older men, the Osteoporotic Fractures in Men (MrOS) study. Bone 2016;90:181–4.
[20] Wima Lawansa SJ. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. J Steroid Biochem Mol Biol 2018;175:177–89.
[21] Park SK, Garland CF, Gorham ED, BuDoff L, Barrett-Connor E. Plasma 25-hydroxyvitamin D concentration and risk of type 2 diabetes and pre-diabetes: 12-year cohort study. PLoS One 2018;13(4):e0193070.
[22] Gagnon C, Daly RM, Carpentier A, et al. Effects of combined calcium and vitamin D supplementation on insulin secretion, insulin sensitivity and β-cell function in multi-ethnic vitamin D-deficient adults at risk for type 2 diabetes: a pilot randomized, placebo-controlled trial. PLoS One 2014;9(10):e109607.
[23] Teegarden D, Donkin SS. Vitamin D: emerging new roles in insulin sensitivity. Nutr Res Rev 2009;22(1):82–92.
[24] George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabet Med 2012;29(8):e142–50.
[25] Davidson MB, Duran P, Lee ML, Friedman TC. High-dose vitamin D supplementation in people with prediabetes and hypovitaminosis D. Diabetes Care 2013;36(2):260–6.
[26] von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. Br J Nutr 2010;103(4):549–55.
[27] Pittas AG, Harris SS, Stark PC, et al. The effects of calcium and vitamin D supplementation on blood glucose and markers of inflammation in nondiabetic adults. Diabetes Care 2007;30:980–6.
[28] de Boer IH, Tinker, et al. Calcium plus vitamin D supplementation and the risk of incident diabetes in the Women's Health Initiative. Diabetes Care 2008;31:701–7.
[29] Avenell A, Cook JA, MacLennan GS, et al. Vitamin D supplementation and type 2 diabetes: a substudy of a randomized placebo-controlled trial in older people (RECORD trial, ISRCTN 51647438). Age Ageing 2009;38:606–9.
[30] Summary of Revisions. Standards of medical care in diabetes. Diabetes Care. 2018;41(Suppl 1):S4–6.

[31] Alberti KG, Zimmet PZ. Definition diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15(7):539–53.
[32] Orwoll E, Riddle M, Prince M. Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. Am J Clin Nutr 1994;59(5):1083–7.
[33] Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabet Med 2008;25(3):320–5.
[34] Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. Eur J Nutr 2009;48(6):349–54.
[35] Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. Diabetologia 2010;53(10):2112–9.
[36] Autier P, Gandini S, Mullie P. A systematic review: influence of vitamin D supplementation on serum 25-hydroxyvitamin D concentration. J Clin Endocrinol Metab 2012;97(8):2606–13.
[37] Holick MF, Biancuzzo RM, Chen TC, Klein EK, Young A, Bibuld D, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab 2008;93(3):677–81.