Clinical research

No effect of vitamin D supplementation on cardiovascular risk factors in subjects with metabolic syndrome: a pilot randomised study

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Abstract

Introduction: Patients with metabolic syndrome (MetS) may have lower 25-hydroxyvitamin D (25(OH)VitD) serum levels compared with non-MetS individuals. Vitamin D (VitD) deficiency is associated with various cardiovascular disease (CVD) risk factors. Yet, the effect of VitD supplementation on MetS remains uncertain. Our aim was to examine the effect of VitD supplementation on CVD risk factors in MetS subjects.

Material and methods: This pilot study had a PROBE (prospective, randomised, open-label, blinded end-point) design. Fifty patients with MetS were included and randomised either to dietary instructions (n = 25) (control group) or dietary instructions plus VitD 2000 IU/day (n = 25) (VitD group) for 3 months. This study is registered in ClinicalTrials.gov (NCT01237769).

Results: In both groups a similar small weight reduction was achieved. In the VitD group serum 25(OH)VitD levels significantly increased by 91% (from 16.0 (3.0–35.0) to 30.6 (8.4–67.0) ng/ml, $p < 0.001$), while in the control group no significant change was observed (from 10.0 (4.0–39.6) to 13.0 (3.5–37.0) ng/ml). In both groups triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, fasting glucose, haemoglobin A_{1c}, homeostasis model assessment index and diastolic blood pressure did not significantly change. Systolic blood pressure decreased by 3.7% (from 134 ±14 to 129 ±13 mm Hg, $p = 0.05$) in the VitD group, while it decreased by 1.5% (from 132 ±13 to 130 ±16 mm Hg, p = NS) in the control group (p = NS between groups). In the VitD group serum 25(OH)VitD increase was negatively correlated with SBP decrease ($r = -0.398$, $p = 0.049$).

Conclusions: VitD supplementation (2000 IU/day) did not affect various CVD risk factors in patients with MetS.

Key words: vitamin D, metabolic syndrome, hypertension, cholesterol, triglycerides, glucose.

Introduction

During the last few years vitamin D (VitD) has attracted the interest of the scientific community since apart from its central role in bone homeostasis, recent studies suggest its implication in various cell func-

tions. Indeed, VitD receptors (VDRs) may control about 0.5–5.0% of the total human genome, i.e. 100–1250 genes [1].

VitD deficiency (defined as serum 25(OH)VitD < 20 ng/ml, according to the Institute of Medicine (IOM) estimations [2]), has emerged as a new "pandemic", as it may affect up to 50% of young adults and healthy children, accounting overall for up to one billion people worldwide, even in sunny areas. We also considered our patients as VitD deficient when having serum 25(OH)VitD < 20 ng/ml, although the "cut-point" value is a matter of discussion and some researchers regard it a common misconception [3]. This deficiency can be attributed to the modern sedentary lifestyle with reduced sun exposure [4], while obesity may be associated with one third of VitD deficient cases [5].

Interestingly, VitD deficiency has been associated with a wide range of diseases, such as cardiovascular disease (CVD), diabetes and malignancies as well as infective, autoimmune, and neurodegenerative diseases and even with overall mortality [6, 7].

In particular, metabolic syndrome (MetS) is often accompanied by VitD deficiency and low levels of serum 25(OH)VitD have been associated with metabolic disturbances in this setting [8–11], though not consistently [12–15]. Moreover, research regarding the effect of VitD supplementation on CVD risk factors in MetS subjects has not reached definite conclusions.

In this context we performed a pilot study testing the effect of VitD supplementation on various CVD risk factors (weight, body-mass index (BMI), waist circumference, systolic and diastolic blood pressure (BP)) and biochemical parameters (glucose homeostasis, lipids) in persons with MetS. This study is registered in ClinicalTrials.gov (NCT01237769).

Material and methods

Study population

This study had a PROBE (prospective, randomised, open-label, blinded end-point) design. All participants gave written informed consent before any clinical or laboratory evaluation and any dietary or drug therapeutic intervention. The study protocol was approved by the ethics committee of our institution and was conducted following the guidelines outlined in the Declaration of Helsinki.

Consecutive subjects with MetS were included. These subjects were diagnosed by fulfilling 3 or more of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) [16] criteria, when they visited the Outpatient Metabolic and Obesity Clinic of the University Hospital of Ioannina, Ioannina, Greece. Patients with dia-betes, chronic kidney or liver disease, triglycerides > 500 mg/dl (5.65 mmol/l) and intake of calcium and/or VitD supplements as well as lipid-lowering medications were excluded from the study. Patients with elevated blood pressure (BP) who did not receive treatment participated in the study, as well as patients with hypertension who received stable treatment for at least 3 months and whose BP levels normalised during the intervention.

Eligible patients were randomly allocated (through a computer-generated sequence of random numbers) by sex and age as baseline factors to either only dietary instructions (n = 25, Non-Suppl group) or to receive 2000 IU VitD/day (Vitamin D$_3$, Lamberts) along with dietary instructions (n = 25, VitD Suppl group) for 3 months. We administered 2000 IU VitD/day, a higher than usual dose but within safety limits, since former studies failed to find any significant changes in CVD risk factors when using usual VitD doses (400–800 IU/day). Supplementation of up to 2000 IU VitD daily has been deemed by the U.S. Food and Drug Administration's nutritional guidelines as more effective and safe [17]. Similarly, Endocrine Society clinical practice guidelines conclude that to raise serum 25(OH)VitD levels above 30 ng/ml, intakes of 1500 to 2000 IU/day may be required [18]. All patients (n = 50) followed a 12-week dietary intervention programme according to NCEP ATP III guidelines [16]. The compliance with dietary instructions was assessed by completing food diaries and through discussion during follow-up visits, while compliance with study medication was assessed by tablet count at week 12; patients were considered compliant if they took 80–100% of the prescribed tablets.

In order to minimise the effect of sunlight on 25(OH)VitD levels, all specimens were collected during March to September, a season during which the duration of sunlight is approximately similar in Greece. Blood pressure was measured 3 times in the right arm of patients after a 10-min rest in a sitting position using an electronic sphygmomanometer (WatchBP Office, Microlife WatchBP AG, Widnau, Switzerland) and calculated as the average of the second and third measurement.

Laboratory measurements

All laboratory assays were performed after an overnight fast and were blindly assessed regarding treatment allocation at baseline and 12 weeks after study onset. Serum 25(OH)VitD levels were measured quantitatively by an enzyme immunoassay method using the reagents from DRG Instruments GmbH kit (DRG, Marburg, Germany). The method's analytical sensitivity is 1.28 ng/ml and the intra- and inter-assay variation is 13% for each at the level of 18 and 16 ng/ml, respectively.

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Total cholesterol (TCHOL), triglycerides (TGs) and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically on an Olympus AU600 Clinical Chemistry analyser (Olympus Diagnostica, Hamburg, Germany). The Friedewald formula was used to calculate serum low-density lipoprotein cholesterol (LDL-C) (when triglycerides were < 350 mg/dl; 3.95 mmol/l). Serum insulin was determined on an AXSYM analyser by a microparticle enzyme immunoassay (Abbott Diagnostika, Wiesbaden-Delkenheim, Germany) with a coefficient of variation of 4.2% to 9.0%. The homeostasis model assessment (HOMA) index was calculated using the formula: fasting insulin (mIU/l) × fasting glucose (mg/dl)/405. Serum apolipoproteins AI (ApoAI) and B (ApoB) were measured by immunonephelometry on a Behring Nephelometer BN ProSpec (Dade-Behring, Lieberbach, Germany). The determination of HbA_{1c} was based on a latex agglutination inhibition assay (Randox Laboratories Ltd., Antrim, UK). HbA_{1c} values are expressed as percentage of the total haemoglobin concentration. The sensitivity of the method is 0.25 g/dl of HbA_{1c} and the within-run and between-run precision < 6.67% and < 4.82%, respectively.

Finally, serum parathyroid hormone (PTH) was measured by IMMULITE 2500 Intact PTH, a solid-phase, two-site chemiluminescent enzyme-labelled immunometric assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA 90045-6900 USA).

Statistical analysis

This was a pilot study and therefore formal power calculations were not performed. The evaluation of the distribution of each variable (Gaussian or not) was done with the Kolmogorov-Smirnov test. For the variables with a Gaussian distribution data are presented as mean and standard deviation and for those with a non-Gaussian distribution they are presented as median (range, min-max). For variables that did not follow a normal distribution log-transformation was first applied. However, a normal distribution was not obtained for any variable and therefore non-parametric tests were used. The paired samples *t*-test or the Wilcoxon signed ranks test was used to assess the effect of treatment in each group. For comparisons between treatment groups we used the analysis of covariance (ANCOVA) or the Kruskal-Wallis test for non-parametric variables, adjusted for baseline values, as appropriate. Correlations between parameters were evaluated using Spearman's correlation coefficient [19]. The significance was set at $p < 0.01$ due to multiple comparisons. All analyses were performed through the SPSS 18.0 statistical package for Windows (SPSS Inc., 1989-2004, Chicago, IL).

Results

The clinical and laboratory characteristics of study participants ($n = 50$) are shown in Table I.

No significant differences in baseline characteristics were noted between the 2 groups. There were also no differences in dietary intake between the groups at baseline or after the intervention (data not shown). Seventy-four percent of the participants were VitD deficient at baseline (25(OH)VitD < 20 ng/ml).

Three months after the intervention, a similar small weight reduction (1–2 kg) was achieved in both groups (Table II), implying poor compliance with dietary intervention in both groups (a 500 kcal/day reduction of energy for 3 months is expected to decrease body weight by up to 5–7 kg). In the VitD group, 25(OH)VitD levels increased by 91% (from 16.0 (3.0–35.0) to 30.6 (8.4–67.0) ng/ml, $p < 0.001$), while in the control group a non-significant increase by 30% (from 10.0 (4.0–39.6) to 13.0 (3.5–37.0) ng/ml) was seen. In both groups TCHOL, TGs, HDL-C, LDL-C, ApoA1, ApoB, fasting glucose, fasting insulin, HbA_{1c}, HOMA index and diastolic blood pressure did not significantly change. Systolic blood pressure (SBP) decreased by 3.7% (from 134 ±14 to 129 ±13 mm Hg, $p = 0.05$) in the VitD group, while it decreased by 1.5% in the control group (from 132 ±13 to 130 ±16 mm Hg, p = NS) (Table II). In the VitD group the increase of 25(OH)VitD levels was negatively correlated with the decrease of SBP ($r = –0.398$, $p = 0.049$).

Discussion

In this pilot study we found that VitD supplementation (2000 IU/day) in MetS subjects was not associated with any significant change in various CVD risk factors.

Several epidemiological studies have indicated an association between low 25(OH)VitD serum levels and MetS and/or its components [8–10, 20, 21], while others did not confirm these associations [12–15, 22]. Our MetS population was 74% VitD deficient at baseline (25(OH)VitD ~ 13 ng/ml). A recently published study in diabetic patients with MetS showed a high prevalence of VitD deficiency and an inverse correlation with glycaemic control and CVD risk factors, except for HDL-C, insulin resistance and obesity. The SBP was the only factor which could be predicted from VitD concentrations [23]. Moreover, a recent study concluded that the active VitD metabolite, 1,25(OH)$_2$VitD, acting like a potent hormone that binds to VDRs and regulates transcription of several genes, is also associated with MetS and its components (i.e. high triglycerides and low HDL-C) [24]. The same study showed inverse associations between 25(OH)VitD and MetS, triglycerides and waist circumference [24].

Table I. Baseline characteristics of study participants

Parameter	VitD suppl group	Non-suppl group	*P*-value
N	25	25	NS
Age [years]	52 ±9	51 ±12	NS
Sex (m/f)	15/10	11/14	NS
Smoke (yes/no)	4/21	6/18	NS
Weight [kg]	89 ±16	89 ±13	NS
BMI [kg/m²]	31.0 ±5.0	33.4 ±6.0	NS
Waist circumference [cm]	107 ±13	111 ±10	NS
SBP [mm Hg]	134 ±14	132 ±13	NS
DBP [mm Hg]	85 ±6	85 ±9	NS
TCHOL [mg/dl]	219 ±36	231 ±34	NS
HDL-C [mg/dl]	48 ±10	50 ±9	NS
LDL-C [mg/dl]	140 ±35	147 ±26	NS
TGs [mg/dl]	150 (56–336)	146 (84–339)	NS
Apo A1 [mg/dl]	136 ±26	143 ±13	NS
Apo B [mg/dl]	92 ±25	107 ±16	NS
Fasting glucose [mg/dl]	103 ±15	97 ±11	NS
Fasting insulin [mg/dl]	10.5 (5.9–19.7)	9.2 (2–19.8)	NS
HOMA index	2.5 (0.4–6.6)	2.6 (1.5–4.6)	NS
HbA$_{1c}$ (%)	6.2 ±0.8	6.0 ±0.5	NS
25(OH)VitD [ng/ml]	16 (3–35)	10 (4–40)	NS
PTH [pg/ml]	56 ±27	58 ±20	NS
MetS criteria (number of components)	3.4 ±2.0	3.0 ±2.0	NS

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCHOL – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, TGs – triglycerides, Apo – apolipoprotein, 25(OH)VitD – 25-hydroxy vitamin D, PTH – parathyroid hormone, MetS – metabolic syndrome, HOMA index = fasting insulin × fasting glucose/405. To convert values for triglycerides to mmol/l multiply by 0.01129. To convert values for cholesterol to mmol/l multiply by 0.02586. To convert values for glucose to mmol/l multiply by 0.05551. To convert values for 25(OH)VitD to nmol/l multiply by 2.5.

However, the "VitD-CVD hypothesis" in MetS subjects has not been confirmed by reversal of CVD risk factors through VitD supplementation in a number of studies. In our study a 91% increase in 25(OH)VitD serum levels was not associated with changes in lipids, carbohydrate metabolism parameters or DBP, while a 3.7% decrease in SBP (*p* = 0.05) was observed in the intervention group. In a study of 80 MetS subjects randomized to receive 50,000 IU VitD/week for 16 weeks a significant change was found in triglycerides but not in any other metabolic or anthropometric parameters [25].

A recent large study showed that oral high-dose VitD supplementation (initial dose of 200,000 IU followed a month later by 100,000 IU monthly or placebo) for a median of 3.3 years did not prevent CVD events. Researchers pointed out that a monthly dose may be less effective than daily or weekly doses in CVD prevention [26]. The Vitamin D and Omega-3 Trial (VITAL), a 5-year, randomized, placebo-controlled trial involving 20,000 U.S. people, is the only large study to date aiming to examine whether VitD supplementation (2000 IU/day) with or without addition of ω-3 fatty acids could play a role in the primary prevention of cardiovascular disease and cancer [27].

A small non-significant drop in SBP was noted in the VitD group and the increase in 25(OH)VitD levels was marginally associated with the decrease of SBP in this group. However, no difference was noted compared with controls. Observational studies have shown that the prevalence of hypertension is lower in sunny regions, while it increases with increasing distance from the equator [28]. In a meta-analysis, levels of 25(OH)VitD

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Table II. Clinical and laboratory characteristics at baseline and 3 months after intervention

Parameter	Baseline	3 months	Change (%)	P-value* vs. baseline	P-value* change between groups
Weight [kg]:					
VitD suppl group	89 ±16	88 ±17	−1.1	NS	NS
Non-suppl group	89 ±13	87 ±12	−2.2	0.01	
BMI [kg/m²]:					
VitD suppl group	31.0 ±5	30 ±5	−3.2	NS	NS
Non-suppl group	33.4 ±6	32 ±5	−4.1	0.008	
Waist circumference [cm]:					
VitD suppl group	107 ±13	106 ±13	−0.9	NS	NS
Non-suppl group	111 ±10	107 ±9	−3.6	0.002	
SBP [mm Hg]:					
VitD suppl group	134 ±14	129 ±13	−3.7	NS	NS
Non-suppl group	132 ±13	130 ±16	−1.5	NS	
DBP [mm Hg]:					
VitD suppl group	85 ±6	83 ±6	−2.3	NS	NS
Non-suppl group	85 ±9	82 ±10	−3.5	NS	
TCHOL [mg/dl]:					
VitD suppl group	219 ±36	224 ±37	+2.3	NS	NS
Non-suppl group	231 ±34	232 ±42	+0.4	NS	
HDL-C [mg/dl]:					
VitD suppl group	48 ±10	49 ±9	+2	NS	NS
Non-suppl group	50 ±9	49 ±10	−2	NS	
LDL-C [mg/dl]:					
VitD suppl group	140 ±35	145 ±34	+3.5	NS	NS
Non-suppl group	147 ±26	152 ±37	+3.4	NS	
TGs [mg/dl]:					
VitD suppl group	150 (56–336)	136 (46–261)	−9.3	NS	NS
Non-suppl group	146 (84–339)	131 (73–307)	−10.3	NS	
Fasting glucose [mg/dl]:					
VitD suppl group	103 ±15	102 ±23	−0.9	NS	NS
Non-suppl group	97 ±11	96 ±14	−1.0	NS	
Fasting insulin [μU/ml]:					
VitD suppl group	10.5 (5.9–19.7)	9.3 (3.1–27.9)	−11.4	NS	NS
Non-suppl group	9.2 (2–19.8)	8.4 (4.6–15.9)	−8.6	NS	
HOMA index:					
VitD suppl group	2.5 (0.4–6.6)	2.3 (0.7–11.5)	−8	NS	NS
Non-suppl group	2.6 (1.5–4.6)	1.8 (1.0–4.5)	−3	NS	
HbA$_{1c}$ (%):					
VitD suppl group	6.2 ±0.8	6.2 ±0.7	0	NS	NS
Non-suppl group	6.0 ±0.5	5.6 ±0.5	−6.6	NS	
25(OH)VitD [ng/ml]:					
VitD suppl group	16.0 (3.0–35.0)	30.6 (8.4–67.0)	+91	0.000	0.007
Non-suppl group	10.0 (4.0–39.6)	13.0 (3.5–37.0)	+30	NS	
PTH [pg/ml]:					
VitD suppl group	56 ±27	51 ±19	−9	NS	NS
Non-suppl group	58 ±20	48 ±19	−17	NS	

*P was considered significant if < 0.01. BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCHOL – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, TGs – triglycerides, Apo – apolipoprotein, 25(OH)VitD – 25-hydroxy vitamin D, PTH – parathyroid hormone, MetS – metabolic syndrome. To convert values for triglycerides to mmol/l multiply by 0.01129. To convert values for cholesterol to mmol/l multiply by 0.02586. To convert values for glucose to mmol/l multiply by 0.05551. To convert values for 25(OH)VitD to nmol/l multiply by 2.5.

were inversely associated with hypertension [29]. However, prospective studies show conflicting results, with some reporting that 25(OH)VitD levels could serve as a predictor of future hypertension, while others do not confirm this speculation [30, 31]. The recently published Kailuan study, involving 2456 underground miners, concluded that lower 25(OH)VitD levels were not related to a greater risk of incident hypertension [32]. However, a meta-analysis by these authors, including 7 prospective studies with 53,375 participants, showed a significant association between VitD deficiency and incident hypertension [32]. Various mechanisms have been proposed for interpreting the possible anti-hypertensive effect of VitD. Previous laboratory and animal experiments associated hypovitaminosis D with hypertension possibly through renin-angiotensin-aldosterone system (RAAS) activation (due to insufficient suppression of renin gene expression [33]). Results from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study showed that lower 25(OH)VitD and 1,25(OH)$_2$VitD levels are independently associated with up-regulated circulating RAAS [34]. Also, VitD may have a direct vascular effect, as implied by the presence of 1α-hydroxylase activity in vascular smooth muscle and endothelial cells, the presence of VDRs in endothelial cells and its protective role against calcification in vascular smooth muscle cells in mice models [35]. However, data from interventional studies and especially from RCTs investigating the effect of VitD supplementation on blood pressure are conflicting. A meta-analysis of 4 RCTs found a reduction of SBP by –2.44 mm Hg, but no effect on DBP [36], similarly to our findings. However, a meta-analysis by Witham *et al.* of 11 RCTs showed that administration of VitD and ultraviolet A and B radiation was associated with a non-significant SBP reduction by –3.5 mm Hg and a significant DBP reduction by –3.1 mm Hg [37]. On the other hand, a meta-analysis of 51 RCTs did not find significant effects of VitD supplementation on SBP or DBP [38]. In line with this is another meta-analysis of 46 RCTs (4541 participants) in which there was no significant effect of VitD supplementation on BP [39]. Larger RCTs targeting hypertensive patients with profound VitD deficiency are needed [40].

We found no significant change in carbohydrate metabolism indexes (fasting glucose, HbA$_{1c}$, HOMA index) after VitD supplementation in patients with MetS. The association of insulin resistance with hypovitaminosis D has been extensively studied, with contrasting findings. Some observational studies showed a positive correlation between 25(OH)VitD levels and insulin sensitivity [8, 41], while others did not [42]. The recently published IRAS Family Study cohort showed that plasma free 25(OH)VitD (the very small fraction of total VitD circulating unbound from VitD binding protein) appeared to have a modestly stronger relation to insulin sensitivity than the total form of serum 25(OH)VitD [43]. Most prospective studies found an inverse association between 25(OH)VitD levels and risk of insulin resistance and dysglycaemia [44–47]. Meta-analyses of prospective studies also argue for a significant association between hypovitaminosis D and incident diabetes [48, 49]. Several mechanisms have been proposed to explain the alleged association, including possible local regulation of pancreatic beta cell function [50] due to the presence of VDRs and 1α-hydroxylase, indirect effects through calcium homeostasis [51] or even VitD-induced stimulation of osteocalcin, which may improve insulin sensitivity [52]. Overall, studies have not proved a beneficial effect of VitD supplementation on optimizing glucose metabolism parameters [53]. Some but not all studies have shown that the potential benefits of VitD supplementation could be more prominent among pre-diabetic individuals [54, 55]. Moreover, most studies in type 2 diabetes found no effect of VitD supplementation on glycaemic outcome measures [56–58]. Overall, current literature does not support the use of VitD supplements for the prevention and/or treatment of diabetes.

In our study VitD supplementation did not have any effect on serum lipids or apolipoproteins. Several cross sectional studies have demonstrated an inverse relationship between VitD deficiency and lower HDL-C as well as higher triglyceride levels [59, 60]. Investigators speculated that 25(OH)VitD could affect lipid metabolism either directly or indirectly through alterations in parathyroid hormone and/or calcium concentrations [59]. Yet, interventional studies with VitD supplementation have led to conflicting results, with most showing that VitD supplementation might not be translated into clinically meaningful changes in lipid concentrations [61, 62]. A meta-analysis of 19 RCTs found no beneficial effect of VitD supplementation on lipid profile parameters [63].

To explain the difference between results of observational versus interventional studies it has been suggested that these associations may be confounded by shared metabolic risk factors rather than a causal relationship. Obesity alone is regarded as a causal risk factor for VitD deficiency [5]. The fat-soluble VitD could be sequestrated in the excessive body fat of obese persons, which could reduce the detectable serum levels of 25(OH)VitD [64]. In addition, obese patients have generally reduced physical activity and as a result limited sun exposure, which may lead to inadequate VitD skin synthesis. Moreover, some data suggest that an inflammatory process, which is

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usually present in these patients, might decrease 25(OH)VitD levels [65] and indirectly affect various metabolic parameters.

We noticed that parathyroid hormone (PTH) levels did not significantly change in either group (Table II). This finding is consistent with current literature which questions the utility of PTH measurements for identification of optimal VitD levels [3] and considers that the serum 25(OH)VitD and PTH relationship is inconsistent [2].

This study has certain limitations. It was a pilot study with a small number of participants. Therefore, unequivocal conclusions cannot be reached. Another limitation is that the supplementation dose (2000 IU/day) and duration (3 months) may be inadequate to treat VitD deficiency given that subjects had very low 25(OH)VitD levels at baseline. According to previous suggestions, concentrations of at least 35–60 ng/ml would be necessary [66], while in our active treatment group VitD levels only reached 30.6 ng/ml.

In conclusion, we found that oral VitD supplementation (2000 IU/day) in patients with MetS did not affect various CVD risk factors, in line with most previous interventional studies.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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