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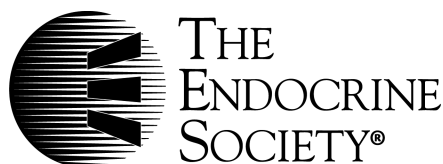
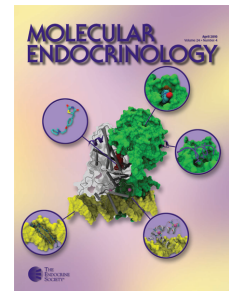
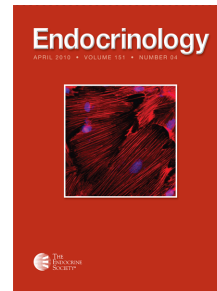
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Effect of a Single Oral Dose of 600,000 IU of Cholecalciferol on Serum Calciotropic Hormones in Young Subjects with Vitamin D Deficiency: A Prospective Intervention Study

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Context: Effects of vitamin D repletion in young people with low vitamin D status have not been investigated so far.

Objective: We evaluated the effect of a single massive dose of cholecalciferol on calcium metabolism at 3, 15, and 30 d, compared to baseline.

Design and Setting: We conducted a prospective intervention study in an ambulatory care setting.

Participants: Forty-eight young subjects with vitamin D deficiency participated in the study.

Intervention: A single oral dose of 600,000 IU of cholecalciferol was administered to each subject.

Main Outcome Measures: We evaluated serum changes of 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D, calcium, and PTH induced by a single load of cholecalciferol.

Results: The 25(OH)D level was 15.8 ± 6.5 ng/ml at baseline and became 77.2 ± 30.5 ng/ml at 3 d ($P < 0.001$) and 62.4 ± 26.1 ng/ml at 30 d ($P < 0.001$). PTH levels concomitantly decreased from 53.0 ± 20.1 to 38.6 ± 17.2 pg/ml at 3 d and to 43.4 ± 14.0 pg/ml at 30 d ($P < 0.001$ for both). The trends were maintained in a subgroup followed up to 90 d ($P < 0.001$). Mean serum Ca and P significantly increased compared to baseline, whereas serum Mg decreased at 3 d. 1,25-Dihydroxyvitamin D significantly increased from 46.8 ± 18.9 to 97.8 ± 38.3 pg/ml at 3 d ($P < 0.001$) and to 59.5 ± 27.3 pg/ml at 60 d ($P < 0.05$).

Conclusions: A single oral dose of 600,000 IU of cholecalciferol rapidly enhances 25(OH)D and reduces PTH in young people with vitamin D deficiency. (*J Clin Endocrinol Metab* 95: 4771–4777, 2010)

Vitamin D depletion has been reported as a very common condition worldwide, with many implications for health (1–4). Although elderly institutionalized patients are at high risk, several studies have also shown a high prevalence of vitamin D deficiency among healthy postmenopausal free-living women and among the gen-

eral adult population (5–13). Well-known consequences of hypovitaminosis D are secondary hyperparathyroidism, bone loss, proximal muscle weakness, increase in body sway, falls, and fractures (7). Moreover, nonskeletal consequences of vitamin D deficiency have been associated with an enhanced risk of chronic disease and cancer

(1–4, 14), and the association between intake of vitamin D supplements and decrease in all-cause mortality has been reported (15). Sunlight exposure is widely recognized as the main source of vitamin D, but prolonged sun exposure is usually discouraged because of an increased risk of skin cancer. A recent evidence-based review (16) actually recognized that a threshold of sun exposure sufficient to maintain a healthy vitamin D status without measurable cancer risk is difficult to define. Hence, current guidelines recommend a daily intake of at least 1000 IU of vitamin D. To reach this supplementation, an adequate food fortification and oral supplementation are widely suggested (1, 17). However, clinical recommendations concerning the optimal dose and the frequency of administration of vitamin D to achieve and maintain the target vitamin D serum level [usually expressed in terms of serum 25-hydroxyvitamin D or 25(OH)D] and to decrease the PTH secretion are still lacking. The majority of the studies (18–22) investigated the response of serum 25(OH)D to large doses of cholecalciferol in the elderly, which are considered the population at highest risk of vitamin D deficiency. Trivedi *et al.* (18) found that a single oral 100,000 IU dose of cholecalciferol is safe and effective in reducing fractures in community-dwelling people over age 65 yr. Ilahi *et al.* (19) suggested that a single oral dose of 100,000 IU of cholecalciferol every 2 months is useful in increasing and maintaining 25(OH)D concentration above baseline in the elderly. Recent data from our group (20) reported the greater potency (and the safety) of a single, large, oral 300,000-IU dose of cholecalciferol, compared with ergocalciferol, in enhancing serum 25(OH)D concentration with concomitant decrease in PTH secretion. Bacon *et al.* (22) reported in the elderly a normalization of 25(OH)D values 1 month after an oral dose of 500,000 IU of cholecalciferol. However, to our knowledge, despite several papers carried out in elderly subjects, data on younger people are still limited. In particular, Tangpricha *et al.* (23) observed an increase of 150% in serum 25(OH)D and a decrease of 25% in serum PTH concentration from baseline to the 12th week in healthy adults who daily received orange juice fortified with 1000 IU of vitamin D₃. We instead performed a prospective intervention study to evaluate the effect of a single loading oral dose of cholecalciferol (600,000 IU) on the vitamin D-PTH axis in a sample of young subjects with vitamin D deficiency.

Subjects and Methods

We studied 48 free-living subjects (35 females and 13 males; mean age \pm SD, 36.04 \pm 8.46 yr; age range, 25–56 yr; body mass index, 24 \pm 3.52 kg/m²) with limited sun exposure because of poor tolerability, skin cancer, or other skin diseases. None of

them had diseases or took drugs affecting bone metabolism, nor did they consume calcium or vitamin D supplements. The study was performed between January and March 2009. All subjects were placed on a standardized diet with 1000–1500 mg of elemental calcium per day starting 2 months before the beginning of the study. All participants received a single oral dose of 600,000 IU of cholecalciferol, which equals two vials of commercially available cholecalciferol in Italy. Fasting blood samples were collected at baseline and 3, 15, and 30 d after cholecalciferol administration in all participants. A subgroup of 20 female subjects (mean age, 33.2 \pm 6 yr; range, 25–50 yr; body mass index, 23.8 \pm 4.01 kg/m²) agreed to continue the follow-up and were also assessed at 60 and 90 d. In all subjects, the following serum biochemical parameters were measured: total calcium (Ca), phosphorus (P), magnesium (Mg), 25(OH)D, and PTH. In the subgroup of 20 subjects who were followed for 90 d, serum 1,25-dihydroxyvitamin D [1,25(OH)₂D] levels were also assessed. Moreover, in this subgroup of subjects, serum 25-hydroxyvitamin D₃ [25(OH)D₃] was measured with HPLC at baseline and at 3 and 60 d to selectively measure 25(OH)D₃, but not other vitamin D metabolites. In 28 participants, a morning fasting 3-h urinary collection was also taken to measure calcium and magnesium excretion; tubular reabsorption was expressed as excretion per unit of creatinine clearance [mg/dl of glomerular filtration rate (GFR)]. Serum 25(OH)D concentrations were measured by RIA (Diasorin Inc., Stillwater, MN); the intra- and interassay coefficients of variation (CVs) were 8.1 and 10.2%, respectively. Serum 25(OH)D₃ level was measured by solid phase extraction/isocratic HPLC-reverse phase with spectrophotometric detection at 265 nm UV (Eureka Lab Division, Chiaravalle, Ancona, Italy). The sensitivity of the method was 2 ng/ml (24). Serum 1,25(OH)₂D concentrations were determined by RIA (IDS; Nordic, Herlev, Denmark); the intra- and interassay CVs were 9.3 and 9.6%, respectively. Serum PTH levels were assessed by IRMA (N-tact PTHSP; Diasorin Inc.); the intra- and interassay CVs were 3 and 5.5%, respectively. Blood samples were then stored at –70 C, and the assays were performed in one batch at the end of the study.

Written, informed consent was obtained from all participants. The protocol was approved by the “Sapienza” University of Rome Ethics Committee.

Statistics

Patient's biochemical measures according to time from vitamin D load were reported as mean \pm SD, and differences were assessed via repeated measurements ANOVA models. Comparisons between groups at baseline and between baseline and follow-up values at each time point were performed by unpaired and paired *t* test. Significance was set at a *P* value of 0.05. SigmaStat version 3.5 (Systat Software, London, UK) was used for statistical calculations.

Results

Biochemical parameters of all the participants at baseline and at all time points are reported in Table 1. Basal values of 25(OH)D and PTH did not differ between the whole group and the 20 subjects followed up to 90 d.

Figure 1A shows changes in serum 25(OH)D levels induced by vitamin D supplementation in the whole sample.

TABLE 1. Biochemical parameters of all subjects at each time point

Parameter		Baseline	3 d	15 d	30 d	60 d	90 d	P ^a
Ca (mg/dl)	A	9.3 ± 0.4	9.5 ± 0.3	9.4 ± 0.3	9.4 ± 0.3			<0.05
	B	9.3 ± 0.3	9.5 ± 0.3	9.4 ± 0.3	9.4 ± 0.3	9.4 ± 0.3	9.5 ± 0.4	NS
P (mg/dl)	A	3.8 ± 0.6	4.0 ± 0.6	3.8 ± 0.5	3.8 ± 0.6			<0.01
	B	3.5 ± 0.4	3.8 ± 0.6	3.6 ± 0.4	3.6 ± 0.6	3.4 ± 0.5	3.5 ± 0.5	<0.01
Mg (mg/dl)	A	2.0 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	2.0 ± 0.2			<0.001
	B	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.2	1.8 ± 0.1	<0.05
25(OH)D (ng/ml)	A	15.8 ± 6.5	77.2 ± 30.5	76.5 ± 27.9	62.4 ± 26.1			<0.001
	B	17.2 ± 6.3	73.7 ± 16.9	70.9 ± 14.9	63.5 ± 12.5	42.8 ± 8.9	31.9 ± 12.6	<0.001
PTH (pg/ml)	A	53.0 ± 20.1	38.6 ± 17.2	40.6 ± 15.8	43.4 ± 14			<0.001
	B	57.0 ± 21.6	39.2 ± 15.9	36.7 ± 14.5	39.2 ± 13	42.8 ± 19.1	37.7 ± 15.1	<0.001
1,25(OH) ₂ D (pg/ml)	A							
	B	46.8 ± 18.9	97.8 ± 38.3	90.7 ± 46.9	74.9 ± 36.8	59.5 ± 27.3	52.9 ± 23.3	<0.001
CaEx (mg/dl GFR)	C	0.08 ± 0.06	0.10 ± 0.08	0.10 ± 0.05	0.08 ± 0.04			<0.05
MgEx (mg/dl GFR)	C	0.04 ± 0.02	0.06 ± 0.03	0.05 ± 0.02	0.05 ± 0.03			NS

All values are expressed as mean ± SD. A, Whole sample (n = 48); B, subgroup of subjects followed up to 90 d (n = 20); C, subgroup of subjects collecting urine samples (n = 28); CaEx, calcium excretion; MgEx, magnesium excretion; NS, not significant.

^a RM ANOVA.

We found a significant change in 25(OH)D levels throughout the entire observation period [*P* < 0.001, by repeated measures (RM) ANOVA]. In particular, a sharp and significant increase was observed already at 3 d, attaining average values of 77.1 ± 30.5 ng/ml (*P* < 0.001), with an absolute increment above baseline of 61.3 ± 28 ng/ml (*P* < 0.001). Subsequently, there was a slow but not significant

decrease; at the end of the observation period, mean 25(OH)D serum levels, as well as the 30-d basal difference, remained significantly higher than baseline (*P* < 0.001) (Fig. 1, A and B). Noteworthy, the highest 25(OH)D level achieved at the third day in a female subject was 136 ng/ml.

Vitamin D load induced a significant decrease in serum PTH concentration (*P* < 0.001, by RM ANOVA), which

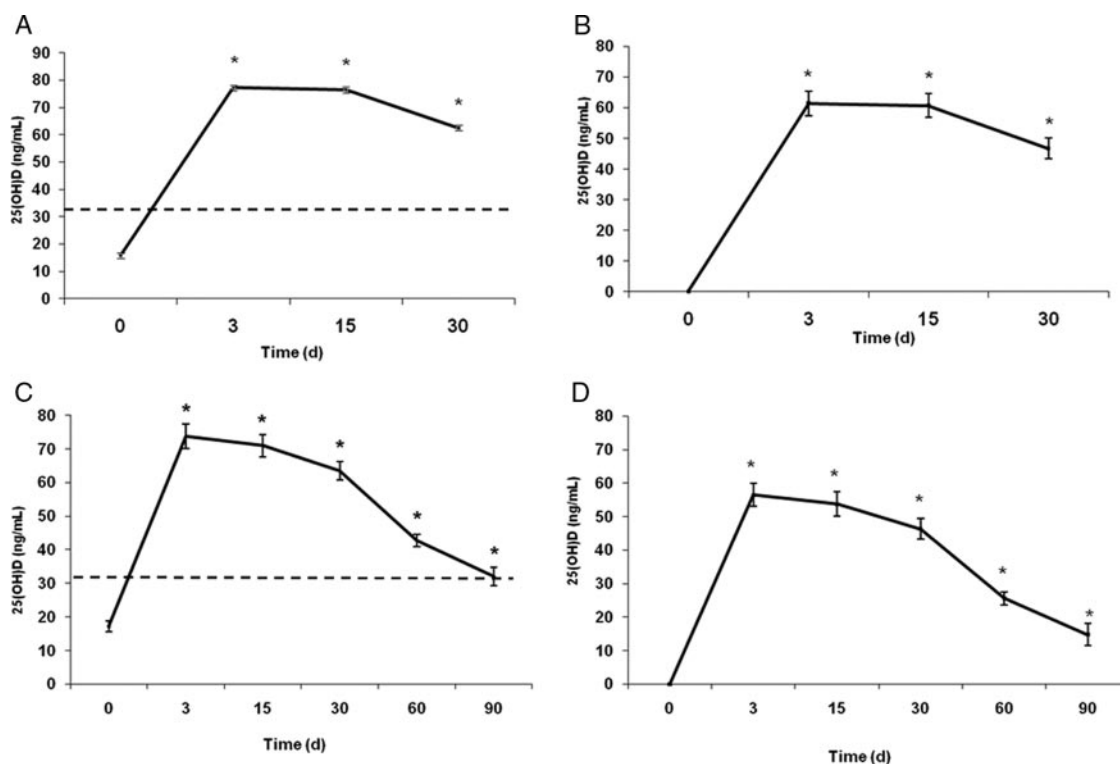


FIG. 1. Effect of vitamin D supplementation on 25(OH)D serum changes. A and B, Mean values and respective basal difference of serum 25(OH)D at each time point in the whole sample (n = 48). B, Mean ± SE values of 25(OH)D basal difference at 3, 15, and 30 d were 61.4 ± 4, 60.7 ± 3.8, and 46.7 ± 23.9 ng/ml, respectively. *, *P* < 0.001 vs. baseline. C and D, Mean values and the respective basal difference of serum 25(OH)D at each time point in the subgroup (n = 20). D, Mean ± SE values of 25(OH)D basal difference at 3, 15, 30, 60, and 90 d were 56.5 ± 3.4, 53.7 ± 3.6, 46.3 ± 3, 25.6 ± 2, and 14.7 ± 3.3 ng/ml, respectively. *, *P* < 0.001 vs. baseline. The dashed lines (A and C) represent the threshold level for vitamin D adequacy, settled at 32 ng/ml.

was already significant at 3 d ($P < 0.001$). Thereafter, PTH values slowly increased, but at each time point they remained significantly lower compared with baseline ($P < 0.001$) (data not shown).

Serum Ca and P levels significantly changed throughout the entire period ($P < 0.05$ and $P < 0.01$, respectively, by RM ANOVA) (Table 1). However, the increase with respect to baseline was significant only at 3 d after supplementation (Ca, 0.1 ± 0.3 mg/dl; P, 0.2 ± 0.5 mg/dl; $P < 0.01$ for both). Moreover, there was a significant change in Mg serum levels ($P < 0.001$), whose values were significantly reduced only on the third day (-0.05 ± 0.1 mg/dl, $P < 0.01$). In subjects collecting urine samples, calcium excretion significantly changed ($P < 0.05$). The increase of calcium excretion was significant both 3 d (0.03 ± 0.04 mg/dl GFR; $P < 0.01$) and 15 d (0.02 ± 0.04 mg/dl GFR; $P < 0.05$) after cholecalciferol supplementation. Magnesium excretion was significantly increased only at 3 d (0.02 ± 0.02 mg/dl GFR; $P < 0.01$).

The changes of serum 25(OH)D levels in the subgroup of 20 subjects followed up to 90 d were similar to those found in the sample as a whole (Fig. 1, C and D). In particular, mean values of 25(OH)D were still significantly higher with respect to baseline at both 60 and 90 d. Noteworthy, at 90 d, nine of 20 patients had 25(OH)D levels over the threshold of vitamin D sufficiency. The increase of serum 25(OH)D at 3 d was confirmed using a HPLC assay, which separates 25(OH)D₃ from all other vitamin D metabolites. Indeed, 25(OH)D₃ serum levels at 3 d were significantly higher with respect to baseline (56.6 ± 15.9 vs. 12.7 ± 6.9 ng/ml; $P < 0.001$); at 60 d serum levels decreased, mean values being still significantly higher than baseline (28.5 ± 8.1 mg/dl; $P < 0.001$). Percentage changes with respect to baseline between the two methods were not significantly different at 3 and 60 d (data not shown). A significant positive correlation was found between HPLC and RIA assays ($r = 0.81$; $P < 0.0001$) (Fig. 2).

The concomitant changes of PTH and 1,25(OH)₂D serum levels, expressed as absolute differences from basal values, are reported in Fig. 3. We found a significant de-

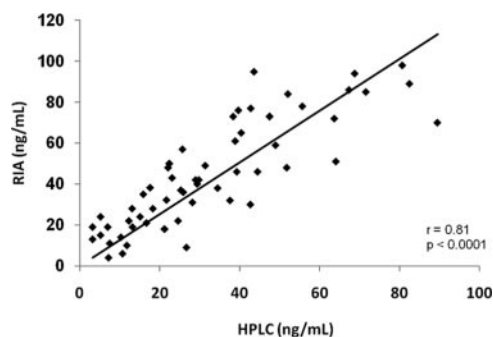


FIG. 2. Correlation between the concentration of 25(OH)D assessed by HPLC method and RIA assay.

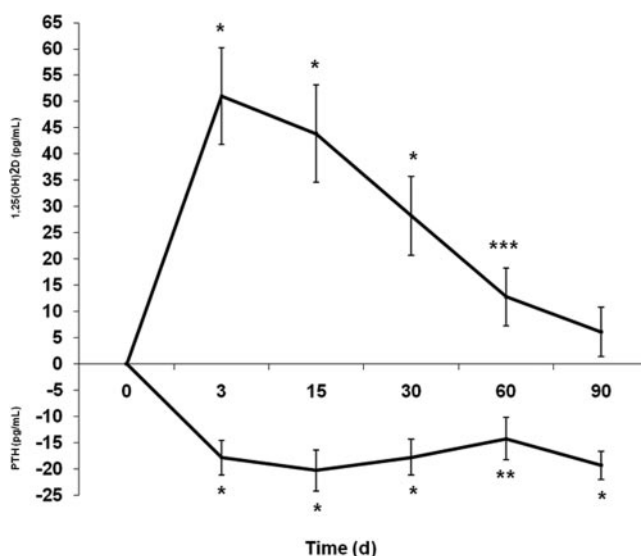


FIG. 3. Effect of vitamin D supplementation on the basal difference of serum 1,25(OH)₂D (upper panel) and of PTH (lower panel) in the subgroup of 20 subjects. Mean \pm SE values of 1,25(OH)₂D basal difference at 3, 15, 30, 60, and 90 d were 51 ± 9 , 43.8 ± 9.3 , 28.2 ± 7.5 , 12.7 ± 5.5 , and 6.1 ± 4.7 pg/ml, respectively. Mean \pm SE values of PTH basal difference at 3, 15, 30, 60, and 90 d were -17.8 ± 3.3 , -20.3 ± 4 , -17.8 ± 3.4 , -14.2 ± 4 , and -19.3 ± 2.7 pg/ml, respectively. *, $P < 0.001$; **, $P < 0.01$; ***, $P < 0.05$.

crease of serum PTH already at 3 d ($P < 0.001$). The lowest values were reached at 15 d (-20.3 ± 17.8 pg/ml; $P < 0.001$). The decrease was statistically significant throughout the entire period of observation. The reduction of serum PTH was mirrored by the concomitant increase in 1,25(OH)₂D values (Table 1). In fact, we observed a rapid increase of 1,25(OH)₂D levels at 3 d ($P < 0.001$). This increase was statistically significant up to 60 d, whereas at 90 d 1,25(OH)₂D levels returned to baseline.

Changes in Ca, P, and Mg levels in the subgroup followed up to 90 d were similar to those observed in the whole sample, with a significant increase in Ca and P levels after 3 d ($P < 0.01$) and a concomitant significant Mg decrease ($P < 0.50$) (data not shown).

Discussion

Available evidence in the elderly suggests that at least 800–1000 IU of vitamin D per day are needed to achieve mean serum 25(OH)D levels of 32 ng/ml (25). This value, associated with the optimal calcium absorptive performance (26), is currently considered by many authors as the threshold of adequate vitamin D status (25, 27–29). However, there are limited and nonconclusive data about the optimal dose and dosing intervals needed to achieve and maintain these levels and to reduce secondary hyperparathyroidism (30). Several studies reported safety and efficacy of supplementation with large doses of vitamin D,

particularly in the elderly (18–22, 31). It has been suggested that a single high dose of cholecalciferol is effective in rapidly increasing 25(OH)D levels, namely in patients with severe vitamin D deficiency. Moreover, higher intermittent doses of cholecalciferol were proposed to improve adherence to treatment (20, 22, 25). New guidelines claimed the reevaluation of the upper limit of safe vitamin D daily intake, with a trend to shift to 2000 IU/d (1).

In this study, we evaluated the effect of a single very large oral dose of cholecalciferol (600,000 IU) on serum levels of 25(OH)D and other calciotropic hormones in young subjects with vitamin D deficiency. Our results demonstrate that an oral dose of 600,000 IU of cholecalciferol is able to rapidly increase serum 25(OH)D levels in patients with severe vitamin D depletion. A peak of 25(OH)D concentration was already attained as soon as 3 d after vitamin D administration, probably due to the strikingly higher dose employed compared with previous reports (20). Remarkable increments of 25(OH)D serum levels were also attained at 3 d when 300,000 IU of cholecalciferol were given to elderly subjects (20). Noteworthy, the highest value attained in the present study by an individual subject was 136 ng/ml, which is well below the widely accepted toxic blood levels of 200 ng/ml (32). This result was likely due to the low pretreatment vitamin D status of our sample. Moreover, 25(OH)D levels remained significantly higher than baseline up to 30 d, and in the subgroup of 20 subjects followed up to 90 d, 25(OH)D basal difference was still significant at 90 d. In this group, at the end of observation, half of the patients had 25(OH)D levels still over 32 ng/ml. Overall, our findings demonstrate that a high oral dose of cholecalciferol rapidly enhances and maintains adequate 25(OH)D levels up to 90 d after administration in young subjects with vitamin D deficiency. These results are in line with data obtained in elderly subjects and probably rely on a rapid absorption and conversion of the oral load of cholecalciferol to the 25-hydroxy metabolite. Heaney *et al.* (31) demonstrated that vitamin D administration induces a biphasic response, with a rapid increase of serum 25(OH)D at low serum vitamin D₃ concentrations and a slower response at higher levels. Hence, we can hypothesize that the huge increase of 25(OH)D levels after supplementation could probably be due to the low basal vitamin D₃ concentration. Ilahi *et al.* (19) also reported a peak of 25(OH)D concentration 7 d after a single oral dose of 100,000 IU of cholecalciferol, whereas others obtained similar results 1 month after giving an oral dose of 500,000 IU of cholecalciferol to subjects with initial 25OHD levels of 20 ng/ml or less (22, 33). However, the authors did not give any data at 3–10 d after cholecalciferol loading. On the other hand, a single im dose of 600,000 IU of cholecalciferol may en-

hance 25(OH)D levels above the threshold of sufficiency only at 4 months in subjects with vitamin D deficiency (34). This finding confirms that, faced with the physiological skin production, the oral administration is a valuable alternative route of vitamin D supplementation. This is also supported by previous results from our group showing that a dose of 300,000 IU of cholecalciferol given orally but not im can sharply and significantly increase 25(OH)D levels in vitamin D-deficient elderly people (20). In that study, the highest 25(OH)D concentration was found at 30 d, and it remained significantly higher than baseline up to 2 months. We can therefore conclude that both 600,000 and 300,000 IU of oral cholecalciferol determine a sharp and significant increase in 25(OH)D levels, maintaining adequate vitamin D status for at least 2 months. Moreover, a single oral dose of 600,000 IU administered to younger and less deficient subjects induced a faster increase of 25(OH)D levels, and a good vitamin D status was maintained up to 90 d.

The results obtained by the RIA method were further strengthened by those obtained with HPLC, separating 25(OH)D₃ from all other vitamin D metabolites. Indeed, the huge increase measured by RIA at 3 d is confirmed by the HPLC results. However, we found an apparent discrepancy between the mean 25(OH)D levels measured by RIA and 25(OH)D₃ assessed by HPLC. Indeed, looking at Fig. 2, it is apparent that RIA measurement overestimated values below 40 ng/ml, whereas it underestimated values above 40 ng/ml with respect to HPLC. Such a difference was not significant, namely at 3 d, and it might be due to the small size of the sample assessed by both methods. Also, considering this limit, we showed that a loading dose of oral cholecalciferol rapidly and significantly improved vitamin D status as soon as 3 d.

The significant increase in serum calcium levels 3 d after supplementation could probably depend on an increase of calcium absorption due to the rise in 25(OH)D values (26). This hypothesis is confirmed by a significant increase in urinary calcium excretion at 3 d, because intestinal calcium daily absorption implicitly equates to urinary calcium daily excretion (32). However, urinary data were not collected in all the participants and unfortunately were not informative about the effect of a very large dose of cholecalciferol on 24-h calcium excretion, which is considered a more sensitive indicator of vitamin D adverse effects (35). It was also shown that a loading dose of 600,000 IU of cholecalciferol rapidly enhanced magnesium urinary excretion, probably because of the concomitant increase in serum and urinary calcium levels. Indeed, renal magnesium reabsorption is proportional to calcium excretion and dependent on calcemia. Alternatively, the reduction in renal Mg reabsorption could be due to suppressed levels of

PTH. However, noteworthy, serum Ca and Mg levels remained in the physiological range during the entire observation period.

Significant reduction of serum PTH was observed already 3 d after administration of 600,000 IU of cholecalciferol and persisted throughout the entire period of observation (30 d); in the group followed up to 90 d, serum PTH levels were suppressed up to 3 months. On the other hand, Diamond *et al.* (34) reported a significant decrease in PTH levels only 12 months after administration of the same amount of cholecalciferol by im route. In line with our previous study, our current results confirm the greater potency of an oral cholecalciferol load in rapidly reducing PTH levels (20).

Also concerning 1,25(OH)₂D serum changes, the current results confirm our previous data (20). This finding could be explained by a rapid conversion of the 25-hydroxy metabolite to 1,25(OH)₂D because the initial secondary hyperparathyroidism promotes a marked increase of 1,25(OH)₂D levels when these patients ingest vitamin D (36). It is also possible that a high concentration of serum vitamin D metabolites may displace 1,25(OH)₂D from the circulating vitamin D-binding protein (32). However, we cannot exclude that the observed relevant change of 1,25(OH)₂D levels at 3 d could depend on the relatively low specificity of the employed assay, and these results will require verification with a more specific assay.

We believe that our results have some clinical implications. The potency of a very large dose of cholecalciferol, given orally, is an important finding in patients with high risk of vitamin D deficiency and high fracture risk. A rapid vitamin D repletion is also desirable to prevent hypocalcemia in vitamin D-deficient patients before treatment with iv bisphosphonates. Moreover, we believe that a loading intermittent dose of cholecalciferol could also be associated with a better adherence to treatment, also in young people with vitamin D deficiency.

Our data regarding PTH changes also deserve emphasis because elevated PTH secretion due to vitamin D deficiency is not only associated with increased bone turnover and fracture risk, but may also result in increased mortality, at least in elderly vitamin D-deficient people (37). Although our study was performed in young people, a rapid reduction of PTH levels could be expected to occur also in elderly patients (38). Moreover, the time-related pattern of PTH change deserves interest because a marked decrease was already obtained at the third day and maintained until 90 d, despite no concomitant changes of serum calcium levels. At the latter time point, the reduction of PTH serum levels still persists, although Ca and 1,25(OH)₂D levels returned to baseline values. This finding, in line with previous results from our group (20, 39), supports the hypoth-

esis that 25(OH)D could also have a direct role in modulating PTH secretion, regardless of both 1,25(OH)₂D and calcium levels.

In conclusion, our results demonstrate that the administration of a single very large oral dose of 600,000 IU of cholecalciferol is useful in rapidly and safely enhancing 25(OH)D levels and in reducing serum PTH in young people with vitamin D deficiency.

Acknowledgments

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Disclosure Summary: The authors have nothing to disclose.

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