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Original article

The effect of calcium and vitamin D supplementation on body composition and weight reduction: a randomized, triple-blind, controlled trial

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Summary. *Background and aims:* Multiple studies have suggested that calcium can supplement diet-induced weight loss, and that vitamin D is the most important regulator of intestinal calcium absorption. The aim of this study was to compare the effects of calcium, vitamin D, and calcium and vitamin D (Ca + Vit D) supplementation on body composition and weight loss in overweight or obese women. *Methods:* In this triple-blind, randomized, parallel, placebo-controlled trial, 100 overweight or obese premenopausal women were randomly allocated to one of the following 4 treatment groups for 8 weeks: 1) calcium supplement (2 tablets per day, each containing 500 mg calcium carbonate), 2) vitamin D supplement (2 tablets per day, each containing 200 IU vitamin D_3), 3) Ca + Vit D supplement (2 tablets per day, each containing 500 mg calcium carbonate plus 200 IU vitamin D_3), or 4) placebo (2 tablets per day, containing micro-cellulose). All groups received a2093 kJ (500 kcal) energy-restricted diet. Anthropometric measurements, body composition and serum vitamin D levels were measured before and after the intervention. Physical activity and 24 h dietary records were taken at baseline, week 4 and week 8 of the intervention. *Results:* There were significant reductions in body weight, body mass index, waist circumference (p<0.001 for all), and body fat mass (p<0.05) in all treatment groups after the intervention. However, the results were not significantly different among the four treatments groups after adjusting for potential covariates. *Conclusion:* Calcium, vitamin D, or Ca + Vit D supplements had no effect on body composition and weight loss even in low vitamin D and calcium consumers.

Key words: calcium, vitamin D, overweight, obese

Abbreviations:

ANCOVA; Analysis of Covariance
WHO; World Health Organization
CVD; cardiovascular diseases
PTH; parathyroid hormone
1,25-(OH)2-D; 1,25-dihydroxyvitamin D
25(OH)D; 25-hydroxy vitamin D
FAS; fatty acid synthase
BMI; body mass index
WC; waist circumference
BIA; bioelectric impedance analysis
MET; metabolic equivalent task
EIA; enzyme immunoassay
DXA; dual energy X-ray absorptiometry
CLA; conjugated linoleic acid
ITT; intentionto-treat
PP; per-protocol

Introduction

Obesity is one of the most significant public health problems in the world (1). The World Health Organization (WHO) estimated thatthe rate of worldwide obesity had more than dou bled from 1980

to 2011 (2).The WHO also estimated that by 2015, about 2.3 billion adults will be overweight and more than 700 million obese (3).

Overweight and obesity are known risk factors for Cardiovascular Diseases (CVD), hypertension, stroke, type 2 diabetes, certain cancers, and numerous other disorders (4). However, the risk of these chronic conditions may be reduced through weight loss.Although some studies have investigated the effects of macronutrients on weight control, the role of micronutrients has not been well studied (5).

Clinical trials have shown that supplemental or dietary calcium can enhance diet-induced weight loss (6). Zemel et al (7) proposed that low-calcium diets can decrease extracellular Ca^{2+}concentrations, which leads to an increase in Parathyroid Hormone (PTH) production and stimulates 1,25-dihydroxyvitamin D(1,25-(OH)2-D) synthesis. Both PTH and 1,25-(OH)2-Dstimulate calcium influx into adipocytes. Increases in intracellular Ca^{2+}concentration reducesthe expression of Fatty Acid Synthase (FAS) – a key regulator enzyme in lipid deposition – which stimulates adipose tissue lipolysis.

Therefore, increased dietary calcium intake can suppress 1,25-(OH)2-D concentration, inhibit adiposity, and increase weight loss (8). Moreover, dietary calcium increases fat oxidation and thermogenesis through up-regulation of uncoupling proteins (UCP) (8-10), decreases hunger, reduces energy/macronutrient intake (10), and has an effect on fecal-fat excretion (8). Although several Randomized Controlled Trials (RCTs) support the beneficial effects of high calcium intake on weight loss in obese individuals (11), results are few studies and inconsistent (12-14).

Serum vitamin D metabolites are the most important regulators of intestinal calcium absorption (15), but the existing RCTs do not consistently support the beneficial effect of calcium and vitamin D supplements on weight and body-fat loss (10, 11). Moreover, in addition to low calcium intakes, serum 25-hydroxy vitamin D (25(OH)D) concentration has been inversely associated with body weight in obese adults (16), and Body Mass Index (BMI) and fat mass in both adults(17) and children (18). *In vivo,*vitamin D receptors are involved in energy metabolism via β-oxidation regulation and UCP expression (19).

It has been suggested that vitamin D may have a potential role in improving insulin sensitivity, which could affect food intake and substrate oxidation (10). In one clinical trial, overweight and obese premenopausal women with a higher baseline vitamin D status lost more body fat while ona hypcaloric diet (20). However, there are very limited RCTs on vitamin D supplements without added calcium to draw any specific conclusions (8, 10).

Inconsistent results of calcium supplementation and weight loss (21) may be due to the baseline vitamin D status, which could further affect calcium absorption and its physiologic functions. Since vitamin D deficiency is common among Iranian females (22, 23), our objective was to assess the effects of calcium, vitamin D, and Ca+VitD supplements on body composition and weight loss in over weight or obese premenopausal Iranian women over 8 weeks.

Methods

Participants

Sample size was calculated on the basis of the primary outcome measures (i.e. visceral fat mass) and by use of the standard deviations reported in a previous study (23). Using Power SSC software and based on the mean difference between independent groups equation, a minimum sample size of 21 participants per group was calculated (statistical power=80%, type I error=5%, mean difference=0.35, SD=0.4). To account for attrition rate of approximately 20%, 25 participants per group were recruited.

Participants were otherwise healthy overweight or obese premenopausal women recruited from Shiraz University of Medical Sciences (SUMS) clinics via invitation. A total of 180 women were evaluated for study eligibility, which were determined through the following inclusion criteria: (i) age range between 20-50 years; (ii) report having regular menstrual cycles; (iii) BMI between 25-40 kg/m^2; (iv) has not been clinically diagnosed with any cancer or severe endocrine, mental, hepatic, renal, gastrointestinal, cardiovascular, neurologic, rheumatologic, hematologic, skeletal, and eating disorders; (v) not taking any medications, nutritional supplements, or herbal supplements in thelast 12 weeks, which could affect body weight, calcium

and/or vitamin D status; (vi) not reporting any history of adverse reactions to the study supplements; (vii) consume dairy product of ≤3 serving/d; (viii) not being pregnant or lactating; (ix) not smoking or drinking alcohol; (x) not being a participant in other trials in the last 6 months; (xi)and not reporting greater than 3 kg of body weight change for the past 3 months.

Participants who did not meet the inclusion criteria during the study period or did not comply with the study protocol were excluded from the study. This study was approved by the Ethics Committee of the Shiraz University of Medical Sciences, Shiraz, Iran (code#:92-6836), and registered to Iranian Registry of Clinical Trial (registration no IRCT2014021116555N1). All participants were informed of the potential risks and benefits of the study, and written consent was obtained from them.

Study design

This randomized, parallel, triple-blind, placebo-controlled trial was conducted from September to November, 2014. Recruitment began in May 2014 and ended in August 2014.

Participants' baseline dietary intakes and physical activity (PA) were assessed during a 2-week run-in period. Using a balanced, blocked randomization method, in a 1:1:1:1 manner, participants were allocated to one of the 4 treatment groups as follows: 1) calcium supplement (2 tablets per day; each containing 500 mg calcium carbonate), 2) vitamin D supplement (2 tablets per day; each containing 200 IU vitamin D_3), 3) Ca + VitD supplement (2 tablets per day; each containing 500 mg calcium carbonate plus 200 IU vitamin D_3), 4)placebo (2 tablets per day, each containing microcellulose). In addition, participants in all groups were placed on a 2093 kJ (500 kcal) energy-restricted diet based on their daily energy requirements.

An independent statistician at SUMS generatedthe randomization sequence, which was used by the study dietitian to allocate the participants into four different treatment groups and to prescribe individual dietary regimens. Participants' enrollment and eligibility assessments were performed by the study clinician who was blinded to the treatment allocation.

Daily energy requirements were calculated using Estimated Energy Requirement (EER) equation (EER (kcal/day)=Total Energy Expenditure (TEE)=448 - 7.95 x Age (yr) + PA x (11.4 x weight [kg] + 619 x Height [m]) (24), and 2093 kJ (500 kcal) deficit balance diets (55% carbohydrate, 18% protein and 27% fat) were designed for each participant by a trained dietitian. All of the participants received a diet sheet containing standard recipes, diet recommendations, and a portion-size booklet of common foods. Participants were asked not to change their physical activity during the study.

Supplements were purchased from Iran Daru Company, Tehran, Iran. All ofthe tablets were similar in shape and color and were placed in identical and opaque pill bottles. The pill bottles were coded with the letters A, B, C and D by a person not involved with the all steps of study (i.e. intervention, biochemical and anthropometric measurement and data analysis). Participants received their supplements every 2 weeks, and researchers contacted all participants on a weekly basis to ensure dietary regimens and supplement intakes.

The study defined participants' compliance to protocol asconsuming ≥80% of the provided supplements. Participants were asked to return the pill bottles at each visit, every two weeks, in order to use the number of remaining supplements to assess compliance. If the participants did not consume their supplements for two consecutive weeks or more, they were excluded from the study.

Assessment

Anthropometrics including height, weight, Waist Circumference (WC), percent fat mass, and percent fat-free mass were measured before and after intervention. Physical activity and 24-h dietary records (2 weekdays and 1 weekend day) were taken at baseline, week 4 and week 8 of the study. Height was measured to the nearest 0.1 cm using a stadiometer (Seca 214 portable stadiometer) without shoes. Weight was recorded to the nearest 0.1 kg in light indoor clothes, using a digital scale (Seca 881, Germany).

BMI was calculated as body weight divided by height squared (kg/m^2). Waist circumference was measured to the nearest 0.1 cm at the end of a normal expiration, by a plastic measuring tape in a standing position, at the narrowest point between the lowest rib

and iliac crest on the body surface without any clothes. Body composition was assessed using a Bioelectric Impedance Analyzer (BIA; Body Stat Quad Scan, model 4000; British Isles) five minutes after a resting period. According to the BIA testing guideline, participants were advised to 1) not eat or drink within 4 h of the test, 2) maintain normal body hydration, 3) not consume caffeine or alcohol within 12 h of the test, 4) not exercise within 12 h of the test, 5) not take diuretics within 7 d of the test and 6) urinate within 30 min of the test (25).

Toassess the physical activity, the average of Metabolic Equivalent Task (MET) hours per day was calculated by multiplying the time of each physical activity by its relative METaccording the International Physical Activity Questionnaires (IPAQ) (26). 24 h dietary records were analyzed by Nutritionist IV software (First Databank, Hearst Corp, San Bruno, CA, USA).

Blood sampling and biochemical measurement

Before and after the intervention, a blood sample was taken from each participant after an overnight fast. The whole blood was centrifuged and serum was stored in −70˚C until the further analysis. Serum 25(OH)-D was measured usingEnzyme Immunoassay (EIA) (Immunodiagnostic Systems Ltd, Boldon, UK) by IDS 25-hydroxy vitamin D EIA kit with CV= 5%.

Statistical analysis

Two populations were used in analyses:the intentionto-treat (ITT) population (n=100) included all randomized participants, and the per-protocol (PP) population (n=81) included all study completers. All data were analyzed using SPSS (version 19; SPSS Inc, Chicago, IL) and P values <0.05 were considered statistically significant. The normal distribution of all continuous variableswas examined by Kolmogorov-Smirnov test. Descriptive statistics were expressed as mean±standard deviation.One-way ANOVA was used to compare the normally distributed variables including general characteristics, anthropometric indices, energy and nutrient intakes, andphysical activity of participants among the four groups at baseline, and to compare the mean totalenergy and nutrient intake, physical activity and serum vitamin D_3 level of participants during the intervention among the four groups. Kruskal-Wallis H test was used to compare serum vitamin D_3concentration of participants among the four groups at baseline (non-normally distributed variable).

Mean differences of anthropometric indices and body composition in both ITT and PP populations were compared among the four groups using One-way ANOVA and analysis of covariance (ANCOVA) modelwith treatment groups (vitamin D, calcium, Ca+VitD, and control) as main effect, and baseline values, BMI, body fat mass and physical activity as covariates. A paired sample t-test was used to estimate the effect of intervention in each group.

Results

A total of 180 women volunteered for this study. After assessing the volunteers for study eligibility, 100 women met the inclusion criteria of whom 81 (placebo: n=19, calcium: n=21, Ca+VitD: n=21, vitamin D: n=20) completed the study. Reasons for dropouts included adverse side effects such as constipation (calcium: n=2, Ca+VitD: n=2), study protocol violation (placebo: n=4, vitamin D: n=3, Ca+VitD: n=2), and participant decision (placebo: n=2, calcium: n=2, vitamin D: n=2) (Figure 1).

Table 1 shows baseline characteristics, anthropometric measurements, dietary intake and physical activity of participants. There were no significant differences in age, weight, WC, fat mass (kg), serum vitamin D_3, calcium, and energy intake of participants among the four groups at baseline. Baseline body fat mass, BMI, and physical activity were significantly different among the four groups.

According to Table 2, there were no significant differences in mean total energy, macronutrient, calcium, and fiber intake among the four groups during the study. The macronutrient distribution was very similar to our recommendations (carbohydrates=55%, protein=17%, fat=28%). Physical activity levels were not significantly different within and between groups.

As shown in Table 3, there was an increase in serum vitamin D levels in both vitamin D and Ca + VitD

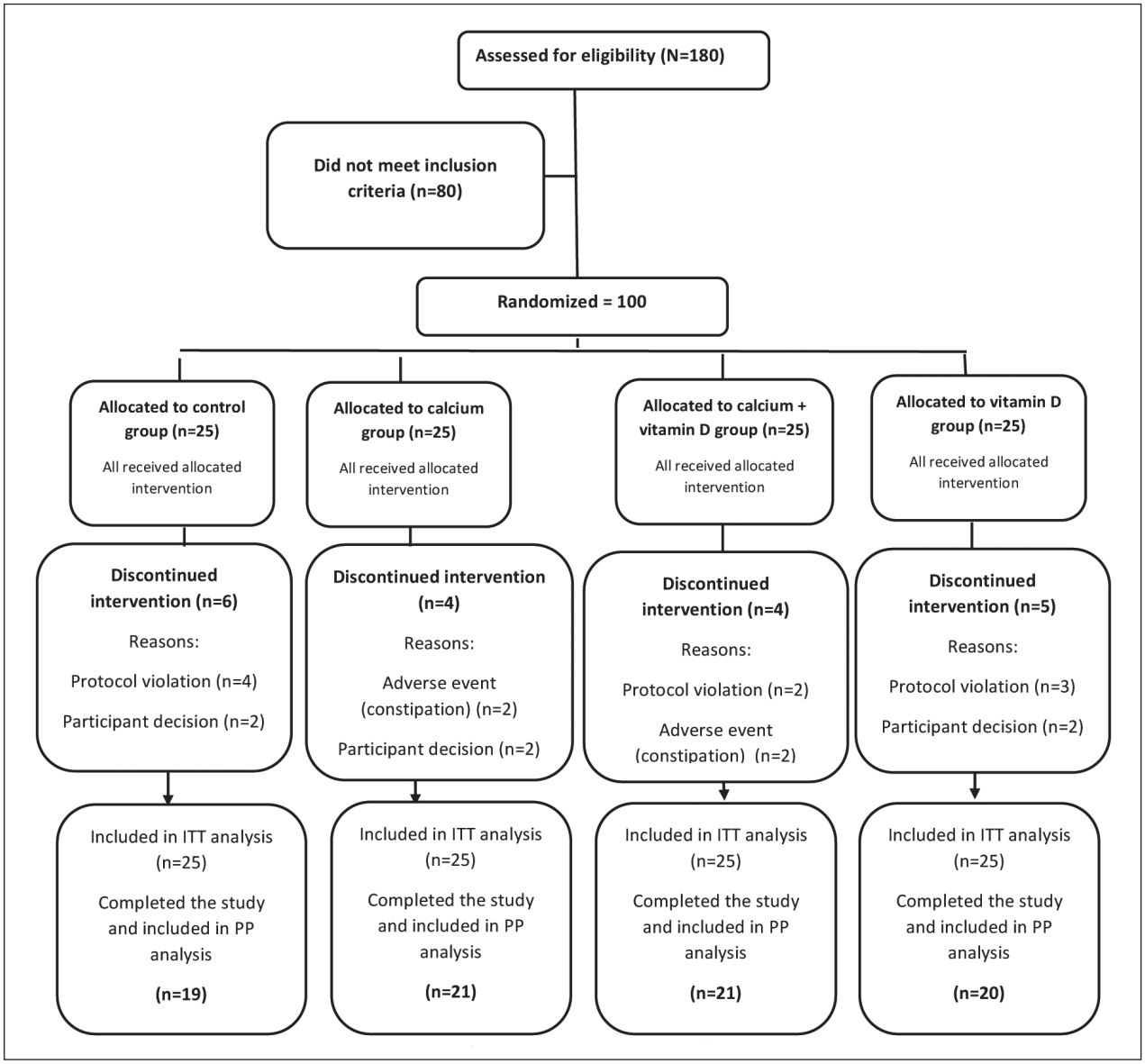

Figure 1. The flow diagram of participants throughout the study

groups which was statistically different from 2 other groups (p<0.001). Also according to percent of taken tablets, there was a compliance of more than 90% in all groups (placebo=91.92±19.81, calcium=95.62±4.48, vitamin D=93.40±9.54, Ca+ Vit D=91.53±12.69).

Table 4 shows baseline and post-intervention values of anthropometric indices in PP and ITT populations. In both PP and ITT populations, significant reductionswere observed in weight, BMI, WC(p<0.001 for all), and body fat mass (p<0.05) in all groups after the 8-wk intervention.

There were significant differences in post-intervention values of BMI (of PP population) and body fat mass (of PP and ITT populations) among the four groups. Butafter adjusting for potential covariates (baseline values, BMI, body fat mass and physical activity) in ANCOVA models, there wereno significant differences.

Table 1. General characteristics, anthropometric indices, dietary intake and physical activity of participants at baseline

	PP population (n=81)					ITT population (n=100)				
	Placebo (n=19)	Calcium (n=21)	Vitamin D (n=20)	Calcium + Vitamin D (n=21)	P-value*	Placebo (n=25)	Calcium (n=25)	Vitamin D (n=25)	Calcium + Vitamin D (n=25)	P-value*
Age (year)	38.2±5.9	39.0±7.6	39.3±5.9	39.1±6.1	0.95	38.2±5.7	38.9±7.5	39.5±5.9	38.4±5.8	0.90
Weight (kg)	79.8±14.1	77.6±9.0	76.7±8.6	82.5±10.4	0.31	81.3±12.8	77.51±8.4	76.8±8.1	82.6±9.8	0.11
BMI (kg/m²)	31.3±4.1	30.0±2.6	29.7±3.1	32.4±3.4	0.05	31.8±3.9	30.1±2.5	29.44±2.9	32.43±3.1	0.003
Waist circumference (cm)	90.1±9.3	87.5±5.6	87.3±7.8	90.9±8.3	0.36	90.7±8.3	87.36±5.3	87.0±7.0	87.3 5.3	0.13
Body fat mass (kg)	27.5±8.2	26.4±6.3	24.0±5.2	29.5±6.6	0.06	24.3±5.0	26.1±6.0	28.2±7.5	26.9±6.0	0.01
Body Fat free mass (kg)	50.8±6.2	51.7±4.5	50.8±6.9	52.9±5.1	0.72	51.8±6.2	51.7±4.5	50.8±6.9	52.9±5.1	0.69
Energy (kJ/day)	7456.8±3110.9	7076.2±2551.9	7577.6±1749.4	7570.1±2465.8	0.60	6798.1±3441.7	5916.1±2623.3	6824.5±2170.6	6982.2±2470.6	0.50
Dietary Calcium (mg/d)	494.0±308.4	380.3±241.5	523.0±271.5	444.1±171.4	0.28	527.8±249.2	380.3±220.4	533.3±245.46	444.1±156.5	0.36
Dietary vitamin D (µg/d)	0.5±0.6	0.3±1.0	0.5±0.7	0.4±0.6	0.99	0.6±0.6	0.3±0.9	0.9±1.0	0.4±0.6	0.45
Serum vitamin D3 (nmol/l)	25.5±9.5	24.5±8.2	26.2±12.3	25.9±7.7	0.47**	25.9±8.4	24.5±7.5	26.3±11.0	26.6±7.2	0.72
Physical activity (MET.h/day)	29.1±5.9	28.9±4.1	27.5±8.2	26.7±3.9	0.04	31.4±8.2	33.3±9.3	27.3±7.6	26.7±3.5	0.005

ITT, intention-to-treat; PP, per-protocol;BMI, body mass index; MET, metabolic equivalent task units; WHR, waist-to-hip ratio.
** Obtained by One way ANOVA. ** obtained by Kruskal–Wallis H test. All values are mean±Standard deviation*

Discussion

The present studyshowed that a 2093 kJ (500 kcal) energy-restricted dietfor 8 weeks significantly reducedweight, BMI, WC, and body fat massin pre-menopausaloverweight or obese women.However, there was no beneficial effect of calcium, vitamin D, or Ca+VitD supplements on weight, BMI, WC, body fat mass, and fat-free mass in the participants.

This study was specifically designed to compare the individual effects of calcium, vitamin D, andCa+VitD supplements on body composition and weight reduction. Results of previousstudies on the effects of both calcium and vitamin D on body weight and body composition have been inconsistent (11, 27-30). Consistent with our study results, multiple clinical trials failed to show a beneficial effect of vitamin D (16, 31-33), calcium (6, 14, 34-38) and Ca+VitD (28, 29) supplementation on body weight and fat mass (31, 33) in overweight and/or obese participants.

Some studies have shown that supplemental(34) or dairy calcium (34, 35, 39, 40) can induce weight loss.Zemel et al. (35) studied the effects of dietary calcium intake in a 24-week long calorie restricted intervention RCT on weight loss in obese adults and found that the control group lost 6.4±2.5 percent (%) of their initial body weight, which was 26% (8.6±1.1% of body weight) lower compared to the high-calcium diet group and 70% (10.9±1.6% of body weight) lower in the high-dairy diet group (p<0.001) (34).

Table 2. Physical activity and key characteristics of diets achieved by study completers (n=81)

Variables	Placebo (n=19)		Calcium (n=21)		Vitamin D (n=20)		Calcium + Vitamin D (n=21)		P*
	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8	
Energy (kJ/day)	7456.8±3110.9	5139.1±1720.0	7076.2±2551.9	5457.8± 1574.9	7577.6±1749.4	4887.1±1573.3	7570.1±2465.8	5923.8±2099.9	0.34
Carbohydrate (% of total energy intake)	52.5±13.3	53.6±3.9	56.3±6.0	55.5± 6.4	55.6±6.0	54.9± 4.1	56.9±10.0	56.4± 6.4	0.73
Protein (% of total energy intake)	12.9±3.9	15.0±2.1	13.4±1.5	16.2±5.2	13.4±2.1	15.5± 1.9	13.52±2.9	14.9± 2.8	0.70
Fat (% of total energy intake)	34.3±15.9	31.2± 2.8	30.1±6.4	28.2±3.9	31.0±4.8	29.7±4.3	29.5±7.6	28.5±5.0	0.69
Fiber (g/day)	11.1±4.3	9.4 ±3.6	9.6±4.2	9.9±4.0	12.4±5.2	9.8±4.3	11.4±5.9	11.4±5.4	0.32
Calcium (mg/day)	495.8±299.8	477.3±158.9	380.3± 241.5	457.8±226.9	523.0±271.5	472.5±238.5	444.1±171.4	518.5±209.6	0.40
Dietary vitamin D (µg/d)	0.5±0.6	1.0±0.7	0.3±1.0	0.4±0.5	0.5±0.7	0.4±0.6	0.4±0.6	0.9±0.9	0.09
Physical activity (MET.h/day)	31.4±9.5	27.8±4.7	33.3±10.2	28.09±3.12	27.3±8.5	25.6±5.8	26.7±3.9	26.1±4.5	0.88

**Obtained by One way ANOVA,*
All values are mean±Standard deviation

Table 3. Comparison of serum vitamin D changes and consumed tablets among the 4 groups by study completers (n=81)

Variable	Placebo (n=19)	Calcium (n=21)	Vitamin D (n=20)	Vitamin D+ calcium (n=21)	P*
Serum vitamin D3 (week 0 – week 8) nmol/l	-0.6±4.5	3.1±15.2	47.2±30.4	35.9±23.0	<0.001
Percent of taken tablets	91.92±19.81	95.62±4.48	93.40±9.54	91.53±12.69	0.73

** Obtained by One way ANOVA, significant different increase in serum vitamin D levels between four groups at p<0.001. according to post hoc test results, increase in serum vitamin D levels in both vitamin D and Ca + VitD groups was statistically different from 2 other groups.*
All values are mean±Standard deviation

Compared to supplemental calcium, dietary calcium intake particularly through dairy consumption has been shown to be more effective in reducing body weight (15, 34), which might be due to the other components of dairy products, including conjugated linoleic acid (CLA) or bioactive peptides found in whey proteins (7, 34). Furthermore, some studies have reported that calcium supplementation without energy restriction may only have small beneficial effects on weight loss (30).

Consistent with our findings, Shalileh et al. (42) examined the effect of 1000 mg calcium/day supplement with a 2093 kJ (500 kcal) energy-restricted diet and found no effects on weight and body composition in obese adults over 2-weeks (41). Other studies, however, suggested that substantial increase in calcium intake may result in more than 0.5kg of weight loss per year (30, 36,42). In a two-year trial examining 340 overweight or obese adults on calcium carbonate (1500 mg Ca/day) and a placebo supplement found no sig-

Table 4. Comparison of changes in anthropometric indices and body composition among the 4 groups after the intervention

PP population (n=81)	Placebo (n=19)			Calcium (n=21)			Vitamin D (n=20)			Vitamin D+ calcium (n=21)				
	week 0	Week 8	P **	week 0	Week 8	P **	week 0	Week 8	P **	week 0	Week 8	P **	P*	P***
Body weight (kg)	79.8±14.1	77.3±14.4	<0.001	77.6±9.0	75.8±8.8	<0.001	76.7±8.6	73.7±8.6	<0.001	82.5±10.4	80.0±10.6	<0.001	0.30	0.26
BMI (kg/m2)	31.3±4.1	30.3±4.3	<0.001	30.0±2.6	29.3±2.6	<0.001	29.7±3.1	28.6±3.2	<0.001	32.4±3.4	31.4±3.3	<0.001	0.05	0.40
Waist circumference (cm)	90.1±9.3	86.7±9.7	<0.001	87.5±5.6	84.7±5.2	<0.001	87.3±7.8	83.4±6.9	<0.001	90.9±8.3	88.1±8.9	<0.001	0.24	0.32
Body fat mass (kg)	27.5±8.2	25.6±8.9	0.001	26.4±6.3	24.3±5.4	0.001	24.0±5.2	21.7±4.8	0.003	29.5±6.6	27.7±6.6	<0.001	0.03	0.15
Body Fat free mass (kg)	50.8±6.2	51.3±6.4	0.11	51.7±4.5	51.4±4.6	0.23	50.8±6.9	51.3±5.4	0.69	52.9±5.1	52.3±5.5	0.03	0.88	0.68
ITT population (n=100)	**Placebo (n=25)**		P **	**Calcium (n=25)**		P **	**Vitamin D (n=25)**		P **	**Vitamin D+ calcium (n=25)**		P **	**P***	**P*****
Body weight (kg)	81.3±12.8	79.4±13.3	<0.001	77.5±8.4	76.0±8.3	<0.001	76.8±8.1	74.4±8.3	<0.001	82.6±9.8	80.5±10.0	<0.001	0.12	0.50
BMI (kg/m2)	31.8±3.9	31.0±4.1	<0.001	30.0±2.5	29.4±2.5	<0.001	29.4±2.9	28.5 2.9	<0.001	32.4±3.1	31.5±3.14	<0.001	0.004	0.80
Waist circumference (cm)	90.7±8.3	88.1±8.9	<0.001	87.3±5.3	85.0±4.9	<0.001	87.0±7.0	83.9±6.4	<0.001	90.6±7.8	88.3±8.3	<0.001	0.08	0.16
Body fat mass	24.3±5.0	22.5±4.8	0.001	26.1±6.0	24.4±5.1	0.001	28.2±7.5	26.8±8.2	0.003	26.9±6.0	28.0±6.1	<0.001	0.01	0.43
Body Fat free mass (kg)	51.8±6.2	51.3±6.4	0.11	51.7±4.5	51.4±4.6	0.23	50.8±6.9	51.3±5.4	0.69	52.9±5.1	52.3±5.5	0.03	0.92	0.81

*ITT, intention-to-treat; PP, per-protocol; BMI, body mass index. Obtained by ANOVA, ** Obtained by paired sample t-test, ***Obtained by ANCOVA adjusted for baseline values, BMI, body fat mass (kg) and physical activity. All values are mean±Standard*

nificant differences in weight loss between two groups (6). Caan et al. (31) also found that daily supplementation of 1000 mg calcium plus 400 IU vitamin D_3 for 7 years had little influence on weight gain preventionin postmenopausal women (30). So that the short period of present study in compare to these studies seems to be the cause of inconsistency.

The different dosage of calcium and study duration may also explain the inconsistent results. Oneproposed reason is the threshold hypothesis, which states that a calcium supplemented weight-loss program may be more effective when the initial calcium intake of participants is under the cut-off value of 500-600 mg/d (43). In a 12-week Ca+VitD supplemented weight-reducing program, 53 healthy overweight or obese college students that had very low initial calcium intake showeda significantly greater fat mass loss with VitD supplementation compared to the control group (29). In our study, the mean intake of calcium at baseline was under the recommended dietary allowances (RDA) too (494.06±308.42 mg/day, 380.33±241.50 mg/day, 523.00±271.57 mg/day and 444.12±171.44 mg/day for the control, calcium, vitamin D and Ca+D group, respectively). The difference in age group of the participants compared to the current study may explain the inconsistent results even (29) because age has been suggested to influence the calcium bioavailability and absorption within the gut lumen (44-46).

Strengths of our study include using a triple blind, randomized, placebo-control trial design, evaluating the participants compliance through measuring serum vitamin D concentrations (before and after the intervention), tablet counting (at 2-week follow up visits), and taking 24 hdietary recall, and physical activity records (at baseline and at the end of each month). Our study had some limitations including its short duration, relatively small sample size, and theuse of BIA rather than dual energy X-ray absorptiometry (DXA) to measure the body composition due to financial limitations.

In conclusion, the present study showed no beneficial effects of calcium, vitamin D, or Ca+VitD supplementation on body composition and weight loss in overweight or obese women with low serum vitamin D and calcium intake.

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