Short-Term Effects on Bone Turnover Markers of a Single High Dose of Oral Vitamin D₃

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Context: Vitamin D deficiency is often treated or prevented by high intermittent doses of vitamin D to achieve a better treatment adherence, but treatment outcomes were contradictory, and even a transient increase in fracture and fall risk was reported.

Objective: The objective of the study was to investigate the short-term effects on bone turnover markers of a single bolus of vitamin D_3.

Design, Setting, Patients, and Intervention: Twelve elderly subjects (eight women, four men; mean age 76 ± 3 yr) were given a single oral bolus of 600,000 IU vitamin D_3. Blood samples were taken at baseline and 1, 3, 7, 14, 30, 60, and 90 d after vitamin D_3 administration. Twenty-four subjects served as controls.

Main Outcome Measures: Changes in serum levels of 25-hydroxyvitamin D (25OHD), 1,25-dihydroxyvitamin D, PTH, C-terminal-telopeptides of type I collagen, cross-linked N-telopeptide of type I collagen (sNTX), osteocalcin, and bone-specific alkaline phosphatase.

Results: No relevant changes in 25OHD and bone turnover markers were observed in the controls. In treated subjects, serum 25OHD attained a peak increment to 67.1 ± 17.1 ng/ml ($P < 0.001$) at d 3. Subsequently it slowly decreased to 35.2 ± 5.8 ng/ml ($P < 0.01$ vs. a baseline value of 21.7 ± 5.6 ng/ml). Mean serum PTH concentration decreased by 25–50% and serum 1,25-dihydroxyvitamin D rose by 25–50%. Serum CTX and sNTX rose significantly at d 1 ($P < 0.01$), they attained a peak increment greater than 50% at d 3, and they subsequently decreased almost back to baseline values at d 90. Serum osteocalcin slightly rose within the first 3 d and then declined by d 60. No changes were observed in serum bone-specific alkaline phosphatase.

Conclusions: Our results indicate that the use of large doses of vitamin D may be associated with acute increases in C-terminal-telopeptides of type I collagen and sNTX, which may explain the negative clinical results obtained by using intermittent high doses of vitamin D to treat or prevent vitamin D deficiency. (*J Clin Endocrinol Metab* **97: E0000–E0000, 2012**)

Vitamin D deficiency is extremely common among elderly subjects (1), and it is thought to contribute to bone loss by stimulating PTH secretion, resulting in increased bone resorption (2). Increasing 25-hydroxyvitamin D (25OHD) serum levels improves intestinal calcium absorption, suppresses PTH levels, reduces fall frequency, lowers osteoporotic fracture risk, and, finally, enhances muscle strength (3). Although it is generally accepted that greater than 29 ng/ml represents the threshold level for vitamin D sufficiency (4), today it is still poorly understood which form of vitamin D, doses and dosing intervals, and routes of administration are warranted to reach and maintain this level.

Most people requiring vitamin D supplementation follow daily regimens, but treatment adherence may be low, resulting in suboptimal 25OHD levels. For this reason

Abbreviations: BAP, Bone-specific alkaline phosphatase; CV, coefficient of variation; 25OHD, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; sCTX, serum C-terminal-telopeptides of type I collagen; sNTX, serum cross-linked N-telopeptide of type I collagen.

higher intermittent doses have been proposed (5, 6), which should improve treatment adherence and outcomes (7). The rationale for using vitamin D bolus was supported by fracture-reduction benefits using 100,000 IU vitamin D3 every 4 months (8) or using 150,000 IU ergocalciferol as a single annual dose in elderly subjects with vitamin D deficiency (9). Nevertheless, it was recently reported that among older community-dwelling women, annual oral administration of high-dose Vitamin D₃ (500,000 IU) resulted in an increased risk of falls and fractures, with the greatest increase occurring during the first 3 months after dosing (10).

The biological plausibility of these unexpected findings remains speculative. The present study seeks to address this issue by measuring 25OHD, 1,25-dihydroxyvitamin D [1,25(OH)₂D], PTH, and bone turnover marker changes in response to a large bolus of vitamin D₃.

Materials and Methods

Subjects

Home-dwelling subjects attending our osteoporosis center aged greater than 70 yr were sequentially assigned to active or control groups. The sun exposure of all participants was less than 10 h/wk, and none had been taking vitamin D supplements or drugs known to interfere with bone or mineral metabolism for the last 12 months. Exclusion criteria were also acute or chronic conditions that affect mineral metabolism or complete immobi-

lization. Twelve subjects (eight women, four men; mean age 76 ± 3 yr; mean body mass index 27.8 ± 2.2 kg/m²) were given a single large dose of vitamin D₃. The study had two control groups. The first group (group A, Table 1) included 10 subjects (seven women, three men; mean age 76 ± 4 yr; mean body mass index 27.2 ± 2.6 kg/m²), and it was expected to provide only information regarding the seasonal changes of serum 25OHD and bone markers. For this control group, only monthly blood samples were obtained. The second control group (group B, Table 1) was shared with a different study run by the same time and requiring frequent blood sampling over the first 2 wk of observation. This was made of 14 subjects (11 women, three men; mean age 71 ± 8 yr; mean body mass index 26.8 ± 4.2 kg/m²). All subjects were recruited from April to May 2010 and were placed on a standardized diet with greater than 1000 mg elemental calcium per day starting 2 months before the beginning of the study.

Study design

The 12 participants in the treated group received soon after breakfast a single oral dose of 600,000 IU vitamin D3, which equals two vials of commercially available vitamin D₃ in Italy (DiBase; Abiogen Pharma, Pisa, Italy). Fasting blood samples were collected at baseline and 1, 3, 7, 14, 30, 60, and 90 d after vitamin D₃ administration in the active group and at baseline and 3, 7, and 14 d or monthly in the control groups.

The study protocol was approved by the local ethical committee and an informed consent was obtained by all participants.

Biochemical measures

Total calcium and phosphate were measured by colorimetric methods (BioAssay Systems, Hayward, CA). Serum 25OHD

TABLE 1. Biochemical parameters (mean ± SD) of subjects of the treated group and control groups at each time point

	Baseline	1 d	3 d	7 d	14 d	30 d	60 d	90 d
Treated patients (N. 12)								
Ca (mg/dl)	9.8 ± 0.4	10.1 ± 0.5	9.9 ± 0.4	9.6 ± 0.6	9.8 ± 0.5	9.9 ± 0.6	9.9 ± 0.5	9.8 ± 0.7
Phosphate (mg/dl)	3.6 ± 0.7	3.7 ± 0.5	3.5 ± 0.6	3.7 ± 0.5	3.6 ± 0.6	3.5 ± 0.5	3.4 ± 0.5	3.6 ± 0.8
25OHD (ng/ml)	21.7 ± 5.6	46.8 ± 7.5[a]	67.1 ± 17.1[a]	62.2 ± 12.5[a]	60.9 ± 13.3[a]	51.6 ± 11.9[a]	43.1 ± 10.3[a]	35.2 ± 5.8[a]
1.25(OH)₂D (pg/ml)	39.6 ± 12.6[a]	65.1 ± 24.7[a]	53.3 ± 23.4[a]	55.7 ± 14.4[a]	53.4 ± 18.0[b]	52.4 ± 16.9[b]	48.6 ± 14.7[b]	56.2 ± 23.0[b]
PTH (pg/ml)	35.0 ± 8.7	32.0 ± 9.5	25.5 ± 7.4[a]	23.4 ± 6.4[a]	25.8 ± 7.8[a]	27.0 ± 9.8[b]	29.3 ± 6.0[b]	28.3 ± 6.1[b]
sCTX (ng/ml)	0.27 ± 0.10	0.41 ± 0.22[a,c]	0.41 ± 0.24[b,c]	0.36 ± 0.14[b,c]	0.37 ± 0.15[b,c]	0.37 ± 0.13[a,c]	0.35 ± 0.14	0.30 ± 0.14
BAP (ng/ml)	15.9 ± 6.5	16.2 ± 6.0	15.9 ± 6.1	15.8 ± 5.8	15.6 ± 5.7	15.3 ± 5.2	15.4 ± 5.1	16.3 ± 5.3
Osteocalcin (ng/ml)	13.6 ± 5.9	13.0 ± 5.8	14.6 ± 6.7[b,c]	14.0 ± 6.1	13.3 ± 5.9	13.2 ± 5.2	12.0 ± 4.4[b,c]	12.5 ± 5.0
sNTx (nmol/liter)	15.8 ± 7.0	24.1 ± 14.0[b,c]	23.2 ± 13.2	21.0 ± 7.8[b,c]	21.5 ± 9.2[b]	21.7 ± 8.1[a]	19,7 ± 7.7	17.7 ± 8.3
Control group A (N.10)								
25OHD (ng/ml)	22.1 ± 7.1					21.8 ± 6.3	24.2 ± 6.9	23.0 ± 5.9
sCTX (ng/ml)	0.27 ± 0.14					0.26 ± 0.17	0.24 ± 0.10	0.22 ± 0.13
Osteocalcin (ng/ml)	12.9 ± 4.0					13.1 ± 4.1	13.1 ± 4.2	12.6 ± 4.3
sNTx (nmol/liter)	16.5 ± 9.1					15.7 ± 10.3	15.1 ± 6.9	13.2 ± 7.5
Control group B (N.14)								
25OHD (ng/ml)	19.1 ± 5.3							
sCTX (ng/ml)	0.29 ± 0.09		0.28 ± 0.11	0.29 ± 0.09	0.25 ± 0.18			
Osteocalcin (ng/ml)	13.4 ± 3.2		12.4 ± 2.8	14.1 ± 3.0	11.8 ± 2.9			
sNTx (nmol/liter)	17.8 ± 8.6		19.1 ± 7.9	16.3 ± 7.8	20.0 ± 9.0			

[a] $P < 0.01$ *vs.* baseline.

[b] $P < 0.05$ *vs.* baseline.

[c] Absolute changes significantly ($P < 0.05$) different from those observed in the control groups. *P* values were adjusted for multiple comparisons.

concentrations were determined by ELISA (IDS Ltd., Boldon, UK); the intra- and interassay coefficients of variation (CV) were 5.9 and 6.6%, respectively. Serum $1,25(OH)_2D$ levels were measured by immunoextraction followed by quantification by ELISA (IDS); the intra- and interassay CV were 10.2 and 18.1%, respectively. Intact 1–84 PTH was assayed by a two-site immunoenzymometric assay (IDS); the intra- and interassay CV were 3.4 and 5.8%, respectively. The biochemical markers of bone turnover included serum bone-specific alkaline phosphatase (BAP), serum C-terminal telopeptides of type I collagen (sCTX), N-MID osteocalcin (osteocalcin; IDS), and serum cross-linked N-telopeptide of type I collagen (sNTx; Osteomark; Unipath Ltd., Bedford, UK), all measured by ELISA. The intraassay CV in our laboratory were ranging from 2 to 4% for BAP, osteocalcin, and sCTX, and the corresponding interassay CV from 6 to 8%. The intra- and interassay CV for sNTX were 12 and 16%, respectively. For all these ELISA, two internal standards, with values below the normal range and by the upper part of the normal range, were measured for each commercial kit. Blood samples were stored at -70 C, and the assays were performed in one batch at the end of the study.

Statistical analysis

Data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL) and are expressed as mean $\pm$ SD and percentage changes $\pm$ SEM. Comparisons between baseline and follow-up values at each time point were performed by ANOVA and then by paired t test. Significance was set at a $P = 0.05$. A Spearmen correlation test was used. To compare independent groups, Student's t test and one-way ANOVA were calculated.

Results

All subjects completed the study. In control subjects mean changes in serum 25OHD across the period of the study of $+0.9 \pm 1.2$ ng/ml, and no significant changes in bone turnover markers were observed (Table 1). Because the changes in 25OHD were both small and not significantly different from zero, no attempt was made to adjust the values of the treated subjects. The CV percentage of the two internal standards, particularly for the two control groups, was always lower than the CV percentage claimed by the commercial kit dealer.

Biochemical parameters of the treated participants at baseline and at all time points are listed in Table 1. A sharp and significant increase in 25OHD levels was observed at d 1, attaining a peak increment above baseline to 67.1 ± 17.1 ng/ml ($P < 0.001$) at d 3. Subsequently there was a slow decrease; at the end of the observation period (90 d), mean 25OHD serum levels remained significantly higher than baseline ($P < 0.01$) (Fig. 1).

The vitamin D load was associated with a significant decrease in mean serum PTH concentration, which was significant at d 3 ($P < 0.01$) and with a nadir at d 7 (Fig. 1). Thereafter PTH values slowly increased, but at d 90
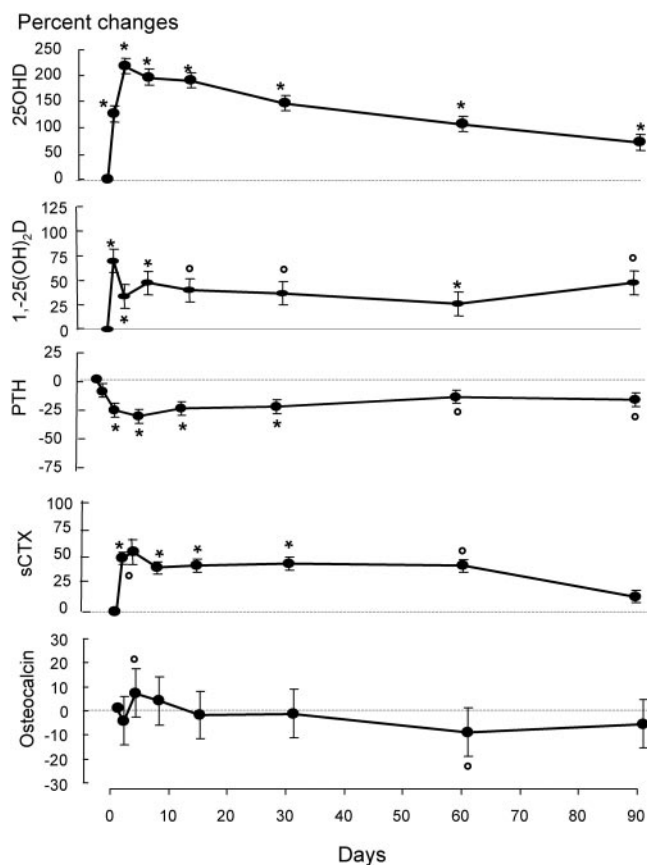

FIG. 1. Effects (percent changes $\pm$ SEM *vs.* baseline) of a single oral bolus of 600,000 IU vitamin D_3 on 25OHD, $1,25(OH)_2D$, PTH, sCTX, and osteocalcin serum levels. *, $P < 0.01$; °, $P < 0.05$.

they remained significantly lower compared with baseline ($P < 0.01$).

The reduction of serum PTH was mirrored by the concomitant increase in $1,25(OH)_2D$ values (Fig. 1), with a rapid increase of $1,25(OH)_2D$ levels at d 1 ($P < 0.05$). This increase remained statistically significant up to 90 d. The increase of $1,25(OH)_2D$ levels at d 1 was inversely correlated with baseline 25OHD serum levels ($P = 0.06$, results not shown).

A sharp and significant increase of sCTX was observed already at d 1 ($P < 0,01$) (Table 1), attaining a percent peak increment of greater than 50% above baseline at d 3 (Fig. 1). Subsequently there was a slow decrease and at the end of the observation period (90 d) mean sCTX levels were not significantly different from baseline. The mean increase in sCTX levels was significant higher in the four subjects with baseline 25OHD serum levels lower than 20 ng/ml ($+0.32 \pm 0.20$ *vs.* $+0.05 \pm 0.13$ ng/ml, respectively; $P = 0.018$, data not shown) than in those with higher starting levels. No significant correlations were observed between changes in sCTX and 25OHD or $1,25(OH)_2D$ serum levels at each time points.

The changes in sNTX mirrored those seen in sCTX, but the level of significance was somewhat less consistent (Ta-

ble 1). Serum osteocalcin increased at d 3 and decreased at d 60 significantly ($P < 0.05$) *vs.* both baseline and control groups (Table 1 and Fig. 1).

Changes of serum calcium, phosphate, and BAP were not statistically significant.

Discussion

In this study we have shown for the first time that the oral administration of a bolus of 600,000 IU vitamin D₃ is associated with a statistically and clinically significant increase in two serum markers of bone resorption, *i.e.* sCTX and sNTX. The changes in the latter were somewhat less consistent, possibly for the lower precision of the available commercial assays (11).

The consequences of administration of similar oral doses of vitamin D₃ on serum levels of 25OHD, calcium, and PTH have been reported by a number of authors (5, 6, 12–14). It appears clear that these megadoses given once a year are unlikely to prevent vitamin D deficiency for more than 3–6 months and this might explain the long-term poor response in terms of fracture risk of these treatment regimens. However, in these previous studies (5, 6, 12–14), no safety issues were reported.

The rapid (within the first day) increase in serum 1,25(OH)₂D was observed also by others (5, 12, 13), and it was explained by a rapid conversion of the 25OHD to 1,25(OH)₂D in subjects with baseline deficiency of vitamin D and secondary hyperparathyroidism (15). The negative correlation ($P = 0.06$) we observed between the increases of 1,25(OH)₂D and the baseline 25OHD serum levels is in line with this reading, together with the observation of large increases in 1,25(OH)₂D serum levels described in subjects with severe vitamin D deficiency after vitamin D₃ supplementation (12, 13). The persistence of high levels of 1,25(OH)₂D over the 90 d of the observation remains unclear because PTH levels were slowly declining. Unfortunately, the assay for 1,25(OH)₂D in the control group could not be done for the inadequate amount of spared serum aliquots, and this does not allow to exclude an unlikely assay drift. We cannot also rule out the possibility of a lack of specificity of the 1,25(OH)₂D assay with an interference of the high levels of other vitamin D metabolites, such as 24,25(OH)₂D, possibly associated with the administration of the vitamin D bolus.

At variance with previous studies, we also measured for the first time the acute changes in bone turnover markers: the results are somewhat surprising. In fact, the decreases in PTH after vitamin D administration would be expected to be associated by gradual reductions in bone turnover markers, whereas we observed a statistically and clinically

significant increase (ca. +50%) in the sCTX and also in sNTX, two markers of bone resorption already 24 h after the administration of high doses of vitamin D, persisting for more than 2 months. To the best of our knowledge, we found description of changes of bone turnover markers only after at least 3 months of vitamin D supplementation. In all these studies, bone turnover markers were found to decrease or to remain unchanged (16–18).

There is not an obvious explanation for our findings. 1,25(OH)₂D stimulates the synthesis and secretion by osteoblasts of a cytokine known as receptor activator of nuclear factor-κB ligand, which has a key role in osteoclastogenesis and bone resorption (19, 20). We hypothesize that the marked and rapid increase of 1,25(OH)₂D after high dose of vitamin D administration might stimulate osteoclastic bone resorption despite the concomitant decrease in serum PTH, which might be responsible of the later reduction of bone resorption. To verify this hypothesis, serum receptor activator of nuclear factor-κB ligand should also be measured in future studies.

If the initiating event is a stimulation of bone resorption, then its duration was possibly too short to be associated in changes in bone formation markers. Indeed BAP did not change, but osteocalcin exhibited small changes with a significant increase at d 3 and a significant decrease at d 60. These changes might be a random observation, but they could possibly reflect a direct stimulatory effect of vitamin D metabolites on osteoblast function (21) and then a late trend for a suppression of bone turnover.

The clinical implications of our observation remain uncertain, but it might explain the lack of benefits observed in some studies or even the unexpected increase in fracture rate reported shortly after the administration of a high dose of vitamin D3 (10). There are no studies on bone fragility or bone microarchitecture occurring shortly (within 2–3 months) after a rapid change in bone turnover. However, the immediate diverging of the incidence in fracture rate in clinical trials with antiresorbers has been attributed to the rapid decrease in bone turnover rather than to the negligible effects on bone mass (22), and an increase in bone turnover level is a well-recognized independent risk factor for fractures, possibly for the associated increase in remodeling sites and then stress risers (11).

Conclusion

Our results clearly indicate that the use of oral megadoses of vitamin D may be counterproductive and that the safety issues about vitamin D dosing should not be limited to hypercalcemia and hypercalciuria. The acute increase in the sCTX and sNTX levels we observed in subjects with vitamin D insufficiency is also challenging the common clinical practice of treating vitamin D-deficient patients

with loading doses of vitamin D at the outset of repletion. Because in our study we investigated only a bolus of 600,000 IU vitamin D, further studies are warranted to identify what initial therapeutic dose can be safely administered in vitamin D-deficient subjects.

Acknowledgments

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