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Original Article

Effect of vitamin D supplementation on anthropometric indices among overweight and obese women: A double blind randomized controlled clinical trial

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ABSTRACT

Aims: The aim of this study was to investigate effect of vitamin D supplementation on anthropometric indices among women with overweight and obesity.

Methods: This double blind randomize clinical trial was conducted on 66 overweight and obese women. Those in intervention group received oral supplement of vitamin D 50,000 IU (1250 mcg) per 25 day and in control group participants received placebo for 3 months. Anthropometric indices were measured before and after 3 months intervention. Before the intervention a 24-h dietary recall (3 days) were used to assess dietary intake of individuals. Independent t test and multivariate repeated measure were used to data analysis.

Results: The mean difference of anthropometric indices, serum calcium, 25 (OH) D₃ and serum PTH between the intervention and control groups were significant ($P < 0/05$). However, no significant differences in serum phosphorus between the intervention and control groups were seen.

Conclusion: Supplementation with vitamin D 50 µg for each day for 3 months resulted in a significant reduction in anthropometric indices in women with obesity and overweight with normal primary 25(OH) D₃ serum levels.

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1. Introduction

Obesity, which is defined as the extra accumulation of fat in body, can increase the risk of many diseases such as cardiovascular disease, blood lipid disorders, type II diabetes mellitus, and hypertension [1–3]. In Iran by the year 2013 the prevalence of overweight and obesity has been reported as 38.8% and 10.2% for male and 34.5% and 18.5% for female respectively [4]. Because of vitamin D storage in deep parts of fat masses in obese people which can make difficult releasing this vitamin into blood circulation, obesity decrease the vitamin D serum level in those people [5]. The association between increased body mass index

(BMI) and low serum 25-hydroxyvitamin D reported in previous studies [6–8]. Obesity leads to low serum vitamin D (OH) 25 followed by secondary hyperparathyroidism [9–11]. Increased intracellular calcium by PTH leads to inhibition of lipolysis and stimulating the fatty acid synthesis and consequently lipid accumulation in adipocyte will increase [12,13]. In the other hand, based on some studies, treatment by vitamin D supplementation through the facilitation of the calcium entrance into adipocyte can stimulate lipogenesis and accumulation of fat in body [14,15]. Therefore, the role of vitamin D in body weight regulation is unclear, as several studies have demonstrated using of vitamin D supplementation cause to weight loss in obese people, whereas some studies show no effect [16,17]. Regarding this point both healthy and vitamin D deficient subjects have participated in previous studies it might affected the results of study, since it is possible that vitamin D supplementation only

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cause to improve of vitamin deficiency and rebuilding of its supply, and doesn't show the expected effect on anthropometric indices. Therefore, according to what was mentioned, the purpose of this study is to determine the effect of vitamin D supplementation on body weight, BMI, serum levels of calcium, phosphorus, vitamin D, and PTH hormone in overweight and obese women with normal level of serum vitamin D.

2. Subjects, materials and methods

This study was conducted as a controlled double blind randomized clinical trial. The sample size was calculated by Type I error (α) = 0.05, Type II error (β) and effect size = 0.6 for each group equal to 38 individuals. Subjects were selected voluntarily through advertisement on hospital, clinic and medical colleges and among overweight and obese women visited the specialty nutrition clinics of Khorramabad Shahid Rahimi hospital.

The eligible criteria were BMI over 25 kg/m² and the age ranging from 18 to 70 years old. The exclusion criteria were: history of diseases such as bone diseases, diabetes, thyroid diseases, kidney or liver diseases, cardiovascular disease, also consuming vitamins and minerals, consuming anticonvulsant drugs, drugs containing estrogen and progesterone, corticosteroids, having special diet, hormonal therapy and weight loss to 10% within the three months before study. At the beginning of the study an efficient medical history including the history of heart diseases and consuming drugs was done. The criteria for inclusion and exclusion to this study were considered. Then, height and weight were measured and body mass index was calculated. After confirming the overweight and obesity in these subjects, other anthropometric indices including waist and hip circumference also were measured.

Subject's blood samples were collected after 12 h fasting and serum 25-hydroxyvitamin D3 levels were determined for all of them. Those who had vitamin D deficiency (serum vitamin D levels <30 ng/mL) were excluded and were introduced to endocrinologist for treatment. In subjects with serum vitamin D $\geq$ 30 ng/mL, other required variables including serum calcium, serum phosphorus and PTH hormone were measured by using the same blood samples.

Subjects having vitamin D deficiency were called and consulted about their treatment. Among 210 referred participants, 76 subjects who met the above inclusion criteria were selected and randomly divided into two groups; vitamin D supplementation group and placebo group. The intervention group took vitamin D supplementation in the form of pearls 50,000 UI colecalciferol, once per 25 days in four different doses. The placebo group took placebo for three month (from the beginning of September to the beginning of December 2013). To get assured from using the supplementation and placebo in appointed time, the subjects were called.

The subjects in control group received placebo containing lactose exactly the same as the pearls of vitamin D in size, form, packing and name. The number of consuming and time span of using the placebo in control group was exactly the same as pearl vitamin in intervention group. At the beginning of the study, a dietary intake data was collected with 3 day 24-h dietary recall (including 2 weekdays and 1 weekend). The dietary data were converted to energy and nutrients using the software Nutritionist IV (version 4.1, 1997; First DataBank, The Hearst Corporation, San Bruno, CA).

2.1. Blinding method

Both researchers and participants did not know about allocation in treatment group.

After 3 months intervention, biochemical and anthropometric indices were recalculated.

The study was confirmed in the Committee of Ethics of Lorestan medical university. Also written informed consent was obtained from participants.

2.2. Measuring the variables

About anthropometric data; weight was calculated with the least clothing and without shoes by Seca scale with the precision of 0.1 kg and height was measured by Seca stadiometer, without shoes and in standing position while the shoulders were in a normal position. BMI was calculated through dividing the weight to the square of height (kg/m²). Waist circumference (WC) at the level of umbilicus and hip circumference at the point of maximum circumference over the buttock were measured in centimeters by using a non-stretch tape meter. All measurements were recorded with the precision of 0.1 cm.

For measuring biochemical data, after 12 h overnight fasting, 10 cc vein bloods were taken from all participants at baseline and after intervention. The serum of received blood samples were segregated after centrifuging for 10 min at 3000 rpm. The serum samples were kept at -70°C for biochemical measurement of 25-hydroxyvitamin D, PTH, calcium, phosphorus and lipid profile. Measuring serum 25-hydroxyvitamin D levels was done via ELISA (Enzyme-Linked Immunosorbent Assay) and by IDS kit (25. OH.D. EIA. KIT. IDS. UK). Measurement of Parathyroid hormone serum levels was done via ELISA and by HOSOT kit made from Novin-Azma Co.

Calcium and phosphorus serum level was measured through colorimetric method and by the Pars Azmoon Co. kits (Tehran, Iran) and via TB autoanalyser machine. Data analysis performed by Statistical Package for Social Sciences (SPSS version 19). To compare the Means of BMI in intervention and control groups, independent *t*-test, and to compare these indices before and after treatment in each group ANCOVA were used. $P < 0.05$ was considered statistically significance.

This clinical trial was recorded in IRCT (Iranian Registry of Clinical Trial). The registry code of this study is IRCT2013072814195N1.

3. Results

During the study four subjects, one for pregnancy & three for personal reasons withdrew from the intervention group. Also six subjects withdrew for personal problems in placebo group. Finally, 34 subjects in intervention group and 32 subjects in control group continued their cooperation for three months. No side effects for vitamin D supplementation or lactose-contained placebo were reported in this study. Table 1 shows the baseline characteristics of study participants. Data had shown no difference in the average intake of protein, vitamin D, calcium and phosphorus between two groups. However, the average intake of energy and fat in vitamin D group were significantly higher than control group (Table 1).

Means $\pm$ SD of anthropometric and biochemical indices in both groups before and after treatment, and anthropometric and biochemical indices changes between vitamin D group and placebo group are presented in Table 2. For assessing the interaction between weight and energy intake in each group after treatment the regression analyses was used. Since the interaction wasn't significant ($P = 0.17$), analysis of covariance was used for remove the effect of energy intake in two groups. According to the results of this analysis and after adjusting of energy intake in both groups, the mean weight of the two groups after vitamin D and placebo intake wasn't statistically significant ($P = 0.72$).

Table 1

Primary information of Vitamin D group and placebo group.

Indices	Vitamin D receiver SD $\pm$ Mean N = 34	Placebo receiver SD $\pm$ Mean N = 32	P
Age (year)	41 $\pm$ 12	38 $\pm$ 9	0.12
Weight (kg)	81.4 $\pm$ 9.1	78.0 $\pm$ 10.4	0.16
Waist circumference (cm)	102 $\pm$ 9.8	100.3 $\pm$ 9.8	0.46
Hip circumference (cm)	111.9 $\pm$ 8.1	110.5 $\pm$ 7.5	0.45
BMI (kg/ m ²)	31.8 $\pm$ 3.6	30.58 $\pm$ 3.4	0.15
25(OH)D ₃ (ng/ ml)	73.6 $\pm$ 35.6	69.8 $\pm$ 45.9	0.71
Serum Parathormone (pmol/L)	41.2 $\pm$ 16.8	38.2 $\pm$ 13.8	0.44
Serum Calcium (ml/dl)	9.3 $\pm$ 0.4	9.2 $\pm$ 0.3	0.97
Serum Phosphor (ml/dl)	3.8 $\pm$ 0.4	3.7 $\pm$ 0.3	0.7
Received energy (kcal)	2324.8 $\pm$ 395.9	2053.3 $\pm$ 386.9	0.006
Received protein (gr)	85.6 $\pm$ 15.4	80.2 $\pm$ 23.6	0.27
Received Fat (gr)	98.7 $\pm$ 32.7	77.8 $\pm$ 20.2	0.003
Received calcium (mg)	1306.1 $\pm$ 242.9	1270.6 $\pm$ 365.7	0.64
Received vitamin D (μ g)	1.4 $\pm$ 1	1.8 $\pm$ 2.5	0.14
Received phosphor (mg)	853 $\pm$ 330.4	986.2 $\pm$ 391.3	0.13

Table 2Comparison of mean anthropometric indices and biochemical changes in vitamin D group and placebo group before and after intervention.^a

		Before intervention (Mean $\pm$ SD)	After intervention (Mean $\pm$ SD)	Change	P within the groups	P between the groups
Weight (kg)	Vitamin D group	81.14 $\pm$ 9.14	78.31 $\pm$ 9.16	-3.13 $\pm$ 1.62	<0.001	<0.001
	Placebo group	78.05 $\pm$ 10.35	77.91 $\pm$ 10.36	-0.14 $\pm$ 1.06	0.46	
Waist circumference (cm)	Vitamin D group	102.06 $\pm$ 9.81	100.15 $\pm$ 10.30	-1.91 $\pm$ 1.70	<0.001	<0.001
	Placebo group	100.26 $\pm$ 9.83	100.31 $\pm$ 10.06	0.05 $\pm$ 1.04	0.80	
BMI (kg/m ²)	Vitamin D group	31.83 $\pm$ 3.61	30.61 $\pm$ 3.60	-1.23 $\pm$ 0.65	<0.001	<0.001
	Placebo group	30.59 $\pm$ 3.36	30.54 $\pm$ 3.40	-0.05 $\pm$ 0.41	0.49	
25(OH) D3(ng/ml)	Vitamin D group	73.6 $\pm$ 35.6	92.8 $\pm$ 34.9	19.2 $\pm$ 10.5	0.0001	<0.001
	Placebo group	69.8 $\pm$ 45.9	53.5 $\pm$ 45.8	-16.2 $\pm$ 18	0.0001	
PTH(pmol/L)	Vitamin D group	41.2 $\pm$ 16.8	39.9 $\pm$ 22.2	-1.27 $\pm$ 11.4	0.52	P < 0.001
	Placebo group	38.2 $\pm$ 13.8	47.5 $\pm$ 12.4	12.44 $\pm$ 14.45	0.001	
Serum Ca (mg/dl)	Vitamin D group	9.25 $\pm$ 0.41	9.32 $\pm$ 0.39	0.07 $\pm$ 0.27	0.15	0.02
	Placebo group	9.26 $\pm$ 0.30	9.15 $\pm$ 0.29	-0.11 $\pm$ 0.28	0.03	
Serum P (mg/dl)	Vitamin D group	3.79 $\pm$ 0.35	4.01 $\pm$ 0.27	0.22 $\pm$ 0.26	<0.001	0.94
	Placebo group	3.75 $\pm$ 0.33	3.98 $\pm$ 0.67	0.23 $\pm$ 50	0.01	

^a Multivariate repeated measure was used.

Also after showing no significance interaction between BMI and energy intake ($P=0.55$), analysis of covariance was done for adjusting the effect of energy intake in two groups, results show no significant difference for mean BMI after treatment in vitamin D and placebo groups ($P=0.74$). To evaluate of interaction between weight and fat intake after intervention in each group, the regression analyses for being homogenized was conducted, Since interaction wasn't significant ($P=0.17$), analysis of covariance was used for adjusting the effect of fat intake for two groups, results demonstrate that there was no statistically significant difference ($P=0.56$) for the mean weight of two groups after vitamin D and placebo intake. As interaction between BMI and fat intake wasn't significant ($P=0.59$), for adjusting the effect of fat intake in two groups, analysis of covariance was used and results indicate no significant difference ($P=0.49$) for the mean of BMI after vitamin D and placebo intake.

4. Discussion

In this study, anthropometric indices such as weight, waist and hip circumference and BMI were significantly reduced in intervention group compared with the placebo group. Theoretically, vitamin D affects weight through different mechanisms.

Various clinical studies indicate the reduction risk of overweight by increasing the calcium intakes [18], whilst vitamin D increases the absorption of calcium from intestine. In contrast, the previous studies have proved that PTH hormone leads to the accumulation of adipose tissue in body through increasing the intercellular absorption of calcium, whilst supplementation with vitamin D leads to reduction of serum PTH [12]. Also it was shown that Supplementation with vitamin D via increasing the 1,25-dihydroxyvitamin D causes the elevating of intercellular absorption of calcium and therefore can culminate to overweight [19].

Zitterman et al. assessed the effect of vitamin D (83 μ g per day) comparing to a placebo on weight loss and risk of cardiovascular diseases in overweight subjects. The duration of this clinical trial was 12 months. At the end, the results demonstrated that although weight loss wasn't clearly influenced by vitamin D supplementation, but waist circumference was overtly decreased (from 101 to 96.2 cm) in intervention group [20]. Also in our study, waist circumference was overtly reduced after 3 months treatment in intervention group that this finding is in agreement with Zittermans et al. Zitterman's study was done on overweight subjects with the serum 25-hydroxyvitamin D levels 12 ng/ml, whilst the participants in our study had no vitamin D deficiency and the average amount of their serum 25-hydroxyvitamin D was

73.56 ng/ml before the study. It can be the reason for the lack of weight loss in Zitterman's study in response to the vitamin D supplementation is against the current study.

In another study, Mahdavi et al. assessed the effect of vitamin D supplementation on serum leptin levels and the amount and composition of body fat on 53 diabetic patients during 12 weeks. They used of 400 IU vitamin D with 1000 mg calcium for supplementation. At the end of study, BMI in intervention group reduced from 27.2 ± 8.5 to 26.2 ± 8.3 , and in control group BMI increased just a bit, but there wasn't any significant difference before and after treatment neither within nor between groups [21]. In our study, BMI and weight decreased significantly after 3 months in intervention group. The difference in the amount of received vitamin D supplementation can justify the outcomes in both studies. It's also merit to mention that our study was done on more subjects and without any background diseases that can justify why the results of these two studies are different.

The effect of vitamin D supplementation on weight and adipose tissue investigated by Salehpour et al., and 77 participants were randomly divided into vitamin D (25 µg per day) and control group (placebo) for 12 weeks. At the end of intervention, weight loss in vitamin D group and control group were (-0.3 ± 1.5) and (-0.1 ± 1.7) respectively. But, there wasn't statistically significant difference between the two groups. They also found that waist circumference was reduced in vitamin D group but increased in control group, for Hip circumference; although decreased in vitamin D group, but there was not significant difference before and after intervention. Their results indicated that vitamin D supplementation (daily intake for 12 weeks) doesn't affect waist and hip circumference [22]. In our study, there was a statistically obvious difference between the two groups in weight and waist and hip circumference. One reason for the difference between these two studies can be the lower dose of vitamin D as supplementation in Salehpour's study, whereas the vitamin D dose is two times more in our study. Also the participants in Salehpour's study had the average serum 25-hydroxyvitamin D levels = 16.72 µg/ml and were vitamin D deficient. But the participants in our study had normal vitamin D level (73.56 µg/ml). It seems that vitamin D supplementation has more effects on weight loss in participants with normal serum levels of 25-hydroxyvitamin D.

In study that conducted by Sneve et al. the effect of vitamin D and calcium supplementation on weight loss in 334 overweight and obese subjects assessed. In this study 20,000 IU vitamin D with 500 mg calcium was used twice a week and for 12 months. At the end, there wasn't any significant difference in weight, waist and hip circumference before and after treatment in both groups. The serum levels of 25-hydroxyvitamin D increased and PTH level decreased in intervention group, but serum calcium did not change [14]. Participants in Sneve's et al. study had the average of serum 25-hydroxyvitamin D levels equal to 53.1 ± 16.9 before treatment that wasn't in normal range (less than normal) whereas participants in our study had normal serum 25-hydroxyvitamin D levels. Due to this finding it seems that weight gain occurred in vitamin D deficiency status.

Caan et al. in a controlled double blind randomized clinical trial, evaluate the effect of calcium and vitamin D supplementation anthropometric indices of 50–79 years old women. In this study 400 IU vitamin D was used with 1000 mg calcium per day. After three years treatment, women in intervention group showed a little but desirable and stable change in weight compared with the control group (average change = -0.13 ; $P = 0.001$). Then participants were followed for 7 years; women with lower age (50–54) had the most weight gain (2.1 kg) and were the only group which permanently had weight gain. In contrast older women (70–79) were the only group who had

weight loss permanently and had the most changes in their weight (-2.5 kg). Generally, women's weight increased until the age of 60 a little but permanently, then it would be fixed for a period of time and then again decreased from the middle to the end of 60s and will be continued to the 70s. As a result the intervention group had less weighing than the control group and weren't at the risk of being overweight [23]. Regarding to time period of our study which lasted after three months, if our study continued the same results could be achieved similar to Caan study between intervention and control groups.

To control the dietary intake during the current study, for each person prescribed own diet and for assessing the diet adherence, they were asked and their answer basically was positive. It was asked from participants to have the same physical activities as before treatment. In subsequent visiting, participants were asked about increasing or decreasing their physical activities, so that those with change were been omitted from the study.

Some limitation of this study include: the lack of any practical supervision on participants about alterations in their life styles and adherence to their prescribed diet, and another limitation is that our population study weren't checked about their sunlight exposure.

Our data indicate that daily supplementation of 50 µg Vitamin D for 3 months leads to a significant reduction in weight, BMI, waist and hip circumference and weight-hip ratio in obese and overweight women with normal serum levels of 25-hydroxyvitamin D. we also found a significant increasing of serum calcium and serum 25-hydroxyvitamin D, and a significant reduction in PTH hormone level at the end of study.

Conflicts of interest

None.

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