The Effect of Vitamin D Supplementation on Adiposity, Blood Glycated Hemoglobin, Serum Leptin and Tumor Necrosis Factor-α in Type 2 Diabetic Patients

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ABSTRACT

Background: Since tumor necrosis factor-α (TNF-α) could be one of the risk factors at the development of diabetes complications; as well as serum leptin deficiency is related to increased susceptibility to infections in diabetic patients, they are potential indices from the preventive medicine viewpoint. This study was conducted to represent the effect of supplemental vitamin D3 on serum leptin, TNF-α and adiposity in type 2 diabetic patients.

Methods: In this randomized double-blind placebo-controlled trial, study sample was selected through type 2 diabetic patients (n = 51). A total of 26 patients were orally supplemented by vitamin D3 (400 IU/d) (vitamin D group) and 25 patients by placebo (placebo group) for 14 weeks. The blood glycated hemoglobin (HbA1c) and the serum ionized Ca, leptin, TNF-α, and serum 25-hydroxyvitamin D (25[OH] D) were measured at the two groups in the baseline and postintervention stages.

Results: It was shown that despite of theplacebo group, serum 25(OH) D and serum leptin was significantly increased (P = 0.001 and P = 0.002, respectively), while serum TNF-α was decreased significantly (P = 0.001) in vitamin D group. The remaining parameters, including body fat mass and HbA1c had no alterations between baseline and postintervention stages in vitamin D group.

Conclusions: This study may advocate vitamin D supplementation among type 2 diabetic patients due to its beneficial effects on prevention of diabetes complications.

Keywords: Adiposity, leptin, supplemental vitamin D3, tumor necrosis factor-α, type 2 diabetes

Original Article

INTRODUCTION

Type 2 diabetes mellitus could apparently be influenced by vitamin D through different mechanisms. Contrary to type 1 diabetes, the relationship between vitamin D and type 2 diabetes is less clear.[1] It has been well-corroborated that reducing of total body fat, including visceral fat is one of the strategies in the management of type 2 diabetes. Both in small case–control studies and in some large population-based studies have been

shown total body fat was inversely associated with 25-hydroxyvitamin D (25[OH] D) levels.[2,3] On the other hand, there were some evidences implying persons suffering from diabetes, have lower serum concentrations of vitamin D.[4]

Vitamin D replenishment corrects glycemia and insulin secretion in diabetic type 2 patients with established hypovitaminosis D. Since the vitamin D receptors and vitamin D–binding proteins (DBP) are found in pancreatic tissue as well as a relationship between certain allelic variations in the vitamin D receptor and DBP genes with glucose tolerance and insulin secretion has been shown, it has been suggested a role for vitamin D in the pathogenesis of type 2 diabetes.[5] Abnormalities in many systemic inflammation markers such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and its receptor, C-reactive protein and plasminogen activator inhibitor-1 have been reported in type 2 diabetes. Some of these immune mediators, such as TNF-α can directly intervene to insulin-signaling, causing insulin resistance through several mechanisms.[6] In adipocytes or surrounding skeletal muscle cells, TNF-α may elevate serine phosphorylation of the insulin receptor and also of insulin receptor substrate-1 (IRS-1) and possibly other proteins that mediate intracellular insulin-signaling. Serine-phosphorylated IRS-1 has been shown to prevent insulin receptor tyrosine kinase activity, which results in impaired downstream insulin-signaling.[7] Interestingly, it has been reported that vitamin D downregulate the synthesis of several cytokines including IL-2, interferon-γ, TNF-α, and TNF-β.[8,9] In addition, lipodystrophy is associated with lowered insulin sensitivity, suggesting that an adipocyte factor may be involved as a signal in insulin sensitivity, perhaps via leptin or TNF-α.[10] Leptin also collaborates to reduce intracellular content of triglycerides. Leptin resistance can probably reduce these physiological functions of leptin and assist in maintain excessive free fatty acid flux and intracellular accumulation, leading to insulin resistance and also contributing to beta-cell dysfunction.[11] Moreover, high blood levels of leptin seem to play a role in the appearance of hypertensive and diabetic retinopathy as well as in detachment of the retina.[12,13]

The purpose of the study reported here was to assess the probable effect of vitamin D3 supplementation on the amount and percent of total body fat, whereby it may affect on serum levels of leptin and TNF-α. The amount of blood glycated hemoglobin (HbA1c) was also measured as a tool for monitoring blood sugar control in patients with diabetes mellitus.

METHODS

Patients and study design

The present randomized double-blind placebo-controlled trial was conducted in diabetes clinic of Taleqani hospital located at Urmia city, Iran, which affiliated to Urmia University of Medical Sciences. According to the method proven at the medical researches committee of the university, we invited type 2 diabetic patients randomly through existing over 1800 documents of diabetic patients. Later, we illustrated our goals and methods would be performed in the survey for all of the invited patients. The patients invited were ones that put on glucose lowering agents, – but not insulin - to managing their diseases. The participants were not suffering from other illnesses (such as cardiovascular diseases, renal failure, and/or inflammatory diseases) except diabetes. Furthermore, we included only nonsupplemented patients with vitamin D and/or Ca. All of the subjects pertained to the same ethnicity. Totally, the sum of 51 patients could achieve to final analysis stage of the study in two groups: Group supplemented with 400 IU/d vitamin D3 ($n = 26$) for 14 weeks (vitamin D group) and group administered daily placebo for 14 weeks ($n = 25$) [Figure 1]. We used two kinds of edible drops in this study. Vitamin D drops were composed of vitamin D3 (cholecalciferol) 400 IU/ml (10 mcg/ml) plus thin vegetable oil (purified component of coconut and palm oil). There are no other chemicals or additives. They were tasteless and odorless. Apparently, the placebo drops are composed of above-mentioned vegetable oil alone.

Vitamin D3 or placebo was given to each of subjects by investigator assistant in terms of they belong to which of the studied group. The matching was carried out between subjects of two groups with a view to their sex and age. The subjects included survey sequentially in a course of several days at baseline and postintervention stages of study. To eliminate the seasonal changes in vitamin D nutritional status, we did

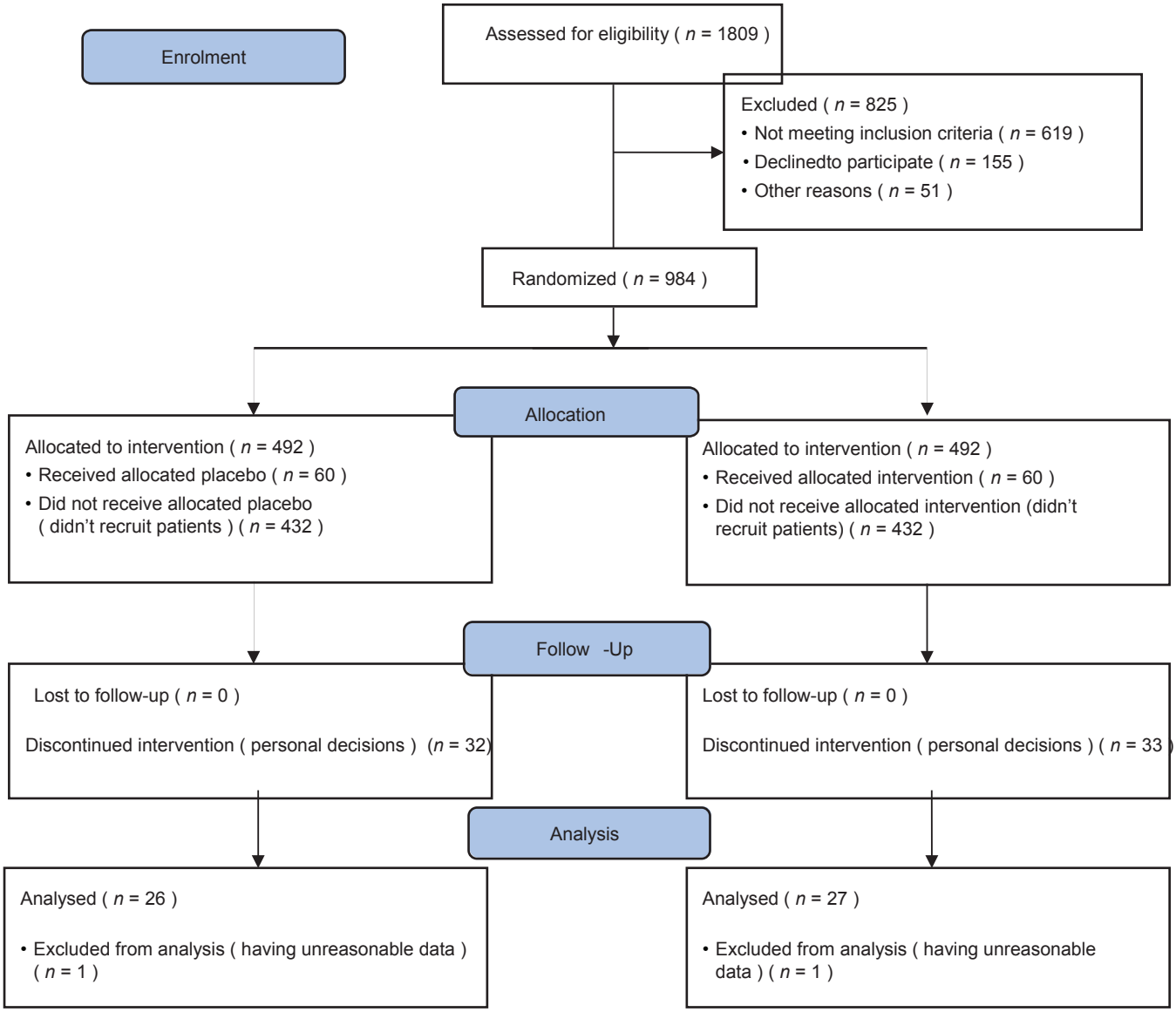

Figure 1: Flow of groups from recruitment to analysis based on CONSORT 2010 flow

the baseline and postintervention steps during 4-month (hence mid-autumn to late winter). There were two questionnaires for anyone that each of which were filled by investigator respectively after interview with them. After almost 6-7 weeks, we called the participants up to confirm their collaborations and to consume their drops correctly. At 14th week of intervention, we invited again all of subjects separately to consider them and to study the parameters at postintervention stage at the same above-mentioned manner. This study was conducted according to the guidelines laid down in medical ethics committee regulation and all procedures involving human subjects were approved by the Tabriz University of Medical Ethics Committee. Written informed consent was obtained from all patients.

Anthropometric and body composition measurements

The weight measurements were fulfilled with the lowest clothing in both baseline and postintervention stages. Waist circumference was measured as triplet measurements to assessing fat distribution at visceral region. The statures of subjects were dipped by a mural stadiometer. Body composition including total body fat, fat-free mass and total body water was measured by bioelectrical impedance analyzer equipment (MALTRON-907, 4 electrodes, 50 kHz, UK).

Dietary assessment

We assessed dietary intake of subjects using three-dimensional 24-h recall questionnaires, which the subjects completed over 2 weekdays and 1 weekend days. This method allowed us to estimate daily nutrients intake of each subject before and after the intervention. The daily intake of the energy, protein, vitamin D and Ca were assessed to this purpose. Nutrients were analyzed by Nutritionist III software, version 7.0 (N-Squared computing, Salem, OR, USA), which was modified for Iranian foods.

Laboratory tests

Sample collection procedure

In each of baseline and postintervention stages, serum collected on sterile, clean and dry tubes rapidly separated after blood sample coagulation. In order to collecting of blood sample, we poured the samples in heparinized tubes for measuring HbA1c. Afterwards, both serum and blood samples were immediately transferred to a freezer and kept frozen (−80°C) until assaying were done (6-10 d later).

Measurement of serum parameters

Leptin levels in serum samples were assessed via ELISA using commercial DRG Leptin sandwich ELISA EIA-2395 Kits (DRG Diagnostics®, Marburg, Germany).[14] Serum TNF-α was measured by an immunoradiometric assay method (Biosource, Medgenix®-TNF-α-IRMA, Belgium), according to the manufacturer recommendations.

Serum 25(OH) D levels were determined in subjects by the competitive chemiluminescence immunoassay procedure using LIAISON 25(OH) D total assay kit (No. 310600, DiaSorin Inc., USA).

Serum concentration of ionized Ca (iCa^{2+}) was measured by using ion selective electrode procedure. The equipment we used was KONE®; USA. The samples were taken at the gel barrier tubes within 24-h without being frizzed. Ion selective electrodes accuracy of direct potentiometry is generally ± 2-4% for monovalent ions and ± 4-8% for divalent ions.

The blood HbA1c levels were measured automatically by using high pressure liquid chromatography procedure within 24-h. The name of the equipment we used for this purpose was DREW-DS5 Analyzer; England. Coefficient of variation for each of normal subjects and abnormal ones were reported as 3.72% and 3.70%, respectively.

Statistical analysis

Statistical analyses were performed using the SPSS version 11.5 (Chicago, IL, USA) statistical package. The data are expressed as mean ± standard error of the means and confident interval of 95%. Chi-square test and correlation test was used to determination of relations between qualitative and quantitative data respectively between studied groups. We utilized Kolmogorov–Smirnov test to trying for normality of the distributions. The groups' means were compared by independent *t*-test to comparing the means of two studied groups at baseline and postintervention and to compare the means of the after-before difference means at the two groups. The means of vitamin D group at baseline and post intervention stages were compared by paired *t*-test for measurements with normal distribution. In order to testing differences between baseline and postintervention stages of nonparametric data, we used Wilcoxon Signed Rank Test.

RESULTS

On the whole, 58.82% of the participants ($n = 30$) were female and 41.18% of those ($n = 21$) were male without no significant differences in two groups. The mean and standard error of age in the placebo group were 49.28 ± 2.00 and those of vitamin D group were 52.26 ± 2.09, without any significant differences in two groups.

A total of 62.75% of the patients ($n = 32$) were retired and the remaining (37.25% i.e. $n = 19$) were occupied or were housewives without any significant correlations in two groups.

The dietary and physical activity characteristics of both studied groups participants at baseline and postintervention stages are presented in Table 1.

Based on Table 1, none of the dietary and physical activity variables were changed at baseline and postintervention stages in both groups of study.

Table 2 shows there was no significant alteration in adiposity and the other body composition characteristics, including waist circumference between two groups at the baseline as well as at the postintervention stages of study. Independent *t*-test also showed no statistically significant differences

Table 1: The status of dietary intake, physical activity and daily exposure to sunlight among the studied groups at baseline and postintervention stages

Studied variables	Baseline		*P**	Postintervention		*P*†	*P*§	*P*#
	Placebo group (*n*=25)	Vitamin D group (*n*=26)		Placebo group (*n*=25)	Vitamin D group (*n*=26)			
Energy intake (kcal/day)	2054±83.67	1984±78.83	0.244	2062±95.94	1964±76.84	0.165	0.215	0.529
Protein intake (g/day)	67.30±5.20	64.42±4.96	0.191	66.90±5.10	65.06±5.05	0.271	0.176	0.533
Ca intake (mg/day)	639.20±67.74	626.3±69.89	0.508	644.9±75.72	611.9±72.04	0.196	0.344	0.289
Vitamin D intake (µg/day)‡	2.96±0.76	3.06±0.48	0.288	3.09±0.62	3.12±0.46	0.144	0.195	0.456
Physical activity duration (min/day)	36.4±4.71	36.2±5.42	0.418	34.2±4.65	32.9±4.92	0.156	0.314	0.144
Daily exposure to sunlight (min/day)	16.2±4.24	22.6±12.7	0.081	14.4±3.91	20.8±5.4	0.106	0.816	0.367

Data are presented as mean±standard errors. **P* value of independent t-test for placebo and vitamin D groups at baseline stage, †*P* value of independent *t* test for placebo and vitamin D groups at postintervention stage, §*P* value of independent *t* test for postintervention-baseline difference means, #*P* value of paired *t* test for vitamin D group at baseline and postintervention stages, ‡The vitamin D intake of the participants belonged vitamin D group was calculated without estimating added vitamin D due to intervention

Table 2: The comparison of the anthropometric and body composition characteristics between the studied groups at baseline and postintervention stages

Studied variables	Baseline		*P**	Postintervention		*P*†	*P*§	*P*#
	Placebo group (*n*=25)	Vitamin D group (*n*=26)		Placebo group (*n*=25)	Vitamin D group (*n*=26)			
Weight (kg)	73.1±2.73	74.9±2.12	0.738	73.6±2.96	75.4±2.23	0.645	0.812	0.568
BMI (kg/m^2)	27.9±0.93	28.9±0.86	0.886	28.1±0.99	29.2±0.77	0.701	0.449	0.538
Waist circumference	97.6±2.14	94.5±3.24	0.360	98.4±2.16	98.6±1.81	0.106	0.098	0.411
Fat mass (kg)	25.9±1.59	27.3±1.39	0.297	26.7±1.67	27.9±1.23	0.225	0.385	0.311
Fat mass (%)	35.3±1.49	36.2±1.39	0.637	36.0±1.42	36.6±1.31	0.389	0.345	0.103
Fat free mass (kg)	47.18±1.96	48.1±1.71	0.446	46.94±1.96	47.9±1.69	0.416	0.576	0.901
Fat free mass (% w/w)	64.7±1.49	63.8±1.29	0.314	64.0±1.42	63.3±1.22	0.396	0.298	0.101
Total body water (L)	34.5±1.44	35.1±1.28	0.236	34.8±1.52	35.1±1.24	0.881	0.256	0.888
Total body water (% v/v)	47.3±1.09	46.7±0.95	0.365	46.9±1.04	46.4±0.89	0.312	0.624	0.108

Data are presented as mean±standard errors. **P* value of independent *t* test for placebo and vitamin D groups at baseline stage, †*P* value of independent *t* test for placebo and vitamin D at postintervention stage, §*P* value of independent *t* test for postintervention-baseline difference means, #*P* value of paired *t* test for vitamin D group at baseline and postintervention stages. BMI=Body mass index

between fat mass means differences in the baseline with the postintervention stages. The comparison among the two stages of the survey in vitamin D group, with regard to amount of body adipose tissue in terms of kilogram had the same results.

Serum iCa^{2+} levels were not changed during period of study in none of two groups (1.173 ± 0.018 mmol/l vs. 1.157 ± 0.057 mmol/l for vitamin D group with $P = 0.231$ and 1.144 ± 0.021 mmol/l vs. 1.147 ± 0.017 mmol/l for placebo group with $P = 0.779$).

Table 3 shows that there was a significant increase in serum 25(OH) D in postintervention phase in comparison with baseline phase in vitamin D group ($P = 0.001$). Independent *t*-test for the postintervention-baseline difference means of serum 25(OH) D revealed a significant difference between the studied groups ($P = 0.008$). However, the difference of postintervention-baseline's means of serum HbA1c, did not achieve to statistically significance level.

Table 3 also shows an increase in serum leptin ($P = 0.046$) and a decrease in serum TNF-α ($P = 0.012$) during the intervention period by comparing the means of placebo and vitamin D groups differences.

According to the Table 3, we did not observe any significant differences between two studied

Table 3: The comparison of the serum biochemical variables between the studied groups at baseline and postintervention stages

Studied variables	Baseline		*P**	Postintervention		*P*†	*P*§	*P*#
	Placebo group (*n*=25)	Vitamin D group (*n*=26)		Placebo group (*n*=25)	Vitamin D group (*n*=26)			
Serum 25(OH) D (ηmol/L)	22.16±5.32	21.46±4.65	0.442	20.92±7.23	46.39±6.89	0.001	0.008	0.001
Serum HbA1c (%)	7.36±0.56	6.78±0.43	0.085	8.42±0.67	6.6±0.46	0.048	0.242	0.246
Serum leptin (ηg/ml)	11.20±1.96	11.96±2.18	0.196	14.95±2.59	25.27±4.97	0.001	0.046	0.002
Serum TNF-α (ρg/ml)	11.91±1.03	10.43±1.12	0.156	10.26±1.46	4.89±1.24	0.014	0.012	0.001

Data are presented as mean±standard errors. **P* value of independent *t* test for placebo and vitamin D groups at baseline stage, †*P* value of independent *t* test for placebo and vitamin D at postintervention stage, §*P* value of independent *t* test for postintervention-baseline difference means, #*P* value of Paired *t* test for vitamin D group at baseline and postintervention stages. TNF-α=Tumor necrosis factor-α, 25(OH) D=25-hydroxyvitamin D

groups in the four studied serum factors at the baseline stages.

DISCUSSION

Our findings revealed that added vitamin D had not any effect in amount of fat mass of body in diabetic type 2 patients. An inverse association between various anthropometric measures including body mass index (BMI) as well as body fat mass and intake of vitamin D or circulating concentrations of vitamin D has been reported by a number of studies.[2,15,16] An epidemiologic study reported vitamin D intake and BMI were inversely associated in both sexes ($P < 0.001$).[2] The principal limitation of this study was its cross-sectional design; hence the causative nature of the association cannot be established. Furthermore, although it was included a large number of subjects as sample population, the vitamin D nutritional status were determined by food frequency questionnaire alone in healthy males and females. In spite of the fact that there are some cross-sectional and case–control studies concerning correlations of vitamin D and adiposity in diabetic type 2 patients[17-19] however a well-controlled clinical trial study is really rare in this context as yet. In a double-blind randomized controlled trial,[20] to determine the short-term effect of vitamin D3 supplementation on insulin sensitivity in apparently healthy, middle-aged, centrally obese men, no changes in secondary outcome measurements (including BMI and waist circumference) were similarly found with supplementation. None of the studies evaluated subjects' physical activity as a confounder. Thus, we may conclude there are influences of fat-free compartments of bone, muscle[21] and abdominal organs (liver, kidney, gut)[22] on body composition to explain the lack of such effects.

Our results could not detect a significant deference in HbA1c between the two stages of the survey in group vitamin D. Although we could not find a statistical difference between postintervention baseline difference means, we found a significant reduction in HbA1c in vitamin D group in comparison with the placebo group at the post intervention stage implying the supplementation of the subjects' diet with vitamin D3 in nutritional doses may potentially be effective in repelling their blood HbA1c rise. It should not forget that one of possible reasons for this phenomenon could be elevated levels of the blood HbA1c at the baseline; although it did not reach to a statistical significance ($P = 0.85$). There are some studies conducted on persons to determine the effect of vitamin D on HbA1c. Most of these were limited by a small sample size. One of the earliest studies was carried out by Ljunghall *et al.*[23] who studied 65 middle-aged men with impaired glucose tolerance. They found that serum concentrations of vitamin D were not associated with a change in HbA1c; nevertheless, their subjects were at normal range of vitamin D. Recent evidences have demonstrated that persons with type 2 diabetes who have hypovitaminosis D are more likely to have increased HbA1c ($P < 0.02$) compared with those persons with diabetes who do not.[17,24-26] In one of the most recent studies Jorde and Figenschau[27] reported in a randomized, placebo-controlled study after 6-month, HbA1c levels were not significantly different from baseline values in patients with type 2 diabetes who had normal serum 25(OH) D levels. Similar findings were seen in another recent investigation in this regard.[28] According to the authors' emphasis, this study[27]

has several limitations and there could be some elucidations for the lack of effect of vitamin D on glucose metabolism observed. Firstly, the number of patients was too low to draw definitive conclusions. In addition, the included subjects were treated with metformin and insulin, which might have masked any subtle effects of vitamin D. In general, we can say the supplementation of the diet with vitamin D3 in type 2 diabetics could correct their HbA1c levels more likely in patients with sub-optimal amounts of serum 25 (OH) D. Our data indicate the subjects' intakes of vitamin D are lower than that of recommended daily allowance recommendations. Hence, our results seem to be rational in this context.

In our study, a statistical significant increase in serum leptin and decrease in serum TNF-α were indicated after 14 weeks supplementation with 400 IU vitamin D3 in group vitamin D. It has been seen anti-TNF-α antibodies improve insulin sensitivity in obese rodents and TNF-α-deficient mice are protected from obesity-induced insulin resistance on a high fat diet.[29] It has been documented that anti-TNF-α therapy may be beneficial in reducing the complications of diabetes.[30] In a study on four women (52-76 years) with type 2 diabetes in danger of vision loss due to severe macular edema on hypoglycemic therapy, two infusions of the monoclonal anti-TNF antibody, infliximab, (5 mg/kg) in 1-month intervals intravenously showed significant regression in macular edema.[31] Hence, we assume based on our results, the supplementation of the diabetic type 2 patients' diet with 400 IU/d vitamin D may potentially have beneficial effects at least on prevention of their disease long-term complications due to its decreasing effect on serum TNF-α.

Despite of a few reports implying vitamin D inhibit *in vitro* leptin secretion by human adipose tissue,[32,33] some experimental studies indicate different results. Tarcin *et al*.[34] recently reported a significant elevation in serum concentrations of leptin after therapy with 300,000 IU vitamin D in 23 asymptomatic vitamin D-deficient subjects. Lately, it is found that the leptin dose of 12.5 ηg/h significantly lowers blood glucose in mice and that 25 ηg/h of leptin normalizes plasma glucose and insulin without significantly reducing body weight, establishing that leptin exerts its most potent effects on glucose metabolism.[35] Therefore, we can suppose when vitamin D intake is below, the serum leptin level will be low, which it could be followed by increasing appetite and weight gain. Vitamin D supplementation may cause serum leptin increment that it would be lead to weight reduction.

We have to admit that our study was restricted by no measurements of serum parathyroid hormone, serum 1,25-dihydroxy vitamin D and by the absence of homeostasis model assessment of insulin resistance mensuration. In addition, we used a supplemental dose of vitamin D3 (400 IU/d) for our trial; whereas the doses used for some of above-mentioned clinical trials, were mostly more (sometimes up to 40,000 IU/week).

In summary, we were not able to demonstrate an effect on body fat mass in subjects with type 2 diabetes after supplementation with vitamin D, but the effectiveness of the supplementation was seen in attenuation of one of the markers of systemic inflammation, TNF-α, as well as in enhancement of the serum leptin levels. There is a need for larger studies with longer duration and graded doses of vitamin D.

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