European Journal of Clinical Nutrition (2006), 1–7
 0954-3007/06 $30.00
www.nature.com/ejcn

ORIGINAL ARTICLE

Vitamin D_2 dose required to rapidly increase 25OHD levels in osteoporotic women

SR Mastaglia[1], CA Mautalen[2], MS Parisi[1] and B Oliveri[2]

Sección Osteopatías Médicas, Hospital de Clínicas, Universidad de Buenos Aires, Buenos Aires, Argentina

Objective: Assessment of the effectiveness and safety of high daily 125 μg (5000 IU) or 250 μg (10 000IU) doses of vitamin D_2 during 3 months, in rapidly obtaining adequate 25 hydroxyvitamin D (25OHD) levels.
Design: Longitudinal study.
Subjects: Postmenopausal osteopenic/osteoporotic women ($n=38$) were studied during winter and spring. Median age (25–75th percentile) was 61.5 (57.00–66.25) years, and mean bone mineral density (BMD) was 0.902 (0.800–1.042)g/cm^2. Subjects were randomly divided into three groups: control group ($n=13$): no vitamin D_2, 125 μg/day ($n=13$) and 250 μg/day ($n=12$) of vitamin D_2 groups, all receiving 500 mg calcium/day. Serum calcium, phosphate, bone alkaline phosphatase (BAP), C-telopeptide (CTX), 25OHD, mid-molecule parathyroid hormone (mmPTH), daily urinary calcium and creatinine excretion were determined at baseline and monthly.
Results: For all subjects ($n=38$), the median baseline 25 hydroxyvitamin D (25OHD) level was 36.25 (27.5–48.12) nmol/l. After 3 months, 8% of the patients in the control group, 50% in the 125 μg/day group and 75% in the 250 μg/day group had 25OHD values above 85 nmol/l (34 ng/ml). Considering both vitamin D_2 groups together, mmPTH and BAP levels diminished significantly after 3 months ($P<0.02$), unlike those of CTX. Serum calcium remained within normal range during the follow-up.
Conclusions: The oral dose of vitamin D_2 required to rapidly achieve adequate levels of 25OHD is seemingly much higher than the usual recommended vitamin D_3 dose (20 μg/day). During 3 months, 250 μg/day of vitamin D_2 most effectively raised 25OHD levels to 85 nmol/l in 75% of the postmenopausal osteopenic/osteoporotic women treated.
European Journal of Clinical Nutrition advance online publication, 4 January 2006; doi:10.1038/sj.ejcn.1602369

Keywords: menopause; osteopenia; osteoporosis; vitamin D; vitamin D insufficiency; vitamin D requirement

Introduction

Vitamin D insufficiency – determined by 25 hydroxyvitamin D (25OHD) serum levels– is common in elderly populations (particularly in institutionalized subjects) (Mc Kenna, 1992) and leads to secondary hyperparathyroidism, high bone turnover, bone loss and osteoporotic fractures (Brazier *et al*., 1995; Chapuy *et al*., 1996; Dawson-Hughes *et al*., 1997; Boff *et al*., 1999; Oliveri *et al*., 2004). The threshold of serum 25OHD that separates vitamin D sufficiency from insufficiency has largely been defined by its biological effect, primarily by the increase in serum parathyroid hormone (PTH).

Reports in the literature have established serum 25OHD levels below which PTH begins to rise to be between 30 and 50 nmol/l (12–20 ng/ml) (Bouillon *et al*., 1987; Gloth *et al*., 1995; Ooms *et al*., 1995; Mc Kenna and Freaney, 1998). Other authors have proposed higher levels of 25OHD as limits of insufficiency: 62.5, 77.5 or 110 nmol/l (Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1997; Haden *et al*., 1999). Prior studies performed on elderly people in our country established a cutoff level of 67.5 nmol/l (Oliveri *et al*., 2004). Furthermore, administration of vitamin D supplements to attain mean 25OHD levels between 72.5 and 110 nmol/l decreased the

Correspondence: SR Mastaglia, Córdoba 2351, 8° piso (1120) Buenos Aires, Argentina.
E-mail: osteologia@ciudad.com.ar
[1]Fellow of the National Council for Scientific and Technologic Research (CONICET).
[2]Researcher of the National Council for Scientific and Technological Research (CONICET).
Guarantor: SR Mastaglia.
Contributors: SRM participated in the recruitment of subjects, the statistical analysis and the preparation of the manuscript. MSP contributed with statistical analysis and preparation of the manuscript. CM and BO participated in the design of the study, the statistical analysis and the preparation of the manuscript.
Received 27 April 2005; revised 21 September 2005; accepted 7 November 2005

incidence of osteoporotic fractures (Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1997; Trivedi *et al*., 2003). A group of experts on vitamin D proposed that patients with minimum desirable 25OHD level clusters between 70 and 80 nmol/l to be at lower risk of fracture (Dawson-Hughes *et al*., 2005).

Heaney *et al*. (2003b) proposed a different parameter to establish adequate 25OHD levels: optimal intestinal calcium absorption. The authors reported that 25OHD levels in the elderly should be above 85 nmol/l. Even though there is no consensus on this 'adequate' level of 25OHD, we considered 85 nmol/l as the desirable level to be achieved, taking into account the importance of calcium intestinal absorption in skeletal balance. The same group of investigators found that a group of young adult men needed an average daily vitamin D intake (supplement food and tissue store) of approximately 95 μg/day to sustain 'normal' 25OHD levels during winter. Administration of 125 or 250 μg/day of oral vitamin D_3 per day during 20 weeks resulted in 25OHD levels between 150 and 200 nmol/l (from a baseline level of 67.5 nmol/l, but no abnormal levels of serum calcium were observed) (Heaney *et al*., 2003a). To our knowledge, there are no previous studies on the dose of oral vitamin D_2 needed to rapidly produce adequate increments in 25OHD levels in postmenopausal women who need to be treated for osteoporosis. This information is of particular importance considering that oral vitamin D_3 treatment regimens are able to induce a significant reduction in bone fractures in men and women over the age of 65years (Chapuy *et al*., 1992; Trivedi *et al*., 2003). In addition, it has been reported that adequate levels of vitamin D enhance response to antiresorptive therapy for osteoporosis (Koster *et al*., 1996; Yamanaka *et al*., 2004).

The aim of our study was to evaluate whether administration of high daily doses (125 and 250 μg) of vitamin D_2 during 3 months allowed to obtain desirable 25OHD levels (above 85 nmol/l) rapidly.

Subjects

Seventy women seen at the outpatient's section of the Hospital de Clínicas, who presented spontaneously for bone mass evaluation, were asked to participate in the study. All subjects were aged between 50 and 70 years, had started menopause at least 1 year prior to the study, and lived in Buenos Aires (latitude 34°S).

Screening

Sixty five women agreed to participate, five of whom were excluded on the basis of the following exclusion criteria: (1) treatment with vitamin D or any other medication known to affect mineral metabolism, within 12 months prior to the study; (2) a health condition which rendered administration of vitamin D unadvisable (renal lithiasis, tumors) or affecting vitamin D metabolism (hepatic disease, renal insufficiency).

Densitometry of the lumbar spine or femoral neck and biochemical determinations of mineral metabolism were performed on the remaining 60 women, after obtaining their written informed consent. Densitometry of the lumbar spine (LS) or femoral neck (FN) was performed by DXA (LUNAR DPX-L) and the results were compared with normal reference values to calculate *T* score (Vega *et al*., 1993).

Five patients presented hypercalciuria and 10 failed to meet densitometric inclusion criteria: bone mineral density (BMD), *T* score of LS or FN below −1.

Randomization and design

We carried out this longitudinal open study during winter and spring (June–November) of 2002. The protocol was approved by the Ethics Committee of the Hospital de Clínicas in agreement with the Declaration of Helsinki (Edinburgh 2000) (World Medical Association, 2000).

The final study population comprised 45 women who were randomly assigned to one of three three treatment groups. All patients received an oral dose of 500 mg/day of calcium in the form of calcium carbonate (1 tablet/day) and they were randomly assigned to receive one of the following treatments for 3 months: control group: no vitamin D_2 ($n=13$); 125 μg/day (5000 IU/day) of vitamin D_2 ($n=13$); and 250 μg/day (10 000 IU/day) of vitamin D_2 ($n=12$). Vitamin D_2 (the only pharmaceutical preparation of vitamin D alone, available in Argentina) was administered in the form of oral drops. The vials containing vitamin D_2 were supplied by Spedrog-Caillon (Buenos Aires, Argentina). The vitamin D_2 solution was analyzed blindly by a laboratory using liquid chromatography; the concentration was found to be 62.5 μg per drop.

Patients in the 125 μg/day group received two daily drops and those in the 250 μg/day group received four daily drops. Compliance with calcium and vitamin D_2 regimens was assessed by pill counts and drop counts in each box and vial returned to us at each monthly visit. Seven patients dropped out of the study during follow-up (two from the control group, two from the 125 μg/day group and three from the 250 μg/day group): four women for personal reasons, one underwent emergency colecystectomy, one was diagnosed with primary hyperparathyroidism – previously masked because of vitamin D deficiency, and one failed to comply with the instructions. We report the results obtained from the 38 patients who completed the treatment, and whose baseline characteristics were (median) (25–75th percentile): 61.5 (57.0–66.2) years old, 65.5 (58.0–72.25) kg weight, 1.55 (1.51–1.58) m height, 26.6 (23.3–30.6) kg/m^2 body mass index (BMI) and 0.902 (0.800–1.042) g/cm^2 BMD.

Materials and methods

Biochemical determinations

Fasting blood and 24 h urine samples were collected from all the patients before the onset of the study and monthly

throughout the study. All serum and urine samples were frozen and stored until processed. The side effects evaluated for safety were the presence of hypercalcemia (serum calcium >10.5 mg/dl) and hypercalciuria (urinary calcium >250 mg/24 h or calciuria/creatininuria ratio >0.37 mg/mg) (Vieth *et al*., 2001). Serum calcium, 24 h urinary calcium and creatinine excretion were determined each month before giving the patient a new vial of vitamin D_2 and a new box of calcium tablets.

Serum calcium, phosphate, urinary calcium and creatinine determinations were performed using standard techniques.

Bone alkaline phosphatase (BAP) (reference range: 31–95 IU/l) was determined by agglutination with wheat germ (Farley *et al*., 1994). Serum levels of 25OHD were determined by radioimmunoassay (DIASORIN). Inter- and intra-assay coefficients of variation (CV) were 19 and 7.6%, respectively. PTH was measured by radioimmunoassay using an antiserum against the mid- and terminal carboxyl groups of the molecule (mmPTH) (reference range: 20–100 pg/ml) (Arnaud *et al*., 1971).

Serum C-terminal crosslinking telopeptide of type I collagen (CTX) (reference range: 14–450 ng/ml) was measured by electrochemical luminescence (Roche Elecsys 1010); intra and interassay CV were 1.6 and 6.1%, respectively. All samples for 25OHD, mmPTH, CTX and BAP determinations were analyzed in the same assay and processed simultaneously at the end of the follow-up period in order to minimize interassay variation.

Statistical analyses

Statistical analysis was performed using SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL, USA). Comparisons between groups, at different time points, were established using a nonparametric unpaired test (Mann–Whitney). Comparison between baseline and follow-up values in each group was performed using a nonparametric paired test (Wilcoxon). A value of P below 0.05 was considered significant.

Results

Table 1 shows the baseline characteristics of the patients arranged according to treatment groups. None of the studied variables showed significant differences among groups.

Compliance

The mean rates of compliance with treatment on the basis of pill and drop counts were ($X\pm$s.d.) 89 ± 11 and 92 ± 10%, respectively.

Changes in serum 25OHD

Table 2 shows 25OHD medians values of the three groups throughout the 3 months follow-up. No changes in 25OHD values were observed throughout the study period in the control group. Values increased significantly in the 125 and 250 μg/day groups after 1 month of treatment ($P<0.01$). The values increased from 42.0 (23.7–45.0) nmol/l at baseline to 77.5 (66.2–56.2) nmol/l, after 3 months treatment in the 125 μg/day group ($P<0.001$ vs baseline), and from 32.5 (27.5–45.0) nmol/l to 97.7 (79.3–123.1) nmol/l in the 250 μg/day group ($P<0.01$). After 2 and 3 months of treatment,

Table 1 Characteristics of the three groups of patients at baseline

Group	*Age (years)*	*Weight (kg)*	*Height (m)*	*BMI (kg/m^2)*	*BMD (g/cm^2)*	*mmPTH (pg/ml)*	*25 OHD (nmol/l)*
Control ($n=13$)	61.9 (54.5–67.0)	60.0 (56.5–70.0)	1.56 (1.52–1.59)	25.8 (23.2–28.6)	0.837 (0.731–0.973)	45.0 (32.5–65.0)	45.0 (31.2–61.2)
125 μg/day ($n=13$)	65.0 (57.0–67.0)	66.0 (60.5–77.0)	1.50 (1.51–1.58)	27.4 (25.0–31.7)	0.973 (0.839–1.103)	65.0 (52.5–90.0)	42.0 (23.7–45.0)
250 μg/day ($n=12$)	60.0 (56.2–66.2)	66.0 (52.7–72.2)	1.53 (1.50–1.57)	25.9 (22.4–30.4)	0.968 (0.817–1.056)	57.5 (36.2–76.2)	32.5 (27.5–37.5)
	NS	NS	NS	NS	NS	NS	NS

Results are expressed as median (25–75th percentile), BMI: body mass index, BMD: bone mineral density, mmPTH: mid-molecule parathormone, 25OHD: 25 hydroxyvitamin D, NS: not statistically significant.

Table 2 Serum values of 25 hydroxyvitamin D (25OHD) and mid-molecule parathormone (mmPTH) of the three groups of patients with and without vitamin D_2 treatment, throughout the study

Month	*25OHD (nmol/l)*			*mmPTH (pg/ml)*		
	Control group	*125 μg/day group*	*250 μg/day group*	*Control group*	*125 μg/day group*	*250 μg/day group*
0	45.0 (31.2–61.2)	42.0 (23.7–45.0)	32.5 (27.5–37.5)	45.0 (32.5–65.0)	65.0 (52.5–90.0)	57.5 (36.2–76.2)
1	42.5 (30.0–67.5)	63.7** (45.6–81.3)	77.5** (70.8–86.8)	60.0 (35.0–75.0)	55.0 (40.0–60.0)	60.0 (40.0–82.5)
2	47.5 (38.7–66.2)	67.5** (57.5–91.2)	97.7** (79.1–111.8)	50.0 (32.5–62.5)	60.0 (45.0–75.0)	50.0 (41.2–72.5)
3	55.0 [72.5–68)	77.5** (66.2–156.2)	97.7** (79.3–123.1)	40.0 (30.0–77.5)	55.0* (37.5–62.5)	50.0 (32.5–67.5)

Results are expressed as median (25–75th percentile), **$P<0.01$ vs baseline, *$0.01>P>0.05$ vs baseline. All groups received 500 mg/day of calcium carbonate.

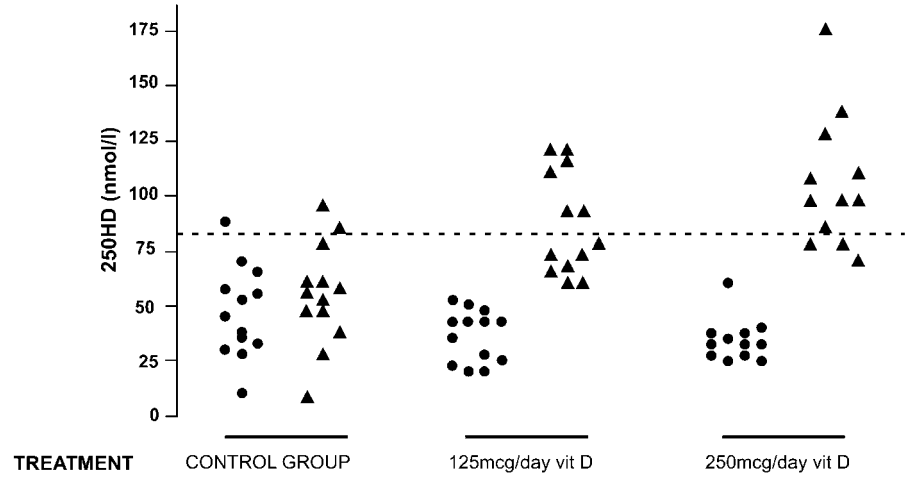

Figure 1 Individual values of 25 hydroxyvitamin D (25OHD) at baseline (black circles) and after 3 months (black triangles) of follow-up in three group. All patients received 500 mg/day of calcium carbonate. The dotted line shows the hypothetical adequate 25OHD level of 85 nmol/l.

average 25OHD levels in the 250 μg/day group were higher than those in the 125 μg/day group, although the difference was not statistically significant ($0.1 > P > 0.05$). Figure 1 shows individual 25OHD serum values at baseline and after 3 months of treatment. Considering a cutoff value of 85 nmol/l, one out of 13 patients in the control group exhibited higher values at baseline and throughout the study; however, none of the remaining patients in this group with lower baseline values reached this level. All baseline values in the 125 μg/day group were below 52.5 nmol/l, yet six out of 12 patients (50%) showed values above 85 nmol/l after 3 months of treatment. Nine out of 12 patients (75%) reached levels of 25OHD above the same limit after 3 months in the 250 μg/day group. If we consider a different cutoff level of 67.5 nmol/l, 77 and 100% of patients in the 125 or 250 μg/day groups, respectively, after 3 months were above this level, whereas the corresponding percentage for controls was only 23%.

Changes in PTH

Serum mmPTH values obtained throughout the study in each of the three groups are shown in Table 2. A decreasing trend in mmPTH levels was observed in each of the groups receiving vitamin D_2. The differences were close to statistical significance in the 125 μg/day group after 3 months of treatment ($0.1 > P > 0.05$). Evaluation of results considering both groups that received vitamin D_2 as a single group showed that mmPTH levels decreased significantly from 60.0 [40.0–60.0] pg/ml to 50.0 [35–62.5] pg/ml after 3 months of treatment ($P < 0.02$).

Changes in bone markers

BAP values tended to decrease as compared to baseline values in vitamin D_2 groups; the decrease was significant in the 125 μg/day group after 3 months of treatment ($P < 0.02$). Evaluation of results considering both groups that received vitamin D_2 as a single group showed that BAP levels decreased significantly from 68.0 [54–78] IU/l to 61.0 [54–71] IU/l after 3 months of treatment ($P < 0.02$). No statistically significant changes in serum CTX were observed throughout the study.

Safety

Average serum calcium levels remained unchanged in the control and in the 125 μg/day group. The average value in the 250 μg/day group was found to increase significantly after 2 months of treatment ($P < 0.05$). Analysis of results, considering both vitamin D_2-treated groups as a whole, showed an increase in serum calcium levels from 9.3 [8.9–9.6] mg/dl at baseline to 9.6 [9.3–9.9] mg/dl after 3 months treatment ($P < 0.05$). However, no individual value in any of the three groups was above the upper limit of the normal range (10.5 mg/dl). After 3 months of treatment, urinary calcium excretion was found to increase in patients receiving vitamin D_2: from 99.0 [69.5–147.5] mg/24 h to 152.0 [102–204] mg/24 in the 125 μg/day group ($P < 0.05$), and from 121.0 [88.7–140.0] mg/24 h to 149.0 [120.7–225.7] mg/24 h in the 250 μg/day group ($P < 0.05$). The control group (500 mg of oral calcium per day) exhibited a nonsignificant increase from 117.0 [88.5–189.5] mg/24 h at baseline to 183.0 [105.0–250.0] mg/24 h after 3 months of treatment.

One patient in the 125 μg/day group exhibited values above 250 mg/24 h at 2 and 3 months (312 and 340 mg/24 h, respectively) compared to a baseline value of 172 mg/24 h. One patient in the 250 μg/day group showed an increase from a baseline value of 229–278 mg/24 h after 3 months treatment. One patient in the control group exhibited hypercalciuria (>250 mg/24 h), with values reaching

358 mg/24 h at 3 months compared to 202 mg/24 h at baseline. Urinary calcium excretion was below 250 mg/24 h in all the remaining cases. There were no differences among groups at any of the studied times points when comparing the number of patients with urinary calcium levels higher than 250 mg/24 h. None of the subjects showed a calciuria/creatininuria ratio >0.37 mg/mg during the follow-up.

Discussion

Vitamin D_3 is an important therapeutic agent used in osteoporosis treatment, since several studies have proven its efficacy in preventing bone fragility-associated fractures (Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1997; Trivedi *et al*., 2003; Bischoff *et al*., 2005). Some studies have suggested that one important effect of vitamin D is that it improves muscular function, which in turn would contribute to reducing falls (Pfeifer *et al*., 2002; Bischoff *et al*., 2003). The mechanisms most frequently associated with vitamin D effects are an increase in intestinal calcium absorption, a decrease in PTH secretion, and a diminution in the rate of bone remodeling (Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1997). However, few studies have attempted to determine the optimal dose of vitamin D required to rapidly achieve the aforementioned changes in patients with osteopenia/osteoporosis without causing hypercalcemia. Heaney *et al*. (2003b) established that serum 25OHD levels should be above 85 nmol/l in order to obtain optimal intestinal calcium absorption. This level of serum 25OHD is not very different from that found in other studies establishing the optimal level of 25OHD to be between 62.5 and 110 nmol/l, in order to avoid increases in PTH serum levels and to reduce the incidence of osteoporotic fractures (Chapuy *et al*., 1992; Dawson-Hughes *et al*., 1997; Haden *et al*., 1999; Trivedi *et al*., 2003; Oliveri *et al*., 2004; Dawson-Hughes *et al*., 2005). The studies performed on Caucasian women who received vitamin D_2 or D_3 to treat and/or prevent osteoporosis have shown wide variations as regards the dose employed and the time points used for follow-up determinations. Chapuy *et al*. (1992) found that administration of 20 μg/day of vitamin D_3 increased 25OHD levels from 40 to 105 nmol/l after 18 months of treatment, but levels of 25OHD at shorter periods are not reported. A 100 μg/day dose of vitamin D_3 have been used by Vieth *et al*. (2001) in young men and women, increasing 25OHD levels from 40 to 97.5 nmol/l at 3 months; the levels remained unchanged after 5 months treatment. In the same study, the author reported that administration of 25 μg/day of vitamin D_3 increased 25OHD levels from 40 nmol/l at baseline to 67.5 nmol/l at 3 months, finding no changes after 5 months of treatment. In older people, the same authors (Vieth *et al*., 2004) administered doses of 15 and 100 μg/day of vitamin D_3 during more than 6 months and observed a higher improvement of well-being score in the higher vitamin D_3 dose group. Other studies using different oral doses (10–20 μg/day) with baseline 25OHD levels <50 nmol/ml (range: 7.5–47.0) failed to achieve average 25OHD levels above 67.5 nmol/l (Brazier *et al*., 1995; Lips *et al*., 1996; Meyer *et al*., 2002). Tangpricha *et al*. (2003) administered 25 μg/day of vitamin D_3 in orange juice to a group of normal young adults, obtaining an important mean increase in 25OHD levels from 37 to 94 nmol/l in 12 weeks. In turn, Malabanan *et al*. (1998) administered 1250 μg of oral vitamin D_2 once a week (equivalent to $\sim$171.5 μg/day) to a group of subjects with a mean age of 67 years and found that average 25OHD baseline level of 42.5 nmol/l rose to a mean value of 87.5 nmol/l after 8 weeks treatment; the authors did not report whether the subjects had normal or diminished bone mass nor the percentage of subjects who reached levels above 85 nmol/l.

Dawson Hughes *et al*. (1997) conducted studies in the US on a population with baseline 25OHD values of approximately 75 nmol/l. Administration of 17.5 μg/day of vitamin D_3 elevated 25OHD levels to 110 nmol/l after 3 months of treatment. It is most likely that the required dose differs according to the baseline level of 25OHD (<or >75 nmol/l). The present study is the first to be conducted in women over the age of 50 years with osteopenia/osteoporosis and with average 25OHD baseline values below 50 nmol/l. These 25OHD baseline values were similar to those previously reported for the Buenos Aires population in a study carried out in winter on subjects in the same age range (Oliveri *et al*., 2004). Administration of a dose of 125 or 250 μg/day of vitamin D_2 during 3 months did not cause hypercalcemia. One patient in each group had hypercalciuria (>250 mg/24 h). After 3 months treatment, 75% of patients receiving 250 μg/day vitamin D_2 and 50% of those treated with 125 μg/day of vitamin D_2 had reached 25OHD values above 85 nmol/l which is probably an adequate therapeutic level for patients with osteopenia/osteoporosis. Given the small number of patients in each group, results were also evaluated considering the groups receiving 125 or 250 μg/day of vitamin D_2 as a whole, and showed significant decreases in serum levels of mmPTH and BAP; both these effects are desirable therapeutic objectives of osteoporosis treatments. The lack of response of serum CTX may be attributed to the marked variation and dispersion of this bone resorption marker or to the small number of patients. The 25OHD values observed at 3 months in both groups treated with vitamin D_2 were higher than those observed at 2 months, so that the present study does not allow establishing the safety of long-term treatment with these doses. However, it is noteworthy that according to Vieth *et al*. (2001), administration of 100 μg/day of vitamin D_3 did not cause 25OHD levels to change between months 3 and 5 of treatment. Both forms of vitamin D, ergocalciferol (D_2) or cholecalciferol (D_3), were generally considered equivalent. However, recent publications (Trang *et al*., 1998; Armas *et al*., 2004) suggest that vitamin D_2 is less effective than vitamin D_3 in raising serum 25OHD levels. Trang *et al*. (1998) administered both

forms in a dose of 100 μg/day during 1 month. The increase in serum 25OHD levels was 1.7-fold higher with vitamin D_3. Armas *et al*. (2004) showed that administration of a single dose of 1250 μg/day of vitamin D_2 or vitamin D_3 produced similar initial increases in 25OHD, but levels were maintained over a longer period with the vitamin D_3 compound, suggesting that potency of vitamin D_2 is less than one-third than that of vitamin D_3. The issue is still controversial with a dissenting observation reported recently (Rapuri *et al*., 2004). Additional studies should be conducted to establish optimal dosing taking into account the form of vitamin D administered.

The limitations of the present study are: (1) all subjects presented spontaneously to our hospital for bone mass evaluation. It is possible, but unlikely, that this group of patients is not a representative sample of the entire postmenopausal population. As mentioned above, baseline 25OHD values were similar to those observed in previous studies (Oliveri *et al*., 2004). (2) We did not study the effect of 20 μg/day of vitamin D_2. However, previous studies, such as Heaney's report, have shown a limited increment in 25OHD levels with a daily dose of 25 μg of vitamin D_3 (Heaney *et al*., 2003a). Further long-term studies – 12 to 24 months – should be performed to asses the safety and efficacy of using elevated doses of vitamin D in osteopenic/osteoporotic patients, and to establish the period throughout which these high doses should be maintained.

This study evidences the need to re-examine the doses used conventionally on these patients. In addition, our results show that with the administration of a dose of 125 or 250 μg/day of vitamin D_2 during 3 months most patients reached adequate levels of 25OHD, without causing any significant adverse effects.

Acknowledgements

This study was supported by the Fundación de Osteoporosis y Enfermedades Metabólicas Óseas (FOEMO). Spedrog Caillon SAIC laboratory supplied the vitamin D_2 and calcium.

References

Armas LAG, Hollis BW, Heaney RP (2004). Vitamin D $_2$ is much less effective than vitamin D_3 in humans. *J Clin Endocrinol Metab* **89**, 5387–5391.

Arnaud CD, Tsao HS, Littlediket T (1971). Radioimmunoassay of human parathyroid hormone in serum. *J Clin Invest* **50**, 21–31.

Bischoff H, Stahëlin H, Dick W, Akos R, Knecht M, Salis C *et al*. (2003). Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res* **18**, 343–351.

Bischoff H, Willett W, Wong B, Giovannucci E, Dietrich T, Dawson-Hughes B (2005). Fracture prevention with vitamin D supplementation. *JAMA* **293**, 2257–2293.

Boff MS, Kohlmeier L, Hurwitz S, Franklin J, Wright J, Glowascki J (1999). Occult vitamin D deficiency in postmenopausal women with acute hip fracture. *JAMA* **281**, 1505–1511.

Bouillon RA, Aurweerch MD, Lissens WD, Pelemans WK (1987). Vitamin D status in the elderly, seasonal substrate deficiency causes 1,25$(OH)_2$ cholecalciferol deficiency. *Am J Clin Nutr* **45**, 755–763.

Brazier M, Kamel S, Maamer M, Agbomson F, Elesper I, Garabedian M *et al*. (1995). Markers of bone remodeling in the elderly subject: effect of vitamin D insufficiency and its correction. *J Bone Miner Res* **10**, 1753–1761.

Chapuy MC, Arlot M, Duboeuf F, Brun J, Crouzet B, Arnaud S *et al*. (1992). Vitamin D_3 and calcium to prevent hip fractures in elderly women. *N Engl J Med* **327**, 1637–1642.

Chapuy MC, Schott AM, Garnero P, Hans D, Delmas PD, Meunier PJ (1996). Healthy elderly French women living at home have secondary hyperparathyroidism and high turnover in winter. *J Clin Endocrinol* **81**, 1129–1133.

Dawson-Hughes B, Harris S, Krall E, Dallal G (1997). Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med* **337**, 670–676.

Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R (2005). Estimates of optimal vitamin D status. *Osteoporos Int* **16**, 713–716.

Farley JR, Hall SL, Ilacas D, Orcutt C, Miller BE, Hill CS *et al*. (1994). Quantification of skeletal alkaline phosphatase in osteoporotic serum by wheat germ agglutinin precipitation, heat inactivation, and a two-site immunoradiomeric assay. *Clin Chem* **40**, 1749–1756.

Gloth FM, Gunberg CM, Hollis BW, Haddad JG, Tobin JD (1995). Vitamin D deficiency in homebound elderly persons. *JAMA* **274**, 1683–1686.

Haden ST, Fuleihan GEH, Angell JE, Cotran NM, LeBoff MS (1999). Calcidiol and PTH levels in women attending an osteoporosis program. *Calcif Tissue Int* **64**, 275–279.

Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ (2003a). Human Serum 25 hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* **77**, 204–210.

Heaney RP, Dowell M, Hale C, Bendich A (2003b). Calcium absorption varies within the reference range for serum 25-hydroxivitamin D. *J Am Coll Nutr* **22**, 142–146.

Koster JC, Hackeng WH, Mulder H (1996). Disminished effect of etidronate in vitamin D deficient osteopenic postmenopausal women. *Eur J Clin Pharmacol* **51**, 145–147.

Lips P, Graafimans WC, Ooms ME, Bezemer PD, Bouyrt LM (1996). The vitamin D supplementation and fracture incidence in elderly people: a randomized placebo-controlled trial. *Ann Intern Med* **24**, 400–406.

Malabanan A, Veronikis IE, Holick MF (1998). Redefining vitamin D insufficiency. *Lancet* **351**, 805–806.

Mc Kenna M (1992). Differences in vitamin D status between countries in young adults and the elderly. *Am J Med* **93**, 69–77.

Mc Kenna MJ, Freaney R (1998). Secondary hyperparathyroidism in the elderly: means to defining hypovitaminosis D. *Osteoporos Int* **8** (Suppl 2), 3–6.

Meyer H, Smedshaug G, Kvaavik E, Falch J, Tverdal A, Pedersen J (2002). Can vitamin D supplementation reduce the risk of fracture in the elderly? A randomized controlled trial. *J Bone Miner Res* **4**, 709–715.

Oliveri B, Plantalech L, Bagur A, Wittich A, Rovai G, Pusiol E *et al*. (2004). High prevalence of vitamin D insufficiency in healthy elderly people living at home in Argentina. *Eur J Clin Nutr* **58**, 337–342.

Ooms ME, Lips P, Roos JC, Van der Vijgh WJF, Popp-Snijders C, Bezemer D *et al*. (1995). Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. *J Bone Miner Res* **8**, 1177–1184.

Pfeifer M, Begerow B, Minne H (2002). Vitamin D and muscle function. *Osteoporos Int* **13**, 187–194.

Rapuri PB, Gallagher JC, Haynatzki G (2004). Effect of vitamins D_2 and D_3 supplement use on serum 25OHD concentration in elderly women in summer and winter. *Calcif Tissue Int* **74**, 150–156.

Tangpricha V, Koutkia P, Rieke SM, Chen TC, Perez AA, Holick F (2003). Fortification of orange juice with vitamin D: a novel approach for enhancing vitamin D nutritional health. *Am J Clin Nutr* **77**, 1478–1483.

Trang HM, Cole DEC, Rubin LA, Pierratos A, Shirley S, Vieth R (1998). Evidence that vitamin D_3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D_2. *Am J Clin Nutr* **68**, 854–858.

Trivedi DP, Doll R, Tee Khaw K (2003). Effect of four monthly oral vitamin D (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomized double blind controlled trial. *BMJ* **326**, 469–474.

Vega E, Bagur A, Mautalen C (1993). Densidad Mineral Ósea en Mujeres Osteoporóticas y Normales de Buenos Aires. *Medicina (Buenos Aires)* **53**, 211–216.

Vieth R, Chan PCR, MacFarlane GD (2001). Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. *Am J Clin Nutr* **73**, 288–294.

Vieth R, Kinball S, Hu Amanda, Walfish PG (2004). Randomized comparison of the effects of the vitamin D_3 adequate intake versus 100 mcg (4000IU) per day on biochemical responses and the well-being of patients. *Nutr J* **3**, 8–17.

World Medical Association Declaration of Helsinki (2000). Ethical principles for medical research involving human subjects. *JAMA* **284**, 3043–3045.

Yamanaka M, Ishijima M, Tokita A, Kitahara K, Enomoto F, Sawa M *et al*. (2004). Importance of the understanding of the 25-hydroxyvitamin D status for the alendronate treatment of postmenopausal osteoporosis. *J Bone Min Res* **19** (Suppl 1), S463, M495.