Original Communication

Vitamin D supplementation has no effect on matrix metalloproteinases-2, -9, and tissue inhibitor matrix metalloproteinase-1 in subjects with metabolic syndrome: A pilot study

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Abstract: The present randomized, double-blind, placebo controlled study aimed to evaluate the effect of vitamin D supplementation on matrix metalloproteinases-2, -9 (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in subjects with metabolic syndrome. Forty-six eligible subjects were randomly assigned to either vitamin D or placebo groups for 16 weeks. The participants were asked to take 50,000 IU vitamin D or matching placebo every week. Metabolic and anthropometric indices, serum MMP-2, MMP-9, TIMP-1 and high-sensitivity C-reactive protein (hsCRP) were assessed before and after intervention. Moreover, dietary intake, sun exposure and physical activity were also determined. The trial was registered at http://www.irct.ir (No. IRCT201409033140N14). Participants were 40.20 ± 4.60 y and 45.50% males. Compared to the baseline values, MMP-9 and TIMP-1 concentrations were decreased after 16 weeks in the intervention group ($p = 0.03$ and $p = 0.04$, respectively). However, the changes of MMP-2, MMP-9, TIMP-1 and hsCRP in the intervention group were not significant compared to the placebo group ($p > 0.05$). Furthermore, the metabolic or anthropometric indices between two study groups remained unchanged ($p > 0.05$). The findings of the present study demonstrated no effect of vitamin D supplementation on MMP-2, MMP-9 and TIMP-1 concentrations in subjects with metabolic syndrome. However, there is a need for more longitudinal trials to investigate the role of vitamin D on atherosclerosis and cardiovascular diseases in subjects with metabolic syndrome.

Keywords: vitamin D, matrix metalloproteinase, tissue inhibitor of matrix metalloproteinase, metabolic syndrome

Introduction

Vitamin D deficiency, measured as 25(OH)D less than 50 nmol/L, is considered as a public health problem [1, 2]. Besides the classical effect of vitamin D in calcium and bone metabolism, recent studies report the association of vitamin D with mortality, cardiovascular diseases, diabetes mellitus, and metabolic syndrome [3–5]. Metabolic syndrome is defined as a cluster of several risk factors such as hyperglycemia, dyslipidemia, hypertension and visceral obesity which contribute to the development of atherosclerosis [6]. Muldowney et al. have recently reported that the increased risk of metabolic syndrome has been correlated to the risk factors of cardiovascular diseases in subjects with 25(OH)D concentration between 40–50 nmol/L [7].

Matrix metalloproteinases (MMPs) are the zinc dependent family of peptidases which are distributed at the cell surfaces. These molecules are involved in the vascular remodeling and stimulation of cytokines secreted by macrophages and endothelial cells [8]. The dysregulation of the activity of MMPs and their inhibitors might contribute in the pathogenesis of cardiovascular diseases [9, 10]. Previous studies have shown the high levels of MMP-2, MMP-9 and tissue inhibitor of matrix metalloproteinase (TIMP-1) in subjects with metabolic syndrome and patients with hypertension [11, 12]. Vitamin D has been shown to down-regulate the production of MMP-9 and MMP-7 in keratinocytes and MMP-2 in vascular smooth muscle cells [13, 14]. In human studies, it has been demonstrated that vitamin D concentration was inversely correlated with MMP-9 [15].

Although, in in vitro studies, it has been shown that vitamin D would regulate the expression of matrix metalloproteinases; the findings of the human studies are sparse. Therefore, this pilot study was designed to assess whether vitamin D supplementation would affect the serum concentration of MMP-2, MMP-9 and TIMP-1 in subjects with metabolic syndrome.

Methods

Study design and population

This study was part of a clinical trial which was performed from October 2014 to June 2015 in Tabriz, Iran. It was a randomized double-blind clinical trial on the effect of vitamin D supplementation on different cardiovascular risk factors in subjects with metabolic syndrome. In this study, we investigate the effect of vitamin D supplementation on MMP-2, MMP-9, TIMP-1 and high-sensitivity C-reactive protein (hsCRP) levels. The details of the study, i.e. inclusion and exclusion criteria, were described elsewhere [16]. Briefly, voluntaries with the age of 30–50 y and body mass index (BMI) 18.5–40 kg/m^2 were recruited through advertisement in Tabriz city (38° N). Subjects were excluded if they had taken supplements such as vitamin D, calcium or omega-3 within the past three months, if they were menopausal, pregnant, lactating, on weight reduction programs, current smokers, or if they had taken antihypertensive, fat–lowering, or drugs interacting with vitamin D metabolism. Then, they were assessed for metabolic syndrome according to the international criteria [17]. The participants with metabolic syndrome were randomly assigned into two groups: vitamin D (Dana pharm. Co; Tabriz, Iran) (50,000 IU /week) or placebo (miglyol, Dana pharm. Co; Tabriz, Iran) for sixteen weeks. The dose of vitamin D was chosen according to guidelines on nutrient effects in clinical studies and is considered safe based on "the 2011 report on dietary reference intakes of vitamin D" [18, 19]. Randomization was done by using Block randomization produced by Random Allocation Software, version 1.0 (M. Saghaei, Department of Anesthesia, Isfahan University of Medical Sciences, Isfahan, Iran) [20] with stratification by age and sex. The pearls were given twice at the beginning and 8-week. Subjects were asked to take their pills with their lunch. The study was approved by the Ethical Committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.439), and was registered on the Iranian Registry of Clinical Trials (http://www.irct.ir) with the identification code. IRCT201409033140N14. At the beginning, the research aims, and approach were explained for the subjects and they all signed the consent form [16].

Laboratory analysis

At the beginning and the end of the study, blood samples were obtained from all participants after 12-hour fast. After sera separation, fasting serum glucose and lipid profiles (Pars Azmun Co., Tehran, Iran) were measured by an auto-analyzer (Hitachi, 717, Boehringer Mannheim, Japan). MMPs, TIMP-1 and hsCRP were determined at the end of the study for all sera which had kept on −80 °C, by using the enzyme-linked immunosorbent assay (ELISA) (Zellbio, Gmbh, Ulm, Germany) according to the manufacturers' instructions. Intra- and inter-assay coefficient variation (CV %) for MMP-9, MMP-2 and TIMP-1 were < 10% and < 12%, respectively. For hsCRP, the sensitivity of the test was 10 ng/ml and the intra assay CV % was < 10%. Serum 25(OH)D concentration was determined by Quantitative chemiluminescent immunoassay (Diasorin, Stillwater, USA). The dynamic range was 10–375 nmol/L and functional sensitivity was $\leq$ 10 nmol/L. Intra- and inter-assay CV % were 20% and 10.6%, respectively.

Anthropometric factors and blood pressure

Anthropometric assessments were performed at the baseline and the end of the study. Weight was measured with light clothes and without shoes using a digital scale with the precision of 0.1 kg (Beurer, GmBH, 89077 Ulm, Germany). Height was measured using a stadiometer with the precision of 0.1 cm. Waist and hip circumferences were determined with the precision of 0.1 cm with a tape at the midpoint between the lower rib and iliac crest in a standing position and as the greatest gluteal circumference, respectively. Body mass index (BMI = weight (kg)/height (m)^2) and waist to hip ratio (WHR) were calculated. Blood pressure measurement was performed by a digital

sphygmomanometer (BC08; Beurer GmbH, Ulm, Germany) twice with 5-min intervals at the beginning and the end of the study. The mean of two measurements was reported [16].

Physical activity and sun exposure

Physical activity levels of the subjects were determined using a short form of the International Physical Activity Questionnaire by an instructed interviewer [21] and analyzed according to the met-min/week [22]. Duration of the exposure to sunlight was measured according to a questionnaire determining the frequency of exposure to sunlight in a usual day in a previous week [23]; the details on sun exposure measurement were given elsewhere [16].

Dietary intake assessments

Dietary intake assessment was determined according to one-day recall and two-day records and was analyzed by the Nutritionist IV software program (First Databank Inc, Hearst Corp, San Bruno, CA, USA) [16].

Statistical analysis

In this pilot study, the sample size was calculated based on 80% power and a 5% significance level, considering hsCRP variable from the study of Shab-bidar et al. [24], which necessitated at least 23 cases in each group. Normal distribution of data was tested by the Kolmogorov–Smirnov test, and also considering the mean and the standard deviation (SD). Data were expressed as mean ± SD, if not otherwise stated. For within group analysis, paired sample t test and Wilcoxon Signed rank test were performed for parametric and non-parametric values, respectively. Furthermore, the changes of the variables across each group were calculated as: means/medians of the end of the study minus baseline values. Independent sample t test and Mann Whitney U tests were used to compare the changes of the variables according to their normal or non-normal distribution. For statistical analysis SPSS (ver. 21; IBM Corp., Armonk, NY, USA) was used.

Results

The flow diagram of the study population was shown in Figure 1. Forty-six subjects completed the study. Table 1 presents the baseline characteristics of the study participants. The mean age of the study population was 39.23 ± 4.91 y and 41.18 ± 5.9 y in the vitamin D and placebo groups, respectively. Approximately, 45.5% of the study subjects were males and 72.7% had high school diploma in both groups. There was no significant difference in sun exposure between the two groups at the beginning and the end of the study ($p > 0.05$).

Anthropometric and metabolic characteristics of the two groups are shown in Table 2. Total cholesterol concentration reduced in the vitamin D group (−5.3%, $p = 0.03$). Moreover, systolic blood pressure was decreased in both groups (vitamin D: −6.2%; $p = 0.005$; placebo group: −2.8%; $p = 0.04$). However, the difference between changes of total cholesterol, systolic blood pressure and anthropometric variables were not significant among two groups ($p > 0.05$, Table 2). Among metabolic variables, there was a significant difference in triglyceride concentration change in the intervention group compared to the placebo group ($p = 0.02$, Table 2).

After sixteen weeks, vitamin D concentration increased in the intervention group (median and 25, 75 percentiles of changes: 71.03 (18.75, 108.25) nmol/L) but decreased in the placebo group (median and 25, 75 percentiles of the changes: −1.76 (−25.50, 15.30) nmol/L) ($p < 0.001$). MMP-2, MMP-9 and TIMP-1 concentrations decreased in both vitamin D and placebo groups (−2.3% and −8.4% for MMP-2, −4.9% and −3.7% for MMP-9, −10.7% and −3.2% for TIMP-1, in vitamin D and placebo groups, respectively). The intra group reduction of MMP-9 and TIMP-1 was significant ($p < 0.04$) and for MMP-2 it was near to significant ($p = 0.055$) in the vitamin D group. However, in between-group analysis, the changes of MMP-2, MMP-9 and TIMP-1 were not significant ($p > 0.05$). HsCRP showed no significant change among two groups ($p > 0.05$, Table 3).

As shown in Table 4, compared with baseline, energy and carbohydrate intakes were decreased in the intervention group (−20.6%, $p = 0.03$ and −23.0%, $p = 0.02$). In between-group analysis, only the change of carbohydrate was significant ($p < 0.04$), while carbohydrate and other dietary assessed parameters were not significant ($p > 0.05$).

Discussion

In the present study, MMP-9 and TIMP-1 concentration decreased in treated group with vitamin D, but this was not significant compared to the placebo one. Furthermore, no changes on the MMP-2 or hsCRP concentrations were shown. In addition, no significant changes in the anthropometric or metabolic factors, except of triglyceride and serum vitamin D, were observed between two study groups.

The association between Vitamin D deficiency and cardiovascular disease has been investigated [25]. Observational and epidemiological studies have been shown relationship between vitamin D deficiency and cardiovascular diseases and atherosclerosis [26–28]; moreover, in

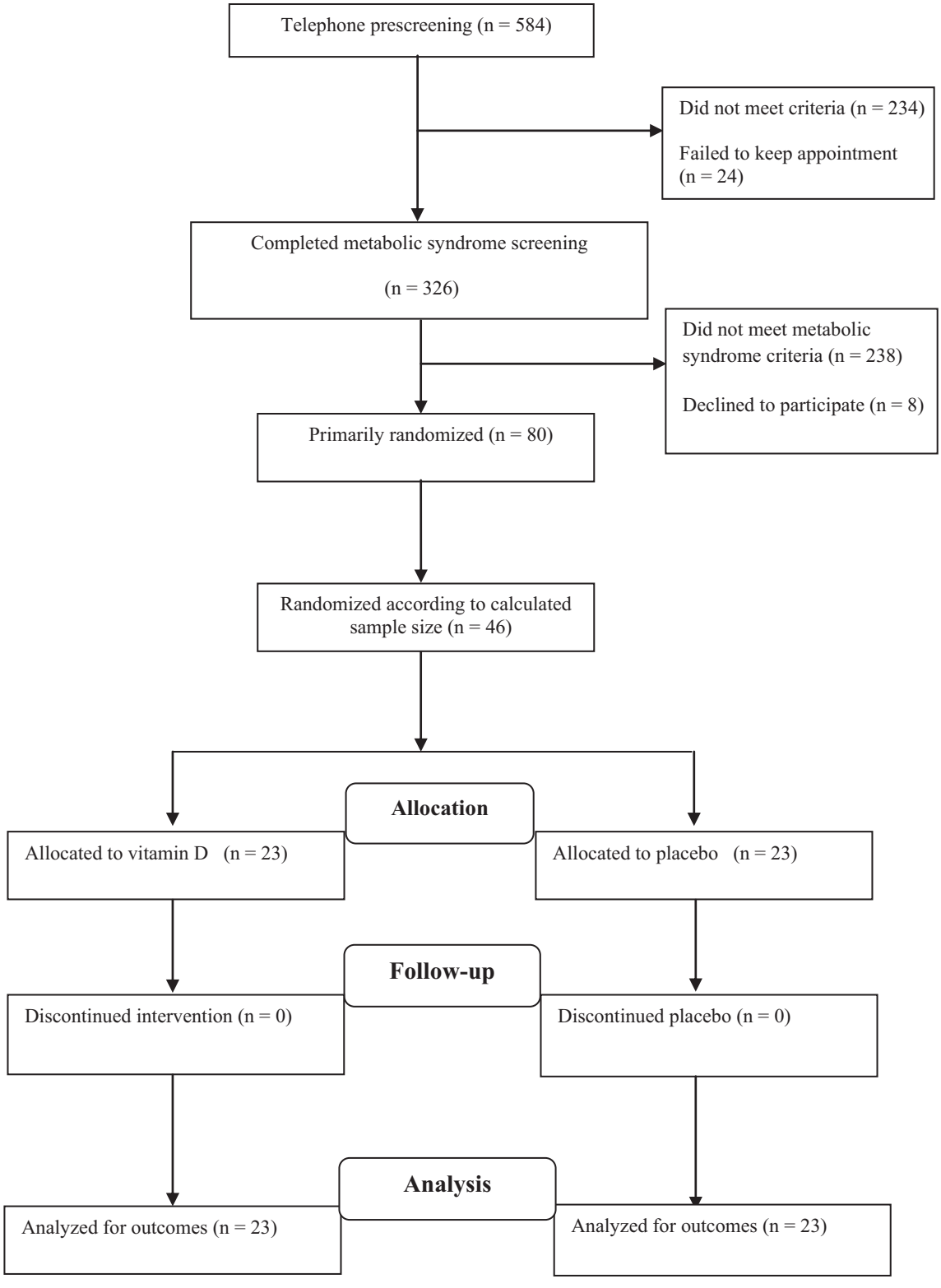

Figure 1. Flow diagram of the study.

animal studies the effect of vitamin D was shown in prevention of atherosclerosis and myocardial ischemia [29, 30]. However, the results of clinical trials are inconsistent. Besides, the underlying mechanisms by which vitamin D could have a protective role in atherosclerosis have not been clearly demonstrated yet.

Table 1. Baseline characteristics of the study groups.

Variable	Vitamin D (n = 23)	Placebo (n = 23)	p
Sex (male); n (%)	10 (45.5)	10 (45.5)	0.61[a]
Age (y)	39.2 ± 4.9	41.2 ± 5.9	0.24[b]
Physical activity (met-minutes/week)	693.0 (132.0, 2487.7)	1014.2 (132.0, 1776.7)	0.98[b]
Education; n (%)			
High school diploma	16 (73)	16 (73)	0.63[a]
College	6 (27)	6 (27)	
Sun exposure			
None	9 (41)	6 (27)	0.61[a]
10 min–1 hour	10 (45)	12 (54)	
1–2 hour	0	1 (5)	
> 2 hour	3 (14)	3 (14)	

[a]Denotes the significance of differences of frequencies between two groups (chi square test).
[b]Denotes the significance of differences between two groups (independent t- test).

Matrix metalloproteinases are important regulatory molecules in inflammation and vasculature by contributing to remodeling of connective tissue and basement membranes and also by acting on pro-inflammatory cytokines in chemokine activity control [31, 32]. The concentrations of these enzymes increase in the inflammatory atherosclerosis progression of vascular wall [12]. Matrix metalloproteinase-9 elevation has been shown to be associated with the development of atherosclerosis and acute myocardial infarction and was responsible for plaque instability and rupture [11, 32]. Matrix metalloproteinase-2 was also more active in atherosclerotic lesions rich in smooth muscle cells [33]. It has been shown that the development of vascular changes was associated with the increase of MMPs and the disturbance of MMP/TIMP ratio [34].

Reduction of MMP-9 concentration in our treated subjects was similar to previous studies. In a prospective study in 171 healthy British Bangladeshi adults, vitamin D levels were shown to be an independent determinant of MMP-9 and hsCRP levels. In this study in subjects who were vitamin D insufficient and with acute cardiovascular events, MMP-9 was reported to increase during five-year follow-up. The authors concluded that the raised levels of MMP-9 might relate to inflammatory vascular damage [15]. In the same study after one-year supplementation by 50,000 IU injection three-monthly, MMP-9 and TIMP-1 levels went down significantly [15]. However, the levels of MMP-2 and hsCRP were also reduced significantly which was in contrast with ours. In a study of diabetes patients, daily consumption of fortified doogh with 1,000 IU vitamin D, reduced the MMP-9 concentration after three months [35]. In contrast, Muldowney et al, found no changes in MMP-9, TIMP-1 and hsCRP concentrations in four study groups with 0–600 IU supplementation of vitamin D after twenty-two weeks [7].

Some mechanisms were suggested for the impact of vitamin D on MMP-9 concentration. Vitamin D would down regulate MMP-9 expression by inhibition of c-Jun-N-terminal kinase (JNK) activation and NFκB signaling pathway [14]. NFκB pathway activation might lead to chronic inflammation and endothelial dysfunction in metabolic syndrome [36]. It was also shown that vitamin D could reduce the MMP9 levels by elevating IL-10 concentration which in turn might decrease MMP-9 secretion [37].

To the best of our knowledge, there is few data from clinical trials to demonstrate the effect of vitamin D on MMP-2 concentration [13]. The results of in vitro studies are conflicting. In a study of vascular smooth muscle cells (VSCM), vitamin D derivatives, maxacalcitriol and calcitriol inhibited MMP-2 mRNA and protein expression which were induced by phosphate and TNF-α. In this study, the authors concluded that vitamin D would have inhibitory effect on the vascular mineralization [15]. In another in vitro study in keratinocytes, vitamin D could suppress the expression of MMP-2 in response to lipopolysaccharide stimulation, suggesting the effect of vitamin D on blunting the abnormal cell growth which causes some skin lesions [38]. The similar inhibitory effect of vitamin D on MMP2 was shown in fibroblasts derived from Taiwanese patients with chronic rhinosinusitis with nasal polyposis [39]. In contrast, Pittarella et al, considered the effect of vitamin D on the endothelial cells proliferation and migration in a human umbilical vein endothelial cells (HUVEC). In this study, MMP-2 activity was significantly increased in Vitamin D-treated samples by inhibiting eNOS activity. The authors concluded that vitamin D might play a role on angiogenesis, suggesting another role for vitamin D in wound healing [40].

While the activity of MMPs increase by the intensity of components of metabolic syndrome related with the development of cardiovascular diseases, it seems vitamin D might lower the risks of cardiovascular diseases by limiting MMP concentrations. In this study, although a significant decrease was observed in intervention group in MMP-9

Table 2. Anthropometric and metabolic characteristics at the baseline and after intervention in subjects with metabolic syndrome

Variable	Vitamin D (n = 23)	Placebo (n = 23)	p[c]
Weight (kg)			
Before	86.7 ± 14.7	89.4 ± 13.3	
After	86.6 ± 14.4	89.3 ± 13.2	
p[a]	0.67	0.84	
Change (95% CI)[b]	−0.1 (−0.75, 0.50)	−0.1 (−0.65, 0.54)	0.86
BMI (kg/m^2)			
Before	33.10 ± 4.83	33.58 ± 4.35	
After	33.14 ± 4.97	33.49 ± 4.28	
p[a]	0.84	0.82	
Change (95% CI)[b]	−0.04 (−0.23, 0.17)	−0.09 (−0.33, 0.15)	0.98
Waist circumference (cm)			
Before	104.9 ± 11.8	104.8 ± 9.3	
After	103.6 ± 11.3	104.9 ± 9.3	
p[a]	0.04	0.87	
Change (95% CI)[b]	−1.3 (−2.65, −0.04)	0.1 (−1.45, 1.68)	0.14
Hip circumference (cm)			
Before	107.9 ± 11.9	111.5 ± 7.8	
After	108.2 ± 12.1	110.8 ± 7.3	
p[a]	0.42	0.91	
Change (95% CI)[b]	0.3 (−0.41, 0.96)	−0.7 (−1.49, 0.13)	0.07
FBS (mg/dl)			
Before	92.5 ± 13.2	95.6 ± 10.2	
After	95.3 ± 8.1	94.3 ± 10.7	
p[a]	0.29	0.52	
Change (95% CI)[b]	2.8 (−2.63, 8.26)	−1.3 (−5.33, 2.79)	0.21
Triglyceride (mg/dl)			
Before	265.9 ± 107.9	181.9 ± 61.1	
After	238.9 ± 86.6	205.3 ± 73.2	
p[a]	0.40	0.05	
Change (95% CI)[b]	−27.0 (−63.79, 9.79)	23.4 (−0.77, 47.50)	0.02
HDL-C (mg/dl)			
Before	46.8 ± 8.3	46.7 ± 10.7	
After	47.1 ± 5.9	46.7 ± 7.8	
p[a]	0.88	0.98	
Change (95% CI)[b]	0.3 (−3.44, 3.99)	0.04 (−3.78, 3.87)	0.93
LDL-C (mg/dl)			
Before	116.7 ± 33.7	113.8 ± 29.4	
After	109.4 ± 23.8	105.3 ± 29.3	
p[a]	0.16	0.01	
Change (95% CI)[b]	−7.3 (−18.03, 3.36)	−8.5 (−15.02, −1.96)	0.85
Total cholesterol (mg/dl)			
Before	216.2 ± 46.1	196.8 ± 39.1	
After	204.7 ± 36.5	193.1 ± 32.1	
p[a]	0.03	0.35	
Change (95% CI)[b]	−11.5 (−22.25, −0.83)	−3.7 (−12.08, 4.53)	0.24
SBP (mmHg)			
Before	133.0 ± 14.8	130.2 ± 10.7	
After	124.7 ± 14.3	126.5 ± 12.7	
p[a]	0.005	0.04	

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Table 2. (Continued)

Variable	Vitamin D (n = 23)	Placebo (n = 23)	p[c]
Change (95% CI)[b]	−8.3 (−13.86, −2.81)	−3.7 (−7.25, −0.15)	0.15
DBP (mmHg)			
Before	83.9 ± 10.8	83.4 ± 8.9	
After	82.1 ± 12.9	82.5 ± 10.4	
p[a]	0.22	0.41	
Change (95% CI)[b]	−1.8 (−5.07, 1.25)	−0.9 (−2.94, 1.26)	0.56

[a]Denotes the significance of within-group changes. [b]Denotes the changes (after minus baseline of the study) and 95% confidence intervals of the variables. [c]Denotes the comparison of the changes between groups (independent sample t-test). BMI: body mass index; DBP: diastolic blood pressure; FBS: fasting blood sugar; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; SBP: systolic blood pressure.

Table 3. Vitamin D status and inflammatory markers at the baseline and after intervention in subjects with metabolic syndrome

Variable	Vitamin D (n = 23)	Placebo (n = 23)	p[c]
25(OH)D (nmol/L)			
Before	11.97 (0.00, 25.93)	21.71 (13.65, 31.43)	
After	83.00 (65.62, 91.87)	19.95 (15.01, 28.93)	
p[a]	<0.001	0.74	
Change (min; max)[b]	71.03 (18.75, 108.25)	−1.76 (−25.50, 15.30)	<0.001
MMP-2 (ng/ml)			
Before	351.50 (334.00,1125.75)	351.50 (332.25,385.00)	
After	343.50 (310.00,1024.75)	322.00 (310.25, 390.25)	
p[a]	0.05	0.36	
Change (min; max)[b]	−8.00 (−342.00, 133.00)	−29.5 (−138.00, 124.00)	0.38
MMP-9 (ng/ml)			
Before	82.00 (74.75, 175.25)	81.00 (74.00, 103.25)	
After	78.00 (67.75, 149.50)	78.00 (71.50, 96.50)	
p[a]	0.03	0.59	
Change (min; max)[b]	−4.00 (−298.00, 14.00)	−3.00 (−383.00, 38.00)	0.28
TIMP-1 (ng/ml)			
Before	297.50 (277.00, 1129,00)	283.5 (255.75, 324.75)	
After	265.50 (242.00,1085.75)	274.5 (259.00, 291.25)	
p[a]	0.04	0.33	
Change (min; max)[b]	−32.00 (−1189.00, 59.00)	−9.00 (−953.00, 80.00)	0.28
hsCRP (mg/L)			
Before	4.86 ± 3.54	6.08 ± 4.48	
After	5.02 ± 3.81	6.10 ± 4.46	
p[a]	0.97	0.55	
Change (95% CI)[b]	0.16 (−0.15, 0.14)	0.02 (−0.18, 0.10)	0.69

Data are presented as median (25, 75 percentiles) except for hsCRP which presented mean ± SD. [a]Denotes non-parametric Wilcoxon singed rank test, except for hsCRP which shows paired-samples t test. [b]Denotes the changes (after minus baseline of the study) and minimum, maximum, except for hsCRP which shows changes (after minus baseline of the study) and 95% confidence interval. [c]Denotes the comparison of the changes between groups (Mann Whitney U-test, except for hsCRP: independent sample t-test). 25(OH) D: 25-hydroxy vitamin D; hsCRP: high-sensitivity C-reactive protein; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinase.

and TIMP-1 concentrations, the difference between the two study groups were not significant which might be due the small sample size of our study. This study opens a window for some other clinical trials with more sample size and long duration to investigate the potential effect of vitamin D in cardiovascular diseases.

Our study is among the first to investigate the effect of vitamin D supplementation on matrix metalloproteinases in subjects with metabolic syndrome. Furthermore, we could determine the dietary intakes of vitamin D and other micronutrients which might influence the results of the study. Despite these strengths, the present study has a few

Table 4. Macronutrient and micronutrient intake at baseline and after intervention in subjects with metabolic syndrome

Variable	Vitamin D (n = 23)	Placebo (n = 23)	p[c]
Energy (kcal/d)			
Before	1965.9 ± 845.8	1712.0 ± 877.2	
After	1560.3 ± 446.9	1794.6 ± 891.2	
p[a]	0.03	0.83	
Change (95% CI)[b]	−405.6 (−763.52, −47.60)	82.6 (−253.58, 418.74)	0.54
Protein (g/d)			
Before	68.9 ± 34.7	61.3 ± 32.1	
After	54.8 ± 17.7	64.6 ± 33.0	
p[a]	0.08	0.63	
Change (95% CI)[b]	−14.1 (−30.33, 2.01)	3.3 (−10.67, 17.41)	0.09
Carbohydrate (g/d)			
Before	293.0 ± 127.7	254.6 ± 152.3	
After	225.7 ± 66.3	264.8 ± 141.5	
p[a]	0.02	0.66	
Change (95% CI)[b]	−67.3 (−125.75, −8.80)	10.2 (−38.45, 59.01)	0.04
Fat (g/d)			
Before	59.9 ± 29.8	51.6 ± 26.2	
After	50.1 ± 18.0	52.4 ± 33.4	
p[a]	0.09	0.91	
Change (95% CI)[b]	−9.8 (−21.46, 1.85)	0.8 (−15.36, 17.03)	0.27
Dietary fiber (g/d)			
Before	11.8 ± 5.6	11.2 ± 5.8	
After	11.2 ± 5.1	12.3 ± 6.7	
p[a]	0.74	0.46	
Change (95% CI)[b]	−0.6 (−4.37, 3.17)	1.1 (−1.99, 4.22)	0.46
Magnesium (mg/d)			
Before	161.1 ± 89.2	158.1 ± 64.3	
After	143.7 ± 45.1	197.2 ± 126.7	
p[a]	0.48	0.17	
Change (95% CI)[b]	−17.4 (−68.19, 33.55)	39.1 (−19.31, 97.60)	0.14
Phosphorous (mg/d)			
Before	695.4 ± 384.1	667.3 ± 288.5	
After	544.8 ± 165.4	733.2 ± 406.1	
p[a]	0.13	0.41	
Change (95% CI)[b]	−150.6 (−349.98, 48.78)	65.9 (−98.34, 230.19)	0.09
Zinc (mg/d)			
Before	5.4 ± 2.9	5.6 ± 2.5	
After	5.5 ± 2.5	6.4 ± 3.7	
p[a]	0.91	0.44	
Change (95% CI)[b]	0.1 (−1.7, 1.9)	0.8 (−1.3, 2.8)	0.44
Copper (mg/d)			
Before	0.8 ± 0.5	0.9 ± 0.2	
After	0.7 ± 0.2	1.0 ± 0.6	
p[a]	0.35	0.39	
Change (95% CI)[b]	−0.1 (−0.4, 0.1)	0.1 (−0.2, 0.4)	0.45
Iron (mg/d)			
Before	11.9 ± 5.8	12.69 ± 7.4	
After	11.0 ± 3.5	12.39 ± 5.0	
p[a]	0.54	0.35	
Change (95% CI)[b]	−0.9 (−4.3, 2.3)	−0.2(−3.1, 2.5)	0.39

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Table 4. (Continued)

Variable	Vitamin D (n = 23)	Placebo (n = 23)	p[c]
Calcium (mg/d)			
Before	516.5 ± 275.7	545.6 ± 306.4	
After	392.2 ± 169.8	528.5 ± 222.24	
p[a]	0.10	0.79	
Change (95% CI)[b]	−124.3 (−245.4, 26.6)	−17.1 (−151.9, 117.8)	0.27
Vitamin D (IU/d)			
Before	0.01 (0.01, 15.70)	0.01 (0.01, 96.16)	
After	0.01 (0.01,4.50)	0.01 (0.01, 12.48)	
p[a]	0.308	0.346	
Change (min; max)[b]	−0.48 (−4.03; 1.31)	−0.29 (−2.40; 1.05)	0.87

Dietary nutrient intake was analyzed by the Nutritionist IV software program. Data presented as mean ± SD, for vitamin D intake presented as median (25, 75%).
[a]Denotes the comparison within groups (paired samples t-test), except for vitamin D which shows non-parametric Wilcoxon singed rank test.
[b]Denotes the changes (after minus baseline of the study) and 95% confidence intervals of variables; for vitamin D: the changes (after minus baseline of the study) and minimum, maximum.
[c]Denotes the comparison of the changes between group (independent sample t-test, except for vitamin D intake: Mann Whitney U-test).

limitations. First of all, the sample size was small. Although the sample size was calculated according to the change of hsCRP concentration, this was a pilot study and to show the clear effects, more sample size would be needed. Secondly, the number of biomarkers that we monitored was limited, due to our budget. Thirdly, the study was conducted in cold seasons; therefore, the results could not be interpreted for the whole year. Therefore, additional studies should be performed to better investigate these inflammatory markers.

Conclusion

The present study showed no effect of vitamin D supplementation on MMP-9, MMP-2 and TIMP-1 concentrations in subjects with metabolic syndrome. Further large-scale clinical trials are needed to demonstrate the possible effect of vitamin D on cardiovascular risk factors in subjects with metabolic syndrome.

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Conflict of interest
The authors declare that there is no conflict of interest.

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