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ORIGINAL CONTRIBUTION

Is a lower dose of vitamin D supplementation enough to increase 25(OH)D status in a sunny country?

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Abstract
Background Calcium and vitamin D are essential nutrients for bone metabolism Vitamin D can either be obtained from dietary sources or cutaneous synthesis. The study was conducted in subtropic weather; therefore, some might believe that the levels of solar radiation would be sufficient in this area.
Aim of the study To evaluate calcium and vitamin D supplementation in postmenopausal women with osteoporosis living in a sunny country.
Methods A 3-month controlled clinical trial with 64 postmenopausal women with osteoporosis, mean age 62 ± 8 years. They were randomly assigned to either the supplement group, who received 1,200 mg of calcium carbonate and 400 IU (10 μg) of vitamin D_3, or the control group. Dietary intake assessment was performed, bone mineral density and body composition were measured, and biochemical markers of bone metabolism were analyzed.
Results Considering all participants at baseline, serum vitamin D was under 75 nmol/l in 91.4% of the participants. The concentration of serum 25(OH)D increased significantly ($p = 0.023$) after 3 months of supplementation from 46.67 ± 13.97 to 59.47 ± 17.50 nmol/l. However, the dose given was limited in effect, and 86.2% of the supplement group did not reach optimal levels of 25(OH)D. Parathyroid hormone was elevated in 22.4% of the study group. After the intervention period, mean parathyroid hormone tended to decrease in the supplement group ($p = 0.063$).
Conclusion The dose given (400 IU/day) was not enough to achieve 25(OH)D concentration, considered optimal for bone health.

Keywords Dietary intakes · Serum 25-hydroxyvitamin D · Vitamin D supplement · Postmenopausal · Osteoporosis

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Introduction

Progressive aging of the world's population contributes to the increase in the prevalence of chronic diseases, such as osteoporosis [14]. Osteoporotic fractures are one of the major causes of disability, morbidity and mortality in older people [12]. Therefore, it is worthwhile to prevent osteoporotic fractures and its complications. Lifestyles changes are advised, especially dietary components: potentially vitamin D and calcium are important for bone health [9, 20].

The micronutrients of greatest importance are calcium and vitamin D [30]. An adequate intake of calcium and vitamin D prevents bone loss by slowing bone turnover, especially bone resorption, and can reduce the risk of fractures in postmenopausal women [10]. Regrettably, there is a significant proportion of the Brazilian population failing to achieve the recommended dietary calcium and vitamin D intakes. According to the Brazilian Osteoporosis Study (BRAZOS, 2007), that evaluated 2,040 participants over 40 years old, calcium intake of 99.2% of the participants were below recommended levels, and 99.3% presented vitamin D intakes below the recommended values proposed by DRIs [33].

Sunlight exposure is responsible for 80–90% of vitamin D storages. Solar ultraviolet B radiation with wavelength between 290 and 315 nm are capable of penetrating the skin and converting 7-dehydrocholesterol to previtamin D_3, which is rapidly converted to vitamin D_3 [18, 19]. Sao Paulo is geographically located at 23°34′S, with subtropical weather, where solar radiation is considered abundant [37]. However, numerous studies conducted in sunny countries such as Australia, Brazil, Lebanon, Tunisia and New Zealand have observed a high prevalence of vitamin D deficiency [16, 25, 26, 37, 41].

Serum 25-hydroxyvitamin D concentration is the best indicator of vitamin D status because the long half-life of about 1–2 months, gives an indication of vitamin D stores obtained from cutaneous synthesis and vitamin D intake over long periods [42]. Furthermore, circulating 25(OH)D serves as substrate not only in the kidney, but also in other sites where 1α-hydroxylase is found, promoting further roles for vitamin D in addition to functions in calcium homeostasis. Therefore, it is a strong indicator of biological effects of vitamin D in epidemiological studies [21].

The majority of the studies that evaluate vitamin D supplementation have been carried out in countries located in higher latitudes [4, 11, 34, 40]. Therefore, it is important to investigate if there is a differential response to vitamin D supplementation in a sunny country.

Considering the high prevalence of osteoporosis worldwide, vitamin D insufficiency, and the low intake of calcium and vitamin D, we aimed to evaluate if the calcium and vitamin D supplementation is sufficient to increase plasma concentrations of 25(OH)D at optimal levels for bone health and how it affects bone metabolism in a sunny country.

Materials and methods

Subjects

The study group comprised of 64 Caucasian postmenopausal osteoporotic women from the outpatient osteoporosis clinic at São Paulo Hospital, Brazil, geographically located at 23°34′S. We excluded women with renal, gastrointestinal, endocrinal and rheumatic disease; cancer; bilateral hip surgery; and cognitive deficit. Women who had received therapy known to alter bone metabolism, such as bisphosphonate, calcitonin, fluoride salts, hormone replacement, estrogen, hydrochlorothiazide, calcium or vitamin D supplement were also excluded.

The ethics committee of the University of São Paulo approved the protocol, and written informed consent was obtained from all study subjects.

Study design and supplements

In this 3-month controlled clinical trial, the subjects were randomly assigned to either the control or supplement group. The participants enrolled to the intervention study as soon as they were diagnosed with osteoporosis. Participants were evenly divided between the intervention and control groups every month of the year.

The supplement group received two pills of 600 mg of elemental calcium in the form of calcium carbonate and 200 IU (5 μg) of vitamin D_3 [a total of 1,200 mg of calcium and 400 IU (10 μg) of vitamin D a day]. The subjects were advised to take the supplement doses separately and at mealtime. The control group received general orientation regarding healthy diet and exercise.

At baseline, women's medical history was assessed, dietary data was collected, bone mineral density and body composition were measured, and biochemical markers of bone metabolism were analyzed. At the end of the study, another bone metabolism and dietary intake assessment was performed.

Dietary intake assessment

Dietary intakes were collected by a 3-day food record for gathering information on macro- and micro-nutrients intake. Dietary data obtained was checked carefully and calculated by nutrition software (Nutrition Data System for Research, University of Minnesota, 2007). Food without fortification was used, since there is no mandatory fortification of calcium and vitamin D in Brazil.

The reference values proposed by the Institute of Medicine (1997) were compared with the actual nutrient intakes [33].

Bone mineral density assessment

Bone mineral density in the femoral neck, spine, and total body was measured by dual energy X-ray absorptiometry with the use of a DPX scanner (Lunar Radiation Corp., Madison, WI). Software version 3.6z was used for data acquisition and analysis. The coefficients of variation (CV) for the measurements were 3.0% (femoral neck), 2.0% (spine), and 0.6% (total body). Osteoporosis was defined according to the World Health Organization criteria [45].

Biochemical measurements

Data was collected through all four seasons. Blood was drawn between 7:00 and 9:00 a.m. after the subjects had fasted for at least 8 h. All samples were kept frozen at −70 °C until analysis. Total calcium, phosphorus, and

magnesium were measured by standard laboratory methods. Serum 25-hydroxyvitamin D was measured by radioimmunoassay kit DiaSorin (Stillwater, MN). The inter-assay CV was 12%. The cutoff value for vitamin D insufficiency used was 75 nmol/l. Serum intact parathyroid hormone (PTH) was measured by chemiluminescence immunoassay kit (Nichols Institute, San Juan Capistrano, CA). The intra-assay CV was 7% and inter-assay CV was 9.2%. The normal range for adults is 15–65 pg/ml. Bone alkaline phosphatase (BAP) was measured by enzyme immunoassay kit Metra (Quidel, San Diego, CA), with inter- and intra-assay variations of 5.2 and 5.8%, respectively. The normal range for postmenopausal women given by the manufacture of the kit is 14.2–42.7 U/l.

Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate variables' distributions. General characteristics and dietary patterns presented normal distribution and independent-sample *t* test was used for comparison between groups. The effects of intervention in the same group were evaluated by paired-sample *t* test. The results are expressed as mean ± standard deviation.

For biochemical parameters repeated measures ANCOVA, followed by Tukey–Kramer multiple comparison test was used to evaluate the differences between two groups. The profile test by contrasts was then performed to evaluate the effects of intervention in the same group. Variables were transformed in ranks due to the absence of normal distribution. All variables were adjusted for season of the year when blood was collected, age and fat mass.

Oneway ANCOVA was used to compare delta percentage, followed by Tukey–Kramer multiple comparison test, adjusted for season of the year when blood was collected, age and fat mass. Variables were transformed in ranks due to the absence of normal distribution.

Analyses were conducted with software Statistical Package for the Social Science SPSS vs. 11.5 (SPSS Inc., Chicago, IL) and Statistical Analysis System vs. 8.02 (SAS Institute, Cary, NC). Statistical significance was assumed when $p < 0.05$.

Results

Sixty-four postmenopausal women with osteoporosis were originally evaluated. There were six dropouts during the 3 months intervention. Three subjects did not complete because of illness, one subject could not be contacted and two subjects dropped out for personal reasons. Thus, the results of 58 subjects who provided complete data were included in the final analyses.

There were no statistically significant differences at baseline in anthropometric characteristics or bone mineral density between the control group and the supplement group (Table 1). A high fat body mass was observed in both groups. The loss of bone mass was predominant in the lumbar spine (L1–L4).

The intervention results for nutrient intake that are most relevant to bone and calcium economy are shown in Table 2. The baseline intakes did not differ between groups. Both mean daily intakes of calcium and vitamin D were far below recommended nutrient intake of 1,200 mg/day for calcium and 10–15 μg/day for vitamin D, according to the Dietary References Intake (Institute of Medicine, 1997) for age and gender. Considering all participants at baseline, 98.3% of the subjects presented calcium intake under the adequate intake (AI), and none of the participants reached the AI for vitamin D.

No change was observed in the control group for the nutrient intakes after the intervention period. As expected, the supplement use led to a significant increase in calcium and vitamin D intake levels in the supplement group. The other nutrients did not differ after the 3-month period.

Results of the biochemical markers of bone metabolism at baseline and post-intervention for both groups are presented in Table 3. At baseline, there was no significant difference in any laboratory variable between the control and supplement groups. Serum levels of calcium, phosphorus and magnesium were within the normal range in both groups, and were not significantly altered after the intervention. Mean PTH was in the normal range

Table 1 Baseline characteristics of the study subjects according to treatment group

Characteristics	Control ($N = 29$) mean (SD)	Supplement ($N = 29$) mean (SD)	*p*
Age (year)	63.25 ± 8.8	61.30 ± 8.3	0.393
Weight (kg)	58.75 ± 11.1	60.30 ± 8.7	0.537
Height (cm)	152.44 ± 5.3	150.46 ± 6.3	0.202
BMI (kg/m^2)	25.25 ± 4.4	26.71 ± 3.7	0.185
Lean body mass (kg)	14.28 ± 2.1	14.41 ± 1.9	0.820
Fat body mass (%)	39.86 ± 6.9	41.70 ± 6.4	0.300
Bone mineral density (g/cm^2)			
Total body	1.01 ± 0.19	0.98 ± 0.07	0.483
Spine (L1–L4)	0.84 ± 0.10	0.84 ± 0.07	0.973
Femoral neck	0.74 ± 0.10	0.74 ± 0.08	0.851
T-score			
Total body	−1.68 ± 1.31	−1.69 ± 0.94	0.986
Spine (L1–L4)	−2.78 ± 0.91	−2.83 ± 0.68	0.819
Femoral neck	−2.08 ± 0.86	−1.97 ± 0.81	0.618

Independent-sample T Test

Table 2 Nutrient intake according to treatment group at baseline and after 3 months

Nutrient intake	Control ($N = 29$)		Supplement ($N = 29$)	
	Baseline	3 months	Baseline	3 months
Energy (kcal/day)	1,253 ± 382	1,322 ± 414	1,392 ± 488	1,426 ± 504
Carbohydrate (%)	54.3 ± 7	53.2 ± 8	50.3 ± 5	51.4 ± 5
Fat (%)	30.1 ± 7	31.7 ± 7	34.2 ± 7	33.0 ± 4
Protein (%)	15.5 ± 6	14.9 ± 5	15.3 ± 4	15.5 ± 4
Calcium (mg/day)	559.6 ± 214	587.9 ± 277	568.9 ± 287	1,700.4 ± 376*$^{\perp}$
Vitamin D (μg/day)	2.8 ± 1.4	3.2 ± 2.1	3.5 ± 1.6	12.7 ± 2.6*$^{\perp}$
Phosphorus (mg/day)	800.9 ± 271	833.1 ± 285	886.4 ± 337	805.0 ± 317
Magnesium (mg/day)	196.0 ± 61	195.8 ± 58	212.0 ± 101	198.7 ± 100

* $p < 0.05$ paired-sample t test; $^{\perp}$ $p < 0.05$ independent-sample t test

Table 3 Biochemical parameters according to treatment group at baseline and after 3 months

Biochemical exams	Control ($N = 29$)		Supplement ($N = 29$)	
	Baseline	3 months	Baseline	3 months
Calcium (mg/dL)	9.26 ± 0.75	9.28 ± 0.55	9.33 ± 0.47	9.20 ± 0.80
Phosphorus (mg/dL)	3.70 ± 0.43	3.71 ± 0.49	3.67 ± 0.41	3.54 ± 0.52
Magnesium (mg/dL)	1.98 ± 0.27	1.92 ± 0.32	2.02 ± 0.29 ($N = 28$)	1.91 ± 0.20 ($N = 28$)
PTH (pg/ml)	58.18 ± 20.60	57.35 ± 18.19	54.36 ± 15.28	50.61 ± 12.32
25(OH)D_3 (nmol/l)	52.87 ± 21.40	58.8 ± 24.72	46.67 ± 13.97	59.47 ± 17.50*
BAP (U/l)	30.2 ± 17.6 ($N = 28$)	29.5 ± 11.9 ($N = 28$)	26.0 ± 11.8 ($N = 28$)	25.2 ± 9.2 ($N = 27$)

Analyses were adjusted for season of the year of blood collection, age and fat mass. When there were missing data, the number of subjects for whom data were available is shown in parentheses

* $p < 0.05$ ANCOVA repeated measures

(15–65 pg/ml), but 22.4% of the participants were above the upper limit.

Taking all participants into consideration at baseline, 56.9% of the participants presented 25(OH)D concentrations below 50 nmol/l. The prevalence of vitamin D insufficiency in accordance with a cutoff level of 75 nmol/l, considered optimal for bone health, was an alarming 91.4%. Furthermore, 3 subjects (6.9%) were deficient, according to the cutoff level of 25 nmol/l.

In the supplement group, the concentration of serum 25(OH)D increased significantly after 3 months intervention ($p = 0.023$). The control group did not change significantly its mean vitamin D concentration from baseline. Over this same period, parathyroid hormone tended to decrease in the supplement group; however, it was not significant ($p = 0.063$). There were no significant differences in BAP after intervention in both groups.

Figure 1 represents the percentage change of the biochemical parameters after the intervention. Serum levels of vitamin D in the supplement group was the only one with significant increase of 32.7% ($p = 0.017$), even when adjusted for season of the year, age and fat mass.

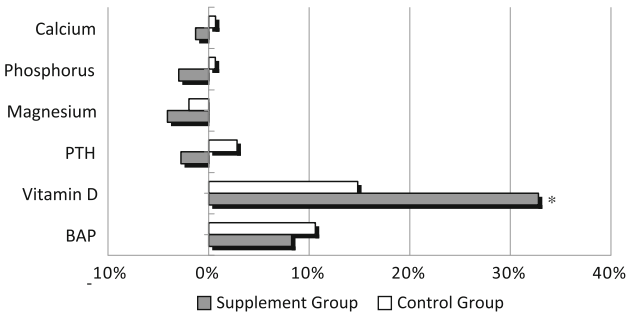

Fig. 1 Mean percentage change from baseline for biochemical parameters, according to control and supplement groups. *$p < 0.05$ Oneway ANCOVA. Analyses were adjusted for season of the year of blood collection, age and fat mass

Discussion

The present study demonstrated that supplementation of 400 IU per day (10 μg) is not enough to raise serum 25(OH)D concentrations to levels considered optimal for bone health (≥75 nmol/l) in postmenopausal women with osteoporosis, living in a subtropical climate. In addition,

the supplementation of calcium and vitamin D did not significantly affect bone turnover markers.

Low calcium intake is a worldwide problem [31]. In the present study, 98.3% of the participants did not meet requirements of 1,200 mg/day for calcium intake. One of the reasons for such a low intake is the high cost of natural sources of calcium. Data from household food budget surveys in Brazil showed a positive relation in the consumption of dairy foods, fruits and vegetables with the family budget [23]. The use of supplements can be effective in the optimization of calcium intake, mainly in groups with a high risk for fractures. However, possible side effects have to be considered. A study conducted by Bolland et al. [7] suggests that high calcium intakes might have an adverse effect on vascular health, especially for participants over 80 years, and with calcium intake above 1,900 mg/day. In the present study, the supplement group after the intervention had a mean daily calcium intake of 1,700 ± 376 mg, with no report of previous cardiovascular event. The literature suggests that a high calcium intake might be a concern for populations with elevated basal calcium intake, individuals with poor renal function, and with report of cardiovascular event [36].

Inadequacies of vitamin D intake are also observed in several countries [8, 28, 32]. In the present study, none of the subjects reported an adequate vitamin D intake, with a mean daily intake of 2.87 ± 1.4 μg and 3.52 ± 1.6 μg in the control and supplement groups, respectively. Similarly, in Europe, the vitamin D intake is approximately 2–3 μg/day, except in Norway and Sweden where fish intake and the contribution from fortified foods are high [32]. Mean intakes of vitamin D in the United States, using NHANES 1999–2000 consumption data, were estimated at 4.7 μg/day among older female adults. Considering the use of dietary supplement, the average rose to 9.5 μg/day in the same group [28]. These findings reflect the importance of food fortification and supplementation practices, especially considering the evidence that adequate vitamin D intake is associated with a lower occurrence of osteoporotic hip fractures in postmenopausal women [15].

Controversy exists in determining an optimal serum 25(OH)D concentration. However, there is an emerging consensus for vitamin D sufficiency cutoff at ≥ 75 nmol/l (30 ng/ml), based on a threshold required for maximal suppression of circulating PTH, greatest calcium absorption, highest bone mineral density, and prevention of fractures [13].

Besides the low vitamin D intake, we also observed a mean baseline plasma 25(OH)D concentration of 52.87 ± 21.40 nmol/l in the control group and 46.67 ± 13.97 nmol/l in the supplement group, indicating sub-optimal concentrations for bone health (<75 nmol/l) in 91.4% of the participants. Our results are comparable with studies conducted in regions of higher latitudes. Neuprez et al. [29] studied the vitamin D inadequacy in Belgian postmenopausal osteoporotic women, the mean serum 25(OH)D concentrations reached 48.7 (20.7) nmol/l in women without vitamin D supplements.

The major reason for low vitamin D status in urban dwellers is inadequate UVB exposure, given that the majority of vitamin D is gained from cutaneous synthesis; hence, such a high prevalence of vitamin D inadequacy in a subtropical climate would not be expected. However, a number of factors can influence the cutaneous synthesis and can partially explain the inadequate levels of vitamin D. Two of them are usually found in Sao Paulo; clouds and aerosol are known to attenuate radiation in the atmosphere, including UVB [44]. Another factor is the decreased capacity of the skin to produce vitamin D due to age-related changes. As well as behavioral factors such as the amount of time spent outdoors, home confinement, and amount of clothing and sunscreen usage [19, 32, 44]. Our data met up with other studies that indicate that low latitude does not prevent vitamin D insufficiency and that other factors must be considered [2, 22, 32, 41].

As expected, in the present study, daily supplementation with 400 IU (10 μg) of vitamin D_3 for 3 months significantly increased vitamin D status. However, the dose given was limited in effect, as 86.2% of the supplement group did not reach concentration levels of 25(OH)D, considered optimal for bone health (≥75 nmol/l). Barger-Lux et al. [3] dosed the same amount of vitamin D_3 for 8 weeks in healthy adult males, and reported a rise of 11 nmol/l in 25(OH)D concentration from a baseline value of 67 nmol/l. Our study showed a similar increase of 12.8 nmol/l ($\Delta = 32.7\%$). This slightly higher increase might be explained by other authors' findings, who observed that higher increases are seen at lower basal 25(OH)D concentrations [3, 39].

An editorial by Vieth et al. [43] addresses the importance of increasing the recommendation of daily vitamin D intake in order to achieve adequate 25(OH)D levels. Moreover, a meta-analysis of randomized controlled trials demonstrated that nonvertebral fracture prevention with vitamin D is dose dependent, and doses higher than 10 μg of vitamin D are necessary [5].

In our study, we did not find significant differences in serum PTH and in the biochemical markers of bone formation, BAP, after intervention in both groups. In agreement with our results, Rapuri et al. [34], in a study with elderly women, did not observe a significant effect of vitamin D supplement use on serum PTH. On the other hand, vitamin D supplementation has been shown to decrease plasma levels of PTH, besides it is well established that serum PTH correlate negatively with serum 25(OH)D in elderly and postmenopausal women [1, 6, 24,

38]. In addition, Riggs et al. [35] observed a significant increase of almost 20% (0.002) in serum PTH after 4 years of calcium supplementation. In the current study PTH concentrations in the supplement group tended to decrease, however, it was not significant. Possibly, higher doses of vitamin D supplementation would have the expected effects on PTH levels.

For biochemical markers of bone turnover, Grados et al. [17] observed a decrease in BAP although not significant in the supplement group that received 500 mg of calcium and 400 IU of vitamin D daily for 1 year. Mezquita-Raya et al. [27] also observed no significant differences in postmenopausal women with high and low vitamin D status. According to Barnes et al., several factors can contribute to the non-significant effect of vitamin D supplementation on bone turnover. First, their variation over time, second, vitamin D might play a more active role in bone mineralization rather than bone remodeling, and lastly there is evidence that a serum 25(OH)D level between 78 and 95 nmol/l is the threshold for effects on bone metabolism [4].

One limitation of our study might be the duration of supplementation (3 months). Though, it takes around 30 days for the mean serum 25-hydroxyvitamin D [25(OH)D] concentration to achieve plateau when vitamin D_3 is administered orally. For that reason, studies should evaluate vitamin D supplementation for a minimum of 4 weeks [42]. Additionally, calcium and vitamin D supplementation prevents bone loss mainly by slowing bone resorption; therefore, further studies should also investigate bone resorption biomarkers.

In conclusion, our study population of postmenopausal women with osteoporosis did not reach adequate intake of calcium and vitamin D. In addition, 91.4% of the participants presented sub-optimal vitamin D status, indicating that the geographical location did not influence as much as was expected. A daily supplement dose of 10 μg (400 IU) vitamin D_3 for 3 months significantly increased vitamin D status. However, this augment was not sufficient to achieve optimal concentrations of serum 25(OH)D. In view of our results, it is important to consider increasing the availability of higher vitamin D supplement doses in sunny countries.

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