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Effect of vitamin D3 supplementation and influence of BsmI polymorphism of the VDR gene of the inflammatory profile and oxidative stress in elderly women with vitamin D insufficiency Vitamin D3 megadose reduces inflammatory markers

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ABSTRACT

Objective: This study aimed to evaluate the effect of vitamin D3 megadose supplementation and influence of BsmI polymorphism in the VDR gene on the inflammatory profile and oxidative stress in elderly women with vitamin D deficiency.
Methods: A double blind, randomized, placebo-controlled trial was conducted with 40 elderly women (aged 68 ± 6 years) diagnosed with vitamin D insufficiency (24.7 ± 3.1 ng/mL). Participants were distributed into a supplementation group that received 200,000 IU of vitamin D3 (SG; n = 20) and a placebo group (PG; n = 20). Blood samples were collected at baseline and after intervention to analyse the 25(OH)D, parathyroid hormone, serum calcium, ultra-sensitive C-reactive protein (us-CRP), alpha 1-acid glycoprotein (AGP-A), total antioxidant capacity (TAC), and malondialdehyde (MDA) levels, as well as the renal and hepatic function, and genotyping was performed for BsmI polymorphism.
Results: Four weeks after supplementation, elderly women in the SG group showed a significant increase in the serum concentration of 25(OH)D (25.29 ± 2.8 to 31.48 ± 6.0; p = 0.0001), which was followed by increased TAC (65.25 ± 15.66 to 71.83 ± 10.71; p = 0.03) and decreased serum PTH (46.32 ± 13.2 to 35.45 ± 11.0; p = 0.009), us-CRP (0.38 ± 0.3 to 0.19 ± 0.1; p = 0.007) and AGP-A levels (75.3 ± 15.4 to 61.1 ± 5.9; p = 0.005). Changes in BP, ANAC and MDA were not observed. The 25(OH)D and PTH, us-CRP and AGP-A levels of participants with the BB/Bb genotype were more responsive to supplementation, but their other markers did not change.
Conclusions: Supplementation with a vitamin D3 megadose reduced inflammatory markers and increased the total antioxidant capacity in elderly women with vitamin D insufficiency. The 25(OH)D, PTH, us-CRP and AGP-A levels of elderly patients with the BB/Bb genotype were more responsive to supplementation compared with those with the bb genotype.

1. Introduction

Researchers have described vitamin D deficiency as a worldwide epidemic (Hossein-nezhad and Holick, 2013). The elderly are most at risk for this deficiency due to special features (van Schoor and Lips, 2011). According to Holick et al. (2011), 20% to 100% of American, Canadian and European elderly suffer from vitamin D deficiency, and this condition is also common in Australia, Middle East, India, Africa and South America.

Vitamin D deficiency is classically associated with diseases related to bone metabolism. Over the past few years, studies have identified associations between low levels of vitamin D and extra-skeletal conditions, such as cardiovascular diseases, hypertension, diabetes and insulin metabolism, autoimmune disorders, metabolic syndrome and obstructive sleep apnoea (Dobnig, 2011; Christakos et al., 2013; Song and Park, 2013; Mete et al., 2013).

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The nuclear receptor of vitamin D (VDR) is a member of the transcription factor superfamily (Haussler et al., 1998) that binds to 1,25-dihydroxyvitamin D (1,25(OH)2D3) and mediates its biological processes. The discovery that most body tissues and cells contain vitamin D receptors (VDR) and some contain enzymatic equipment for converting the inactive form (25(OH)D) into the active form (1,25(OH) 2D) has implicated new functions for this vitamin (Christakos et al., 2013). Polymorphisms in the VDR have been studied as possible factors involved in the predisposition to diseases.

The VDR rs1544410 is an adenine/guanine (A/G) substitution located in the 3′UTR region of intron 8 that does not alter the structure and function of the VDR but is strongly related to the poly (A) tail, potentially affecting the stability of mRNA (Vuolo et al., 2012). This polymorphism has been examined as a possible genetic marker in several important clinical conditions, such as type 1 diabetes mellitus (Xiao et al., 2006; Panierakis et al., 2009), obesity (Al-Daghri et al., 2014) and some specific cancers (Bai et al., 2012; Mostowska et al., 2013). Polymorphisms of the VDR gene, including BsmI, are also associated with different responses to vitamin D supplementation (Elnenaei et al., 2011).

Recently, some studies have shown that vitamin D supplementation beneficially affects blood pressure (Witham et al., 2009; Judd et al., 2010), improves insulin sensitivity (Talaei et al., 2013), and reduces inflammation and oxidative stress biomarkers (Chen et al., 2014; Sharifi et al., 2014). However, these data remain controversial, and other studies have demonstrated the negative effects of supplementation on extra-skeletal conditions (Ponda et al., 2012; Witham et al., 2013).

With regard to the beneficial effects of vitamin D supplementation on inflammatory and oxidative stress biomarkers, a study of colorectal cancer patients showed reduced serum levels of CRP, TNF, IL6 and IL8 after vitamin D supplementation (Hopkins et al., 2011). Vitamin D supplementation also reduced the us-CRP and malondialdehyde (MDA) levels in patients with non-alcoholic liver disease (Sharifi et al., 2014). Studies that included older adults have shown conflicting results. In haemodialysis patients, supplementation with cholecalciferol resulted in decreased us-CRP levels (Matias et al., 2010); however, the inflammatory markers did not improve in non-diabetic individuals (Pittas et al., 2007).

Although the effects of vitamin D on human health have received considerable attention in recent years, available data are limited and controversial, which precludes the assessment of the extra-skeletal effects of this vitamin in the elderly.

In this sense, the present study aimed to evaluate the effect of vitamin D3 megadose supplementation and the influence of the BsmI polymorphism of the VDR gene on the inflammatory and oxidative stress biomarkers in elderly women with vitamin D insufficiency.

2. Materials and methods

2.1. Subjects

The serum vitamin D levels of 142 elderly individuals belonging to social groups at the Centres for Reference and Citizenship, Program of Care for the Elderly (PAPI) of the City Hall of João Pessoa/PB, Brazil were evaluated. Of these subjects, 58 (54 females and 4 males) were diagnosed with vitamin D insufficiency. We selected to monitor the elderly women to maximize the homogeneity of the evaluated group and increase the rate of participation. The study was conducted from December 2013 to March 2014. Older women diagnosed with vitamin D insufficiency according to criteria adopted by the Endocrine Society (Holick et al., 2011) were selected to participate in a phase II, double-blind, randomized and placebo-controlled clinical trial.

The inclusion criteria were the following: female; age ≥ 60 years; diagnosis of vitamin D insufficiency/deficiency; no use of vitamin D supplements, anti-inflammatory medications, anticonvulsants or HIV/AIDS treatments; no diagnosis of nephrotic syndrome, acute or chronic renal failure, liver disease, hypothyroidism, hyperthyroidism, history of cerebrovascular accident (CVA) or acute myocardial infarction (AMI) in the last six months; not an alcoholic or chronic smoker; preserved cognitive status and agreement to participate in the study. Volunteers who ingested vitamin D supplements or other antioxidant vitamins; initiated anti-inflammatory drug, anticonvulsant or HIV/AIDS drug treatment; changed food intake or were exposed to sun during the study were excluded from the study.

The clinical trial was approved by the Research Ethics Committee of the Federal University of Paraíba (UFPB), Centre for Health Sciences (CCS) under protocol number 0374/12. All participants provided informed consent by signing the free and informed consent form (IC) of the National Health Council according to Resolution 466 of December 12, 2012. The trial was registered at ClinicalTrials.gov under identification number CT02222649.

2.2. Sample calculation

The sample size was calculated based on a sample calculation for experimental studies proposed by Eng (2003). To this end, the effect of vitamin D supplementation on the us-CRP level described in the study by Hopkins et al. (2011) was used, which evaluated the effect of vitamin D and calcium supplementation on inflammatory biomarkers of colorectal adenoma patients and found an effect size of 0.66. By adopting an alpha error of 0.05 and beta statistical power of 0.80, a minimum sample size of 16 subjects per group was determined.

This study included 40 elderly women who were randomly allocated into 2 groups of 20 subjects; one group received vitamin D3 megadose supplementation (69.35 ± 6.6 years) and the other received a placebo (67.30 ± 5.0 years). All participants completed the study.

2.3. Study design

The study was monitored in two stages. At baseline, a questionnaire was administered to collect information on the skin phototype and sun exposure and conduct a blood pressure (BP), cardiac autonomic nervous (ANAC), nutritional assessment. Blood was also collected to measure the serum 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (PTH), ultra-sensitive C-reactive protein (us-CRP), alpha 1-acid glycoprotein (AGP-A), total antioxidant capacity (TAC), malondialdehyde (MDA), alanine transaminase (ALT), aspartate transaminase (AST), urea, creatinine, and calcium levels at baseline. Capsules containing the supplement or placebo were then distributed to the groups. The same variables were evaluated four weeks after the intervention.

2.4. Supplementation protocol

The vitamin D and placebo supplements were formulated by Herva LTDA (CNPJ 70.109.475/0001-04), which provided a certificate of quality control analysis. The vitamin D and placebo capsules were identical in appearance and could only be differentiated by the package coding. Each vitamin D capsule delivered 50,000 IU of cholecalciferol; thus, four capsules were administered for a total dose of 200,000 IU cholecalciferol per day. The same procedure was adopted for the placebo supplementation.

Participants were told to not change their usual food intake, sun exposure and physical activity, and they were also instructed not to ingest any supplement containing vitamin D or other antioxidant vitamins until the end of the study period. They were also told to inform the research team about the diagnosis of new diseases and the use of new medical therapies.

2.5. Skin phototype and sun exposure

The skin phototype was stratified according to Fitzpatrick's (1988) classification, which classifies the tolerance of the skin to ultraviolet light from white (I) to black (VI) based on a personal interview. Sun exposure was defined as the average time of sun exposure per day, and seasonal variations were not considered because the study was conducted during the summer.

2.6. Blood pressure and cardiac autonomic nervous assessment

The blood pressure was measured with an indirect method, i.e., the auscultation technique, using a properly calibrated Welch Allyn DS44 aneroid sphygmomanometer (New York, USA). Three measurements were performed with an interval of 5 min between measurements. The mean of the last two measurements was considered, as proposed by the VI Guidelines on Hypertension (SBC/SBH/SBN, 2010).

The autonomic activity was determined by recording the variability of the R–R heart rate interval with a Polar ® heart rate monitor, model RS800CX (Kempele, Finland). The instrument was validated by recording an electrocardiogram at rest and during exercise (Nunan et al., 2008; Porto and Junqueira, 2009). The R–R heart rate intervals were recorded with participants in the resting condition for 10 min, and the measurement lasted at least 5 min. The data were analysed using the HRV Kubios Software version 2.0 (University of Kuopio, Finland).

2.7. Nutritional assessment

The patients were weighed using an electronic digital scale (Filizola, Luminamea 02 550). The procedure was performed in triplicate. The height was determined with accuracy of 1 mm and precision of 0.5 cm using a stadiometer (Sanny®, Caprice ES2060). As proposed by the World Health Organization (WHO, 1998) the Body Mass Index (BMI) was adopted to assess the nutritional status. The waist circumference (WC) and hip circumference (HC) were measured in duplicate (Callaway et al., 1988; Keenan et al., 1992).

The dietary intake of participants was assessed at baseline and four weeks after supplementation based on a quantitative food frequency questionnaire validated using three 24-hour recalls applied at different time intervals to a population of women in the city of João Pessoa/PB (Lima et al., 2007, 2008). This questionnaire was used to quantify the weekly frequency of consumption of fish and whole and skim milk. The consumption of fish was classified as follows: never, sometimes (1–3×/week) and frequently (>4×/week). The consumption of milk was categorized on a daily basis (Neves et al., 2012).

2.8. Biochemical analysis

Fifteen millilitres of blood was collected in the morning after a 12-hour fasting period to analyse the 25(OH)D, PTH, us-CRP, AGP-A, MDA, TAC, calcium, urea, creatinine, ALT and AST levels. The blood samples were centrifuged at 3000 rpm for 15 min, and the supernatant was transferred to micro-tubes and frozen at −20 °C until the analyses.

The serum 25(OH)D level was determined using HPLC (high-performance liquid chromatography), and the PTH dosage was determined using an immunometric chemiluminescent assay. The serum us-CRP and AGP-A levels were measured using the immunonephelometric method. The oxidant activity was determined based on the plasma malondialdehyde (MDA) levels, which were measured by reacting thiobarbituric acid (TBARS) with products from the decomposition of hydroperoxides, as described by Ohkawa et al. (1979). The total antioxidant capacity was evaluated with the DPPH method as described by Brand-Williams et al. (1995). The calcium, urea, creatinine, AST and ALT levels in the serum samples were measured using commercial kits from Labtest (Minas Gerais, Brazil) according to the manufacturer's recommendations in automatic LabMax 240 premium analyser (Lagoa Santa — MG, Brazil).

2.9. Determination of BsmI genotype of the VDR gene by CRP/RFLP

Buccal epithelial cell samples were obtained with a 3% sucrose rinse. The genomic DNA was extracted according to a method described by Aidar and Line (2007).

The BsmI genotype of the VDR gene was determined by CRP/RFLP using 5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′ and 5′-AACCAG CGGGAAGTCAAGGG-3′ primers. The annealing temperature was 58 °C. The resulting fragment of 870 bp was digested with BsmI at 37 °C for 12 h. The fragments were visualized on a 1.5% agarose gel. The B allele was not cleaved and remained with the 870 bp fragment. The presence of the restriction site generates two fragments of 640 and 230 bp (b allele). The BsmI polymorphism was analysed in 38 individuals, and 2 samples were lost due to problems with DNA extraction.

2.10. Statistical analysis

The data are presented as the mean and standard deviation. The normality and homogeneity of variance were analysed using the Shapiro–Wilks and Levene tests. An independent Student's t test was used to compare the baseline and final values between the vitamin D3 and placebo groups. A paired t test was used to evaluate possible differences between the pre- and post-intervention periods. Whenever necessary, independent and paired t tests were replaced with nonparametric Mann–Whitney and Wilcoxon tests, respectively. Statistical significance was defined as $p < 0.05$. Statistical analyses were performed using the GraphPadInstat software version 3.0 (GraphPad San Diego, CA, USA).

3. Results

The vitamin D3 (Vit D3) and placebo (PLA) groups were statistically similar in age, weight, BMI and basal serum 25(OH)D levels. The distribution of skin phototypes and sun exposure was also similar between groups. Volunteers from both groups had normal blood pressure, were overweight and had a high waist circumference, as shown in Table 1. Food intake remained unchanged during the intervention period, and the intake of foods fortified with vitamin D was not reported.

Table 1
Baseline characteristics of vitamin D3 and placebo groups.

	VIT D3 (n = 20)	PLA (n = 20)	p value
25(OH)D (ng/mL)	25.29 ± 2.8	26.16 ± 3.4	0.39
Age (years)	69.35 ± 6.6	67.30 ± 5.0	0.28
Weight (kg)	62.99 ± 11.7	65.69 ± 11.0	0.46
Stature (m)	1.51 ± 0.05	1.50 ± 0.04	0.69
BMI (kg/m^2)	27.58 ± 4.9	29.06 ± 5.0	0.10
WC (cm)	88.74 ± 9.9	92.05 ± 10.8	0.32
HC (cm)	101.44 ± 10.3	104.74 ± 8.9	0.22
WHR	0.87 ± 0.05	0.87 ± 0.07	0.90
WSR	0.58 ± 0.06	0.61 ± 0.07	0.25
SBP (mm Hg)	133.15 ± 15.02	126.35 ± 18.23	0.20
DBP (mm Hg)	77.25 ± 10.30	79.1 ± 9.00	0.55
Skin phototype (%)			
I, II and III	60	60	
IV, V and VI	40	40	
Mean sun exposure (min/day)	35.5 ± 17.5	37 ± 17.5	0.67

Data shown as the mean ± standard deviations. VIT D3 — group supplemented with vitamin D3; PLA — placebo group; 25(OH)D — serum 25-hydroxyvitamin D; BMI — body mass index; WC — waist circumference; HC — hip circumference; WHR — waist to hip ratio; WSR — waist to stature ratio; SBP — systolic blood pressure; DBP — diastolic blood pressure. There is no significant difference between groups (independent t test or Mann–Whitney).

Importantly, Brazilian public policy does not establish vitamin D fortification in foods.

The supplementation protocol did not alter the resting blood pressure or autonomic modulation in both the vitamin D3 and PLA groups (Table 2).

Vitamin D3 megadose supplementation did not produce hepatic or renal disorders. The baseline values were similar between the supplemented and placebo groups, and the ALT, AST, urea, creatinine and uric acid levels did not statistically differ (Table 2).

As shown in Table 2, the serum (OH)D levels significantly increased in the VIT D3 group compared with the PLA group four weeks after intervention ($p = 0.0001$). Additionally, this value remained higher in the supplementation groups at the end of the study ($p = 0.0003$). This increase in the serum 25(OH) levels in the supplemented group was accompanied by a significant decrease in the serum PTH levels ($p = 0.009$), which did not occur in the placebo group. Intervention did not significantly affect the serum calcium levels in either of the groups.

Regarding inflammatory markers, the serum us-CRP ($p = 0.007$) and AGP-A ($p = 0.005$) levels significantly decreased in the VIT D3 group and did not change in the PLA group. The oxidative stress and antioxidant markers, as evaluated by TAC, significantly increased in the VIT D3 group ($p = 0.03$) but not the PLA group. Moreover, the oxidant marker MDA did not significantly change in either group (Table 2).

Individuals were classified according to genotype to evaluate the effect of vitamin D supplementation according to the BsmI polymorphism in the VDR gene. In the supplemented group, 63.2% (n = 12) of elderly patients exhibited the BB/Bb genotype, and 36.8% (n = 7) exhibited the bb genotype. In the placebo group, 47.4% (n = 9) of individuals exhibited the BB/Bb genotype and 52.6% (n = 10) exhibited the bb genotype.

Supplementation significantly increased the serum 25(OH)D levels in BB/Bb individuals ($p = 0.009$) but not bb individuals. The serum PTH levels significantly decreased in BB/Bb individuals ($p = 0.003$) but not bb individuals. The inflammatory markers us-CRP and AGP-A decreased significantly more ($p = 0.04$ and $p = 0.03$, respectively) in BB/Bb individuals. The other variables did not significantly differ (Table 3).

Table 3
Effect of vitamin D3 supplementation according to genotype.

	BB/Bb VIT D3 pre	BB/Bb VIT D3 post	bb VIT D3 pre	bb VIT D3 POST
	(n = 12)		(n = 07)	
25(OH)D (ng/mL)	25.49 ± 2.9	31.98 ± 7.1[a]	25.58 ± 4.6	29.64 ± 3.3
PTH (pg/mL)	51.06 ± 10.5	34.86 ± 11.5[a]	36.6 ± 15.1	39.72 ± 10.1
Calcium (mg/dL)	8.38 ± 1.0	8.9 ± 0.6	8.4 ± 0.9	8.93 ± 0.9
Us-CRP (mg/L)	0.44 ± 0.4	0.2 ± 0.1[a]	0.29 ± 0.2	0.18 ± 0.1
AGP-A(mg/dL)	75.16 ± 17.3	59.5 ± 6.5[a]	75.5 ± 14.5	63.5 ± 4.6
MDA (μmol/L)	3.87 ± 1.9	3.90 ± 1.2	3.45 ± 1.7	4.15 ± 0.8
TAC (% inhibition)	66.65 ± 16.6	70.2 ± 11.1	61.9 ± 16.9	72.61 ± 9.9

Data shown as the mean ± standard deviations. VIT D3 — group supplemented with vitamin D3; PLA, placebo group; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; us-CRP, C-reactive protein ultrasensitive; AGP-A, alpha 1 acid glycoprotein; MDA, malondialdehyde; TAC, total antioxidant capacity.
[a] Intragroup significance — pre and post supplemented groups.

4. Discussion

This study demonstrated that 4 weeks of supplementation with 200,000 IU of vitamin D3 administered as a single dose increased the serum 25(OH)D levels and total antioxidant capacity and significantly reduced the us-CRP and AGP-A levels in elderly women with vitamin D insufficiency. The serum 25(OH)D, PTH, us-CRP and AGP-A levels of individuals with the BB/Bb genotype were more responsive to supplementation.

The high prevalence of vitamin D deficiency and insufficiency reported in several populations (Holick et al., 2011), including in tropical populations (Maeda et al., 2014), seems to indicate that food and sun exposure are not sufficient to maintain adequate levels of this vitamin. The Endocrine Society (Holick et al., 2011) has suggested that supplementation doses should reach 50,000 IU of vitamin D2 or D3 for individuals with vitamin D deficiency. Several studies have proposed much higher doses, which varied between 100,000 and 600,000 IU in single or multiple administrations. The single megadose used in the supplementation protocol of this study corresponds to 200,000 IU and significantly increased the serum 25(OH)D levels in the absence of toxicity effects, corroborating the findings of other studies (Tellioglu et al., 2012; Witham et al., 2013).

Previous studies have shown that vitamin D supplementation can reduce the blood pressure (Witham et al., 2009; Judd et al., 2010) and improve insulin sensitivity (Talaei et al., 2013), phenomena that can be at least partially explained by the reduction of oxidative stress (Sharifi et al., 2014) and systemic inflammation (Chen et al., 2014).

Table 2
Biochemical and haemodynamic data of vitamin D3 and placebo groups at baseline and after intervention.

	VIT D3 pre	VIT D3 post	PLA pre	PLA post
	(n = 20)		(n = 20)	
25(OH)D (ng/mL)	25.29 ± 2.8	31.48 ± 6.0[a,b]	26.16 ± 3.4	24.42 ± 3.8
PTH (pg/mL)	46.32 ± 13.2[c]	35.45 ± 11.0[a]	34.19 ± 10.0	38.15 ± 14.9
Calcium (mg/dL)	8.42 ± 0.4	8.93 ± 0.7	8.73 ± 1.0	8.92 ± 0.7
us-CRP (mg/L)	0.38 ± 0.3	0.19 ± 0.1[a]	0.35 ± 0.3	0.30 ± 0.3
AGP-A (mg/dL)	75.3 ± 15.4	61.1 ± 5.9[a]	70.84 ± 14.0	65.30 ± 11.9
MDA (μmol/L)	3.76 ± 1.76	3.97 ± 1.11	3.91 ± 1.9	4.47 ± 1.17
TAC (%)	65.25 ± 15.6	71.83 ± 10.7[a]	69.10 ± 15.9	69.89 ± 7.4
Urea (mg/dL)	35.8 ± 8.5	32.5 ± 10.4	32 ± 7.6	31.55 ± 6.7
Creatinine (mg/dL)	0.63 ± 0.2	0.67 ± 0.2	0.63 ± 0.2	0.69 ± 0.2
Uric acid (mg/dL)	4.32 ± 1.4	4.13 ± 1.4	4.10 ± 1.4	4.00 ± 1.3
ALT (U/L)	15.95 ± 10.7	16.7 ± 6.4	14.45 ± 8.6	20.35 ± 10.4
AST (U/L)	31.6 ± 11.3	31.8 ± 11.7	30.35 ± 7.5	30.55 ± 12.7
SBP (mm Hg)	133.15 ± 15.0	137.15 ± 21.2	126.35 ± 18.2	135.3 ± 24.9
DBP (mm Hg)	77.25 ± 10.3	76 ± 9.2	79.1 ± 9.0	76.8 ± 9.2
LF (ms^2)	611.4 ± 1614.5	556.1 ± 1791.6	286.1 ± 495.2	365.6 ± 607.6
HF (ms^2)	308.5 ± 681.8	610.15 ± 2072.3	181.5 ± 277.8	268.9 ± 475.5
LF/HF	2.4175 ± 1.7	1.9565 ± 1.7	2.4645 ± 1.6	2.4865 ± 1.8

Data shown as the mean ± standard deviations. VIT D3 — group supplemented with vitamin D3; PLA, placebo group; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; us-CRP, C-reactive protein ultrasensitive; AGP-A, alpha 1 acid glycoprotein; MDA, malondialdehyde; TAC, total antioxidant capacity; ALT, alanine amino transferase; AST, aspartate aminotransferase; SBP, systolic blood pressure; DBP, diastolic blood pressure; LF, low frequency; HF high frequency.
[a] Intragroup significance — pre and post supplemented group.
[b] Significance between groups — post supplemented with placebo.
[c] Significance between pre supplemented and pre placebo.

However, reports have also indicated the ineffectiveness of vitamin D supplementation for these purposes. Moreover, these benefits are not confirmed in the elderly populations investigated in this study.

The effect of supplementation on inflammatory markers was assessed. Matias et al. (2010) observed a significant reduction in the serum us-CRP levels at the one-year follow-up of vitamin D-deficient haemodialysis patients who received high doses of vitamin D3 supplementation. Similarly, Sharifi et al. (2014) administered vitamin D3 doses of 50,000 IU every 14 days for four months to patients with liver disease and found a reduction in the us-CRP levels. Our data corroborate these findings and also demonstrate the anti-inflammatory effect of vitamin D supplementation in the elderly. However, Witham et al. (2013) found no reduction in the us-CRP levels after 8 weeks of supplementation with a single dose of 100,000 IU vitamin D3 in women in southern Asia. Pittas et al. (2007) also found no reduction in the CRP levels, although their study examined smaller daily doses in conjunction with calcium supplementation.

The plasma levels of an acute-phase inflammatory marker, alpha 1-acid glycoprotein (AGP-A), were also analysed, and a significant reduction was observed in the vitamin D3 group. AGP-A is directly associated with the most important pro-inflammatory cytokines because its secretion is regulated by interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumour necrosis factor α (TNF-α), which are important mediators of the inflammatory and immune response (Fournier et al., 2000). In addition, examining this marker is inexpensive, which enables its routine analysis in the evaluation of patients in clinical practice.

The literature is inconclusive about the effect of vitamin D supplementation on the inflammatory process. Experimental protocols have attempted to homogenize the sample, but variations persist, and conflicting results have been found. These discrepant results may be due to the effect of genetic polymorphisms that may modulate the response to supplementation (Gagnon et al., 2014). VDR gene variants have been linked to different response patterns to vitamin D supplementation and other therapies (Poon et al., 2012; Neyestani et al., 2013; Serrano et al., 2013). The differential activity of the VDR protein has been partially related to variations in the VDR gene (Poon et al., 2012). Therefore, we sought a homogenous sample and evaluated the effect of supplementation as a function of the genotype distribution of the VDR BsmI polymorphism.

Corroborating the findings of this study, Elnenaei et al. (2011) found that BB/Bb genotype postmenopausal women were more responsive to vitamin D supplementation. In contrast, the skeletal parameters of young healthy girls treated with vitamin D for one year were lower in the BB genotype (Arabi et al., 2009). Because VDR is involved in the regulation of many genes, different biological responses can be identified for the same genotype (Poon et al., 2012).

The physiological impact of 1,25(OH)2D3 is not restricted to the homeostasis of calcium and phosphate. VDR has been demonstrated to be involved in the decreased activation of the pro-inflammatory transcription factor NF-κB, which suggests that VDR plays an intrinsic inhibitory role in inflammation (Szeto et al., 2007; Wu et al., 2010; Chen et al., 2013). Some studies have demonstrated that the VDR BsmI genotype may influence the inflammatory marker profile and the anti-inflammatory response to treatment with vitamin D. In Italian patients undergoing haemodialysis and active vitamin D (calcitriol) treatment, Pacini et al. (2008) demonstrated that the presence of a BsmI restriction site (b allele) was more frequent in patients with elevated serum CRP levels and suggested that the presence of this allele could be considered a risk factor in the pathogenesis of inflammation. Similar findings were described by Punzi et al. (2012): the b allele was associated with higher CRP levels in cachectic cancer patients, which is an early clinical predictor of the development of the most aggressive form of cachexia.

Recently, 25(OH)D3 supplementation was shown to significantly inhibit the differentiation of healthy and T1DM human monocytes into dendritic cells and increase the number of intermediate cells (IC) in vitro, demonstrating an immunomodulatory effect rs1544410 genotype dependent; bb leads to a smaller increase in IC after supplementation with 25(OH)D3 compared to Bb and BB. Therefore, sensitivity to 25(OH)D3 increases in the following order: bb, Bb and BB (Mauf et al., 2014). Our results are in line with this assumption; both inflammatory markers, CRP and AGP-A, were less responsive to supplementation in elderly women with the bb genotype. This finding is the first from a clinical trial to support the hypothesis that the VDR genotype affects the vitamin D anti-inflammatory effect. However, one of the limitations of this study was the sample size. Therefore, additional studies should be conducted to better assess these inflammatory markers and genotypic profiles in a broader sample.

Wiseman (1993) first described the antioxidant role of vitamin D in 1993, showing that vitamin D3 and its active form 1,25(OH)2D3 inhibited iron-dependent liposomal lipid peroxidation. Other studies have recently demonstrated the antioxidant properties of this vitamin. Tarcin et al. (2009) demonstrated that the administration of 300,000 IU of vitamin D3 per month for 3 months in patients with vitamin D deficiency significantly decreased the MDA levels. Sharifi et al. (2014) evaluated the effect of supplementation with 50,000 IU of vitamin D3 every 14 days for four months in adults with non-alcoholic fatty liver disease and also observed a reduction in the plasma MDA levels. In these studies, high-dose or high-frequency supplementation resulted in decreased lipid peroxidation. Our data show that the single administration of a high dose does not seem to be sufficient to promote a reduction in lipid peroxidation.

Despite the absence of a reduction in lipid peroxidation, apparently contradictory results were found: the total antioxidant capacity of supplemented elderly women significantly increased. This result corroborates the findings of Asemi et al. (2013), who found an increase in the total antioxidant capacity and total glutathione in pregnant women in response to supplementation. The increase in the total antioxidant capacity without the expected reduction in lipid peroxidation is difficult to explain based on the data of this study. However, Sharifi et al. (2014) observed a reduction in the MDA levels with no change in the total antioxidant capacity. Thus, this relationship should be further investigated.

Vitamin D supplementation has been associated with beneficial effects on blood pressure. Forman et al. (2013) studied the effect of supplementation with 1000, 2000 and 4000 IU/day of vitamin D3 for three months in 250 black adults and observed −0.66 mm Hg, −3.4 mm Hg and −4.0 mm Hg decreases in the systolic blood pressure, respectively. Nasri et al. (2014) evaluated the effects of vitamin D3 supplementation on blood pressure in diabetic patients receiving 50,000 IU/week of vitamin D3 for 12 weeks. This treatment significantly reduced the systolic and diastolic blood pressures compared with the control group.

Judd et al. (2010) compared the effects of a similar vitamin D3 megadose of 200,000 IU for three weeks and 0.5 μg of calcitriol twice per day for one week. The blood pressure did not decrease in the placebo and vitamin D3 groups; individuals who received calcitriol showed a 9% reduction in the systolic blood pressure (SBP) compared with the placebo group, but the SBP returned to baseline pre-treatment levels one week after the completion of calcitriol therapy.

Corroborating data obtained by Judd et al. (2010), this study showed that vitamin D3 megadose supplementation did not affect the resting blood pressure. Similarly, Witham et al. (2013) evaluated vitamin D3 supplementation with 100,000 IU and also found no reduction in blood pressure.

These differences in the responses cannot be conclusively explained based on the present study. However, these results suggest that the continuous long-term supplementation with smaller vitamin D doses would be more effective in lowering the blood pressure than a single megadose, and further studies should be carried out to examine this issue.

In short, data from this study showed that vitamin D supplementation increased the serum 25(OH)D levels, and individuals with the BB/

Bb allele were more responsive to supplementation. Reductions in the us-CRP and AGP-A levels and increases in the total antioxidant capacity were also observed. The anti-inflammatory effect was significant in BB/Bb carriers. Despite the limitations of our study, we demonstrated connections between genetic and nutritional factors, as evidenced by the different responses to supplementation, and these findings may contribute to the adoption of more individualized treatments.

Considering the beneficial effects of vitamin D supplementation and the influence of the BsmI polymorphism of the VDR gene on the response to this supplementation, further long-term studies of larger sample sizes distributed by genotype should be conducted to elucidate the genetic interactions and inflammatory and oxidative stress markers after vitamin D supplementation in the elderly.

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