The increased awareness of vitamin D deficiency and its consequences on optimal health requires an appropriate evaluation of vitamin D repletion regimens for deficient patients. Vitamin D deficiency leads to secondary hyperparathyroidism, increased bone turnover and bone loss, predisposing individuals to osteoporosis and osteoporotic fractures. In addition, it is associated with a variety of chronic diseases such as certain cancers, cardiovascular diseases, autoimmune diseases, muscle weakness and chronic pain, diabetes (types 1 and 2), schizophrenia, and depression. Despite ongoing controversy, many experts agree that vitamin D deficiency is defined as circulating levels of 25-hydroxyvitamin D (25(OH)D) less than 20 ng/mL. For the correction of vitamin D deficiency, many treatment modalities that are heterogeneous with respect to dose, dosing interval, and formulation of vitamin D supplementation are available. Although humans produce vitamin D3 (cholecalciferol) endogenously, vitamin D supplements exist in 2 distinct forms. Vitamin D2 (ergocalciferol) is manufactured from irradiation of ergosterol from yeast.
Vitamin D3 is manufactured by irradiating 7-dehydrocholesterol obtained from the lanolin in sheep’s wool with ultraviolet B radiation. Despite both forms being used interchangeably for vitamin D repletion, there have been conflicting data published about the equipotency of vitamins D2 and D3; there is also a concern that vitamin D2 might increase catabolism of 25(OH)D3. Several studies comparing the 2 forms in a head-to-head manner suggest that they are equally effective in raising total serum 25(OH)D, while others suggest that vitamin D2 is less effective.

The present study sought to compare 25(OH)D responses with 2 high-dose vitamin D2 and vitamin D3 regimens, and to observe the change in serum 25(OH)D3 levels following the supplementation of an ergocalciferol mega-dose.

PATIENTS AND METHODS

The subjects were 89 women and 16 men aged between 18 and 79 years, in good general health, who attended Jordan University Hospital (JUH) outpatient clinics (orthopedics, rheumatology, endocrinology, surgery, and family medicine) between September 2010 and February 2011. Exclusion criteria included a serum 25(OH)D level >20 ng/mL, creatinine clearance <20 mL/min, pregnancy and lactation, presence of hepatic disease, malabsorption states and granulomatous conditions, history of nephrolithiasis, current glucocorticoid use >6 months, treatment with oral vitamin D supplementation >400 IU/d, and disorders that might influence vitamin D or parathyroid hormone (PTH) metabolism. Patients who were prescribed medications that may interfere with vitamin D metabolism, including anticonvulsants, verapamil, statins, thiazide diuretics, orlistat, and estrogen-containing medications, were also excluded. The inclusion criterion was a baseline serum 25(OH)D <20 ng/mL. Of the 131 eligible participants, 109 agreed to participate in the study. Four of those who agreed to participate were excluded. Two subjects were found to be non-compliant with the regimen, reporting that they took less than 100% of vitamin D3 that was prescribed. Another subject reported that he was taking vitamin D supplements on a weekly basis, and 1 subject disclosed that he was taking oral prednisone.

Sample size

Sample was not statistically calculated because of the following:

(a) There was no available mean value for 25-OH D (vitamin D level) in the Jordanian population. This mean value and its standard deviation are essential for calculating any sample size needed to assess the vitamin D level. Furthermore, this research is a clinical study to assess the mean change in the status of vitamin D deficiency after receiving 2 different high-dose vitamin D regimens (vitamin D2 vs vitamin D3).

(b) Subjects were recruited from those coming to general laboratory at JUH for vitamin D assessment as referred from rheumatology, diabetes, family medicine, and orthopedics outpatient clinics.

(c) Time frame for the study was 90 days (recruitment phase). During this period, any result <20 ng/mL was considered “vitamin D deficient” (n=158); those who met the eligibility criteria (n=131) were invited to participate in the study. A total of 109 subjects consented and 105 completed the study. Thus, the final sample size was 105 subjects.

Randomization Method

(a) Systematic randomized method was used to place every nth subject into the 2 treatment arms (vitamin D2 vs vitamin D3). (Every nth=every second person).

(b) A list of subjects was prepared and numbered from 1 to 109.

(c) The starting point for the placement of subjects was chosen blindly by placing a finger on the list of numbered subjects. This location represented the starting point. In the case of this study, it was subject number 47 who was placed in the vitamin D2 group. The following subject, i.e., number 48 was placed in the vitamin D3 group, and so on.

Treatment

Participants were assigned to 1 of the 2 treatment groups using a minimization algorithm to ensure balanced numbers of men and women between groups. One group (vitamin D3: Biotech vitamin D3-50 [50 000 IU 100 caps]) took 50 000 IU vitamin D3 per day for 10 consecutive days. The second group (vitamin D2: Sterogyl 15 “A” oral solution 600 000 IU/1.5 mL
CORRECTING VITAMIN D DEFICIENCY

[alcohol-based, colorless]) took a 600,000 IU vitamin D2 single bolus dose. The baseline doses were administered by study personnel, resulting in 100% compliance. Compliance with subsequent doses was assessed by tablet counts and participants self-reporting.

Vitamin D deficiency was defined as a serum 25(OH)D level <20 ng/mL. Efficacy for each treatment modality was defined as its ability to raise or maintain 25(OH)D levels above 20 ng/mL during the winter months. Serum 25(OH)D2, 25(OH)D3, and total 25(OH)D levels were determined using the liquid chromatography-mass spectrometry method developed and validated at King Abdullah University Hospital laboratories.26

Venous blood samples were collected from each subject between February 28 and May 15, 2011, to test for vitamin D deficiency before administering treatment (vitamin D2 or D3). The samples were protected from light, centrifuged, and stored at −78°C until analysis. Participants were not required to fast for blood collection, which was performed by routine venipuncture. Samples were collected in plain gel tubes to separate serum from red cells and allowed to clot for 30–45 min at room temperature. They were then centrifuged at 1792×g for 5 min, before the serum layer was transferred to glass tubes. All specimens were shipped frozen on dry ice to King Abdullah University Hospital and stored at −78°C until thawed for analysis.

Clinical assessment
Details regarding current medication(s) and diseases, and inclusion-exclusion criteria were acquired from patients’ hospital records. Other information about subjects was collected through direct interviews using a non-validated questionnaire. The questionnaire contained questions about age, gender, education, (indoor/ outdoor) occupation, and dress style. One post-treatment blood sample was collected from each subject 30 to 45 days post-treatment, as it was the most reasonable prearranged period to meet the patients at the same time while they were visiting their attending physicians. This was the most effective and convenient approach for the patients who come from distant geographic locations as well as for the researchers. This is for time, cost, and logistics constraints.

The analysis of all collected samples was completed in 3 days in May 2011. Compliance with vitamin D3 and vitamin D2 regimens was assessed by participants self-reporting. Compliance with vitamin D3 was defined as taking 100% of the tablets prescribed, and for vitamin D2 as taking the single bolus dose of vitamin D2 as prescribed.

Statistical analysis
Data were statistically analyzed using Microsoft Excel (2007) software. A t test was used to test the difference between 2 means, a chi-square test was used to test the association between categorical variables, and an analysis of variance (ANOVA) test was used for the analysis of variance between several means. Data were reported as mean (standard deviation [SD]). All tests were 2-tailed, and a P value <.05 was considered significant.

RESULTS
A total of 105 participants were enrolled in the study: 51 subjects received 600,000 IU vitamin D2 and 54 subjects received 500,000 IU vitamin D3. The participants were 66.3% female and 33.7% male. The females were divided according to their dress style: Hijab (uncovered face and hands) and Niqab (covering all of their bodies) (80.4%) and Western (4.37%). The mean ages were 45.2 (12.4) and 47.4 (14.9), for vitamin D2 and vitamin D3 groups, respectively. Socio-demographic data of the study participants are illustrated in Table 1. There was no significant difference between the mean ages of the 2 groups. The mean age for the vitamin D2 group was 47.35 (14.90), CI [43.28–51.42] and for the vitamin D3 group 45.22 (12.36), CI [41.74–48.7]. The mean total 25(OH)D increment from baseline was 10.33 (5.68) ng/mL over a mean of 43.08 (2.81) days for the vitamin D2 group. In the vitamin D3 group, the mean increment in 25(OH)D was 23.67 (9.29) ng/mL over a mean of 36.9 (2.9) days. The difference between the 2 mean increments was highly significant: P=3.15×10−18. Because the mean change in 25(OH)D level was measured over several days (32–45 days for the vitamin D2 group and 30–40 days for the vitamin D3 group), an ANOVA was applied to evaluate this source of variation. For the vitamin D3 group, the ANOVA for the difference between the means did not show any significant difference between the mean changes in serum 25(OH)D (total post-total pre) observed by duration (P=.65 for the vitamin D3 group and P=.42 for the vitamin D2 group).

Only 40% (20/51) of subjects treated with vitamin D2 attained a level of ≥20 ng/mL compared with 96.3% (52/54) treated with vitamin D3. In addition, only 8% of subjects treated with 600,000 IU vitamin D2 (4/51) attained an optimal level of 25(OH)D ≥30 ng/mL, compared with 92.6% (50/54) treated with 500,000 IU vitamin D3. Of the 51 subjects treated with 600,000 IU vitamin D2, 25(OH)D serum levels remained <10 ng/mL in 10% of subjects (5/51); they remained classified as severely deficient, despite receiving treatment. The vitamin D status of subjects after treatment with
CORRECTING VITAMIN D DEFICIENCY

600 000 IU of vitamin D2 and 500 000 IU of vitamin D3 is provided in Tables 2 and 3, respectively.

The influence of a single bolus dose of 600 000 IU vitamin D2 on the ratio of 25(OH)D3:D2 metabolites in 37 of 51 subjects was studied. All values below the limit of quantification (<4 ng/mL) were excluded. A total of 28 subjects (75.7%) showed a negative change, with a mean decrease of 6.75 ng/mL in the serum 25(OH)D3 level. However, 9 subjects (24.3%) displayed a positive change, with a mean increase of 4.47 ng/mL in the 25(OH)D3 level. Treatment with 600 000 IU vitamin D2 decreased 25(OH)D3 levels by a mean of 4 ng/mL in all subjects (n=37).

DISCUSSION

Several studies have evaluated the efficacy and safety of high-dose vitamin D regimens for correcting vitamin D deficiency.7-31 In the present study, the patients supplemented with 500 000 IU vitamin D3 had a significant increase in their serum 25(OH)D level, compared with those supplemented with a single mega-dose of 600 000 IU vitamin D2. In the vitamin D3–treated group, the average total serum 25(OH)D level increased by 47.03 (23.67), and more than 92.6% (50/54) of subjects reached the optimal vitamin D status (25(OH)D ≥30 ng/mL) over 36.9 (2.9) days. Conversely, in the vitamin D2–treated group, only 8% (4 subjects) achieved optimal levels. Furthermore, only 40 % (20/51) attained a level ≥20 ng/mL in the vitamin D2 group, whereas 96.3% (52/54) of subjects attained a level ≥20 ng/mL in the vitamin D3 group.

The results obtained with the vitamin D3–treated group in this study are comparable with previous studies that applied the 10-day 50 000 vitamin D3 regimen. Hackman et al30 showed that ≥90% of subjects treated with 500 000 IU achieved levels of >20 ng/mL. Bacon et al28 showed that 500 000 IU is safe and effective in older women with serum 25(OH)D levels <10 ng/mL, and that it produces a 15% reduction in PTH concentrations. Wu et al25 demonstrated that the high-dose regimen is safe and effective in raising mean serum 25(OH)D levels by 13 (4) ng/mL from baseline after 4 months.

However, the mean increment of serum 25(OH)D following supplementation with a single bolus dose of 600 000 IU vitamin D2 was only 10.33 (5.68) ng/mL over 43.08 (2.81) days. The 600 000 IU vitamin D2 single dose failed to correct vitamin D deficiency in 62% of subjects (31/51). Furthermore, serum 25(OH)D levels in 10% of subjects (5/51) remained ≤10 ng/mL.

The influence of a vitamin D2 mega-dose on 25(OH)D3 levels was studied. The level of 25(OH)
D3 metabolite increased in 24.3% of studied subjects (9/37); however, the increment in 25(OH)D3 level was modest (4.47 ng/mL), whereas the 25(OH)D3 level decreased in 75.7% (28/37) of subjects. The average decrease in 25(OH)D3 level (6.75 ng/mL) in 28 subjects in the vitamin D2–treated group was consistent with prior reports.18,31,32 The mechanism(s) and potential importance of this remain unclear. It is possible that this decline simply reflects competition for available 25-hydroxylase activity; however, in vivo regulation of vitamin D 25-hydroxylation in humans is not entirely understood.33 Of more importance is the impact of a vitamin D2-induced reduction in circulating 25(OH)D3 on clinical outcomes. To the best of our knowledge, there is no evidence that suggests that 25(OH)D2 has different biological effects than 25(OH)D3. The mechanism(s) by which vitamin D2 administration decreases 25(OH)D3 levels and the clinical importance of this, if any, requires elucidation.

The findings of this study reveal that vitamin D2 when given as a single 600 000 IU dose was not effective in correcting the vitamin D status. It is possible that the administration of such mega-doses interferes with homeostatic control mechanisms, possibly hepatic, and induces a competition for available 25-OH hydroxylase activity. This may lead to a decrease in the efficiency of vitamin D2 conversion to its active hydroxymetabolites. Nevertheless, studies that used a total of 600 000 IU of vitamin D2 (12 doses of 50 000 IU) over a period of 4 weeks showed that all individuals in the vitamin D2–treated group achieved a serum 25(OH)D concentration above 30 ng/mL, a commonly recommended goal of vitamin D repletion therapy.31 Another study, demonstrated that 500 000 IU vitamin D2 over 5 weeks was effective in increasing the 25(OH)D level by 24 ng/mL from baseline.27 This may imply that vitamin D2 when given in weekly doses is more effective than if it is given in a single bolus dose of 600 000 IU.

The half-life of 25(OH)D in serum is about 22 to 28 days. Thus, the period of this study is equivalent to 1 to 2 half-lives for the 2 treatment groups. However, a minimum period of 3 to 4 half-lives is necessary to accurately study steady state levels.30 Thus, results obtained in this study may not correspond to levels at the steady state. Instead, they may reflect the change in serum 25(OH)D level by 24 ng/mL from baseline.27 This study may have the following limitations. First, the study was conducted in an open-label design. In addition, the claims of the participants could not confirm that they were 100% compliant, although they were urged to be adherent, and were provided comprehensive counseling and education on the biological significance of vitamin D and its role in disease prevention. Second, this study had no placebo-only group. Not providing any vitamin D treatment to people who were deficient was considered unethical, especially given that 91.4% of the participants had 25(OH)D levels <12 ng/mL, suggesting they had “severe vitamin D deficiency”. Since clothing can prevent sun exposure, and thus vitamin D synthesis and status, Mulla et al showed that females dressing in Hijab or Niqab have lower 25(OH)D plasma levels than their counterpart Western style-dressed females living in Jordan. In addition, sun exposure to uncovered face and hands as in Hijab-dressed females is not sufficient for vitamin D synthesis.34 It is, therefore, possible that some of the changes in 25(OH)D levels were caused by factors other than the interventions provided, such as altered behavior relating to sun exposure or dietary modification. If this was the case, it is likely that the effect would be consistent across the 2 treatment groups. Furthermore, it is unlikely that increased ultraviolet exposure would have affected the results relating to changes in serum 25(OH)D levels.
since most of the participants of this study were women who wore conservative-style dress, shielding most of their skin, worked indoors or were housewives who had limited sun exposure.

The study sample size was sufficient. It included 109 treated subjects, from which 4 subjects were excluded following assessment, resulting in a final sample of 105 subjects. All participants fulfilled the requirements of the study, and the response rate was 83.2% (109 subjects responded, out of 131 potential candidates).

The effect of vitamin D on parathyroid hormone, phosphate, and calcium was not investigated in this study due to the limitation of resources. The present study demonstrated that the 10-day vitamin D3 regimen rapidly normalized 25(OH)D to optimal levels in the majority of subjects. This high-dose regimen may be an effective and cheap alternative for patients with vitamin D deficiency.

REFERENCES

1. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr 2004; 80 (Suppl 6):1678S-85S.
2. Holick MF. The vitamin D epidemic and its health consequences. Nutr [AUTHOR: If the journal name comprises single word, then it should not be abbreviated. Please check and make it full, if so.] 2005; 20(5):135-39.
3. KJ Pepper, SE Judd, MS Nanes, V Tangpricha. Evaluation of vitamin D repletion regimens to correct the vitamin D status in adults. Endocr Pract 2009; 15 (2): 159-103. [AUTHOR: Please check if the page range should be 95-103 instead of 159-103.]
4. Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative Meta-analysis. Am J Prev Med 2007; 32(3): 210-16.
5. Peterlik M, Cross HS. Dysfunction of the vitamin D endocrine system as common cause for multiple malignant and other chronic diseases. Anticancer Res 2006; 26 (4A): 2581-88.
6. Forman JP, Bischoff-Ferrari HA, Willett WC, Stampfer MJ, Curhan CG. Vitamin D intake and risk of incident hypertension: results from three large prospective cohort studies. Hypertension 2006; 48 (4):876-82.
7. Mathieu C, Gysemans C, Giulietti A, Bouillon R. Vitamin D and diabetes. Diabetologia 2005; 48 (7):1247-57.
8. Holick MF. Vitamin D deficiency.N Engl J Med 2007; 357(3): 266–81.
9. McGrath JJ, Kimlin MG, Saha S, Eyles DW, Parish AV. Vitamin D insufficiency in south-east Queensland. Med J Aust 2007; 174 (3): 150-51
10. Glith FM III, Lindsay JM, Zelesnick LB, Bre- enough WB III. Can vitamin D deficiency produce an unusual pain syndrome? Arch Intern Med 1991; 151(8): 1662-64.
11. Aufer P, Gandini S. Vitamin D supplementation and total mortality: a meta-analysis of randomized controlled trials. Arch Intern Med 2007;167(16):1730-37.
12. Zittermann A. Vitamin D in preventive medi- cine: are we ignoring the evidence? Br J Nutr 2006; 95(3):552-72.
13. Dawson-Hughes B, Heaney RP, HolickMF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. Osteoporos Int 2006; 16 (7): 713-16.
14. Przybelski P, Agrawal S, Krueger D, Engelke JA, Walburn F, Binkley N. Rapid correction of low vitamin D status in nursing home residents. Osteoporos Int 1991; 2008:1621-28.
15. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supple- mentation on fractures and mortality in men and women living in the community: randomized dou- ble blind controlled trial. BMJ 2003; 326(7387):469- 74.
16. VandeGriend JP, McQueen RB, Linnebur SA, Vondracek SF. Prescription ergocalciferol dosing for vitamin D repletion: a retrospective evaluation. Pharmacotherapy 2012; 32 (10):135-41.
17. Romagnoli E, Massia ML, Cipriani C, Fassino V, Mazzei F, D’Erraso E et al. Short and long-term variations in serum calcium hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. J Clin Endocrinol Metab 2008; 93(8):3015-20.
18. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. J Clin Endocrinol Metab 2004; 91(11): 5387-91.
19. Trang HM, Cole DE, Rubin LA, P ierratos A, Siu S, Veth R. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2, Am J Clin Nutr 1998; 68(4): 854-58.
20. Holick MF, Biancuzzo RM, Chen TC, Klein EK. Young A, Bibuld D, Reitz R, Salameh W et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab 2008;33(3): 677–81.
21. Gordon CM, Williams AL, Feldman HA, May J, Sinclair L, Vasquez A et al. Treatment of hypovita- minosis in infants and toddlers. J Clin Endocrinol Metab 2008; 93(7): 2716-21.
22. Glendenning P, Chew GT, Seymour HM, Gil- lett MJ, Goldswain PR, Ingerd JAC et al. Serum 25-hydroxyvitamin D levels in vitamin D-insuffi- cient hip fracture patients after supplementation with ergocalciferol and cholecalciferol. Bone 2008;45(5): 870-75.
23. Thacher TD, Obadofin MO, O’Brien KO, Abrams SA. The effect of vitamin D3 and vitamin D3 on intestinal calcium absorption in Nigerian children with rickets. J Clin Endocrinol Metab 2009; 94(9):3314-21.
24. Biancuzzo RM, Young A, Bibuld D, Cai MH, Winter MR, Klein EK et al. Fortification of orange juice with vitamin D2 or vitamin D3 is as effective as an oral supplement in maintaining vitamin D status in adults. Am J Clin Nutr 2010; 91(8):1621-6.
25. Wu F, Stakyova T, Horne A, Clearwater J, Ames R, Mason B et al. Efficacy of an oral, 1-day course of high-dose calcitriol in correcting vita- min D deficiency. NZ Med J 2003; 116(1179):U536.
26. Melhem SJ, Aiedeh KM, Haddi KA, Alali F. The Determination of 25-OH Vitamin D (D2/D3) in Human Serum by Liquid Chromatography Tandem Mass Spectrometry with Comparison to IDS Enzyme Immunoassay, JUPS 2013; 8(2):203-22.
27. Adams JS, Kantorovich V, Wu C, Javanbakht M, Hollis BW. Resolution of vitamin D insuffi- ciency in osteoporotic patients results in rapid re-covery of bone mineral density. J Clin Endocrinol Metab1999; 84(8):2729–30.
28. Bacon CJ, Gamble GD, Horne AM. High dose oral vitamin D3 supplementation in the elderly. Os- teoporos Int 2009 (8); 20:1407-15.
29. Cipriani C, Romagnoli E, Scillitani A, Chiodini I, Clerico R, Carnevale V et al. Effect of a single oral dose of 600,000 IU of cholecalciferol on se- rum calcitriol hormones in young subjects with vitamin D deficiency: a prospective intervention study. J Clin Endocrinol Metab 2010; 95(10):4771- 77.
30. Hackman KL, Gagnon C, Briscoe RK, Lam S, AnpalahanM, Ebeling PR. Efficacy and safety of oral continuous low-dose versus short-term high-dose vitamin D: a prospective randomized trial conducted in a clinical setting. Med J Aust 2010;192(12): 868-89.
31. Przybelski P, Agrawal S, Krueger D, Engelke JA, Walburn F, Binkley N. Rapid correction of low vitamin D status in nursing home residents. Os- teoporos Int 2008; 19(11):1621–28.
32. Tjellesen L, Hummer L, Christiansen C, Rad- bro P. Serum concentration of vitamin D metabo- lites during treatment with vitamin D2 and D3 in normal premenopausal women. Bone Miner1986(15):407–13.
33. Gascon-Barro M, Dancers C, Mirshahi A, Néron S, Zalats S, Nanci A. The normal liver harb-ors the vitamin D nuclear receptor in nonparen- chymal and biliary epithelial cells. Hepatology 2003; 37(5):1304-42.
34. Mallah EM, Hamad MF, Elmansareae MA, Qinnna NA, Idaikae NM, Arafat TA et al. Plasma concentrations of 25-hydroxyvitamin D among Jordanians: Effect of biological and habitual fac- tors on vitamin D status. BMC Clin Pathol 2011 Aug 4;11:8. doi: 10.1186/1472-6890-11-8.

CORRECTING VITAMIN D DEFICIENCY

Original article