



## A double blind randomized clinical trial to investigate the effect of vitamin D supplementation on metabolic and hepato-renal markers in type 2 diabetes and obesity

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### Abstract

**Background:** According to the recent studies, vitamin D deficiency has been correlated with progress in type 2 Diabetes and Metabolic Syndrome. The aim of this study was to assess the effect of vitamin D supplementation on glucose and lipid profiles, blood pressure, and biomarkers of liver and kidney in type 2 diabetic patients.

**Methods:** In this Double blinded randomized clinical trial, 90 patients with type 2 diabetes and serum 25-Hydroxy vitamin D levels of less than 30 ng/ml recruited from “Besat Diabetes Clinic” in Rasht, North of Iran. The subjects took 50000 IU vitamin D supplements or placebo for 8 weeks. We assessed the levels of serum 25 (OH) vitamin D, glucose and lipid profiles, oxidative and inflammatory indices, liver and kidney biomarkers, blood pressure, and sun exposure time, physical activity before and after intervention, and compared them between cases and controls.

**Results:** Vitamin D supplementation significantly increased serum vitamin D level, Superoxide Dismutase (SOD) activity, and significantly decreased serum HbA1C (Glycosylated Hemoglobin) level ( $p < 0.001$ ). High Density Lipoprotein (HDL) Cholesterol increased significantly ( $p = 0.016$ ), and Erythrocyte Sedimentation Rate (ESR) significantly decreased ( $p = 0.039$ ) after the intervention.

**Conclusion:** Our results represented that weekly supplementation with 50000 IU vitamin D for 8 weeks may be effective by improving HbA1C and lipid profile in type 2 diabetes mellitus.

**Keywords:** Vitamin D, Glucose, Diabetes, Lipid, Diet, Nutrition, Liver, Kidney, Type 2 diabetes, Inflammation

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### Introduction

Diabetes is defined as a metabolic disease related to insulin secretion, sensitivity, dysfunction and resistance, leading to increased serum glucose (1). Type 2 Diabetes Mellitus (T2DM) may have been the result of several pathogenic mechanisms such as pancreatic Beta cell dysfunction, increased serum insulin level, and insulin resistance (2). One of the major risk factors of T2DM is obesity which leads to cellular oxidative stress, insulin resistance, and Beta cellular dysfunction (3). Furthermore, chronic increase in serum glucose due to T2DM is closely related to disrupting the normal function of organs such as

kidney, heart, liver, central and peripheral nervous system, and cornea during long time (4).

Cardiovascular disease (CVD) is the main cause of mortality among people with T2DM reportedly 2-4 times more prone to CVD risk comparing those without T2DM. Furthermore, high serum lipid profiles is the main risk factor for CVDs among T2DM cases (5, 6). On the other side, vitamin D deficiency has been considerably increased among all age groups, and it has been reported that several cities in Iran vitamin D deficiency is highly prevalent. Besides Iran, Pakistan has reported 90% overall

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#### ↑What is “already known” in this topic:

While observational studies suggest that there is an inverse relationship between vitamin D levels in T2DM, but its relationship with insulin resistance is still unclear, and the results of interventional studies are generally contradictory.

#### →What this article adds:

Weekly supplementation with 50000 IU vitamin D for 8 weeks may be effective by improving HbA1C, lipid profile and inflammatory factor of ESR in type 2 diabetes mellitus.

prevalence of vitamin D deficiency, 48-60 % and 40-70% among Chilean and Brazilian healthy postmenopausal women respectively. Less than 50% of world's population have been reported with sufficient serum vitamin D (7-10). According to a meta-analysis by Akbari and coworkers, vitamin D supplementation may significantly decrease Insulin resistance markers (HOMA-IR and HOMA B), and LDL cholesterol levels. However, they did not observed significant increase in fasting glucose and HDL serum levels (11)

Insulin resistance is one of the main causes of T2DM, and according to recent studies, vitamin D has had inverse association with insulin resistance which may include vitamin D deficiency as a risk factor for incident T2DM (10-13). Moreover, vitamin D deficiency and T2DM have common risk factors such as obesity, age, and sedentary life style, and serum level of 25(OH) vitamin D has been reported significantly low comparing those without T2DM (14,15). Some studies have found direct relationship between 25 (OH) vitamin D levels and Insulin secretion in Pancreatic Beta cells, and some studies have demonstrated that vitamin D has anti-inflammatory effects (16).

In some degenerative diseases such as Insulin Dependent Diabetes Mellitus (IDDM), active oxygen metabolites can deteriorate the disease by increasing oxidative stress if they are not scavenged by body's antioxidant defense system (17). Besides, hyperglycemia, hyperlipidemia, and increased inflammatory cytokines strengthen the risk of inflammation and metabolic risk (18).

Since recent studies are controversial about the effect of

vitamin D on glycemic control, we aim to find the effect of vitamin D on lipid and glycemic profile, antioxidant factors, liver and kidney biomarkers in T2DM cases. In our double blinded randomized Clinical Trial, we give vitamin D supplements to the intervention group to find out if vitamin D improves insulin resistance, and glycemic index in T2DM.

### Methods

Current study is a double blinded randomized Clinical Trial, of the random assignment type, on 90 subjects with T2DM who visit "Besat" Specialist Clinic in Rasht; Gilan province, North of Iran, from the beginning until the end of autumn in 2016 .

As it is shown in **Diagram 1**, after admitting the patients to the clinic, they were asked to participate in the project if they were interested. After signing the "written informed" consent, we randomly divided them into 2 groups of intervention and control. Intervention was implied by eight 50000 IU vitamin D pearls, and the placebo included oral paraffin manufactured by Zahravi Pharmaceuticals-Iran. We asked the subjects to take one tablet per week for 8 weeks. During the intervention period, we had a weekly phone call follow-up with each subject and asked them if they took their supplement, and also about the probable side effects and complications of using supplements. We recorded the number of used pearls as well as returned blisters and packs.

The inclusion criteria were Diagnosed T2DM in both males and females, 25-65 year old, Serum 25 (OH) vita-

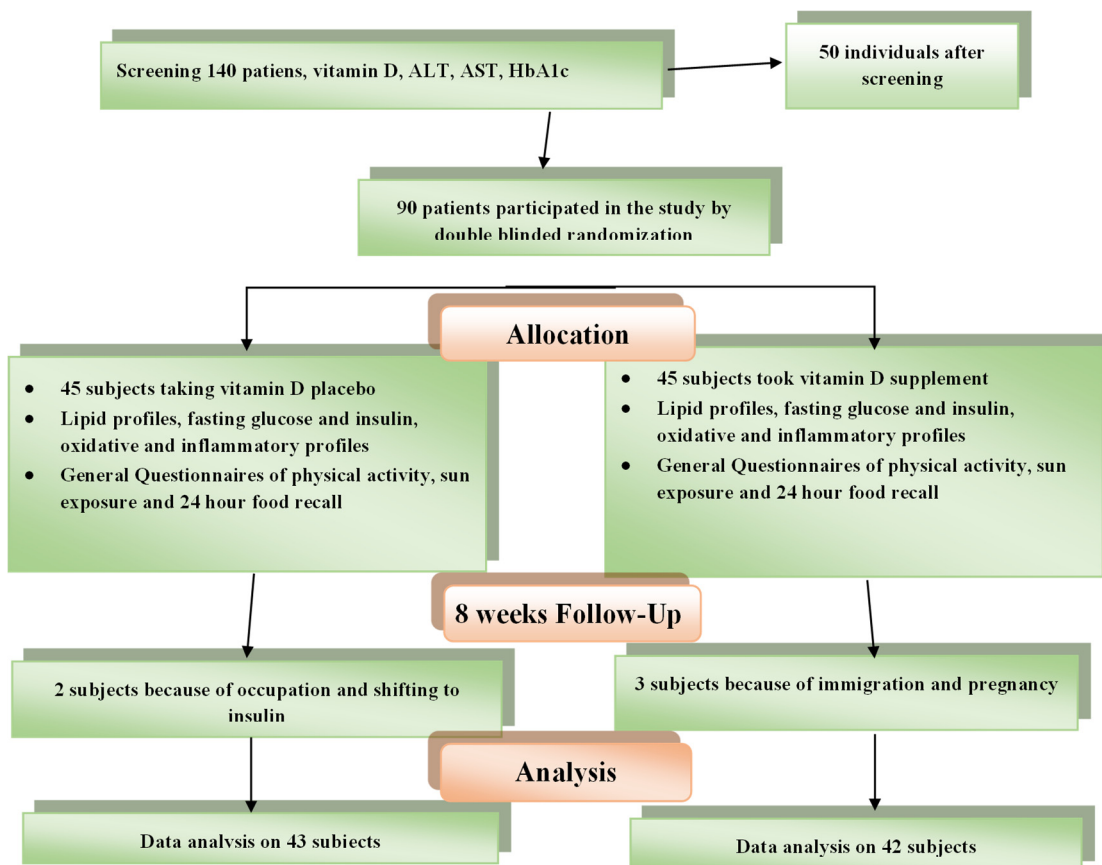


Diagram 1. According to flow diagram of participants, 3 patients in the intervention group and 2 patients in the placebo group were excluded

min D < 30 ng/ml, Body Mass Index (BMI) between 25 to 35 kg/m<sup>2</sup>, HbA1c < 8.5%. Exclusion Criteria were frequent consumption of supplements, any acute disease, modification in type or dose of hyperglycemia medication, and decision to discontinue compliance.

We measured anthropometric characteristics, systolic and diastolic blood pressure, in the beginning and at the end of study. Subjects were weighted for being fasted, and not being in first 5 days of menstrual cycle to eliminate possible weight changing factors. Subjects were standing without shoes, and minimal cloths, on Seca scales with 100 g accuracy. Height measurement was done in standing position without shoes, straight head, flat feet against wall, arms at sides, looking straight by Seca eca Height easure (0.5 cm accuracy). Body Mass Index (BMI) was calculated by deviding weight to square height (kg/m<sup>2</sup>).

Furthermore, we collected 24 hour food recalls from each subject in 2 non-consecutive days (one week day and one weekend day). We assessed their physical activity via short International Physical Activity Questionnaire (IPAQ) (19), and sun exposure by "sun exposure questionnaire" (20).

We collected 10 ml blood from Brachial vein for measuring serum vitamin D, Glucose, fasting insulin, Glycosylated Hemoglobin, total cholesterol, HDL cholesterol, HDL cholesterol, Triglyceride, SOD and Glutathione Peroxidase as the antioxidant enzymes activity, inflammatory markers including hsCRP and ESR, hepatic enzymes including ALT and AST, and renal biomarkers including Blood Urea Nitrogen (BUN) and Serum Creatinine.

All biochemical tests were conducted by Olympus analyzer and Delta kits. We used Alpha Classic analyzer for rechecking samples with ESR higher than 30. We used serum for all samples except HbA1c and ESR. We used Fiedewald formula for LDL cholesterol measurement. Measuring Glycosilated hemoglobins, we used Nycocard kit in Nycocard Reader (Made in Norway). Insulin was measured by Cobas e 411, with normal range of 2.6-24.9 Uμ/ml and Roche Kit.

SOD was measured by "Zellbio GmbH (Germany), and normal range of SOD activity between U/ml100-5, and also Gluthathione Peroxidaze by "Zellbio GmbH (Germany) and activity range of 20-500 U/ml, Elisa method and Elisa Reader model "Tecan A-5082" (Austria). We measured 25 (OH) vit D by quantitative diagnosis 25 (OH) D<sub>3</sub> kit from ImmunoDiagnostic Systems (IDS), UK which is an Enzyme Immunoassay kit with sensitivity of 5 mmol/l by ELISA method and Elisa Reader, Enzyme Immunoassay model ( Austria). Insulin resistance was assessed by HOMA-IR index and insulin sensitivity was evaluated by QUICKI (21, 22).

Insulin resistance= [fasting serum glucose (mmol/l)\*fasting insulin (μIU/ml)]/ 22.5

Insulin sensitivity=1/ Fasting insulin concentration (μIU/ml) + fasting glucose concentration (mmol/l)]

### Sampling

Since the primary outcomes of this study were serum level of 25-hydroxy vitamin D, Glycosylated hemoglobin (HbA1c), Insulin Resistance (HOMA-IR), and insulin

sensitivity (QUICKI) we calculated the sample size using insulin resistance index based on Baziar and coworkers' study (23). Based on 1.33 unit difference of mean insulin resistance between two study groups after intervention, 1.18 standard deviation for vitamin D group and 2.84 Standard Deviation for placebo group, and 5% probability of type-2 error, and 80% power, we calculated our sample size by G-Power software and sample size calculation formula for comparison of two independent groups. Our sample size was 40 accordingly, and we recruited 45 subjects considering probable withdrawal.

Randomization was implied by 23 quadratic blocks via ([www.sealedenvelope.com](http://www.sealedenvelope.com)) website. For blinding, we used unique codes for each subject produced by software, and subjects were not aware to be either in intervention or placebo group.

### Analysis

We analyzed the data using SPSS24. We described quantitative variables by mean and standard deviation and to describe the qualitative variables, a frequency report (percent) was used.

To compare the mean of quantitative outcomes between the two groups, independent t-test or its non-parametric equivalence, i.e., Mann-Whitney test were used and Chi-square test was used to compare qualitative variables between the two groups.

Also compared the results before and after intervention within each group by Paired t-test. We adjusted baseline characteristics for confounders, and ran ANCOVA models, and considered P-value < 0.05 as the statistical-significant value. Nutritional status and nutrients intake was analyzed by Nutritionist software (version 4).

### Ethics

Iran University of Medical Sciences Ethics Committee approved this clinical trial with Ethics Number of IR.IUMS.REC.1395.9223475201 in 05/09/2016 and record number of IRCT201604202365N11, and the participants signed the consent and participated in the project as volunteers.

Vitamin D intervention dose was equal to the National Institute of Health recommendations for treatment of vitamin D insufficiency and deficiency (50000 IU weekly for 8 weeks). Thus, lowest side effects had been expected after supplement intake (24). They could leave the project whenever they were not interested to continue. Furthermore, all of the ethical obligations were followed according to Helsinki ethical principles during blood sampling, and anthropometric measurements as well as confidentiality of subjects' information (25) also, after termination of intervention we gave vitamin D supplements to placebo group according to the Protocol.

### Results

The mean and standard deviation of BMI in the vitamin D and placebo group were 30.43 (3.2) and 31.37 (3.4), respectively (weight range of 59-115 kg, and height=163.5-184 cm), and mean age of 50 years (age

Table 1. Mean, standard deviation of baseline characteristics among two intervention and placebo groups before intervention

Variable	Category	Group		p
		Vitamin D (N=42)	Placebo (N=43)	
Age (Y)	-	50.36 (10.2)	5.05 (10.7)	0.892*
Weight (kg)	-	77.38 (10.9)	79.27 (11.6)	0.442*
Height (cm)	-	159.35 (8.0)	158.77 (7.6)	0.736*
BMI	-	30.43 (3.2)	31.37 (3.4)	0.194*
Disease period (months)	-	65.52 (38.5)	67.02 (35.4)	0.664*
Medication & daily dose	Metformin (500 mg)	2.11 (1.13)	2.51 (1.26)	0.135*
	Glibenclamide (5mg)	1.31 (0.94)	0.95 (0.78)	
Sex	Male	7.00 (16.7)	8.00 (18.6)	0.815**
	Female	35.00 (83.3)	35.00 (81.4)	
employment	Unemployed	32.00 (76.2)	32.00 (74.4)	0.850**
	Employed	10.00 (23.8)	11.00 (25.6)	

Y: Year, kg: Kilo gram, cm: centimeter, BMI: Body Mass Index, mg: milligram, \* Independent t-test, \*\*Chi-square test

Table 2. Mean and standard deviation of physical activity, weight, and BMI in intervention and placebo groups

Variable		Vitamin D (N=42)	Placebo (N=43)	pvalue*
Physical activity (METminutes/week)	Baseline	685.50 (99-34755)	462.00 (99-28560)	0.346
	After intervention	693.00 (99-33831)	462.00 (99-28659)	0.333
	P-value**	0.104	0.811	
Weight (kg)	Baseline	77.38 (10.92)	79.27 (11.67)	0.442
	After intervention	77.13 (10.81)	78.77 (11.53)	0.502
	P-value***	0.076	0.011	
BMI ( kg/m <sup>2</sup> )	Baseline	30.43 (3.23)	31.37 (3.40)	0.194
	After intervention	30.34 (3.32)	31.18 (3.42)	0.254
	P-value***	0.097	0.009	

kg: Kilo gram; cm: centimeter; BMI: Body Mass Index; mg: milligram, \* Independent t-test, \*\*.Mann-whitney U test, \*\*\*Paired t-test

range of 25-65 years), diagnosed with T2DM since 6 years ago, and used at least two types of hyperglycemia treatment medicines. According to the results of Table 1 there was not a significant difference between intervention and placebo group in terms of baseline characteristics including age, sex, weight, height, BMI, Disease period, type and dose of medications ( between group P-value).

There was not a significant difference in weight, BMI, and physical activity between intervention and placebo group before and after intervention (Between groups P-value). Furthermore, there was no significant difference in each of placebo and intervention group before and after intervention (within group P-value) (Table 2).

Mean and standard deviation of energy intake, macronutrients, and micronutrients from diet was not significantly different between placebo and vitamin D groups before and after intervention, and also between each of placebo and vitamin D before and after intervention. We asked the participants not to change their ordinary diet during 8 weeks of intervention. Since current study was conducted from early fall season until the end of fall, sun exposure did not changed during the study period (Table 3).

According to the findings of Tables 1,2, and 3, we did not find significant difference before and after intervention, in the probable confounding variables comprising age, weight, sex, height, BMI, marital status, education

Table 3. Mean and standard deviation of energy intake, macronutrients, and micronutrients before and after intervention among vitamin D and placebo groups

		Vitamin D (N=42)	Placebo (N=43)	pvalue
Energy (kcal/day)	Baseline	1638.59 (377.82)	1578.38 (399.64)	0.478
	After intervention	1701.36 (485.61)	1564.68 (392.75)	0.157
	P-Value <sup>2</sup>	0.381	0.871	
Protein (g/day)	Baseline	82.27 (23.34)	78.79 (31.82)	0.568
	After intervention	89.31 (35.83)	80.31 (32.16)	0.226
	P-Value <sup>2</sup>	0.190	0.823	
Carbohydrate (g/day)	Baseline	232.69 (67.62)	220.85 (54.71)	0.377
	After intervention	231.41 (62.34)	214.21 (51.19)	0.168
	P-Value <sup>2</sup>	0.891	0.522	
Total fat (g/day)	Baseline	47.61 (11.25)	46.83 (12.22)	0.760
	After intervention	51.31 (17.33)	47.41 (14.13)	0.258
	P-Value <sup>2</sup>	0.199	0.830	
SFA (g/day)	Baseline	16.10 (4.58)	15.35 (3.75)	0.413
	After intervention	16.01 (6.10)	15.74 (4.54)	0.819
	P-Value <sup>2</sup>	0.913	0.639	
MUFA (g/day)	Baseline	16.06 (3.85)	15.59 (5.07)	0.636
	After intervention	17.13 (5.85)	15.56 (4.86)	0.182
	P-Value <sup>2</sup>	0.265	0.976	
PUFA (g/day)	Baseline	9.98 (3.04)	10.04 (3.45)	0.836
	After intervention	10.49 (2.47)	9.97 (3.86)	0.480
	P-Value <sup>2</sup>	0.365	0.919	

1. Independent t-test, 2. Paired t-test, SFA: saturated fatty acid, MUFA: Mono Unsaturated Fatty Acid, PUFA: Poly Unsaturated Fatty Acid.

Table 3. Cntd

Calcium (mg/day)	Baseline	835.33 (279.75)	838.41 (301.51)	0.961
	After intervention	804.44 (324.89)	833.63 (292.95)	0.665
	P-Value <sup>2</sup>	0.487	0.929	
Phosphorus (mg/day)	Baseline	1444.29 (402.18)	1364.80 (372.84)	0.347
	After intervention	1459.15 (467.88)	1355.23 (388.99)	0.268
	P-Value <sup>2</sup>	0.814	0.897	
Vitamin D (ng/ml)	Baseline	1.73 (1.28)	1.84 (1.41)	0.710
	After intervention	1.58 (1.18)	1.64 (1.22)	0.835
	P-Value <sup>2</sup>	0.386	0.334	
Total fiber (g/day)	Baseline	29.23 (10.67)	26.58 (7.56)	0.192
	After intervention	29.96 (8.25)	25.91 (9.05)	0.578
	p <sup>2</sup>	0.127	0.603	

Table 4. Mean and Standard Deviation of primary variable before and after intervention among 2 groups of intervention and placebo

		Vitamin D (N=42)	Placebo (N=43)	Crude pvalue <sup>2</sup>	Adjusted pvalue <sup>3</sup>
VitaminD (ng/ml)	Baseline	17.24 (7.83)	17.56 (7.82)	0.853	<0.001
	After intervention	38.86 (10.76)	14.79 (7.08)	<0.001	
	P-value <sup>2</sup>	<0.001	<0.001		
HbA1c (%)	Baseline	7.51 (0.87)	7.15 (1.12)	0.103	<0.001
	After intervention	6.76 (0.98)	7.21 (1.11)	0.051	
	P-value <sup>2</sup>	<0.001	0.657		
HOMA-IR (N)	Baseline	5.36 (3.23)	5.83 (3.09)	0.499	0.421
	After intervention	6.44 (3.73)	7.39 (4.58)	0.295	
	P-value <sup>2</sup>	0.006	0.003		
Quicki (N)	Baseline	0.76 (0.08)	0.73 (0.06)	0.149	0.645
	After intervention	0.73 (0.07)	0.71 (0.07)	0.217	
	P-value <sup>2</sup>	0.005	0.003		

1. Independent t-test, 2. Paired t-test, 3. ANCOVA

level, duration of diabetes, and nutritional factors (dietary Vitamin D), energy, calcium, phosphorus, Poly Unsaturated Fatty Acid (PUFA), Unsaturated Fatty Acid (SFA), Unsaturated Fatty Acid (MUFA), fiber, carbohydrate, and protein), physical activity, and sun exposure. Therefore, we considered mentioned factors as controlled variables and for both primary and secondary outcomes, we conducted ANCOVA analysis only adjusting the baseline values.

Among all of the primary outcomes, vitamin D and

Glycosylated hemoglobin represented significant difference after intervention comparing baseline (Post intervention between group p-value <0.001). Serum vitamin D level was doubled after intervention, and glycosylated hemoglobin decreased 1% in intervention group (Table 4).

According to the results of Table 5, among all of the secondary outcomes, HDL cholesterol, SOD, and ESR represented significant difference (P<0.05) between vitamin D and placebo group after intervention (Table 5).

Table 5. Mean and Standard Deviation of secondary variable before and after intervention among 2 groups of intervention and placebo

		Vitamin D (N=42)	Placebo (N=43)	pvalue <sup>1</sup>	Adjusted pvalue <sup>3</sup>
FBS (mg/dl)	Baseline	172.48 (62.16)	178.28 (71.74)	0.629	0.130
	After intervention	175.52 (65.44)	162.72 (61.36)	0.355	
	P-value <sup>2</sup>	0.742	0.053		
Insulin (micIU/ml)	Baseline	12.64 (5.94)	14.09 (6.43)	0.284	0.101
	After intervention	15.31 (7.24)	18.77 (9.02)	0.055	
	P-value <sup>2</sup>	0.002	<0/001		
LDL cholesterol (mg/dl)	Baseline	104.60 (42.74)	105.98 (33.43)	0.868	0.086
	After intervention	104.71 (27.94)	95.60 (30.26)	0.153	
	P-value <sup>2</sup>	0.985	0.017		
HDL cholesterol (mg/dl)	Baseline	49.10 (11.31)	46.42 (8.02)	0.211	0.016
	After intervention	51.64 (8.48)	46.28 (9.38)	0.007	
	P-value <sup>2</sup>	0.091	0.905		
TG (mg/dl)	Baseline	180.69 (74.47)	201.72 (125.41)	0.349	0.684
	After intervention	187.14 (85.47)	218.16 (187.68)	0.332	
	P-value <sup>2</sup>	0.574	0.354		
Total cholesterol (mg/dl)	Baseline	190.21 (55.46)	192.63 (46.10)	0.828	0.181
	After intervention	192.10 (35.35)	183.70 (42.93)	0.328	
	P-value <sup>2</sup>	0.809	0.094		
AST (U/L)	Baseline	20.52 (5.73)	21.37 (6.10)	0.511	0.177
	After intervention	19.93 (5.09)	22.09 (7.72)	0.131	
	P-value <sup>2</sup>	0.544	0.451		
ALT (U/L)	Baseline	22.74 (8/93)	23.98 (9/04)	0.527	0.134
	After intervention	22.67 (8/21)	26.02 (11/27)	0.127	
	P-value <sup>2</sup>	0.949	0.118		

1.2Independent t-test, 2. Paired t-test, 3. ANCOVA

Table 5. Cntd

BUN (mg/dl)	Baseline	14.95 (5.31)	15.02 (5.01)	0.950	0.592
	After intervention	13.95 (5.07)	14.44 (5.05)	0.657	
	P-value <sup>2</sup>	0.173	0.299		
Cr (mg/dl)	Baseline	0.94 (0.21)	0.96 (0.18)	0.607	0.700
	After intervention	0.91 (0.24)	0.94 (0.18)	0.523	
	P-value <sup>2</sup>	0.253	0.377		
SOD (u/ghb)	Baseline	49.68 (3.49)	49.42 (3.15)	0.717	<0.001
	After intervention	51.31 (4.34)	46.39 (4.84)	<0.001	
	P-value <sup>2</sup>	0.004	<0.001		
CPx (u/ghb)	Baseline	338.93 (132.60)	357.00 (121.50)	0.514	0.678
	After intervention	523.26 (147.36)	508.16 (139.16)	0.628	
	P-value <sup>2</sup>	<0/001	<0/001		
Hs CRP (mg/dl)	Baseline	2.47 (1.64)	2.75 (2.30)	0.517	0.086
	After intervention	2.22 (1.76)	3.32 (3.74)	0.086	
	P-value <sup>2</sup>	0.386	0.134		
ESR %	Baseline	17.88 (9.79)	22.84 (13.03)	0.051	0.039
	After intervention	17.12 (8.77)	25.93 (17.55)	0.005	
	P-value <sup>2</sup>	0/416	0/192		
SBP (mmHg)	Baseline	12.21 (1.45)	12.14 (1.10)	0.791	0.824
	After intervention	12.26 (1.51)	12.26 (1.21)	0.985	
	P-value <sup>2</sup>	0.796	0.487		
DBP (mmHg)	Baseline	7/88 (1.07)	7.66 (0.88)	0.307	0.194
	After intervention	7.52 (1.03)	7.66 (0.87)	0.505	
	Pvalue <sup>2</sup>	0.057	1.000		

## Discussion

The aim of this study was to assess the effect of vitamin D supplementation on glucose and lipid profiles, blood pressure, and biomarkers of liver and kidney in type 2 diabetic patients.

Weekly intake of 50,000 IU Vitamin D supplement for 8 weeks resulted in descending HBA1c, and ESR, and elevation of HDL cholesterol, and SOD levels.

According to the outcomes of this study, mean serum (OH) vitmin D3 level increased by two times at the baseline after 8 weeks intervention. Whereas, the placebo group represented lower serum vitamin D at the end of the study.

The results of this study is along with some similar studies such as Borissova and coworkers' study in 2003 according which daily intake of 1322 IU Calcitriol supplement for one month, increased insulin secretion and decreased insulin resistance (26). Heshmat and colleagues found that intramuscular injection of 300,000 IU vitamin D did not change anthropometric, HbA1c, and HOMA index (27). In a study by Nasri and coworkers in 2014, dose of vitamin D was similar to our study (weekly 50,000 IU), and they found that vitamin D group had lower HbA1c after intervention (28). SUNNY trial in 2015 represented no significant modification in HbA1c and other glycemic indicators after 6 months of 50000 IU vitamin D supplementation among 275 subjects (29). Al-Daghri and coworkers in 2011 demonstrated that 25 (OH) vitmin D is inversely associated with obesity, insulin resistance and LDL levels among Soudi subjects with T2DM (30). In a cross-sectional study by Nielsen and coworkers in 2016, not only there was no support on the hypothesized positive effect of vitamin D on risk of T2DM, but they also found that higher vitamin D levels were in a weak positive association with fasting serum glucose level (31).

The mentioned controversy among recent studies may have been due to variety of dietary patterns, timing and type of hyperglycemia medications, and the effect of genetic factors among different study populations.

The effect of Vitamin D supplementation on increased fasting serum insulin level was not significant ( $p=0.10$ ) after adjusting the analysis for baseline measurements between intervention and control groups. The mentioned results are along with the results of SUNNY trial (29). Whereas, Eftekhari and coworkers represented that vitamin D supplementation increased Beta Cells function and conclusively insulin secretion ( $p=0.009$ ) (32). However, Tabesh and coleagues studied the effect of calcium and vitamin D supplementation and their intervention had decreased fasting serum insulin levels significantly ( $p=0.01$ ) (33), and similarly, Yazadn-Rad and his colleagues found that 2 months vitamin D supplementation decreased fasting insulin level significantly ( $p=0.048$ ) (34).

The potential mechanisms regarding the effect of vitamin D on insulin level are assumed to be presence of Vitamin D Receptors (VDRs) in pancreas cells, and expression of 1-alpha-hydroxylase in these cells (35). Moreover, there are some elements on human insulin promotors that respond to vitamin D, and can activate transcription of human insulin gene (36, 37).

Modification of insulin sensitivity and resistance after intervention was not significant in our study, which is along with Al-Daghri and colleagues' study in 2010, and SUNNY trial (29, 30). However, Tabesh and coworker found significant decrease in HOMA-IR and significant increase of QUICKI after weekly intake of 50,000 IU vitamin D and 1000mg calcium per day (33). Yazadan-Rad and colleagues intervention with 4000IU vitamin D for 2 months, represented increased insulin sensitivity (34), and in a cross-sectional study in 2016 there was a negative correlation between 25(OH) vitmin D and HOMA-IR after adjusting the analysis for confounders (38).

Modification of insulin resistance and sensitivity after intervention was not significant probably because insulin sensitivity and resistance formulas are dependent on fasting serum glucose and insulin level which increased after intervention.

One of the probable mechanisms of vitamin D associa-

tion with diabetes and its effect on glucose homeostasis may be probably because of vitamin D effect of insulin receptor in pancreatic beta cells. In other words, vitamin D may stimulate expression of insulin receptor gene and increase glucose transfer from intestine (39).

On the other side, vitamin D supplementation led to 1% decrease in HbA1c among intervention group ( $p=0.001$ ). These findings are in accordance with Tabesh and colleagues' study (33), Yazadn-Rad and coworkers, and Nasri and coworkers' project (28). Whereas, in Nasri and coworkers' study HbA1c increased after intervention ( $p=0.013$ ). Al-Sofiani and colleagues demonstrated that HbA1c did not changed considerably after intervention ( $p=0.5$ ) (40). Sheth and colleagues found  $p=0.057$  on the association of serum (OH) vit D and HbA1c (41). SUNNY trial also did not find any improvement in HbA1c after intervention (29). The explanation for this controversy may be the result of unadjusted potential confounders, and reverse causality in observational studies comparing trials. We did not find a particular mechanism in this regard.

Vitamin D supplementation did not impact LDL cholesterol, TG, and total cholesterol. However, HDL cholesterol increased after intervention which is in accordance with Tabesh and colleagues's study (33), Gannage and coworkers' study in 2009 among 381 non-obese University students (42), and Yazadan-Rad and colleagues' project (34). Tabesh and coworkers attained significant results on decreased LDL cholesterol after intervention (33), and in a cross-sectional study by Liu and coworkers, serum (OH) vit D was in non-significant positive association with HDL cholesterol and non-significant inverse association with triacylglycerol (43). The mechanism for increased levels of HDL cholesterol would have been related to anti-inflammatory and anti-oxidative effect of vitamin D by increasing Apo lipoprotein A1 after 8 weeks intervention.

AST, ALT, BUN, and Creatinine remained unchanged after intervention probably because the range of mentioned variables was not out of normal levels among the subjects who participated this study.

Furthermore, vitamin D supplementation significantly increased SOD, whereas, modification of Glutathione Peroxidase (GPx) was not significant. Despite, actual mechanism of vitamin D on oxidative stress biomarkers is not clear yet, improvement of cellular Glutathione levels, attenuation of active oxygen species, and pro-inflammatory markers by vitamin D could be a probable explanation for positive effects of vitamin D on oxidative stress.

Comparing change in ESR and hsCRP inflammatory indices before and after intervention represented significant and nonsignificant differences respectively. These findings are along with Akbarzadeh and coworkers' study (44), and Haidari and colleagues' study (45). But Razzaghi and coworkers' research in 2017 represent different results (46). We may guess the controversy in this area is due to different study designs, fat mass, and variety of inflammation levels, different dose of vitamin D, and intervention period. Inflammatory markers may need time to be improved after vitamin D deficiency.

Systolic and diastolic blood pressure did not change

significantly after intervention which is in accordance with Tabesh and coworkers' study (33), SUNNY trial (29), and Nayak and colleagues' study in 2016 (47). Lee and colleagues conducted a study in 2016, and found different results comparing our study (48). In our study, mean systolic and diastolic blood pressure were normal in both intervention and control groups at the baseline, which can make the results of blood pressure expectable.

The main strength of this study was screening subjects of study not only according to serum vitamin D status, but also for other variables such as HbA1c, ESR, AST and ALT, BUN and Creatinine, and cholesterol and TG levels.

The weaknesses and limitations of our study was that we did not use more robust methods for evaluation of insulin resistance and BMI. Furthermore, we did not measure Parathyroid Hormone (PTH), Apolipoprotein A1, and more sensitive inflammatory markers.

### Conclusion

Vitamin D supplementation for 8 weeks with 50000 IU vitamin D may increase levels of 25 (OH) vitamin D, HDL cholesterol, SOD, and decrease levels of glycosylated hemoglobin, and ESR. However, this intervention did not have significant effect on fasting serum glucose and insulin, liver and kidney biomarkers, blood pressure, insulin sensitivity and resistance.

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### Conflict of Interests

The authors declare that they have no competing interests.

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