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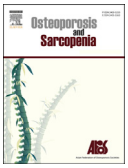

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Original article

"What is an appropriate dosage and interval of vitamin D_2 supplementation to achieve a sufficiency level in postmenopausal women of Thailand?" A randomized, double-blind, placebo-controlled trial

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Abstract

Objective: This study primarily evaluated serum 25-hydroxy vitamin D levels in postmenopausal women with vitamin D insufficiency who received different dosages and intervals of vitamin D_2 supplementation. We secondarily evaluated the percentages of those who achieved vitamin D sufficiency level (Defined as ≥30 ng/ml).
Study design: Randomized double-blind, placebo-controlled trial.
Methods: Postmenopausal women who met the criteria of vitamin D insufficiency (<30 ng/mL) were randomized into 4 groups (N = 25/group). Participants received a 12 week-treatment of different dosages and intervals of vitamin D_2 (placebo, vitamin D_2 20,000 IU/2 weeks, vitamin D_2 20,000 IU/week, and vitamin D_2 40,000 IU/week). Serum total 25-hydroxy vitamin D was determined at baseline, after 4 and 12 weeks of supplementation with electrochemiluminescence immunoassay (Elecys, Roche Diagnostics). Changes of 25-hydroxy vitamin D levels were compared among the groups.
Results: Forty seven percent of postmenopausal women (100/212) screened for study enrolment were found to have vitamin D insufficiency. At 12 weeks, serum 25-hydroxy vitamin D increased significantly from baseline in all groups ($p < 0.01$) (mean serum 25-hydroxy vitamin D level increased from 23.03 ± 4.56 at baseline to 25.60 ± 4.79 ng/ml (placebo), 23.54 ± 5.14 to 27.83 ± 5.27 ng/ml (vitamin D_2 20,000 IU/2 weeks), 22.68 ± 5.21 to 30.50 ± 5.14 ng/ml (vitamin D_2 20,000 IU/week), and 22.88 ± 4.83 to 37.89 ± 5.47 ng/ml (vitamin D_2 40,000 IU/week)). In addition, the 25-hydroxy vitamin D levels were statistically significantly different at 4 and 12 weeks ($p < 0.01$) among all 4 groups. The percentages of those achieving vitamin D sufficiency level after 12 weeks of supplementation were 16% (placebo), 27.3% (vitamin D_2 20,000 IU/2 weeks), 44% (vitamin D_2 20,000 IU/week), and 86.4% (vitamin D_2 40,000 IU/week); statistically significantly different among the four groups ($p < 0.01$). There was no participant with 25-hydroxy vitamin D after 12 weeks of >50 ng/mL in this study.
Conclusion: Vitamin D_2 40,000 IU/week was found to be the most effective dosage for postmenopausal women in this study to achieve serum vitamin D sufficiency level.

Keywords: Postmenopause; Vitamin D_2; Ergocalciferol; Vitamin D insufficiency; Serum total 25-hydroxy vitamin D

1. Introduction

It is well known that adequate levels of circulating 25-hydroxy vitamin D are important for optimal bone health. In addition, recent observational studies appear to suggest a role for vitamin D in the prevention of chronic diseases such as type I diabetes, cardiovascular disease, cancer, cognitive

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decline, pregnancy complication, autoimmunity [1−4]. An international epidemiological investigation on the prevalence of vitamin D inadequacy amongst postmenopausal women with osteoporosis revealed that low levels of serum 25-hydroxy vitamin D were common [5]. Women with 25-hydroxy vitamin D level < 30 ng/mL ranged from 53.4% in Latin American, 57.7% in Europe, 60.3% in Australia, 71.4% in Asia and 81.8% in Middle East region. In Thailand, the prevalence of vitamin D insufficiency (25-hydroxy vitamin D level < 30 ng/mL) was 47% whilst the prevalence of vitamin D deficiency (<20 ng/mL) was 12% [5].

With increasing urbanization, people tend to have more indoor activities, minimal exposure to sunlight due to factory and office lifestyles instead of subsistence farming. As a consequence, people tend to have limited photochemical subcutaneous synthesis of vitamin D. In order to prevent inadequate levels of vitamin D, people need to increase sunlight exposure, take a diet that includes rich sources of vitamin D and/or take vitamin D supplementation [6,7]. Nevertheless, to increase dietary intake to obtain adequate vitamin D is cumbersome and difficult to achieve, while increasing exposure to sunlight may not be acceptable to women due to concerns of photo-degeneration and skin cancer. This is not to mention the increasing use of sunscreen and umbrellas to avoid the heat from sunlight in women from this region. Vitamin D supplementation is deemed to be an easy and practical solution particularly if the cost is low. As a general guide, the recommendation is 600−800 IU vitamin D_3 daily [8], for all healthy postmenopausal women. In Thailand, a cheap and easily available form of vitamin D is the once weekly 20,000 IU of Vitamin D_2 which costs less than 0.04 US$/week. Therefore, the primary aim of this study was to evaluate the efficacy of different vitamin D supplementation regimens on the serum 25-hydroxy vitamin D level in postmenopausal women with vitamin D insufficiency. We secondarily evaluated the percentages of those who achieved vitamin D sufficiency level (≥30 ng/mL) in these different intervention groups.

2. Methodology

2.1. Study population

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University on IRB No. 593/56, Bangkok, Thailand. All subjects provided their written informed consent before recruitment. The study was conducted from March 2014 to February 2015. Pilot study was done to find an appropriate sample size which yielded 25/group (total number = 100) ($\alpha = 0.05$ two-sides, $\beta = 0.2$). One hundred postmenopausal women were recruited from the menopause clinic, King Chulalongkorn Memorial Hospital. The following were the inclusion criteria: 1) age: 50−80 years, 2) amenorrhoea for at least 12 months, 3) being ambulatory and community-dwelling, 4) body mass index (BMI) between 18.50 and 25.00 (kg/m^2), 5) baseline serum 25-hydroxy vitamin D < 30 ng/mL and, 6) willing to give informed consent to participate in the study. Exclusion criteria included those with a history of liver disease or abnormal liver function test, renal disease or abnormal renal function test, a history of malignancy, endocrine disorders, malabsorption or bowel surgery, or use of any vitamin D containing medications within 12 weeks.

2.2. Study design

This study is double-blind randomization design. Participants were divided to 4 groups by "Block-of-four randomization". The first group was assigned to receive two identical placebo pill once weekly. The second group was assigned to take a) vitamin D_2 (ergocalciferol) 20,000 IU and an identical placebo pill once a week and b) two identical placebo pills once a week on an alternate basis. The third group took one vitamin D_2 (ergocalciferol) 20,000 IU and one placebo pill once weekly, the fourth group took two vitamin D_2 (ergocalciferol) 20,000 IU (a total dose of 40,000 IU) once weekly. The pill, manufactured by The British Pensary (L.P) Co., Ltd., is a white, colourless crystal, insoluble in water, soluble in organic solvents and slightly soluble in vegetable oils. Serum 25-hydroxy vitamin D levels were measured at baseline, 4 and 12 weeks after the supplementation (Fig. 1).

2.3. Vitamin D assay

Serum 25-hydroxy vitamin D level was measured using an automated Elecsys (Roche diagnostic) [9], which is an automated assay and measures total 25 hydroxy vitamin D ($D_2 + D_3$). Intra-assay coefficient of variability is 3.57 and inter-assay coefficient of variability is 4.89.

2.4. Statistical analysis

SPSS version 17 was used for statistical analysis. Baseline demographic data were presented as mean and standard deviation for continuous data and percentage for discrete data. Comparison among groups was done using ANOVA for continuous data and Chi square for binary or nominal data. Comparison of the changes of serum 25-hydroxy vitamin D after enrolment at 4 and 12 weeks of study was done using ANCOVA. A p-value of less than 0.05 was considered statistically significant.

3. Results

Forty seven percent of postmenopausal women screened for study enrolment (100/212) had serum 25-hydroxy vitamin D level below sufficiency level (<30 ng/mL). Ninety seven out of 100 participants completed the 12 week study. Two participants in vitamin D_2 (ergocalciferol) 40,000 IU/week (one had urticarial rash, and one relocated to another city) and one participant in the placebo group (had urticarial rash) dropped out of the study. The demographic data revealed no

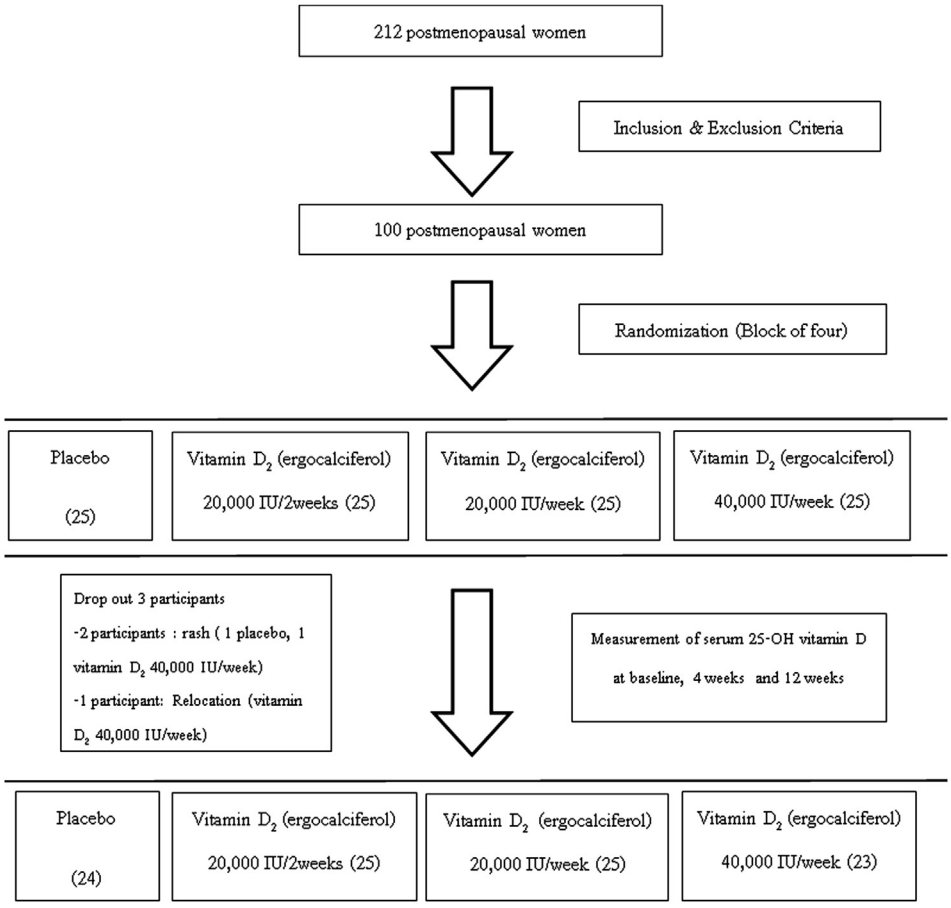


Fig. 1. Trial flowchart summarizing the recruitment and randomization process.

statistically significant difference among the intervention groups (Table 1).

The mean (SD) of serum 25-hydroxy vitamin D at baseline, 4 and 12 weeks of the four groups are shown in Fig. 2. Serum 25-hydroxy vitamin D levels increased significantly from baseline in all groups at 4 and 12 weeks ($p < 0.01$) except the placebo group which showed no significant increase in serum 25-hydroxy vitamin D level at 4 week ($p = 0.10$). There were statistically significant differences in serum 25-hydroxy vitamin D levels among all four groups at 4 and 12 weeks (ANCOVA). Post hoc analysis of serum 25-hydroxy vitamin D levels at 12 weeks (Table 2) showed that there were statistically significant differences in the serum 25-hydroxy vitamin D levels in almost every pair of comparisons except between the placebo and vitamin D_2 20,000 IU/2 weeks ($p = 0.13$).

The percentages of participants in each group with serum 25-hydroxy vitamin D $\geq$ 30 ng/mL, after 12 weeks were 16% in placebo, 27.3% in the vitamin D_2 20,000 IU/2 weeks, 44% in the vitamin D_2 20,000 IU/week and 86.4% in the vitamin D_2 40,000 IU/week ($p < 0.01$) (Fig. 3). There was no statistically significant difference in lifestyle i.e., exercise, sunlight exposure and intake of vitamin D rich diet after 12 weeks (Table 3). No increase in serum 25-hydroxy vitamin D of >50 ng/mL was found in this study.

4. Discussion

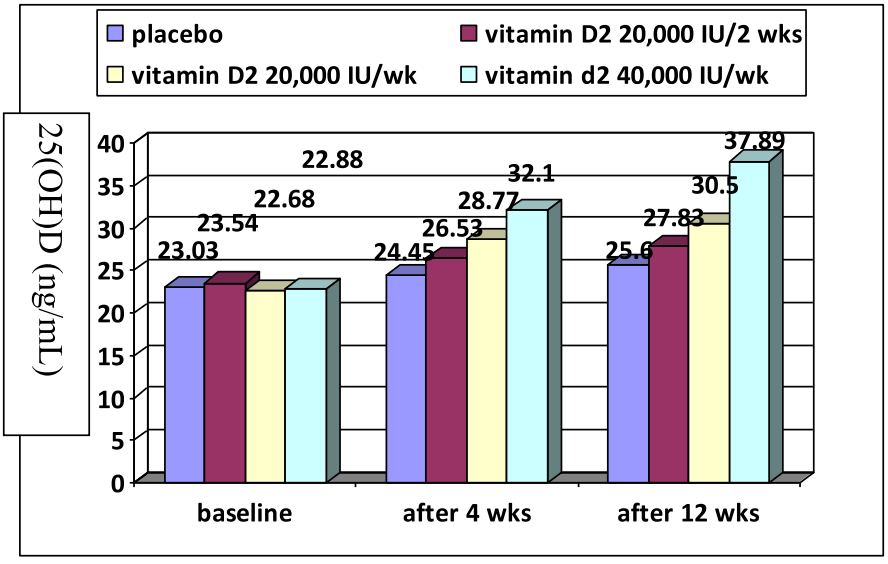
This study revealed that a sizable percentage (47%) of the study population had vitamin D insufficiency when using a cutoff value of $\geq$30 ng/mL to define level of sufficiency [8]. The percentage is exactly the same as the number reported in 2006 by Lipps P., et al. in an international epidemiology investigation of the prevalence of vitamin D inadequacy worldwide though the latter investigated older postmenopausal women with osteoporosis [5]. This is in contrast to a Thai report by Soontrapa S. et al. who found a higher percentage (77%) of hypovitaminosis D in older women [10]. This may be due to the higher cutoff value (>35 ng/mL) used in the latter study compared to ours which adopted the international cutoff value of <30 ng/mL as insufficiency.

From this study, it is found that the once weekly regimen of vitamin D_2 40,000 IU was the most effective regimen to increase 25-hydroxy vitamin D to achieve sufficiency level at 12 weeks (86.4% of the participants in the group). None of the women in this study had 25-hydroxy vitamin D level >50 ng/mL. The once weekly dose of vitamin D_2 20,000 IU is another good option that raised 25-hydroxy vitamin D level in 44% of participants to sufficiency levels. The results of this study are comparable to Joseph P. et al. [11] who conducted a study using a once weekly vitamin D_2 50,000–100,000 IU which

Table 1
Baseline demographic and clinical characteristics of the studied population (N = 100) by randomization.

		Placebo	Vitamin D_2 20,000 IU/2 weeks	Vitamin D_2 20,000 IU/week	Vitamin D_2 40,000 IU/week	*p*-Value
Age (y)		60.40 (7.55)	60.50 (7.18)	62.08 (6.70)	61.52 (6.75)	0.81
BMI (kg/m^2)		22.52 (1.66)	22.41 (1.84)	23.16 (1.60)	22.25 (1.69)	0.26
Age of menopause (y)		48.76 (4.03)	48.70 (4.53)	48.33 (3.96)	50.08 (2.98)	0.42
Baseline 25-hydroxy vitamin D (ng/mL)		23.03 (4.56)	23.54 (5.14)	22.68 (5.21)	22.88 (4.83)	0.94
Occupation	Housewife	48%	58.3%	70.8%	64%	0.81
	Private employee	36%	29.2%	16.7%	24%	
	Business person	8%	8.3%	8.3%	12%	
	Government employee	8%	4.2%	4.2%	0%	
Approximate sunlight exposure (min/d)	≤30 min	76%	83.3%	75%	84%	0.85
	>30 to ≤60 min	12%	12.5%	12.5%	12%	
	>60 to ≤90 min	4%	0%	0%	0%	
	>90 min	8%	4.2%	12.5%	4%	
Sunscreen use	Used	28%	41.7%	41.7%	44%	0.64
	Not used	72%	58.3%	58.3%	56%	
Income (US$/mo)*	≤312	8%	8.3%	12.5%	16%	0.63
	>312 to ≤625	36%	50%	29.2%	44%	
	>625 to ≤937	20%	29.2%	33.3%	24%	
	>937	36%	12.5%	25%	16%	
Education status	Primary	28%	25%	22.2%	20%	0.21
	Secondary	24%	25%	11.2%	40%	
	College diploma	20%	16.7%	25%	28%	
	≥Bachelor's degree	28%	33.3%	41.6%	12%	
Skin pigment	Light	8%	8.3%	8.3%	16%	0.63
	Medium	80%	62.5%	70.8%	56%	
	Dark	12%	29.2%	20.9%	28%	
Cause of menopause	Natural	84%	75%	66.7%	80%	0.52
	Surgical	16%	25%	33.3%	20%	

Data were presented as mean (SD) or percentage, *1 US$ = 32 Thai Bahts.



*Paired t test was used to compare serum 25-hydroxy vitamin D levels from baseline (within group comparison).

Serum 25-hydroxy vitamin D level increased significantly from baseline in all groups at 4 and 12 weeks ($p < 0.01$)

except the placebo group at 4 weeks ($p = 0.10$)

*ANCOVA was used to compare serum 25-hydroxy vitamin D levels between the four groups at 4 and 12 weeks

Fig. 2. Mean serum 25-hydroxy vitamin D levels (ng/mL) among all four groups at baseline, 4 and 12 weeks.

Table 2
Post hoc analysis of serum 25-hydroxy vitamin D levels at 12 weeks.

Group	Group compared	*p* (LSD)
Placebo	Vitamin D_2 20,000 IU/2 weeks	0.13
Placebo	Vitamin D_2 20,000 IU/week	<0.01
Placebo	Vitamin D_2 40,000 IU/week	<0.01
Vitamin D_2 20,000 IU/2 weeks	Vitamin D_2 20,000 IU/week	0.02
Vitamin D_2 20,000 IU/2 weeks	Vitamin D_2 40,000 IU/week	<0.01
Vitamin D_2 20,000 IU/week	Vitamin D_2 40,000 IU/week	<0.01

LSD: Fisher's Least Significant Difference Test.

raised 25-hydroxy vitamin D from 17.7 to 32.9 ng/mL. This is in contrast with a general guideline which recommends 600−800 IU/day (4200−5600 IU/week) of vitamin D for postmenopausal women. The vitamin D supplementation in this study of 20,000−40,000 IU/week are equivalent to daily doses of 2857−5714 IU which are much higher than the general guideline. This may be due to the difference in the effectiveness of ergocalciferol (D_2) used in this study and cholecalciferol (D_3) recommended in the general guideline which is still a controversial issue. Several studies [12,13] suggest that D_3 is more potent in raising and maintaining serum 25-hydroxy vitamin D concentrations and produces a 2- to 3-fold greater storage of vitamin D than does equimolar D_2. Nevertheless, the only available long interval form of inactive vitamin D in Thailand is ergocalciferol (D_2). And it is affordable by most of the population as it costs less than 0.04US$ per week for a 20,000 IU tablet. The regimen of 20,000−40,000 IU/week would be cost-effective though further health economic studies will still be required to confirm its cost-effectiveness.

The association of seasonal changes and 25-hydroxy vitamin D levels was reported in one study which showed no significant differences in the mean total serum 25-hydroxy vitamin D levels among the vitamin D_2 or vitamin D_3 supplemented and unsupplemented groups in summer. But in winter, the mean serum total 25-hydroxy vitamin D levels were higher in women on vitamin D_2 and vitamin D_3 supplements compared with unsupplemented women [14]. Since Thailand is a tropical country, there is no major seasonal change that will have a significant effect on 25-hydroxy vitamin D levels. Our study showed that the regimen of vitamin D_2 20,000 IU every 2 weekly may not be enough to raise serum 25-hydroxy vitamin D level to sufficiency levels. This implies that it may not be appropriate to prescribe this regimen to those who are in real need of vitamin D supplementation e.g., postmenopausal women with vitamin D deficiency. Nevertheless, 16% of participants in the placebo group were found to achieve sufficiency level after 12 weeks. This is probably due to the changes in lifestyle due to increasing health awareness once the group participated in the study. This was indirectly shown by the increase in sunlight exposure and increased intake of vitamin D rich diet.

In this 12 week study, no case of serum 25-hydroxy vitamin D level >50 ng/mL was seen, though this is not yet generally agreeable toxic level. Although the International Osteoporosis Foundation (IOF) has recommended to retest of serum 25-hydroxy vitamin D level after 3 months of supplementation in high risk individuals [15], a longer than 12 week study may be needed to confirm the plateau level with prolonged use of high dose vitamin D_2. Furthermore, it may be more prudent to have a surveillance of vitamin D toxicity such as hypercalcaemia and hypercalciuria, for safety reasons since we did not closely monitor of serum or urinary calcium in this study. Nevertheless, we did not find any cases with clinical symptoms of hypercalcaemia during the 12 week study period.

5. Conclusion

Vitamin D_2 (ergocalciferol) supplementation was found to significantly increase serum 25-hydroxy vitamin D level from baseline. The once weekly regimen of vitamin D_2 40,000 IU was found to be the most effective, raising the serum 25-hydroxy vitamin D level sufficiency level in most of the subjects. The once weekly vitamin D_2 20,000 IU may be another option, although less than half of the participants achieved sufficiency levels. No case of vitamin D intoxication (25-hydroxy vitamin D > 50 ng/mL) was observed in this study.

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Table 3
Changes in lifestyle after 12 weeks.

		Placebo	Vitamin D_2 20,000 IU/2 weeks	Vitamin D_2 20,000 IU/week	Vitamin D_2 40,000 IU/week	*p*
Exercise	Increased	44%	39.1%	24%	28%	0.40
	No	46%	60.9%	76%	72%	
Sunlight exposure	Increased	44%	56.5%	24%	36%	0.19
	No	56%	43.5%	76%	64%	
Intake of vitamin D rich diet	Increased	24%	34.8%	12%	24%	0.32
	No	76%	65.2%	88%	76%	

Data were presented as percentage. Chi-square was used to compare the differences among the groups.

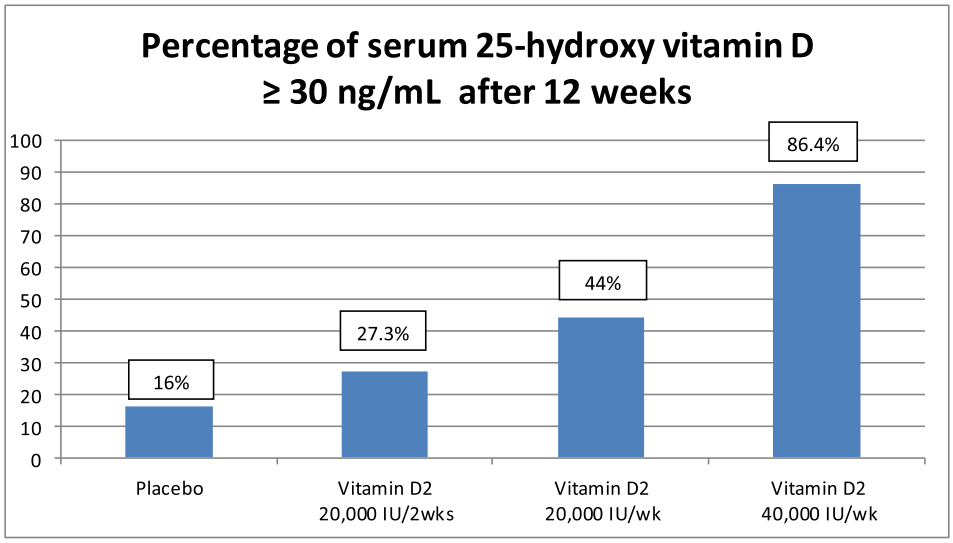

Fig. 3. Percentages of participants who had serum 25-hydroxy vitamin D ≥ 30 ng/mL (sufficiency level) after 12 weeks (Chi-square, $p < 0.01$).

Conflicts of interest

The authors state that there were no conflicts of interest in the conduct of the study. The source of funding in this study was from Ratchadapiseksompotch Scholarship and Menopause Research Unit. The vitamin D assay was supported by Roche Diagnostic Company. The company did not have any involvement in the research and/or manuscript development.

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