Original Article

# Independent and Combined Effects of Calcium and Vitamin D Supplementation on Blood Lipids in Overweight or Obese Premenopausal Women: A Triple-Blind Randomized Controlled Clinical Trial


## Abstract

**Background:** Dyslipidemia is often associated with obesity and contributes to the increased risk of atherosclerosis, heart disease, and stroke. This study was designed to evaluate the independent or combined effect of calcium and vitamin D (Ca + Vit D) supplementation on blood lipid profile in overweight or obese premenopausal women. **Methods:** This study is a triple-blind, randomized, parallel, placebo-controlled trial. About 100 overweight or obese (body mass index (BMI) of 25–40 kg/m$^2$) premenopausal (aged 30–50 years) women, recruited from Shiraz University of Medical Sciences (SUMS) clinics, were allocated into 4 groups: (1) calcium (Ca) supplementation (2 tablets per day; each containing 500 mg calcium carbonate), (2) vitamin D (Vit D) supplementation (2 tablets per day; each containing 200 IU vitamin D3), (3) Ca + Vit D supplementation (2 tablets per day; each containing 500 mg calcium carbonate plus 200 IU vitamin D3), (4) placebo supplementation (2 tablets per day, containing micro-cellulose). All participants received a 500 kcal energy-restricted diet. Blood lipids, serum vitamin D, and anthropometric indices were measured at baseline and after 8 weeks. Physical activity and 3-day dietary records were taken at baseline and every 4 weeks during the intervention. **Results:** At 8 weeks, triglyceride levels were significantly decreased in the Ca group ($P$ = 0.002). Low-density lipoprotein (LDL) levels were decreased in the Ca + Vit D group ($P$ = 0.04) and high-density lipoprotein (HDL) levels decreased in both the Ca and Ca + Vit D groups ($P$ = 0.006, $P$ = 0.004, respectively). The results of one-way ANOVA indicated that changes in the serum lipid profile levels were not significantly different among the four groups ($P$ = 0.90, $P$ = 0.86, $P$ = 0.61, $P$ = 0.27, and $P$ = 0.19, respectively for TG, TC, LDL, HDL, and LDL/HDL). The results were not significant even after adjusting for potential covariates. **Conclusions:** Although the results were not significantly different among the four treated groups at 8 weeks, within-group changes like the reduction in triglyceride and LDL levels, respectively in the Ca group and Ca + Vit D group, and HDL levels in both the Ca and Ca + Vit D groups were significant. These changes may have potentially significant public health implications.


**Keywords:** *Calcium, lipids, obese, overweight, vitamin D, women*


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## Introduction

Obesity-related dyslipidemia is characterized by decreased high-density lipoprotein (HDL), elevated triglycerides (TG), very low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) particles. Dyslipidemia contributes to the increased risk of atherosclerosis, heart disease, and stroke.[1,2] Cardiovascular disease (CVD) in its various forms is a major cause of death worldwide, ranking first in both developing and developed nations.[3] It is estimated that by 2030, nearly 23.6 million people will die from CVD.[4] Dyslipidemia is often associated with obesity while blood lipid disorders are frequently the result of improper diet and lifestyle.[5] Given the role of dyslipidemia as an independent risk factor for CVD, extraordinary efforts have been devoted to understand the role of dietary interventions on lipid profile especially in nutritional studies.

Recently, there are mixed results related to the effect of calcium (Ca) supplementation on blood lipid profile. Some of the studies reported favorable results;[6,7] however, others found weaker[8] effects of Ca supplementation on blood lipids. It has been suggested that Ca supplementation

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may decrease fatty acid absorption through the formation of insoluble calcium–fatty acid complexes resulting in decreased levels of total and LDL cholesterol.[9,10] In contrast, vitamin D (Vit D) plays an important role in increasing the intestinal absorption of Ca thereby regulating Ca homeostasis.[11]

Furthermore, it has been shown that low serum 25-hydroxyvitamin D [25(OH)D], a measure of Vit D status, is associated with an increased risk of CVD.[12,13] Moreover, hypovitaminosis D has been linked to increased total serum cholesterol concentration.[14] It has also been reported that Vit D improves insulin sensitivity and reduces parathyroid hormone, which may, therefore, play a role in improving blood lipid disorders.[4,15]

According to a study conducted in Iran, the prevalence of obesity in women is more than twice than men.[16] In addition, Vit D deficiency is highly prevalent in Iranian people,[17] especially in women[18] and studies have shown that the risk of Vit D deficiency is higher in the obese people.[19] In other words, there is an association between alterations in the Vit D endocrine system and increase in the adiposity or fatty mass storage of Vit D.[20]

Therefore, due to previous contradictory findings of calcium and vitamin D (Ca + Vit D) supplementation trials on lipid profile, and due to the high prevalence of Vit D deficiency in Iranian overweight or obese women,[21,22] this study was designed to evaluate the impact of the independent and combined effects of Ca + Vit D supplementation on lipid profile in overweight or obese premenopausal women.

## Methods

### Study population

A minimum sample size of 21 persons per group was calculated using the Power SSC software and according to the mean difference between independent groups equation (statistical power = 80%, type I error = 5%, mean difference = 0.35, SD = 0.4). Nearly 25 persons in each group were recruited to compensate for a potential attrition rate of approximately 20%, during 8 weeks at followup.

Premenopausal women (aged 30–50 years) participated in this randomized, parallel, triple-blind, placebo-controlled trial. Subjects were chosen from health centers affiliated with Shiraz University of Medical Sciences. Inclusion criteria included females; premenopausal with a body mass index (BMI) of 25–40 kg/m$^2$; not having any type of cancer or severe disease, and generally healthy (any mental, hepatic, renal, gastrointestinal, cardiovascular, neurologic, rheumatologic, hematologic, skeletal, and eating disorders) on the basis of routine clinical and laboratory checkups; not taking any medication, antioxidant, herbal, or mineral/vitamin supplements which could affect body weight, Ca, and/or Vit D status during the last 12 weeks; not reporting any history of adverse reaction to the study supplements; consuming less than 3 servings of dairy products per day; not being pregnant or lactating; not being a regular smoker or consuming alcohol; not being a participant in other clinical trials over the last 6 months; constant body weight (body weight changes less than 3 kg in last 3 months). Participants who consumed less than 80% of the supplements were excluded from the final analysis.

### Experimental design

After a 2 week run-in period, using a balanced block randomization method, in a 1:1:1:1 manner, the participants were allocated to one of the 4 groups as follows: 1) Ca supplementation (2 tablets per day; each containing 500 mg calcium carbonate), 2) Vit D supplementation (2 tablets per day; each containing 200 IU vitamin D3), 3) Ca + Vit D supplementation (2 tablets per day; each containing 500 mg calcium carbonate plus 200 IU vitamin D3) and 4) placebo (2 tablets per day; each containing micro-cellulose). The allocation was concealed by sequentially numbered, opaque, sealed envelope (SNOSE) technique.

Supplements were manufactured by the Iran Drau Company, Tehran, Iran. All groups were placed on a 500 kcal energy-restricted diet. Based on the estimated energy requirement (EER) formula,[23] a balanced diet (55% carbohydrate, 17% protein, and 28% fat) was designed for each participant by a trained dietitian who was blind to study allocation. All of the subjects received a print out of their standard diet and diet recommendations and food quantities were described using household amounts (glass, slice, plates, cups, spoons, etc.). In addition, participants in all four groups were advised not to change their physical activity level during the study.

An independent statistician at Shiraz University of Medical Sciences generated the randomization sequence and the study dietitian at Emam Reza clinic, using this sequence, allocated the subjects into four groups and prescribed the dietary regimens. Participant enrollment and eligibility assessment were performed at Emam Reza clinic by the study clinician who was blinded to the treatment allocation.

All of the tablets were identical in shape and color and were placed in similar and opaque pill bottles. Subjects received their supplements every 2 weeks and monitored for compliance for dietary recommendations and supplement intake. This study was planned as a triple-blind clinical trial in a way that the participants, researchers, and the statistician were blinded to the allocation of 4 groups up to the end of data analysis. The study protocol was registered to the Iranian Registry of Clinical Trial (www.irct.ir) with the ID of IRCT2014021116555N1.

### Anthropometric measurements

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 (personal scale, china). Afterwards, BMI was calculated as weight (kg)/height ($m^2$).

### Dietary intake assessment

Dietary intakes were assessed at baseline, week 4 and week 8 using the 24-h food record method (2 weekdays and 1 weekend). Dietary intake analyses were done by Nutritionist 4 software (First Databank Inc., Hearst Corp., and San Bruno, CA).

### Physical activity assessment

To assess the physical activity, the average of MET.h/day was calculated by multiplying the time of each physical activity by its relative metabolic equivalent task (MET) using the International Physical Activity Questionnaires (IPAQ).[24]

### Biochemical assays

At baseline and after 8 weeks, a 5cc venous blood sample was obtained from each participant between 7:00 to 9:00 AM after an overnight fast. The whole blood was centrifuged and serum was stored in -70°C until the further analyses. Serum 25(OH) D was measured by enzyme immunoassay (EIA) (Immunodiagnostic Systems Ltd, Boldon, UK) using IDS 25-hydroxyvitamin D EIA kit. Plasma lipids (Cholesterol, TG, HDL and LDL) were measured by using colorimetry methods.

### Ethical considerations

The study aims and methods were described to each participant and the signed informed consent forms were obtained from each participant prior to participation. The study protocol was approved by the Ethical Committee in Research of Shiraz University of Medical Sciences (Code number: 92-6836).

### Statistical analysis

The sample size was calculated by using Power SSC software and based on the mean difference between independent groups equation. taking into account a dropout rate of approximately 20%, 100 participants were recruited.

Data were analyzed using IBM SPSS statistics version 19 (IBM SPSS Statistics, Armonk, USA). Normal distribution of variables was tested by the Kolmogorov-Smirnov test. One-way ANOVA was used to compare baseline values of dietary intakes and serum vitamin D3 concentration, also to compare the mean of energy and nutrients intakes of subjects during the intervention among the four groups. The ANCOVA models and intention to treat analysis were used respectively to adjust the potential covariates (baseline values, mean differences of blood lipids, and Vit D among the groups) and to take into account the missing data. We used paired sample *t*-test to estimate the effect of the intervention in each group. $P < 0.05$ was considered statistically significant.

## Results

Of the total 180 women volunteered for this study, 100 met the general eligibility criteria. Eighty-one participants completed the study (Ca supplemented group: 21 persons; Vit D supplemented group: 20 persons; Ca + Vit D supplemented group: 21 persons; placebo group: 19 persons). Protocol violation, participant decision, and adverse events were the main reasons for attrition [Figure 1].

Table 1 illustrates no significant differences in the age, serum vitamin D3, lipid profile and dietary intakes of participants among the four groups at baseline.

Table 2 compares energy, macronutrients, dietary calcium, and fiber intakes as well as a physical activity among

**Table 1: Comparison of age, serum vitamin D3, lipid profile, and dietary intakes of the participants at baseline**

| Variables | Placebo group (*n*=19) | Calcium group (*n*=21) | Vitamin D group (*n*=20) | Calcium-vitamin D group (*n*=21) | *P** |
|---|---|---|---|---|---|
| Age (years) | 38.26±5.92 | 39.09±7.61 | 39.30±5.92 | 39.19±6.12 | 0.95 |
| Serum vitamin D3 (nm/L) | 25.58±9.52 | 24.56±8.28 | 26.27±12.39 | 30.52±18.90 | 0.38 |
| TG (mg/dL) | 142.51±60.24 | 128.37±50.21 | 134.50±83.40 | 148.20±20±100.56 | 0.70 |
| TC (mg/dL) | 172.97±31.06 | 178.00±37.07 | 166.16±39.00 | 179.83±48.23 | 0.69 |
| HDL (mg/dL) | 36.25±9.84 | 39.91±6.93 | 36.72±6.48 | 38.93±8.77 | 0.42 |
| LDL (mg/dL) | 99.02±19.53 | 105.35±25.27 | 101.98±29.87 | 105.35±25.27 | 0.67 |
| LDL/HDL | 2.89±0.88 | 2.65±0.51 | 2.64±0.65 | 2.68±0.77 | 0.65 |
| Energy (kcal/day) | 1781.38±743.18 | 1690.4±609.63 | 1810.23±417.93 | 1808.44±589.08 | 0.93 |
| Carbohydrate (% of energy) | 25.52±13.71 | 56.38±6.02 | 55.60±6.09 | 56.95±10.08 | 0.47 |
| Fat (%of energy) | 34.52±16.36 | 30.19±6.41 | 31±4.83 | 29.52±7.62 | 0.39 |
| Protein (% of energy) | 14.63±2.12 | 16.28±5.25 | 15.10±2.64 | 14.92±2.71 | 0.45 |
| Calcium (mg/day) | 494.06±308.42 | 380.33±241.50 | 523.00±271.57 | 444.12±171.44 | 0.29 |
| Fiber (g/day) | 11.10±4.38 | 9.61±4.26 | 12.49±5.24 | 11.40±5.93 | 0.33 |

All values are mean±standard deviation.*One way ANOVA. TG: Triglycerides, TC: Total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein

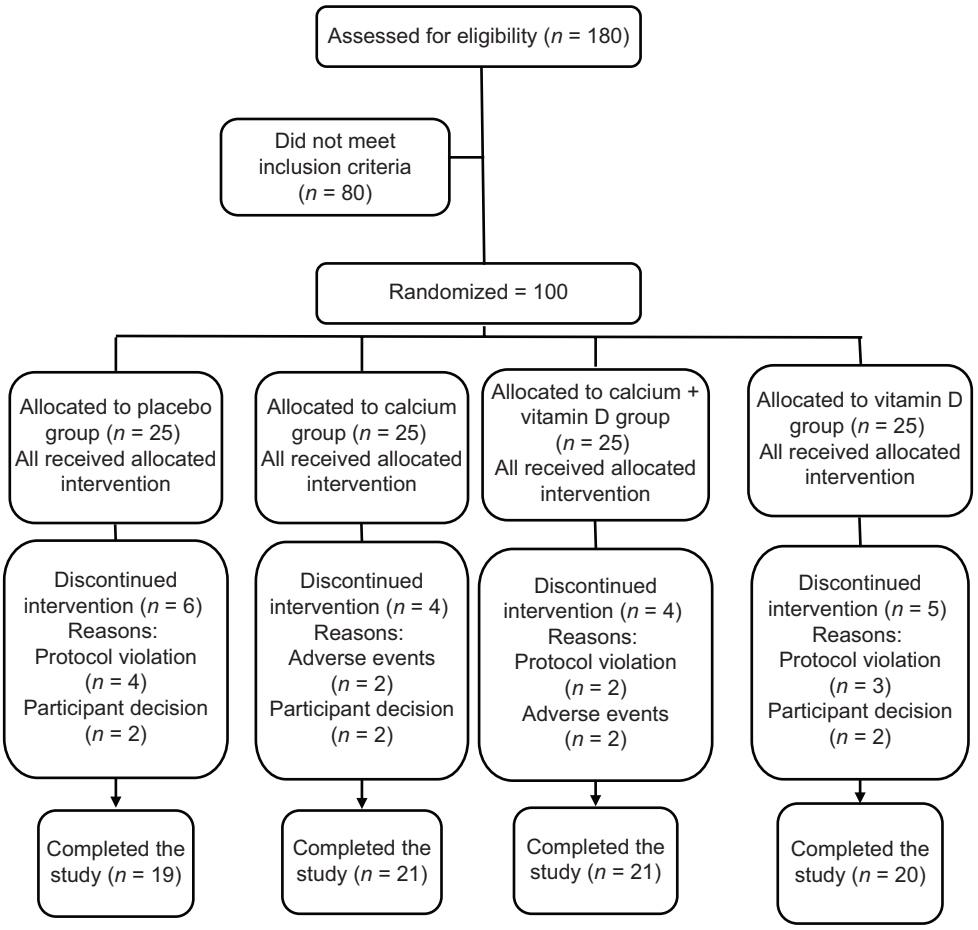


**Figure 1: Participant's flow diagram throughout the study**

**Table 2: Comparison of dietary intakes and physical activity among the groups during the study**

| Variables | Placebo group (*n*=19) | Calcium group (*n*=21) | Vitamin D group (*n*=20) | Calcium-vitamin D group (*n*=21) | *P** |
|---|---|---|---|---|---|
| Energy (kcal/day) | 1227.71±410.90 | 1303.84±376.24 | 1167.50±375.85 | 1415.15±501.76 | 0.35 |
| Carbohydrate (% of energy) | 53.63±3.98 | 55.50±6.49 | 54.95±4.12 | 56.45±6.45 | 0.44 |
| Fat (% of energy) | 31.27±2.89 | 28.21±3.95 | 29.77±4.33 | 28.57±5.06 | 0.10 |
| Protein (% of energy) | 15.08±2.15 | 16.28±5.25 | 15.52±1.91 | 14.97±2.81 | 0.58 |
| Calcium (mg/day) | 477.35±158.97 | 457.84±226.97 | 472.52±238.55 | 518.54±209.62 | 0.81 |
| Fiber (g/day) | 9.46±3.69 | 9.92±4.05 | 9.89±4.33 | 11.49±5.41 | 0.49 |
| Physical activity (MET.h/day) | 27.84±4.77 | 28.09±3.12 | 25.60±5.80 | 26.17±4.52 | 0.24 |

All values are mean±standard deviation.*One way ANOVA

the 4 groups during the intervention. There were no significant differences in mean total energy, macronutrient, fiber, and Ca intake among the four groups during the study. The distribution of macronutrient was similar to our recommendations (carbohydrates = 55%, fat = 28%, protein = 17%,). Physical activity levels were not significantly different within and between groups.

As reported in Table 3, at the end of the study, TG level decreased significantly in Ca treated group ($P$ = 0.002); however, other groups did not have any significant changes in TG level ($P$ = 0.46, $P$ = 0.98, $P$ = 0.38 for placebo, Vit D, and Ca + Vit D groups, respectively). Moreover, significant reductions were found in serum total cholesterol in both the Ca and placebo groups ($P$ = 0.02, $P$ = 0.004, respectively). In addition, LDL levels were significantly decreased in the Ca + Vit D group ($P$ = 0.04), and HDL levels decreased in both the Ca and Ca + Vit D groups ($P$ = 0.006, $P$ = 0.004, respectively) after 8 weeks of intervention.

The results of one-way ANOVA indicated that changes in the serum lipid profile levels were not significantly different

**Table 3: Comparison of lipid profile changes among the four groups after 8 weeks of intervention**

| Variables | Placebo group (19) | | | Calcium group (21) | | | Vitamin D group (20) | | | Calcium-vitamin D group (21) | | | *P** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | Week 8 | *P*** | Baseline | Week 8 | *P*** | Baseline | Week 8 | *P*** | Baseline | Week 8 | *P*** | |
| TG (mg/dL) | 143.89±62.73 | 144.37±75.51 | 0.46 | 111.89±36.23 | 98.54±36.78 | 0.002 | 106.24±50.48 | 106.09±47.55 | 0.98 | 120.64±36.80 | 119.19±50.39 | 0.38 | 0.90 |
| TC (mg/dL) | 174.38±31.33 | 162.77±30.34 | 0.004 | 176.54±37.33 | 164.00±32.45 | 0.02 | 170.13±35.68 | 163.27±29.93 | 0.24 | 169.07±35.36 | 156.72±31.68 | 0.07 | 0.86 |
| LDL (mg/dL) | 99.75±19.83 | 95.25±16.82 | 0.12 | 104.50±25.61 | 97.25±22.13 | 0.06 | 98.74±21.04 | 97.08±19.89 | 0.69 | 98.52±25.98 | 90.83±22.97 | 0.04 | 0.61 |
| HDL (mg/dL) | 36.25±9.84 | 33.65±10.13 | 0.002 | 39.91±6.93 | 37.08±6.25 | 0.006 | 36.72±6.48 | 35.14±5.10 | 0.23 | 38.93±8.77 | 34.37±7.85 | 0.004 | 0.27 |
| LDL/HDL | 2.89±0.88 | 2.99±0.85 | 0.56 | 2.65±0.51 | 2.58±0.59 | 0.44 | 2.64±0.65 | 2.85±0.60 | 0.10 | 2.68±0.77 | 2.76±0.76 | 0.24 | 0.19 |

TG: Triglycerides, TC: Total Cholesterol, LDL: Low-Density Lipoprotein, HDL: High-Density Lipoprotein. All values are mean±standard deviation. *One-way ANOVA. **paired sample *t*-test

among the four groups. The results were not significant even after adjusting aforementioned potential covariates by ANCOVA models and considering the missing data by intention to treat analysis. Therefore, the results of ANCOVA and intention to treat analysis were not provided in the tables.

As shown in Table 4, there was an increase in serum vitamin D levels in both Vit D and Ca + Vit D groups which were statistically different from 2 other groups ($P < 0.001$). It shows the high adherence of participants to administered supplements. In addition, there was also compliance of more than 90% in all groups, resulting from tablet counting.

## Discussion

This was a single-centered, randomized, triple-blind, placebo-controlled, parallel-group trial that evaluated the separate and combined effects of Ca + Vit D supplementation on blood lipid profile in overweight or obese premenopausal women.

Triglyceride levels were significantly decreased in the Ca group ($P = 0.002$). LDL levels were decreased in the Ca + Vit D group ($P = 0.04$), and HDL levels decreased in both the Ca and Ca + Vit D groups ($P = 0.006$, $P = 0.004$, respectively). Although there were several within-group changes, there were no differences among the supplemented groups receiving calcium, Vit D, or Ca + Vit D on blood lipids.

The results of interventional studies with Ca (with or without Vit D) supplementation on lipid profile are contradictory.[4] Zemel *et al*. performed a randomized, placebo-controlled trial on 32 obese adults to evaluate the effects of Ca supplementation on the lipid profile.[25] Their results revealed that Ca supplementation had no significant effects on LDL, HDL, and triglycerides. However, other investigations indicated favorable effects on blood lipids.[26,27] The difference may be the result of prescribing different doses of Ca to participants. On the other hand, Major *et al*. stated that Ca consumption above the dietary reference intakes (RDA)[28] is necessary for individuals with typical inadequate Ca intake to improve lipid profile.

Despite several mechanisms attributed to Ca intake including the formation of insoluble calcium-fatty acid soaps in the gut and reduction of fatty acid absorption,[26,29] the short study duration and inclusion of normal lipemic participants may have contributed to weaker effects in our study as comparison with other surveys.[25,26]

A nonsignificant effect of Vit D supplementation on the blood lipid profile was observed have been reported by others, which are in accordance with our findings[4,30,31] Heikkinen *et al*. suggested that Vit D supplementation may have unfavorable effects on lipids in postmenopausal women including an increase in serum LDL cholesterol;[32] however, other observational studies have revealed that

**Table 4: Comparison of serum vitamin D changes and consumed tablets among the 4 groups**

| Variable | Placebo (*n*=19) | Calcium (*n*=21) | Vitamin D (*n*=20) | Vitamin D+ calcium (*n*=21) | *P** |
|---|---|---|---|---|---|
| Serum vitamin D3 (week 8-week 0) nmol/L | - 0.45±4.51 | 3.13±15.24 | 184.37±157.55 | 111.14±98.12 | <0.001 |
| Percent of tablets taken | 91.92±19.81 | 95.62±4.48 | 93.40±9.54 | 91.53±12.69 | 0.73 |

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

high serum 25(OH) D levels are linked with a favorable lipid profile.[4,33] In addition, it has been reported by some investigators that there are significant positive relations between serum 25(OH) D and total cholesterol, HDL and LDL levels as well as negative associations between serum 25(OH) D and both LDL/HDL and TG.[34,35]

Presumably, improved insulin sensitivity and parathyroid hormone reduction after Vit D intake may have a role in improving blood lipids by decreasing the effect of insulin on the biosynthesis of cholesterol via increased b-hydroxy-b-methylglutaryl coenzyme A reductase (HMG- CoA reductase) activity.[4,15]

The strengths of our study include using a triple-blind, randomized, placebo-control trial design which decrease the potential biases (e.g., selection, detection, and attrition bias) in study implementation and data analysis, evaluating the participants compliance through measurement of serum vitamin D concentrations (before and after the intervention) and tablet counting (at 2-week follow-up visits) also taking 3 day dietary and physical activity records (at baseline and the end of each month) to assess dietary intake and physical activity as potential confounders.

A critical point to consider in conducting a randomized control trial is the trade-off of most external validity (that may be limited with highly selective eligibility criteria) against high internal validity (that is achieved by strict eligibility criteria).[36] In addition, internal validity, itself, is a critical prerequisite for external validity.[37] Hence, we have recruited our study participants with low exclusion criteria as much as possible and from non-referral centers and general practices but our short study duration may have a negative effect on external validity. In addition, the effect of Vit D may depend on latitude,[38] ethnicity, and seasonal effects[39] that may limit the external validity.

Our study limitations include short duration and relatively small sample size due to financial limitations.

This study results may be applicable to premenopausal, healthy, moderately obese white women. However, approving the use of Ca + Vit D supplementation on the improvement of lipid profile may need a longer period of intervention.

## Conclusions

Although the changes in serum lipid profile levels were not significantly different among the four treated groups after 8 weeks of intervention, the present study showed that a 500 kcal energy-restricted diet for 8 weeks reduces serum lipid levels in all groups which many of these alterations were statistically significant especially in the Ca and Ca + Vit D groups. These results may have potentially important public health implications and should be addressed by future clinical trials with longer duration especially among obese and hyperlipidemic individuals.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There are no conflicts of interest.

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