J Nephropharmacol. 2017; 6(2): 74–78.

http://www.jnephropharmacology.com DOI: 10.15171/npj.2017.08

Journal of Nephropharmacology

Calcium-vitamin D supplementation; does it affect lipid profile of menopaused women with normal renal function?

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ARTICLEINFO

Article Type:
Original

Article History:
Received: 5 February 2017
Accepted: 10 April 2017
ePublished: 25 April 2017

Keywords:
Calcium-vitamin D supplementation
Lipid profile
Menopause

ABSTRACT

Introduction: The association between dyslipidemia and cardiovascular disease in women has been proposed and many studies investigated calcium-vitamin D (CaD) supplementation and their effect on lipid concentrations.
Objectives: This randomized single-blind study aimed to evaluate the effect of CaD supplementation on serum lipid levels of menopaused women with normal renal function.
Patients and Methods: In this study, 100 women 50-75 years old with cholesterol of 200-239 mg/dL, triglyceride (TG) of 150-199 mg/dL, low-density lipoprotein (LDL-C) of 100-159 mg/dL and vitamin D level less than 30 ng/mL and normal glomerular filtration rate (GFR) (>90 mL/min/1.73 m^2) were investigated. First, body mass index (BMI) and waist circumference were calculated. The patients were given CaD supplement tablets made by Tehran Shimi Pharmaceutical Manufacturing Co. twice-daily (containing 500 mg calcium and 200 IU vitamin D) before breakfast and before lunch for 6 months. Then, blood lipid levels were collected after 3 and 6 months and were compared with initial data.
Results: One hundred women with average age of 62.76 ± 7.02 years and BMI of 27.98 ± 3.44 kg/m^2 were enrolled. FBS, cholesterol, HDL-C and LDL-C of the patients significantly increased (P < 00.001) but the mean TG and LDL/HDL ratio significantly decreased (P < 00.001). Also, the difference in LDL/HDL ratio was not significant after 3 months, but it was significant after 6 months (P = 00.155 and P < 00.001, respectively).
Conclusion: Supplemental CaD significantly increased HDL-C and decreased TG and LDL/HDL ratio. Thus, it is recommended in menopaused women.

Implication for health policy/practice/research/medical education:
In a study on 100 women aged 50-75 years, CaD supplement tablets was given twice-daily before breakfast and before lunch for 6 months. Lipid profile and FBS were checked at baseline, three and six months and compared. Average age of the patients was 62.76 ± 7.02 years and BMI of 27.98 ± 3.44 kg/m^2. FBS, cholesterol, HDL-C and LDL-C of the patients significantly increased (P < 0.001) but the mean TG and LDL/HDL ratio significantly decreased (P < 0.001). Also, the difference in LDL/HDL ratio was not significant after 3 months, but it was significant after 6 months (P = 0.155 and P < 0.001, respectively).
Please cite this paper as: Javadian M, Yazdani S, Hajian-Tilaki K, Gharouei R. Calcium-vitamin D supplementation; does it affect lipid profile of menopaused women with normal renal function? J Nephropharmacol. 2017;6(2):74-78. DOI: 10.15171/npj.2017.08.

Introduction

Vitamin D is a fat-soluble vitamin with hormonal activity whose role is well known in the homeostasis of calcium, phosphorus and bone metabolism and regulation of the immune system, cell proliferation and survival (1,2). It has many extra-skeletal functions including a positive impact on the cardiovascular system, and has been proposed for mortality risk evaluation in heart failure patients (3). Benefits of vitamin D in immune mediated diseases such as asthma and urticaria, systemic lupus erythematosus, multiple sclerosis, allergic rhinitis, and even some cancers. Adequate intake of calcium is essential for bone such as lung, prostate and ovarian cancer (4,5).

Cardiovascular diseases are the leading cause of death in the world. Thus, identifying effective factors in prevention of atherosclerosis plays an important role in reducing deaths from cardiovascular diseases (6). Given the fact that vitamin D deficiency has been reported in 50% of

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subjects in North America and 60% of elderly people in other countries and the association between vitamin D level and cardiovascular diseases, the use of vitamin D supplements can prevent deaths caused by cardiovascular diseases (7,8).

Previous studies have suggested an inverse association between vitamin D and calcium levels with cardiovascular diseases (9). For bone health, calcium intake should be adequate. Calcium supplements are usually used for people over 50 years. Bone calcium has an inverse relationship with soft tissue calcium. Calcium content in the fat tissue increases following low-calcium diet. One consequence of increased calcium content in the fat tissue is increased lipogenesis (10). Calcium-vitamin D (CaD) supplementation is affordable and available. Also, given that estrogen has a regulatory effect on serum lipid homeostasis and its deficiency may increase serum lipid concentrations, so lipid levels of postmenopausal women are different from premenopausal women (11). This study aimed to investigate the effect of calcium-vitamin D (CaD) supplementation on serum lipid levels of 50-75 years old women.

Patients and Methods

In this randomized single-blind study without placebo control, the effect of calcium-vitamin D supplementation on serum lipid profile of 100 women 50-75 years old with cholesterol level of 200-239 mg/dL, triglyceride (TG) of 150-199 mg/dL, LDL-C (low-density lipoprotein) of 100-159 mg/dL and vitamin D level less than 30 ng/mL was investigated. Inclusion criteria were age between 50-75 years and having normal thyroid hormone levels. Exclusion criteria included consuming calcium-vitamin D supplementation, atorvastatin or drugs which could influence vitamin D and calcium metabolism, underlying disease such as hyperlipidemia, diabetes, heart disease, thyroid diseases and various metabolic bone diseases.

First, the patient's body mass index (BMI) was calculated by dividing weight (kg) by the square of height (cm). After 12 hours of fasting, 4 cc blood sample was obtained and fasting blood sugar (FBS), blood lipid levels including total cholesterol, TG, high-density lipoprotein (HDL-C), and LDL-C were measured. FBS was measured by glucose oxidase method and lipid analysis was done using the enzymatic method on a fully automated analyzer. Serum 25-hydroxy vitamin D level was determined using enzyme immunoassay kit. Renal function was assessed through glomerular filtration rate (GFR) and GFR >90 mL/min/1.73 m^2 was recognized as normal renal function (12). Total serum cholesterol was calculated using the Friedewald equation (13) as following:

LDL = Total cholesterol-TG/5-(HDL).

The patients received CaD supplement tablets (containing 500 mg calcium and 200 IU vitamin D) made by Tehran Shimi Pharmaceutical Manufacturing Co. twice-daily before breakfast and lunch for 6 months. Then, blood lipid levels were measured after 3 and 4 months of supplementation therapy. The patients' weight and height were measured at the end of 6 months and were compared with initial data.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. A written informed consent was obtained from all patients and they were informed that participation in the study was anonymous and voluntary and the results would be confidential. Ethics Committee of Babol University of Medical Sciences approved this study (Ethical code: MUBABOL.REC.1395.183) and it was registered in Iranian Registry of Clinical Trials with IRCT ID: IRCT2017020532406N1 (http://en.search.irct.ir/view/35844).

Statistical analysis

The collected data was analyzed by SPSS version 18.0 for Windows (SPSS Inc, Chicago, Illinois, USA). Descriptive statistical techniques were used to determine the prevalence and the means. Pearson correlation test was used to examine the correlation between the variables. To study the changes of serum lipids, analysis of variance (ANOVA) with repeated measures was performed. A *P* value less than 0.05 was considered as statistically significant.

Results

One hundred women (50-75 years old) with average age of 62.76 ± 7.02 years participated in this study. Of them, 59% aged ≤65 years and 41% were older than 65 years. Thirty-three percent of them were illiterate, while 45% were less than diploma, 12% diploma and 10% were educated higher than diploma. Ninety-two percent were housewife and only 8% were employed.

Forty-two percent of the patients lived in urban and 58% in rural area. The mean body mass index (BMI) of the patients was 27.98 ± 3.44 kg/m^2 and was normal in 32%

Table 1. Characteristics of the patients

Variable	No. (%)
Age (years)	
≤ 65	59 (59)
> 65	41 (41)
Education	
Illiterate	33 (33)
Less than diploma	45 (45)
Diploma	12 (12)
Higher than diploma	10 (10)
Job	
Housewife	92 (92)
Employed	8 (8)
BMI (kg/m^2)	
Normal	32 (32)
Overweight	31 (31)
Obese	37 (37)
Living place	
Urban	42 (42)
Rural	58 (58)

while 31% were overweight and 37% were obese (Table 1). The mean waist circumference was 88.54 ± 9.25 cm and vitamin D level at baseline was 16.73 ± 4.11 ng/dL.

Average serum levels of FBS, cholesterol, HDL-C and LDL-C at various intervals are shown in Table 2. As shown, the mean FBS of the patients significantly increased after 3 and 6 months of CaD supplementation therapy ($P < 0.001$). Also, the mean TG significantly decreased after 3 and 6 months of CaD supplementation therapy ($P < 0.001$). Cholesterol, HDL-C and LDL-C increased significantly after 3 and 6 months of CaD supplementation therapy ($P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively). The mean LDL/HDL ratio decreased significantly at different intervals ($P < 0.001$). Also, the difference in LDL/HDL ratio after 3 months of treatment was not significant, but this difference was significant after 6 months of treatment ($P = 0.155$ and $P < 0.001$, respectively). Patients had a 0.05 ± 0.39 mg/dL significant mean decrease in LDL/HDL ratio after 3 months and 0.15 ± 0.29 mg/dL after 4 months (Table 3).

Discussion

The aim of this study was to evaluate the effect of CaD supplementation on serum lipid levels of postmenopausal women. In this study, the mean serum level of FBS significantly increased after receiving supplemental CaD. Shenoy and colleagues found an inverse association between vitamin D with FBS and total cholesterol and a direct association with HDL-C (14). The study by Moghassemi et al showed that CaD supplementation had no significant difference on FBS of postmenopausal women with and without vitamin D deficiency (15). Vilarrasa et al did not find any association between vitamin D and blood sugar, insulin resistance and lipid profile of fat people (16). There is evidence for the role of vitamin D in maintaining normal glucose homeostasis. By regulating inflammatory and immune processes, vitamin D can reduce insulin resistance and increases insulin secretion (17). In a study using two single oral dose of 100 000 units vitamin D twice a week during 2 weeks, significant changes in blood glucose was not observed (18). There is evidence of the role of vitamin D in maintaining normal glucose homeostasis. Vitamin D can reduce insulin resistance and increase insulin secretion by regulating inflammatory and immune processes (17).

In this study, supplemental CaD significantly decreased the mean serum level of TG but total cholesterol, HDL-C and LDL-C increased significantly. Sai et al found no effects of vitamin D on lipid profile of 489 menopaused women receiving CaD for 3 years (19). In the study of Schnatz et al, higher concentrations of vitamin D were associated with higher HDL-C levels, along with lower LDL-C and TG levels. Women with higher vitamin D concentrations had more favorable lipid profiles, including increased HDL-C, lower LDL-C, and lower TG (20). Chiu and colleagues found no association between vitamin D level with plasma TG and HDL-C in healthy individuals (21). In review study of Jorde et al, in all cross-sectional studies serum vitamin D level was directly related to HDL-C level, improved cholesterol and LDL/HDL ratio. Also, an inverse association was found between serum vitamin D and TG (22). Shahkhalili et al and Denke et al evaluated the effect of food fortification with calcium on blood lipid levels and observed at least one useful effect in one of the components of the lipid profile but Shahkhalili and colleagues found no significant difference in total cholesterol of calcium supplementation group compared to the placebo (23,24). The study of Denke et al is not reliable for some reasons. First, its sample size was very low (13 in total). Second, calcium intake in the intervention group was 2200 mg which is more than 2-fold of DRI. Also, it was performed on hypercholesterolemic patients. It seems that effect of calcium to reduce total cholesterol is higher in this group. But the results of Ditscheid and colleagues is more reliable because their study was crossed and with acceptable calcium dose (1 g) with 31 cases (25). In the study of Karandish et al, calcium supplementation significantly prevented total cholesterol increase

Table 2. Average serum levels of FBS, cholesterol, HDL and LDL at various intervals

Variable	Baseline Mean±SD	3 Months Mean±SD	6 Months Mean±SD	*P* value
FBS (mg/dL)	91.83±6.94	97.85±9.86	98.15±10.26	0.000
TG (mg/dL)	173.73±11.18	164.78±19.75	151.42±20.74	0.000
Cholesterol (mg/dL)	223.26±15.01	228.00±19.25	235.58±23.70	0.000
HDL-C (mg/dL)	45.34±6.99	48.23±7.50	53.53±9.04	0.000
LDL-C (mg/dL)	115.91±8.57	120.32±10.46	125.08±13.05	0.000

Table 3. LDL/HDL ratio at various intervals

Variable	Minimum	Maximum	Mean±SD
Baseline	1.76	3.54	2.62±0.47
Three months	1.61	4.17	2.56±0.52
Six months	1.44	4.83	2.41±0.53
Difference after 3 months	-1.24	0.86	-0.05±0.39
Difference after 6 months	-0.96	1.02	-0.15±0.29

compared with placebo (26). Some studies did not report significant changes in total cholesterol of intervention and control groups of menopause women (6,27,28). Research of Malekzadeh and colleagues showed that different levels of dietary calcium did not significantly affect serum TG and HDL-C concentration of male mice (29). The number of studies with larger sample sizes and controlled studies with smaller sample sizes observing no changes in serum total cholesterol and calcium supplementation is higher and emphasizes that calcium does not have much effect on serum total cholesterol. However, further studies are necessary in order to achieve a definitive conclusion.

In current study, the mean LDL/HDL ratio decreased significantly at different intervals. Also, the difference in LDL/HDL ratio after 3 months of treatment was not significant, but this difference was significant after 6 months. Contrary to us, in the study of Sai et al, LDL/HDL ratio did not change after receiving 20 IU vitamin D/day for 3 years (19). A deficiency of vitamin D can have multiple impacts on the cardiovascular system (30). In the study of Dziedzic et al, women over 70 years showed an inverse correlation of the 25(OH)D level and the stage of coronary atherosclerosis. Vitamin D deficiency affected the levels of total cholesterol, LDL-C and TG (30). Daily supplementation with 400 or 800 IU vitamin D for 12 months did not affect serum total cholesterol, LDL-C, HDL-C, LDL/HDL ratio, or TGs compared to subjects receiving placebo (31). Calcium and vitamin D have metabolic association with each other because vitamin D is essential to maintain intracellular calcium homeostasis (32).

Conclusion

The results of this study can be used in prevention of atherosclerosis and cardiovascular disease patients who are at risk. Supplemental CaD significantly increased HDL-C and decreased TG and LDL/HDL ratio. Vitamin D increases lipoprotein lipase activity in adipose tissue (33) and calcium increases fecal fat excretion (24). Since the increase in TG is known as a risk factor for cardiovascular diseases in women over 50 years, the role of CaD supplement in reducing TG levels should not be underestimated. Also, high level of total cholesterol and LDL-C increases the risk of cardiovascular diseases while increasing HDL-C is protective (34). The reduction of total cholesterol and LDL significantly decreases the cardiovascular risk (30). Hence, supplemental CaD is recommended in menopaused women.

Limitations of the study

Like all studies, we had some limitations. First, we did not have placebo group. Second, our sample size was small. Third, the concomitant use of the vitamin D and calcium, made differentiation between the results and each supplementation hard. Thus, further interventional studies with different doses of vitamin D and calcium to determine the appropriate dose to achieve more accurate results is recommended.

Acknowledgments

The authors would like to thank all women who participated in this study.

Authors' contribution

RG wrote the draft of the manuscript, KH analyzed the data, SY wrote the methodology and approved the draft and MJ finalized the draft.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/Support

This study was part of Obstetrics and Gynecology residential thesis of Dr. Roya Gharouei and funded by Babol University of Medical Sciences and received ethics committee approval (#MUBABOL.REC.1395.183).

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