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Influence of Daily Regimen Calcium and Vitamin D Supplementation on Parathyroid Hormone Secretion

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Abstract. Calcium and vitamin D supplementation has been shown to reduce secondary hyperparathyroidism and play a role in the management of senile osteoporosis. In order to define the optimal regimen of calcium and vitamin D supplementation to produce the maximal inhibition of parathyroid hormone secretion, we have compared the administration of a similar amount of Ca and vitamin D, either as a single morning dose or split in two doses, taken 6 hours apart. Twelve healthy volunteers were assigned to three investigational procedures, at weekly intervals. After a «blank» control procedure, when they were not exposed to any drug intake, they received two calcium-vitamin D supplement regimens including either two doses of Orocal D3 ® (500 mg Ca and 400 IU vitamin D) 6 hours apart or one water-soluble effervescent powder pack of Cacit D3 ® in a single morning dose (1000 mg Ca and 880 IU vitamin D). During the three procedures (control and the two calcium-vitamin D supplementations), venous blood was drawn every 60 minutes for up to 9 hours, for serum Ca and serum PTH measurements. The order of administration of the two Ca and vitamin D supplementation sequences was allocated by randomization. No significant changes in serum Ca were observed during the study. During the 6 hours following Ca and vitamin D supplementation, a statistically significant decrease in serum PTH was observed with both regimens, compared with baseline and with the control procedure. Over this period of time, no differences were observed between the two treatment regimens. However, between the sixth and the ninth hour, serum PTH levels were still significantly decreased compared with baseline with split dose Orocal D3 ® administration, while they returned to baseline value with the Cacit D3 ® preparation. During this period, the percentage decrease in serum PTH compared with baseline was significantly more pronounced with Orocal D3 ® than with Cacit D3 ® ($P = 0.0021$). We therefore conclude that the administration of two doses of 500 mg of calcium and 400 IU of vitamin D3 6 hours apart provides a more prolonged decrease in serum PTH levels than the administration of the same total amount of Ca and vitamin D as a single morning dose in young healthy volunteers. This might have implications in terms of protection of the skeleton against secondary hyperparathyroidism and increased bone resorption and turnover in elderly subjects.

Key words: Calcium — Vitamin D — Parathyroid hormone — Osteoporosis — Treatment

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Osteoporosis of the hip is now widely recognized as a major public health issue. Many reports have carefully evaluated the incidence of fractures in different populations and identified several risk factors for developing osteoporosis. Among them, in the elderly, alteration in vitamin D metabolism and secondary hyperparathyroidism are suggested to play a role in the pathogenesis of age-related osteoporosis [1, 2]. We have recently demonstrated that femoral osteoporosis was largely underestimated in European women, living in both nursing homes or community dwellings [3], and that circulating PTH levels were related to low femoral bone density and osteoporosis.

Adequate vitamin D repletion, particularly in elderly women, is considered essential to avoid secondary hyperparathyroidism [4]. In fact, in elderly women, secondary hyperparathyroidism caused by reduced serum 25-hydroxyvitamin D was shown to be a determinant of an increased bone turnover rate, and hence a risk factor for osteoporotic fractures [5]. Calcium and vitamin D supplementation was shown to reduce bone loss measured at the femoral neck, spine, and total body and to reduce the incidence of nonvertebral fractures in men and women 65 years of age or older who were living in the community [6]. Similarly, in elderly ambulatory women living in nursing homes or apartment houses for elderly people, Ca and vitamin D supplements reduced the risk of hip fractures by 43% and the total number of nonvertebral fractures by 32% by increasing serum 25(OH)D concentration and subsequently decreasing the mean serum PTH concentration by 44% after 18 months of treatment [7].

Though the beneficial effects of calcium and vitamin D supplementation on Ca homeostasis in elderly people are barely challenged [8, 9], few investigations have attempted to identify the ideal regimen for their administration to induce an optimal reduction in PTH secretion. The objective of the present study was to see whether similar effects on serum PTH levels were obtained when Ca and vitamin D supplements were administered as fixed Ca and vitamin D formulations, either in a single dose in the morning, or as two doses separated by a 6 hour gap.

Material and Methods

Subjects

Twelve healthy, male volunteers, 18–35 years old, free of any medication, and without evidence of hepatic, gastrointestinal, renal, or cardiac dysfunctions were included in the present study, after having given fully written informed consent. Subjects who had received, during the last month, hepatic enzymes inductors or inhibitors, vitamin D, digitalis, fluoride, calcitonin, bisphosphonates, glucocorticoids, or, within the last week, any other drug were excluded from the trial. Subjects who were on vegetarian diet or were heavy drinkers or smokers were also excluded. The study had received prior approval of the ethics committee (IRB) of Liège University Medical Center, Liège, Belgium (n° 2000/34 of 20.03.00).

Study Design

This open-label, randomized, cross-over, controlled study was conducted from March 2000 to June 2000. The two study treatments included 1250 mg calcium carbonate and 4 mg cholecalciferol in two tablets containing 500 mg of elemental Ca and 400 IU of vitamin D_3 each (Orocal D3 ®, Theramex, Monaco) (treatment A) and Ca carbonate (2500 mg) and cholecalciferol (8.8 mg) as one water-soluble effervescent powder pack containing 1000 mg of elemental Ca and 880 IU of vitamin D_3 (Cacit D3 ®, Procter & Gamble Pharmaceuticals, Paris, France) (treatment B) were administered on 2 separate days, 1 week apart. The sequence of administration of the 2 treatments A and B was randomized.

At 8:00 a.m., on each treatment day, the subjects ingested, after an overnight fast, treatment B or one of the tablets of treatment A. At 2:00 p.m., the second dose of treatment A was ingested by the patients allocated to this regimen. After taking the study drug, the subjects were allowed to eat only one calcium-free standardized meal, between 11:30 am. and 12:30 p.m., together with 200 ml of apple juice. They were allowed to drink 1.5 l of low mineral residue water (Spa Reine°, Spa Monopole, Spa, Belgium) until the end of the experimental procedure. Venous blood was drawn for analysis prior to the study drug intake (T0) and after 60 (T60), 120 (T120), 180 (T180), 240 (T240), 300 (T300), 360 (T360), 420 (T420), 480 (T480), and 540 (T540) minutes. Seven days prior to the administration of the first sequence, all subjects were exposed to a « blank » control procedure which was similar to what happened at each day of the Ca and vitamin D supplements intake (including venous blood drawn), except that they did not receive any medication. Blood samples were immediately centrifuged and stored at 20°C for the determination of serum Ca and 1–84 intact PTH. Serum calcium concentration was assessed by atomic absorption spectrophotometry without adjustment for serum albumin concentration or specific gravity (normal range: 2.15–2.55 mmol/l). Serum 1–84 PTH concentration was measured by immunoradiometric assay (N°tact, PTH SP kit, Incstar Corporation, Stillwater, MN USA) (normal range: 10–65 pg/ml). Overall tolerance of treatment was assessed after each experimental procedure. Using the Likert scale, acceptability of treatment was assessed after each treatment day by asking the subject's opinion about the treatment and comfort of the administration. Patient response ranged from pleasant to very unpleasant and from very easy to very difficult.

Table 1. Baseline demographics of the population (n = 12)

	Mean ± SD
Age (years)	25.57 ± 3.76
Weight (kg)	75.01 ± 9.64
Height (cm)	179.75 ± 6.56
Systolic arterial pressure (mmHg)	122.91 ± 10.75
Diastolic arterial pressure (mmHG)	74.58 ± 4.98

Statistical Analysis

Descriptive Approach

Quantitative variables (age, weight, height, systolic and diastolic blood pressure) were given in mean ± SE. Serum Ca and PTH concentrations were reported for each dosing schedule and for each period in mean ± SE, and in mean variation percentages compared with those at baseline.

Inferential Approach

For each period (control, treatment A, and treatment B), serum Ca and PTH concentration were compared at each time (T0–T540) by analysis of variance.

For each period (control, treatment A, and treatment B), mean variation percentage kinetics curves were plotted for serum Ca and PTH concentration. Comparison of these curves was made by the nonparametric Zerbe test [10]. Statistical significant thresholds were fixed at 5%. The statistical data were analyzed with SAS 6.12 (SAS Institute Inc., North Carolina, USA).

Results

All subjects completed the entire protocol. Table 1 summarizes the baseline characteristics of the healthy male volunteers. Three subjects, in agreement with the study plan, were dropped from the statistical analysis evaluating the effects of the preparations on serum calcium and parathyroid hormone: two because of baseline serum PTH values below the normal range and one because of a missing value for serum PTH concentration at T120.

There were no significant changes in serum Ca concentration either during the control procedure or following the intake of the two Ca and vitamin D preparations (Table 2 and Fig. 1)

No significant changes in serum PTH concentration were observed during the course of the « blank » control procedure (Table 3 and Fig. 2). Several times both Ca and vitamin D preparations induced significant decreases in serum PTH levels, compared with baseline

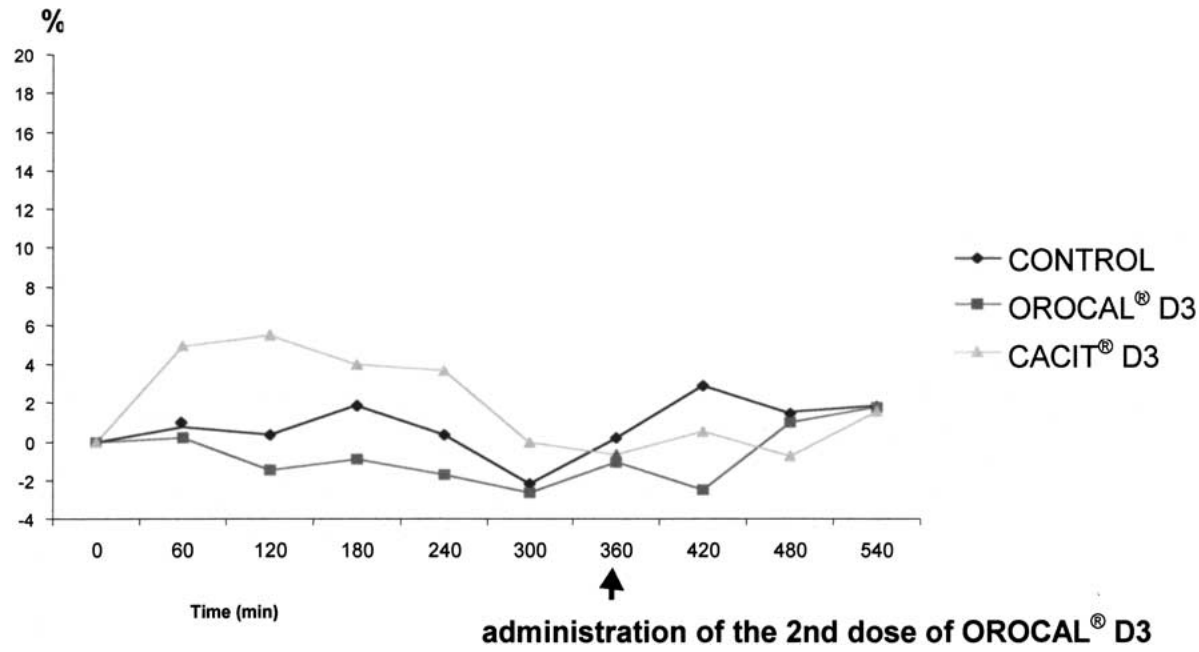

Fig. 1. Relative variations (mean) in serum calcium concentration before and for 9 hours after administration of the morning dose of the two calcium-vitamin D formulations and during blank control procedure.

Table 2. Mean ± SE serum calcium concentration (mmol/L) at 0, 60, 120, 180, 240, 300, 360, 420, 480, and 540 minutes after morning administration of the two calcium-vitamin D formulations and during blank control procedure

	Time (min)									
Treatment	0	60	120	180	240	300	360	420	480	540
Control (1)	2.43 +/−0.028	2.44 +/−0.034	2.44 +/−0.050	2.47 +/−0.025	2.44 +/−0.037	2.37 +/−0.026	2.43 +/−0.022	2.50 +/−0.094	2.46 +/−0.029	2.47 +/−0.041
OROCAL® D3 (2)	2.41 +/−0.040	2.42 +/−0.033	2.38 +/−0.060	2.39 +/−0.047	2.37 +/−0.069	2.35 +/−0.068	2.39 +/−0.044	2.35 +/−0.043	2.44 +/−0.064	2.45 +/−0.035
CACIT® D3 (3)	2.35 +/−0.046	2.46 +/−0.032	2.48 +/−0.029	2.44 +/−0.041	2.43 +/−0.040	2.35 +/−0.053	2.33 +/−0.049	2.36 +/−0.041	2.33 +/−0.045	2.38 +/−0.082
Composition (P)										
(1) vs. (2) vs. (3)	0.330	0.6234	0.3782	0.341	0.6449	0.9242	0.2247	0.1931	0.1310	0.4996
(1) vs. (2)	0.7709	0.5790	0.4802	0.1463	0.4419	0.7656	0.3928	0.1653	0.7188	0.7771
(1) vs. (3)	0.1670	0.6920	0.4989	0.5247	0.9048	0.6463	0.0795	0.1767	**0.0232**	0.3433
(2) vs. (3)	0.3102	0.3364	0.1683	0.4305	0.4986	0.9496	0.3947	0.9267	0.1815	0.4180

and with the values observed during the control procedure.

During the first 6 hours following the administration of the Ca and vitamin D supplements, no statistically significant differences were observed in serum PTH compared with baseline, between treatment A and treatment B ($P = 0.20$) during the whole period. However, from T360 to T540, serum parathyroid hormone levels continued to be suppressed with treatment A but returning to baseline levels with treatment B ($P = 0.0021$).

Overall tolerance was good for the two treatments and they were no differences between A and B. No serious adverse reactions were observed throughout the course of the study. Only one patient reported dizziness while receiving treatment B. This adverse reaction was weak and, in the investigator's judgment, unrelated to study drug intake.

Discussion

In the present study we have demonstrated that administration of calcium and vitamin D supplementation in two doses 6 hours apart induces a more prolonged decrease in serum parathyroid hormone than the same total amount of Ca and vitamin D in a single morning dose in young healthy male volunteers. As Ca and vitamin D metabolism in women depend on the same

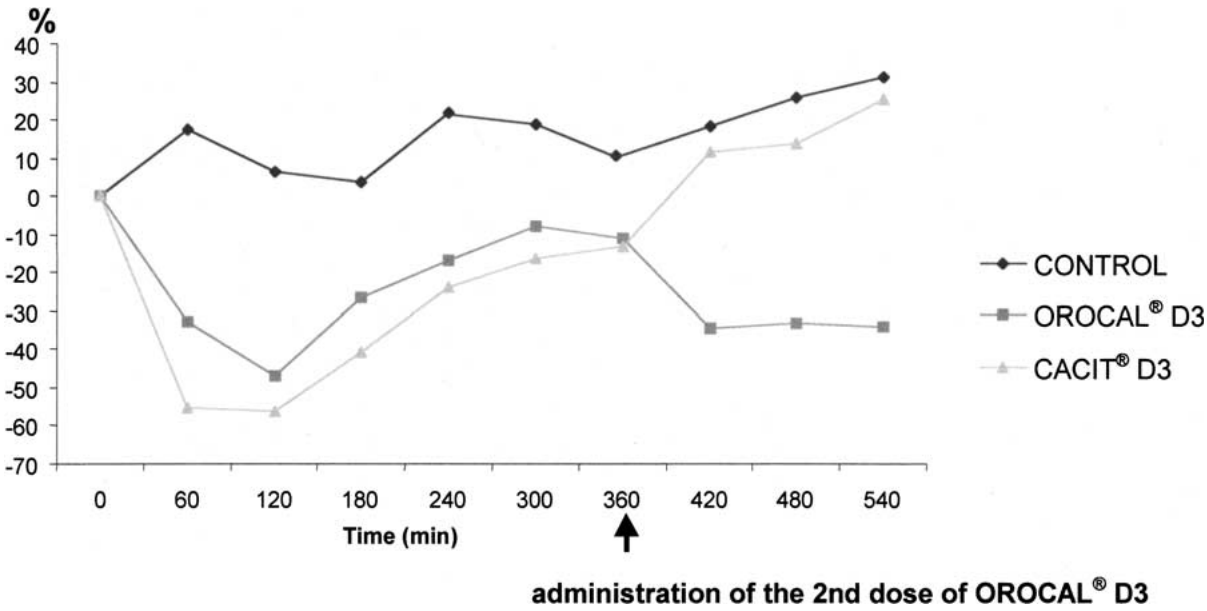

Fig. 2. Relative variations (mean) in serum PTH concentration before and for 9 hours after administration of the morning dose of the two calcium-vitamin D formulations and during blank control procedure.

Table 3. Mean ± SE serum parathyroid hormone concentration (pg/mL) at 0, 60, 120, 180, 240, 300, 360, 420, 480, and 540 minutes after morning administration of the two calcium-vitamin D formulations and during blank control procedure

	Time (min)									
Treatment	0	60	120	180	240	300	360	420	480	540
Control (1)	22.57 +/−1.680	26.53 +/−3.089	22.88 +/−1.874	22.44 +/−1.952	26.86 +/−1.807	26.52 +/−2.067	24.59 +/−1.872	26.37 +/−1.711	27.38 +/−1.333	28.90 +/−2.319
OROCAL® D3 (2)	24.82 +/−2.305	16.46 +/−2.184	12.84 +/−2.055	17.47 +/−1.761	19.11 +/−1.483	21.30 +/−2.205	20.97 +/−1.925	15.59 +/−1.675	16.12 +/−1.813	15.91 +/−1.718
CACIT® D3 (3)	21.49 +/−1.773	9.77 +/−1.363	9.50 +/−1.292	12.94 +/−2.084	16.33 +/−2.782	17.82 +/−2.127	18.48 +/−2.046	23.42 +/−2.367	23.70 +/−2.291	26.32 +/−2.114
Comparison										
(1) vs. (2) vs. (3)	0.4743	**0.0001**	**0.0001**	**0.0076**	**0.0047**	**0.0271**	0.1045	**0.0019**	**0.0009**	**0.0004**
(1) vs. (2)	0.4407	**0.0170**	0.1872	0.0765	**0.0044**	0.1033	0.1962	**0.0004**	**0.0001**	**0.0004**
(1) vs. (3)	0.6649	**0.0001**	**0.0024**	**0.0043**	**0.0059**	**0.0097**	**0.0425**	0.3283	0.1844	0.4235
(2) vs. (3)	0.2685	**0.0194**	**0.0001**	0.1169	0.3913	0.2730	0.3887	**0.0157**	**0.0196**	**0.0015**

mechanism of regulation, similar results would be expected in young, healthy, female volunteers. Male and female elderly subjects are prone to both vitamin D and Ca insufficiency because of a low Ca intake and Ca malabsorption. These lead to secondary hyperparathyroidism and subsequently to increased bone loss. Overall, the rate of both bone formation and bone resorption has been shown to remain high in elderly women as a consequence of secondary hyperparathyroidism [5]. Daily supplementation with vitamin D and calcium, through a dramatic decrease in serum parathyroid hormone levels, normalizes the biochemical markers of bone remodeling, including serum total and bone-specific alkaline phosphatases, serum osteocalcin, and urine pyridinoline and deoxypyridinoline [11]. The role of secondary hyperparathyroidism in the pathogenesis of osteoporotic fractures in the elderly is no longer disputed (3, 4, 12, 13).

Notwithstanding the specific interactions between «osteoblast- and osteoclast-lineage» cells and the mechanism through which PTH affects these interactions are still not fully answered, it seems clear that PTH stimulates osteoclast differentiation *in vivo* and *in vitro*. Osteoclasts are responsible for dissolving the mineral component of bone and hydrolysing the organic matrix. In the basic multicellular units, also called bone remodeling units, osteoclasts are involved during the « resorption phase » of the bone remodelling process. In aging, the decrease in cortical bone results from two processes: cortical thinning and an increase in cortical

porosity, phenomena in which the osteoclast is considered to be the major culprit [15].

The results shown in our study suggesting that the mode of administration of Ca and vitamin D supplementation might have an effect on the duration of the suppression of parathyroid hormone secretion are interesting. By administering Ca and vitamin D in two split doses, the secretion of PTH is decreased for an additional 3 hours, compared with the 6 hour reduction observed when the same amount of Ca and vitamin D is given as a single dose. This may therefore provide more durable protection against secondary hyperparathyroidism and bone resorption.

It has been previously reported that fractional Ca absorption varies inversely with intake [l6, 17]. At a low intake, absorption occurs by two mechanisms: active transport (vitamin D mediated) and passive diffusion. At high intake, active transport probably accounts for very little of the total absorbed. Hence, vitamin D probably plays a small role in absorptive performance of high Ca intakes [16, 17]. These findings are in close accordance with our present observation, suggesting that a high calcium (1000 mg) intake given as a single dose does not provide any substantial benefit, compared with a lower intake (500 mg), on serum parathyroid hormone reduction. The findings also support our hypothesis that Ca and vitamin D taken in split doses is more efficient for reducing serum parathyroid hormone.

In conclusion, taking into account the role of secondary hyperparathyroidism in the pathogenesis of osteoporosis fractures in elderly subjects and the demonstration of a significant reduction in hip and all nonvertebral fractures when elderly subjects are supplemented with Ca and vitamin D preparations, it seems reasonable to suggest that Ca and vitamin D supplementation should be given to elderly people living in both nursing homes and community dwellings. An important issue is constituted by the optimal regimen of Ca and vitamin D supplementation in order to provide a maximal reduction of PTH secretion, hence protecting the skeleton against increase in bone resorption and turnover.

Our findings suggest that due to the well-known kinetics of calcium absorption as a function of the administered dose, split administration of Ca and vitamin D supplements (500 mg of Ca and 400 IU of vitamin D) 6 hours apart leads to significant reduction of parathyroid hormone for an additional 50% duration, compared with a single intake of a similar total amount (1000 mg of Ca and 880 IU of vitamin D). These results should be confirmed in a long-term clinical study with elderly people in order to appreciate also the benefit on bone mineral density.

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