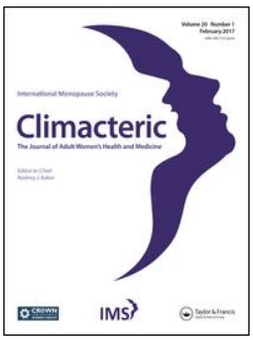

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ORIGINAL ARTICLE

Vitamin D supplementation improves the metabolic syndrome risk profile in postmenopausal women

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ABSTRACT

Objective: This study aimed to evaluate the effect of isolated vitamin D (VD) supplementation on the metabolic syndrome (MetS) risk profile in postmenopausal women.

Methods: In this double-blind, placebo-controlled trial, 160 postmenopausal women aged 50–65 years were randomized into two groups: VD group, supplementation with 1000 IU vitamin D_3/day ($n = 80$); or placebo group ($n = 80$). The intervention time was 9 months, and the women were assessed at baseline and endpoint. Clinical and anthropometric data were collected. Biochemical parameters, including total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, glucose, and insulin, were measured. The plasma concentration of 25-hydroxyvitamin D (25(OH)D) was measured by high-performance liquid chromatography.

Results: After 9 months, there was a significant increase in the 25(OH)D levels for VD group (+45.4%, $p < 0.001$), and a decrease (−18.5%, $p = 0.049$) in the placebo group. In the VD group, a significant reduction was observed in triglycerides (−12.2%, $p = 0.001$), insulin (−13.7%, $p = 0.008$), and the homeostasis model assessment of insulin resistance (−17.9%, $p = 0.007$). In the placebo group, there was an increase in glucose (+6.2%, $p = 0.009$). Analysis of the risk adjusted for age, time since menopause, and body mass index showed that women supplemented with VD had a lower risk of MetS (odds ratio [OR] 0.42; 95% confidence interval [CI] 0.21–0.83), hypertriglyceridemia (OR 0.43; 95% CI 0.22–0.85), and hyperglycemia (OR 0.23; 95% CI 0.10–0.52) compared to the placebo group ($p < 0.05$).

Conclusions: In postmenopausal women with VD deficiency, isolated supplementation with 1000 IU vitamin D_3 for 9 months was associated with a reduction in the MetS risk profile. Women undergoing VD supplementation had a lower risk of MetS, hypertriglyceridemia, and hyperglycemia.

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Vitamin D; supplementation; postmenopausal women; metabolic syndrome

Introduction

Vitamin D (VD), a fat-soluble vitamin, plays an important and recognized role in parathyroid regulation, calcium/phosphorus homeostasis, and bone mineralization[1]. The identification of vitamin D receptor (VDR) in almost all human cells has proved the association of VD deficiency with various non-communicable chronic diseases such as obesity, hypertension, diabetes, metabolic syndrome (MetS), and cardiovascular disease (CVD)[2,3]. In the cardiovascular system, VDRs were found in the vascular smooth muscle, endothelium, and cardiomyocytes. Thus, evidence suggests that VD deficiency is a potential risk factor for CVD[4,5], the main cause of death in postmenopausal women.

Vitamin D deficiency and the increase in the prevalence of obesity are considered important public health issues[6]. Obesity is a proinflammatory state that contributes to insulin resistance, a condition suggested to be the causal factor for dyslipidemia and glucose intolerance[3]. VD deficiency can affect the normal metabolic functioning of adipose tissue, having a significant impact on maintaining metabolic health[3]. MetS is defined by a set of metabolic risk factors that include abdominal obesity, dyslipidemia, arterial hypertension, and hyperglycemia[7]. This syndrome affects approximately 50% of the female population above the age of 50 years and is associated with a three-fold increase in morbidity and mortality risk due to CVD[7–9]. MetS is associated with a metabolic disorder called insulin resistance, in which the normal action of insulin is compromised[9]. In a meta-analysis of 28 studies, elevated serum levels of 25-hydroxyvitamin D (25(OH)D) were associated with a 55% reduction in diabetes incidence, a 51% lower risk of MetS, and a 33% lower risk of CVD[10]. Measurement of 25(OH)D is suitable to evaluate and monitor the VD nutritional status in the human organism since serum levels are the main indicator of body reserves[11].

Vitamin D deficiency plays a key role in the pathophysiology of risk factors for MetS, which affect the cardiovascular system, increase insulin resistance and obesity, and stimulate the rennin–angiotensin–aldosterone system that causes hypertension[10,12]. In a recent review study, the authors concluded that observational and prospective studies, involving the general population of both genders, have indicated that MetS is associated with VD insufficiency. However, the available data from intervention studies were not consistent to

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draw final conclusions on the effect of VD supplementation on the risk factors for MetS. Since there is sufficient biologic plausibility to explain the role of VD in the prevention and treatment of MetS, more studies are required to be carried out to establish the relationship[12]. Moreover, there are few data in postmenopausal women. Some observational studies have demonstrated an inverse relationship between VD and MetS in postmenopausal women[13–15]. On the other hand, to our knowledge there are limited intervention studies with VD supplementation and MetS in younger health postmenopausal women. Based on these data, the aim of this study was to evaluate the effect of isolated VD supplementation on the MetS risk profile in younger postmenopausal women.

Methods

Study design and sample selection

This study was a randomized, double-blind, placebo-controlled clinical trial. The population studied consisted of patients seen at the Climacteric and Menopause Outpatient Clinic of the Botucatu Medical School – UNESP from August 2015 to December 2016. Women aged 50–65 years who have not had a menstrual period for at least 12 months were included. Exclusion criteria were: coronary artery disease (current or previous), cerebrovascular arterial disease, abdominal aortic stenosis or aneurysm, insulin-dependent diabetes, renal failure (creatinine >1.4 mg/dl), liver disorders, cancer, abusive alcohol consumption, grade III obesity, and use of previous VD or hormone therapy. Informed consent was obtained from all participants and the study was approved by the Research Ethics Committee of the Botucatu Medical School, Sao Paulo State University. The study was registered at and approved by the Brazilian Clinical Trials Registry (ReBEC) under registration number RBR-4MHS32. This trial was in accordance with the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement[16].

Randomization and supplementation protocol

After initial screening, all participants were given a number (1–160) according to their order of inclusion in the study. Central computer randomization was conducted using specific software (SAS 9.2 for Windows using Procedure Plan). The women were randomly assigned to two groups in a predetermined sequence: a supplemented group consisting of patients receiving vitamin D_3 supplementation ($n = 80$); and a placebo group consisting of patients receiving placebo ($n = 80$). All participants started simultaneously in February/March 2016. The investigator and the patients were unaware of the group allocation and different numbers (blinding); only the pharmacist responsible for manipulation of the placebo knew to which group the patients belonged. Thus, 80 patients received five oral drops (each drop containing 200 IU, 20-ml flask) of 1000 IU vitamin D_3 (cholecalciferol, DePura®; Sanofi-Aventis, Sao Paulo, Brazil) for 9 months. The remaining 80 patients received five oral drops of placebo with the same characteristics and flavor (1% powdered lemon flavor, 0.2% ethylenediamine tetraacetic acid, liquid flavor qs, and liquid petrolatum qsp in 20 ml). The flasks were identical and were packed and coded by the pharmacist so that the participants could not identify their group. The participants were asked to return the flasks during each visit (every 3 months) to determine the amount of unused medication and compliance. The time of follow-up was 9 months and the patients were submitted to clinical evaluation at baseline and endpoint. The flow chart shows the recruitment and randomization of the participants (Figure 1).

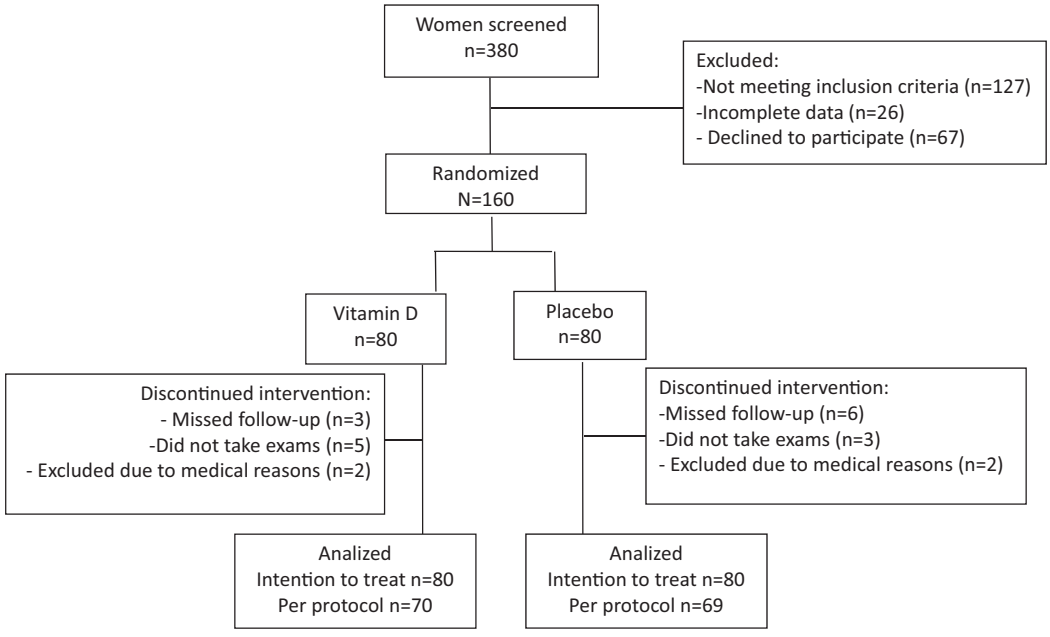

Figure 1. Flow chart of women included in the study.

Clinical and anthropometric measurements

All participants underwent individual interviews in which the following data were collected: age, time since menopause, current smoking, personal history of hypertension, diabetes, and CVD, use of medications, blood pressure, and physical activity. Smokers were defined as people who reported smoking regardless of the number of cigarettes smoked. Women who practiced aerobic physical exercise of moderate intensity for at least 30 min five times a week (150/min/week) or resistance exercise three times a week were considered active. MetS was defined as the presence of three or more of the following diagnostic criteria proposed by the US National Cholesterol Education Program/Adult Treatment Panel III[16]: waist circumference (WC) >88 cm; triglycerides ≥150 mg/dl; high-density lipoprotein (HDL) <50 mg/dl; blood pressure ≥130/85 mmHg or under therapy; and fasting glucose ≥100 mg/dl or under therapy.

The anthropometric data included weight, height, body mass index (BMI = weight/height^2) and WC. The 2002 World Health Organization criteria were used to classify BMI: normal, ≤24.9 kg/m^2; overweight, 25–29.9 kg/m^2; grade I obesity, 30–34.9 kg/m^2; grade II obesity, 35–39.9 kg/m^2; and grade III obesity, ≥40 kg/m^2. WC was measured at the midpoint between the lowest rib and the top of the iliac crest. The patients were advised to remain in the orthostatic position and the reading was performed at the moment of exhalation. This measurement was performed by a single evaluator. Any WC exceeding 88 cm was considered high[17].

Lean body mass and body fat were estimated by total-body dual-energy X-ray absorptiometry at baseline and after 9 months using a Lunar Prodigy Primo densitometer (General Electric®, Madison, WI, USA). The results are reported as gram per area (g/cm^2) or volume (g/cm^3). The precision of the method is 1% for fat-free mass and 2% for fat mass[18]. All tests were performed by the same examiner to minimize interobserver variation. Satisfactory reference values are ≥38.9 kg for total lean mass and <37.3% for total body fat in individuals older than 50 years. Fat mass and fat-free mass were measured in the region extending from the shoulders to the distal phalanx of the feet.

Laboratory assessment

Serum levels of creatinine, triglycerides, total cholesterol, HDL, glucose, and insulin were determined at baseline and after 9 months. Blood samples were collected from each participant after a 12-h fast. Triglycerides, total cholesterol, HDL, and glucose measurements were processed by an automated analyzer (Technicon®, RA-XT System; Global Medical Instrumentation, MN, USA) and quantified by the colorimetric enzymatic method, using specific commercial reagents (Sera-Pak®; Bayer Corporation, Diagnostics Division, New York, USA). Low-density lipoprotein (LDL) was calculated by subtracting total cholesterol from the sum of HDL and triglycerides and dividing the result by 5. The values considered optimal were as follows: total cholesterol level <200 mg/dl, HDL cholesterol level >50 mg/dl, LDL cholesterol level <100 mg/dl, triglyceride level <150 mg/dl, and glucose level <100 mg/dl[16]. Creatinine was measured by dry chemistry in an automated Vitros 950 analyzer (Johnson-Johnson, Rochester, NY, USA). Serum creatinine concentration values were considered within the normal range of 0.7–1.2 mg/dl. Insulin was measured by chemoluminescence with an automated Immulite 2000® immunoassay system (Diagnostic Products Corporation, Los Angeles, CA, USA). The normal range according to the method employed was 6.0–27.0 μIU/ml. Insulin resistance was evaluated based on the statistical measurement of two plasma components (insulin and fasting glucose). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = insulin (mIU/ml) × glucose (mg/dl) / 405. Insulin resistance was defined as HOMA-IR >2.7.

The plasma concentrations of 25(OH)D were measured at baseline and after 9 months for the evaluation of bioavailability and treatment compliance. The 25(OH)D concentrations were determined by high-performance liquid chromatography (HPLC) using an isocratic HPLC system equipped with a Rheodyne® manual injector (model 7725i), a 20-μl loop, and a Waters ultraviolet–visible detector (model M-484). An RP 18 column (4.0 mm × 15 cm, 5-μm particle size; Sigma-Aldrich, St. Louis, MO, USA) was used. The detection limit was 2.5 ng/ml and the coefficient of variation was <7%. Serum 25(OH)D levels were classified as normal (≥30 ng/ml), insufficiency (20–29 ng/ml), and deficiency (<20 ng/ml)[19].

Statistical analysis

Sample size calculation was based on the study by Zittermann *et al.*[20] which demonstrated a reduction in mean triglyceride levels after supplementation with VD (basal 125 ± 59 mg/dl and final 104 ± 50 mg/dl). Assuming a 95% confidence interval (CI) and power of the test of 80%, a minimum of 119 women was estimated. Considering a 20% loss to follow-up, the sample size adopted was 80 women per group. Intention-to-treat analysis was used as the statistical method. The Shapiro–Wilk test was used to determine whether the variables showed a normal distribution and the Levene test was used to determine homogeneity. The mean and standard deviation were calculated for quantitative variables, and the frequency and percentage for qualitative variables. The initial clinical, anthropometric, and biochemical variables were compared between groups by Student's *t*-test and gamma distribution (asymmetric). The frequencies of categorical data were compared by the chi-squared test. To compare the anthropometric and biochemical variables between time points (baseline and after 9 months) and between groups, a repeated-measures design over time (analysis of variance) was used, followed by Tukey's multiple comparisons test adjusted for the group–time interaction. Multivariate analysis by binary logistic regression was performed considering a CI of 95% and calculating the respective odds ratio (OR). The group of women using VD was considered as the response compared to women of the placebo group to evaluate the possible association between the variables influencing the risk for MetS (dependent variables)

and the intervention groups, adjusted for age, time since menopause, and BMI (confounding variables). A level of significance of 5% or the corresponding p value was adopted in all tests. The analyses were performed using the Statistical Analysis System 9.2 program (SAS).

Results

Of 380 invited patients, 160 were included in the study (Figure 1). A comparison of the baseline clinical, anthropometric, and laboratory characteristics between groups, VD supplementation ($n = 80$) and placebo ($n = 80$), is presented in Table 1. The groups were homogeneous in terms of all variables studied ($p > 0.05$). The mean age of the patients included was 58.8 ± 6.6 years in the supplemented group and 59.3 ± 6.7 years in the placebo group, with time since menopause of 12.0 ± 8.8 and 12.3 ± 8.4 years, respectively. In both groups, the patients were classified on average as overweight (BMI 25–29.9 kg/m^2) and as having central fat deposition (WC >88 cm). The mean values of 25(OH)D indicated VD insufficiency (<20.0 ng/ml) (Table 1).

Tables 2 and 3 present a comparison of anthropometric and laboratory parameters between groups and time points. In the comparison of the anthropometric characteristics between groups and between time points, no significant differences were observed in all analyzed parameters ($p > 0.05$) (Table 2). At the final evaluation, after 9 months of treatment, there was a significant increase in the plasma 25(OH)D levels for the group supplemented with VD, with a positive variation of 45.4% ($p < 0.001$). Moreover, for the placebo group there was a decrease of 25(OH)D levels with a negative variation of 18.5% ($p = 0.049$), with a significant difference between the groups and between the final moment (Table 3). In the VD group, a significant reduction was observed in triglycerides (−12.2%, $p = 0.001$), with a significant difference between the groups after 9 months of treatment. There was also a significant decrease in insulin (−13.7%, $p = 0.008$) and HOMA-IR (17.9%, $p = 0.007$) only in the group supplemented with VD, but with no difference between the groups at the end of the intervention. On the other hand, in the placebo group there was an increase in glucose (+6.2%, $p = 0.009$), with a significant difference between the groups and between the final moment ($p < 0.001$) (Table 3).

Table 1. Comparison of initial clinical, anthropometric, and laboratory characteristics among postmenopausal women submitted to vitamin D supplementation ($n = 80$) or placebo ($n = 80$).

Parameter	*Vitamin D*	*Placebo*	p-*Value*[a]
Age (years)	58.8 (6.6)	59.3 (6.7)	0.654[b]
Menopause age (years)	46.8 (6.2)	46.9 (5.6)	0.882[b]
Menopause time (years)	12.0 (8.8)	12.3 (8.4)	0.804[c]
SBP (mmHg)	134.3 (19.8)	136.5 (21.0)	0.499[b]
DBP (mmHg)	81.5 (12.6)	81.0 (10.8)	0.794[b]
BMI (kg/m^2)	29.4 (5.4)	29.9 (4.7)	0.505[b]
WC (cm)	94.0 (12.1)	94.1 (10.3)	0.994[b]
25(OH)D (ng/ml)	15.0 (7.5)	16.9 (6.7)	0.086[b]
Creatinine (mg/dl)	0.7 (0.2)	0.7 (0.1)	0.955[b]
Glucose (mg/dl)	92.5 (10.6)	93.5 (10.9)	0.564[b]
Insulin (μIU/ml)	11.7 (8.1)	10.1 (5.8)	0.085[c]
HOMA-IR	2.8 (2.1)	2.4 (1.5)	0.125[c]
Total cholesterol (mg/dl)	211.3 (48.6)	210.0 (39.7)	0.855[b]
HDL (mg/dl)	51.2 (12.2)	50.6 (12.3)	0.762[b]
LDL (mg/dl)	128.9 (39.7)	129.7 (35.0)	0.885[b]
Triglycerides (mg/dl)	155.5 (92.7)	165.3 (71.1)	0.399[c]
Smoking, *n* (%)	21 (26.2)	19 (23.8)	0.457[d]
Physical exercise, *n* (%)	25 (31.2)	21 (26.2)	0.428[d]
Hypertension, *n* (%)	44 (55.0)	49 (61.2)	0.423[d]
Diabetes, *n* (%)	12 (15.0)	16 (20.0)	0.405[d]
Metabolic syndrome, *n* (%)	23 (28.7)	28 (35.0)	0.063[d]

Data are presented as mean (standard deviation) unless indicated otherwise.
SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; 25(OH)D, 25-hydroxyvitamin D; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Significant difference if $p < 0.05$:
[b]Student *t*-test,
[c]Gamma distribution test, or
[d]Chi-square test.

According to the US National Cholesterol Education Program/Adult Treatment Panel III, which considers the presence of three or more criteria for diagnosis, 24% of the women supplemented with VD and 40.0% of women in the placebo group were classified as having MetS. This difference in the prevalence of MetS between groups was significant ($p = 0.012$) (Table 4). Analysis of the risk adjusted for age, time since menopause, and BMI showed that women supplemented with VD had a lower risk of MetS (OR 0.42; 95% CI 0.21–0.83), hypertriglyceridemia (OR 0.43; 95% CI 0.22–0.85), and hyperglycemia (OR 0.23; 95% CI 0.10–0.52) compared to the placebo group ($p < 0.05$) (Table 4).

For the participants who completed the study, adherence was 92% for the study medication (vitamin D_3 or placebo). No differences in adherence were observed between the treatment groups. Of the 160 women analyzed, 21 discontinued the study before 9 months (Figure 1). Reported adverse events were few, mild, and equally distributed between the supplementation group and the placebo group. Two participants in the VD group and three participants in the placebo group discontinued the study due to gastrointestinal complaints and epigastric pain. No other adverse effects were reported.

Discussion

In this study, isolated supplementation with 1000 IU vitamin D_3 for 9 months was associated with a reduction in the MetS risk profile in younger postmenopausal women with VD deficiency. Women undergoing VD supplementation had a lower risk of MetS, hypertriglyceridemia, and hyperglycemia when compared to women in the placebo group, even after adjusting for different factors. The high prevalence of a poor VD status has gained much public health interest because of its association with CVD conditions, including arterial hypertension, diabetes, and MetS[21,22]. A meta-analysis evaluating the association of VD with MetS and diabetes included 17 prospective studies with 210,107 participants and 15,899 metabolic events. The mean time of follow-up was 10 years. The results showed an inverse association between 25(OH)D concentrations and the risk for diabetes, insulin resistance, and MetS. The authors concluded that interventions designed to maintain serum VD levels within the adequate range might be useful to prevent metabolic diseases[23].

Table 2. Comparison of the anthropometric characteristics among postmenopausal women submitted to vitamin D supplementation ($n = 80$) or placebo ($n = 80$) at baseline and after 9 months of intervention.

Indicator/group	*Baseline*	*9 months*	*Variation (%)*[#]	p-*Value*[a]
SBP (mmHg)				
Placebo	136.5 (21.0)[b]	139.0 (20.3)[b]	2.5 (1.8%)	0.8503
Vitamin D	134.3 (19.8)[b]	130.6 (16.5)[c]	−3.7 (−2.8%)	0.2332
DBP (mmHg)				
Placebo	81.0 (10.8)[b]	81.3 (10.1)[b]	0.3 (0.4%)	0.9978
Vitamin D	81.5 (12.6)[b]	79.8 (11.1)[b]	−1.7 (−2.1%)	0.7732
BMI (kg/m^2)				
Placebo	29.9 (4.7)[b]	30.1 (4.6)[b]	0.2 (0.7%)	0.993
Vitamin D	29.4 (5.4)[b]	29.5 (5.4)[b]	0.1 (0.3%)	0.999
WC (cm)				
Placebo	94.1 (10.3)[b]	94.5 (10.6)[b]	0.4 (0.4%)	0.991
Vitamin D	94.0 (12.1)[b]	92.9 (12.3)[b]	−1.1 (−1.2%)	0.928
Body fat (%)				
Placebo	43.5 (6.4)[b]	43.4 (6.6)[b]	−0.13 (−0.3%)	0.999
Vitamin D	42.6 (7.4)[b]	42.7 (7.8)[b]	0.07 (0.2%)	0.999
Total lean mass (kg)				
Placebo	39.5 (5.3)[b]	38.9 (4.8)[b]	−0.55 (−1.4%)	0.904
Vitamin D	38.4 (5.2)[b]	38.9 (5.4)[b]	0.53 (1.3%)	0.915

Data are presented as mean (standard deviation).
SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference.
[#]Absolute variation: final values subtracted from baseline values.
[a]Significant difference between moments ($p < 0.05$).
[b,c]Significant difference between groups ($p < 0.05$) and [b,b]without significant difference ($p > 0.05$) (analysis of variance in repeated-measures design followed by adjusted Tukey's test).

Table 3. Comparison of the biochemical characteristics among postmenopausal women submitted to vitamin D supplementation ($n = 80$) or placebo ($n = 69$) at baseline and after 9 months of intervention.

Indicator/group	*Baseline*	*9 months*	*Variation (%)*[#]	p-*Value*[a]
25(OH)D (ng/ml)				
Placebo	16.9 (6.7)[b]	13.8 (5.9)[b]	−3.2 (−18.5%)	0.049
Vitamin D	15.0 (7.5)[b]	27.5 (10.4)[c]	12.5 (45.38%)	<0.001
Total cholesterol (mg/dl)				
Placebo	210.0 (39.7)[b]	213.5 (x)[b]	3.5 (1.6%)	0.9693
Vitamin D	211.3 (48.6)[b]	196.3 (x)[b]	−15.0 (−7.1%)	0.2401
HDL (mg/dl)				
Placebo	50.6 (12.3)[b]	50.4 (13.8)[b]	−0.2 (−0.4%)	0.997
Vitamin D	51.2 (12.2)[b]	54.3 (13.0)[c]	3.1 (5.7%)	0.433
LDL (mg/dl)				
Placebo	129.7 (35.0)[b]	126.8 (43.3)[b]	−2.9 (−2.2%)	0.966
Vitamin D	128.9 (39.7)[b]	118.3 (33.6)[b]	−10.6 (−8.2%)	0.331
Triglycerides (mg/dl)				
Placebo	165.3 (71.1)[b]	167.9 (75.2)[b]	2.6 (1.5%)	0.732
Vitamin D	155.5 (92.7)[b]	136.5 (68.1)[c]	−19.0 (−12.2%)	0.001
Glucose (mg/dl)				
Placebo	93.5 (10.9)[b]	99.6 (15.6)[b]	6.1 (6.2%)	0.009
Vitamin D	92.5 (10.6)[b]	92.0 (8.5)[c]	0.5 (0.5%)	0.994
Insulin (μIU/ml)				
Placebo	10.1 (5.8)[b]	10.6 (6.7)[b]	0.6 (4.7%)	0.344
Vitamin D	11.7 (8.1)[b]	10.1 (7.6)[b]	−1.6 (−13.7%)	0.008
HOMA-IR				
Placebo	2.4 (1.5)[b]	2.7 (1.5)[b]	0.3 (11.1%)	0.054
Vitamin D	2.8 (2.1)[b]	2.3 (1.6)[b]	−0.5 (−17.9%)	0.007

Data are presented as mean (standard deviation).
25(OH)D, 25-hydroxyvitamin D; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance.
[#]Absolute variation: final values subtracted from baseline values.
[a]Significant difference between moments ($p < 0.05$).
[b,c]Significant difference between groups and [b,b]without significant difference ($p > 0.05$) (analysis of variance in repeated-measures design followed by adjusted Tukey's test).

There are several possible physiopathological mechanisms that could explain the effect of VD on the components of MetS. The most plausible explanation is that VD influences insulin secretion and sensitivity, which play a major role in MetS. Different biological factors support the association of VD deficiency with insulin resistance and diabetes. Several probable mechanisms of action may explain a possible role for VD to help improve glucose metabolism, including its anti-inflammatory and immunomodulatory effects, the induction of insulin secretion by pancreatic β cells, its indirect effect on regulating calcium concentration in pancreatic β cells, and subsequent insulin secretion[24]. In humans, polymorphisms in the VDR genes have been associated with variations in insulin secretion and sensitivity[25]. The pancreas possesses VDR in insulin-secreting pancreatic β cells and 1α-hydroxylase, and so has the machinery to convert circulating

Table 4. Association between the presence of metabolic syndrome and its clinical and laboratory components among postmenopausal women submitted to vitamin D supplementation or placebo after 9 months of intervention.

Parameter	*Vitamin D*	*Placebo*	*OR (95% CI)*[a]	p-*Value*[b]
Metabolic syndrome	20 (25.0)	32 (40.0)	0.42 (0.21–0.83)	0.012
WC (>88 cm)	49 (61.2)	60 (75.0)	0.53 (0.27–1.04)	0.062
Triglycerides (≥150 mg/dl)	21 (26.2)	37 (46.2)	0.43 (0.22–0.85)	0.014
HDL (<50 mg/dl)	28 (35.0)	34 (42.5)	0.59 (0.30–1.17)	0.132
Glucose (≥100 mg/dl)	9 (11.3)	28 (35.0)	0.23 (0.10–0.52)	0.0003
AP (≥135/85 mmHg)	18 (22.5)	23 (28.7)	0.72 (0.35–1.46)	0.365

Values are expressed as *n* (%).
OR, odds ratio; CI, confidence interval; WC, waist circumference; HDL, high-density lipoprotein; AP, arterial pressure.
[a]Adjusted for age, time of menopause, and body mass index.
[b]Significantly different if $p < 0.05$ (logistic regression).

25(OH)D to 1,25-dihydroxyvitamin D, the active form of VD, and is responsible for physiologic functions[12]. Moreover, VDRs are expressed on various insulin-dependent tissues (including the liver, skeletal muscle, and adipose tissue), suggesting a role for VD in glucose utilization and insulin sensitivity[26]. Also, VD metabolizing enzymes are present in adipocytes and skeletal muscle[27,28]. VD may also affect indirectly insulin sensitivity in skeletal muscle and adipose tissue by regulating the levels of extracellular calcium, which is essential for insulin-mediated intracellular processes[29].

Results from our study suggest that in younger postmenopausal women with VD deficiency, VD supplementation may play a role in the reduction of the MetS risk profile. The women supplemented with VD had a reduction in blood insulin levels (−13.7%) and HOMA-IR (−17.9%). On the other hand, in the placebo group there was an increase in blood glucose levels (+6.2%), with a significant difference between the groups. However, studies in postmenopausal women are scarce to compare with the results of this study. The few randomized controlled trials that are published in this field were conducted in both genders, with patients suffering from type 2 diabetes, elderly and obese individuals, or patients using high therapeutic doses of VD[30]. In a randomized, placebo-controlled trial, supplementation with 4000 IU vitamin D_3 per day for 12 weeks successfully improved VD insufficiency, insulin secretion, and sensitivity in 89 overweight or obese subjects with prediabetes[31]. Improvement in serum insulin and HOMA-IR with a reduction in insulin resistance was reported in 100 patients with type 2 diabetes supplemented with 50,000 IU of VD orally per week for a period of 8 weeks[32]. Another study assessed whether VD supplementation could be used to improve glucose metabolism and components of MetS. Adults ($n = 22$, age 21–75 years) with type II diabetes, hypovitaminosis D, and insulin resistance (HOMA-IR ≥2) were randomly assigned to either supplementation with VD 5000 IU/day or placebo for 12 weeks. A significant improvement was observed in the HOMA-IR with VD supplementation compared to baseline. Changes in BMI, WC, diastolic blood pressure, and lipid profile (HDL, LDL, triglycerides, and cholesterol) did not differ between the groups[33].

In this study, after 9 months of treatment, there was a decrease in blood triglyceride levels (−12.2%, $p = 0.001$), with a significant difference when compared to the placebo group. One possible mechanism underlying the inverse association between serum lipids and VD levels is likely to be related to a reduction in the intestinal absorption and synthesis of lipids, and to a decrease in lipolysis with increasing 25(OH)D concentrations[34]. In a recent cross-sectional study, Schmitt *et al.*[15] evaluated the association between VD deficiency and risk factors for MetS in 463 postmenopausal women. Women with low 25(OH)D levels had higher triglyceride, insulin, and HOMA-IR levels. MetS was detected in 57.8% of women with hypovitaminosis D and in 39.8% of those with sufficient VD. A low 25(OH)D level (<30 ng/ml) was significantly associated with MetS (OR 1.90), high triglycerides (OR 1.55), and low HDL (OR 1.60), compared to sufficient 25(OH)D levels[15]. In a literature review, observational studies showed that subjects with high serum 25(OH)D levels had a more favorable lipid profile than those with deficiency[35]. However, the intervention studies gave divergent results, with some showing a positive effect and some a negative effect of VD supplementation. Similar to our findings, Zittermann *et al.*[20] evaluated 82 subjects (age 47.4 years, 62% women) in a VD group (300 IU vitamin D_3/day) and 83 subjects (age 48.8 years, 72% women) in a placebo group, and observed a significant decrease in serum triglycerides (−13.5%) as compared with the placebo group (+3.0%). Another randomized, controlled, double-blind study assessed the effect of high-dose VD supplementation (50,000 IU weekly) on cardiometabolic risk factors in 80 subjects (age 41 years, 51% women) with MetS. After 4 months, the serum 25(OH)D concentration was increased in the intervention group, while it was decreased in the placebo group. There was a significant decrease in triglyceride concentration. Other metabolic or anthropometric factors did not change significantly[36].

On the other hand, Wood *et al.*[37], in a placebo-controlled trial evaluating 305 postmenopausal women (age 60–70 years), observed that daily supplementation with VD (400 or 1000 IU) had no effect on total cholesterol, HDL, LDL, triglycerides, insulin resistance, or inflammatory markers. Another randomized, placebo-controlled study evaluated the impact of VD on glucose homeostasis in 47 patients with type 2 diabetes mellitus (age 66 years, 53% women), assigned to two groups: oral daily supplementation with VD at a dose of 1000 U/day for 12 months or matching placebo capsules. Glucose homeostasis parameters, leptin, and adiponectin did not change in both groups[38]. A recent randomized placebo-controlled trial evaluated 126 subjects (age 49 years, 46% women) with MetS and VD deficiency, allocated to receive either a daily oral tablet containing 700 IU VD or placebo. There was significantly higher serum 25(OH)D in the treated group, but no significant effect was observed for the metabolic variables which included BMI, blood pressure, blood glucose, and lipids in both the treatment and placebo groups. For those authors, further studies

are warranted in order to elucidate the cause–effect relation between VD status, obesity, and related metabolic disorders[39]. The discrepancies can be explained by the different doses or type of VD given, characteristics or number of subjects included, or duration of the studies.

In the present study, no change in blood pressure and in anthropometric measures was observed between the VD group and the placebo group. Our result was in line with previous clinical trials[33,36,37,39,40]. However, in some other clinical trials, improvement of anthropometric measures and blood pressure occurred. It seems that there needs to be a long time to observe the treatment effect of VD supplementation on anthropometric measures. The inconsistent results could be due to the length of the studies and the type of supplementation. A meta-analysis of four randomized controlled trials found a reduction of 2.44 mmHg in systolic blood pressure in VD-treated subjects without any significant decrease in diastolic blood pressure[41]. Pfeifer *et al.*[42] reported a decrease of 9% in systolic blood pressure in severely VD-deficient women with VD and calcium supplementation. Some physiologic mechanisms for the effects of VD were explained, such as regulation of the renin–angiotensin system, and calcium absorption together with parathyroid hormone[41,43]. Well-designed clinical trials are needed to show whether or not there is a causal role of VD supplementation on decreasing blood pressure.

The strengths of the present study include its prospective, double-blind, placebo-controlled design and the sample metabolic biomarkers that were evaluated in younger healthy women. Consequently, our results may be more relevant to the population of postmenopausal women living in the community than studies focused on people who have specific diseases. The duration of the intervention and the dose administered were also sufficient to cause measurable increases in serum 25(OH)D values. This study also showed high adherence rates (92% of the participants), with no differences between the intervention groups (VD or placebo). Also, the use of HPLC for the measurement of 25(OH)D is more sensitive for detecting VD in plasma. Moreover, the present study has some limitations. The first is related to the representativeness of the sample. Since a group of postmenopausal women attending a public health service were studied, it can be assumed that they are periodically seen by medical professionals and have constant access to general healthcare. The second limitation is that our results cannot be generalized to other racial/ethnic groups, or for postmenopausal women with other diseases or who are frail and institutionalized.

In conclusion, the isolated and daily supplementation of 1000 IU vitamin D_3 for 9 months in younger postmenopausal women with VD deficiency was associated with a reduction in the MetS risk profile. The hypothesis that VD supplementation may lead to a reduction of metabolic biomarkers proposes a potential role for VD in the prevention and treatment of MetS.

Clinical trial registration

The study was registered at and approved by the Brazilian Clinical Trials Registry (ReBEC) under registration number RBR-4MHS32 [UTN number: U1111-1172-6922].

Potential conflict of interest No potential conflict of interest was reported by the authors.

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