

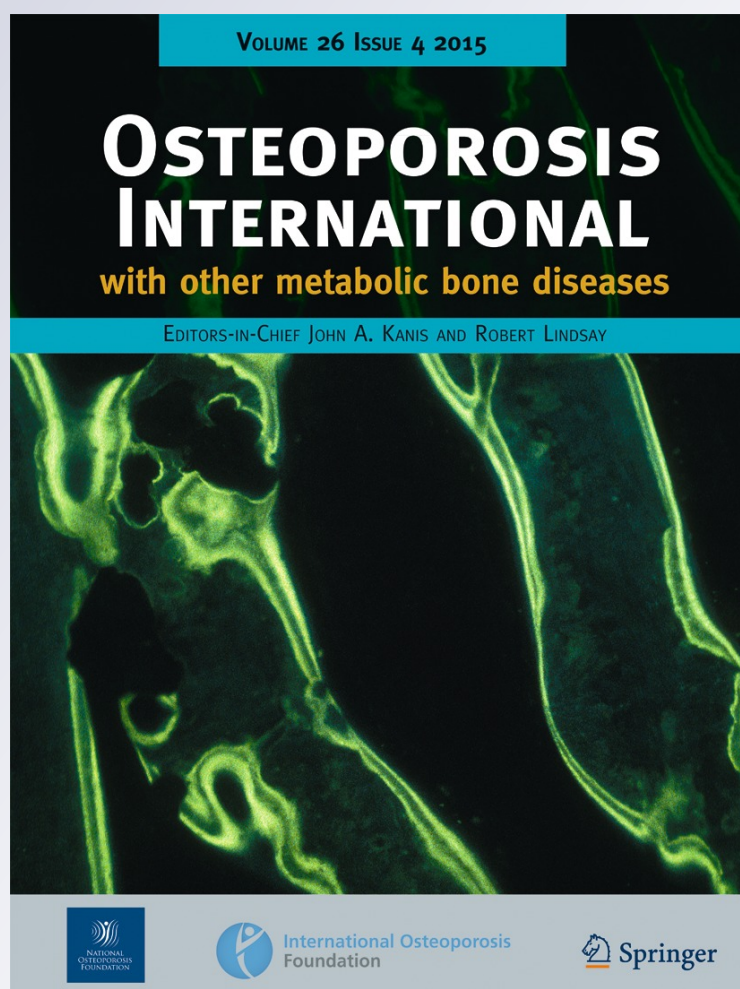
Effects of alendronate and vitamin D in patients with normocalcemic primary hyperparathyroidism

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Abstract

Summary No data on the pharmacological treatment of normocalcemic hyperparathyroidism (NPHPT) are available. We treated 30 NPHPT postmenopausal women with alendronate/cholecalciferol (treated group) or vitamin D alone (control group). Over 1 year, bone mineral density (BMD) increased significantly in treated group, but not in control group. Both treatments did not affect serum or urinary calcium.

Introduction Normocalcemic primary hyperparathyroidism (NPHPT) is defined by normal serum calcium and consistently elevated PTH levels after ruling out the causes of secondary hyperparathyroidism. It is likely that subjects with NPHPT may develop kidney and bone disease. As no data on the pharmacological treatment of NPHPT are available, we aimed

to investigate the effects of alendronate and cholecalciferol on both BMD and bone biochemical markers in postmenopausal women with NPHPT. Safety of vitamin D was evaluated as secondary endpoint.

Methods The study was a prospective open label randomized trial comparing 15 postmenopausal women with NPHPT (PMW-NPHPT), treated with oral alendronate plus cholecalciferol (treated group) and 15 PMW-NPHPT treated only with cholecalciferol (control group). Blood samples were obtained at baseline and after 3, 6, and 12 months. Bone turnover markers (BTM) were measured at baseline, 3, and 6 months, respectively. BMD was assessed at baseline and after 12 months.

Results After 1 year of treatment, BMD increased significantly at the lumbar, femoral neck, and hip level in the treated group, but not in the control group ($p=0.001$). No differences were found between or within groups in serum calcium, PTH, and urinary calcium levels. BTM significantly decreased in the treated group but not in the control group, at 3 and 6 months ($p<0.001$), respectively. No cases of hypercalcemia or hypercalciuria were detected during the study.

Conclusion The results of this study indicate that alendronate/cholecalciferol increases BMD in postmenopausal women with NPHPT. Alendronate/cholecalciferol or vitamin D alone does not affect serum or urinary calcium.

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Keywords Alendronate · Bone mineral density · Bone turnover markers · Normocalcemic primary hyperparathyroidism · Vitamin D

Introduction

Primary hyperparathyroidism (PHPT) is a common endocrine disease characterized by an inappropriate elevation of the circulating parathyroid hormone (PTH) that provides stable

hypercalcaemia [1]. Monoclonal parathyroid adenoma and multi-glandular hyperplasia represent the main causes of this endocrine condition [1]. The disease has been reported to affect the bone, kidney, and cardiovascular system health [2], but in recent years, its clinical scenario has completely changed. The widespread use of multi-channel biochemistry and the increasing attention paid to parathyroid pathologies are the reasons why it is increasingly frequent to find subclinical or asymptomatic forms of PHPT by accident during the biochemical screening of primarily middle-aged or elderly patient [3–5]. This subclinical form of hyperparathyroidism has been recently described as asymptomatic primary hyperparathyroidism. The Fourth International workshop for the management of asymptomatic PHPT stated that it is characterized by the lack of specific sign and/or symptoms that are traditionally associated with PTH excess or hypercalcaemia [6]. A new part of the spectrum of the asymptomatic primary hyperparathyroidism is represented by normocalcemic primary hyperparathyroidism (NPHPT). NPHPT is defined by normal total and ionized serum calcium concentrations and consistently elevated PTH levels after ruling out known causes for secondary elevations of PTH, such as renal disease or vitamin D deficiency [7–12]. It was first formally recognized at the time of the Third International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism in 2008 [7] but, due to the lack of evidence, the expert panel was not able to provide a clear guideline for its management. Although there is contrasting evidence about the metabolic consequences of NPHPT [13, 14], it is likely that subjects with NPHPT can develop kidney and bone involvement [15–20]. Charopoulos et al. demonstrated an impairment of cortical and cancellous skeleton sites in both normocalcemic and primary hyperparathyroidism even though the effect was accentuated in hypercalcemic patients [21].

In the last published guidelines for the management of PHPT, parathyroidectomy (PTX) is recommended for subjects with a BMD T-score at any of the three sites (lumbar spine, proximal femur, or forearm) of less than -2.5 SD [22]. Moreover, in subjects with mild primary hyperparathyroidism, PTX is followed by a decrease in bone turnover with an increase in bone mass, although the cortical compartment does not show any improvement in a short term [23, 24, 5].

No conclusive data are available about the treatment with vitamin D in subjects with PHPT. Although treatment with vitamin D₃ [25–28] and vitamin D₂ [29, 30] slightly decreases the level of PTH in patients with vitamin D deficiency, its effect on bone mineral density (BMD) and bone turnover markers (BMT) has not been well established. Only a randomized placebo controlled trial [28] together with an uncontrolled trial [31] have demonstrated a significant reduction of bone resorption markers with a parallel increase of lumbar spine BMD. Some concerns were raised on the safety of this treatment as other investigators have reported increased

plasma and urine calcium levels after treatment with vitamin D [26, 29].

A few randomized controlled trials have investigated the role of anti-resorptive agents on the management of PHPT. In particular, estrogen therapy [32] and bisphosphonates [33–35, 23] were shown to increase femoral neck and lumbar spine BMD in subjects with mild primary hyperparathyroidism compared to untreated patients.

To date, no data on the pharmacological treatment of NPHPT are available.

Due to this lack of data, our study aimed to investigate the effects of oral treatment with alendronate and vitamin D on BMD and on the biochemical markers of both bone metabolism and turnover in postmenopausal women presenting with NPHPT. As a secondary endpoint we aimed to assess the safety and effects of vitamin D supplements in NPHPT patients.

Material and methods

Study design and population

The study was a prospective investigator-initiated, randomized, open parallel-group trial comparing weekly oral alendronate (70 mg) plus cholecalciferol (2800 IU) (Fosavance® 70 mg/28,000 IU, tablets) with only weekly cholecalciferol (Dibase® 10,000 IU:2800 IU=11 drops). Thirty postmenopausal women (mean age 57 ± 4 years) with NPHPT, as defined by the Consensus Development Conference on the management of primary hyperparathyroidism [7], were enrolled (from January 2009 to March 2013). At baseline, blood samples and information on medical history, socioeconomic factors, and dietary habits (intake of milk and milk products, fish, and fruit) as well as the use of vitamin supplements were collected. In particular, dietary calcium intake was assessed by a questionnaire [36] that was completed after the first visit. Inclusion criteria were a menopausal state of at least 5 years, the presence of osteoporosis (defined by a BMD T-score at one or more skeletal sites lower than -2.5 SD), elevated serum PTH, normal values of calcium after adjustment for serum albumin, and normal vitamin serum levels (>30 ng/ml). Exclusion criteria were secondary hyperparathyroidism, concurrent systematic illness, thyroid disease, hepatic or renal dysfunction, and other disorders known to influence BMD. No patients had received estrogens, bisphosphonates, supplements of calcium and/or vitamin D or other drugs that could interfere with bone or mineral metabolism during the last 12 months. The subjects reported no personal or familial history of recurrent kidney stone disease.

Normocalcemia was defined as an adjusted plasma calcium level below the upper limit of reference range (in our lab, this is set at 10.5 mg/dl). A state of hyperparathyroidism was

Table 1 Biochemical and BMD characteristics of the treated and control patients at baseline. Data are expressed as mean±SD

		Treated group (<i>n</i> =15) mean±SD	Control group (<i>n</i> =15) mean±SD	<i>p</i>
Biochemical data	Age (years)	59±5	57±5	0.343
	Adjusted calcium (mg/dl)	9.9±0.5	9.7±0.4	0.219
	Phosphate (mg/dl)	3.8±0.2	3.9±0.3	0.432
	PTH (pg/ml)	112±21	106±13	0.310
	25-OH-vitamin D (ng/ml)	32±5	34±7	0.500
	CTX (ng/ml)	0.6±0.1	0.7±0.1	0.328
	Osteocalcin (ng/ml)	29±5	27±2	0.402
	24-h urinary calcium (mg/l)	185±57	197±48	0.518
BMD data	Lumbar L1–L4 (g/cm ²)	0.781±0.071	0.772±0.074	0.698
	(T-score)	(−2.3±0.2)	(−2.3±0.2)	
	Femoral neck (g/cm ²)	0.623±0.095	0.643±0.081	0.516
	(T-score)	(−2.0±0.3)	(−1.8±0.2)	
	Total femur (g/cm ²)	0.701±0.112	0.710±0.080	0.782
	(T-score)	(−2.0±0.3)	(−1.9±0.2)	

defined as PTH levels in the upper third of the reference interval or above (>65 pg/ml). Randomization was performed by the investigators using a computer-generated randomization code. The patients were randomized to treatment with Fosavance® (treated group, *n*=15) or with cholecalciferol (control group, *n*=15). To limit the potential difference in terms of bioavailability, all subjects were instructed to take either Fosavance® or cholecalciferol at the same time (in the morning 30 min before breakfast) in a fasting state. Overall dietary calcium intake was not adequate (treated group 685 mg±89 mg; control group 703 mg±12 mg, *p*=ns).

All patients were maintained on a controlled diet with a calcium intake of about 1000 mg/day. The study was

performed in accordance with the Declaration of Helsinki II and guidelines on Good Clinical Practice. Informed consent was obtained from all the patients, and the study was approved by the local ethics committee.

Fasting blood samples for determination of serum PTH, calcium, phosphates, and 25-OH vitamin D were obtained at baseline and after 3, 6, and 12 months. Osteocalcin and urine samples for the measurement of CTX and 24-h calcium excretion were obtained at baseline and after 3 and 6 months. Serum PTH (2.1 % of intra-assay and 2.3 % of inter-assay variability) and osteocalcin (2.4 % of intra-assay and 2.2 % of inter-assay variability) were measured using chemiluminescence immunoassays (Roche Diagnostics, Mannheim,

Table 2 Biochemical parameters of the two patient groups at different time points of the study period. Data are expressed as mean±SD

	Biochemical data	Baseline	3rd month	6th month	12th month	<i>p</i>
Treated group (<i>n</i> =15)	Adjusted calcium (mg/dl)	9.9±0.5	9.5±0.3	9.7±0.4	9.6±0.4	0.100
	Phosphate (mg/dl)	3.8±0.2	3.9±0.3	3.7±0.4	3.8±0.3	0.002
	PTH (pg/ml)	112±21	110±13	112±15	110±13	0.865
	25-OH-vitamin D (ng/ml)	32±5	36±5	35±4	36±5	0.021
	CTX (ng/ml)	0.62±0.09	0.19±0.02	0.29±0.05	–	<0.001
	Osteocalcin (ng/ml)	28±5	15±1	17±3	–	<0.001
	24 h-urinary calcium (mg/l)	185±57	184±61	194±52	182±62	0.769
	Control group (<i>n</i> =15)	Adjusted calcium (mg/dl)	9.7±0.4	9.5±0.3	9.6±0.3	9.5±0.3
Phosphate (mg/dl)	3.9±0.3	3.9±0.4	3.9±0.3	3.7±0.4	0.156	
PTH (pg/ml)	106±13	111±11	105±12	110±14	0.173	
25-OH-vitamin D (ng/ml)	34±7	36±4	34±4	35±4	0.392	
CTX (ng/ml)	0.66±0.11	0.63±0.10	0.69±0.10	–	0.344	
Osteocalcin (ng/ml)	27±2	25±3	27±2	–	0.595	
24 h-urinary calcium (mg/l)	197±48	191±38	211±53	183±63	0.738	

Germany) on a Modular E170. Measurements of serum calcium (1.8 % of intra-assay variability and 2 % of inter-assay variability), phosphate (2.8 % of intra-assay and 2.6 % of inter-assay variability), and 24-h urinary calcium were determined using an Olympus AU 2700 automated multichannel analyzer (Olympus, Tokyo, Japan). Calcium was corrected for serum albumin concentration according the following formula: $[0.8 \times (4.0 - \text{patient's albumin}) + \text{serum calcium}]$. Determination of 25-OH vitamin D (4.6 % of intra-assay variability and 5.1 % of inter-assay variability) was performed using a DiaSorin kit on a LIAISON automated immunoassay analyzer (DiaSorin, Saluggia, Italy). Urinary concentrations of the amino-terminal cross-linked telopeptides of collagen (CTX) were measured with a commercial enzyme-linked immunoassay kit (Cross Laps ELISA, Osteometer, Denmark) (3.6 % of intra-assay and 4.3 % of inter-assay variability). BMD was measured using dual-energy X-ray absorptiometry (DXA) (Hologic Discovery QDR Instrument, MA, USA). DXA was performed at the L1–L4 lumbar spine (postero-anterior projection), total hip, and femoral neck at baseline and after 12 months.

Statistical analysis

Data were analyzed using SPSS version 13.0 for Windows. Variables were expressed as mean \pm SD. BMD was expressed as g/cm^2 and changes in BMD were shown either as percentage change or as absolute difference between values at 12th month and baseline. Differences of data between treated and control patients at baseline were analyzed using unpaired Student's *t* test, as appropriate. Changes over time for each patient were evaluated by repeated measures ANOVA for PTH, calcium, phosphate, 25-OH vitamin D, osteocalcin, urinary calcium, and CTX. Wilcoxon test was used to determine BMD variations. Differences were considered significant when $p < 0.05$.

Results

Baseline biochemical and BMD characteristics of the patients are displayed in Table 1; no significant differences were found between treated and control patients for PTH, calcium, phosphates, 25-OH vitamin D, and osteocalcin concentrations, as well as for 24-h urinary calcium excretion and CTX. BMD at the spine, total hip, and femoral neck was similar in both groups of patients without significant differences (Table 1).

Table 2 shows biochemical data of treated and control patients at different time points. Over all time points, both groups did not show any significant differences in serum and

urinary calcium levels as well as in serum, PTH, and urinary calcium concentrations compared to baseline values (Table 2).

A small, but statistically significant, positive trend was detected for 25-OH vitamin D levels from baseline to the end of the study in the treatment group (Table 2). A similar slightly significant trend was detected for phosphate. No

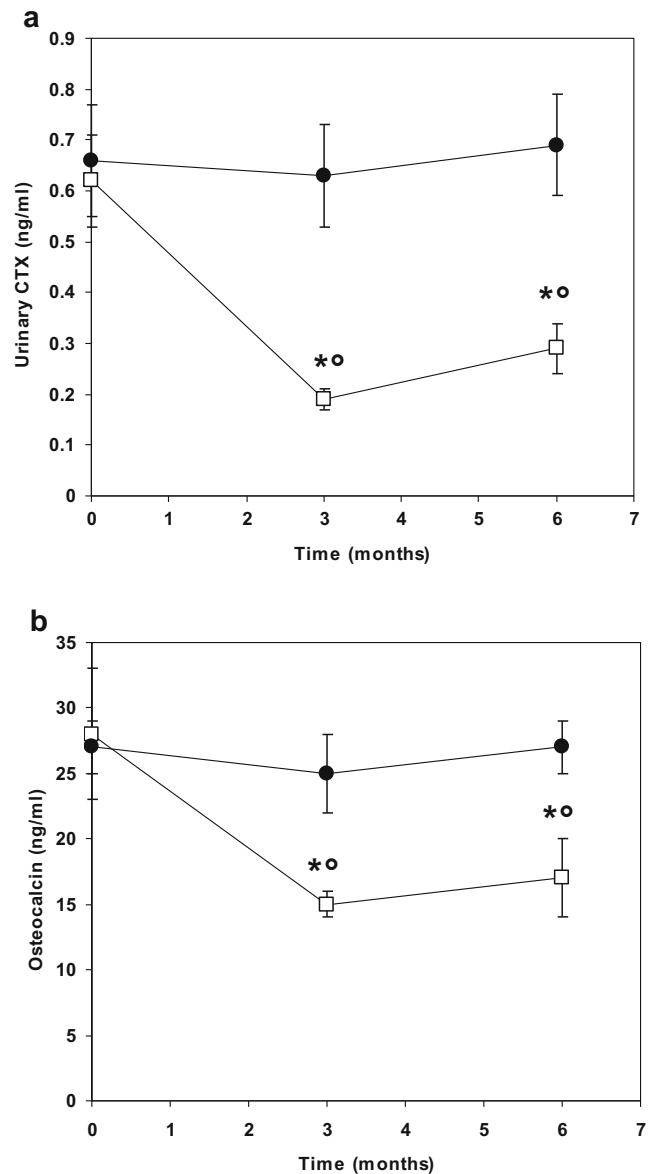


Fig. 1 **a** Urinary CTX concentration at different timing in treated (\square) and control (\bullet) groups. A significant decrement compared to the baseline value (*) is observed in the group under treatment at 3rd and 6th month ($p < 0.001$). A significant decrement of urinary CTX concentration is observed in the group under treatment compared to the control group (\circ) at 3rd and 6th month ($p < 0.001$). **b** Serum osteocalcin concentration at different timing in treated (\square) and control (\bullet) groups. A significant decrement compared to the baseline value (*) is observed in the group under treatment at 3rd and 6th month ($p < 0.001$). A significant decrement of serum osteocalcin is observed in the group under treatment compared to the control group (\circ) at 3rd and 6th month ($p < 0.001$)

difference was detected between groups, at each time point, for each variable.

After 3 months, the treated group showed significantly decreased serum osteocalcin and urinary CTX concentrations (−46.4 and −69.4 %, respectively; $p < 0.001$ vs. baseline) and these values remained reduced after 6 months (−39.3 and −53.2 %, respectively; $p < 0.001$ vs. baseline). The control group did not demonstrate any significant change in osteocalcin and CTX concentration at any time points. A significant difference in CTX and osteocalcin levels was detected between the control and treated group both at 3 and 6 months ($p < 0.001$ for each comparison) (Fig. 1).

BMD values at baseline and after 12 months are reported for both groups of patients in Table 3. After a year of treatment, BMD in the treated group increased significantly at the lumbar, femoral neck, and total femur ($p = 0.001$).

In particular, the greatest increment was at the lumbar spine with a mean BMD increase of 4.7 % (ranging from 3.0 to 8.0 %). In the control group, a significant BMD reduction was recorded at all measured sites compared with baseline ($p = 0.001$). After 12 months, the alendronate plus vitamin D treatment group showed a higher significant increase of BMD (lumbar spine, femoral neck, and total femur) than the control group treated only with cholecalciferol ($p = 0.001$).

Discussion

This is the first study evaluating the effect of alendronate and vitamin D on patients affected by NPHPT. The underlying causes of NPHPT are still unknown but Rao et al. hypothesized that NPHPT and primary hyperparathyroidism are two phases of the same disease: the first would represent the subclinical condition and the second would be the overt disease with elevation of calcium levels [37]. Moreover, Murani et al. have shown that the resistance of kidney and

bone to the actions of PTH could play an important role in the development of this subclinical condition [8].

Although the Fourth International workshop for the management of asymptomatic PHPT tries to clarify its diagnostic criteria and suggests the way to manage subjects with NPHPT, no prospective randomized controlled trials have evaluated the impact of the anti-resorptive therapy in this kind of population.

Few randomized controlled trials have investigated the role of bisphosphonates in the management of PHPT. However, Rossini et al. have demonstrated that 2 years of treatment with alendronate induced significant increases in BMD at the lumbar spine, total hip, and total body compared to control patients which showed a significant BMD reduction [33]. In another placebo-controlled trial, alendronate caused an increase in the lumbar spine and total hip BMD as well as a decrease in BMT without any changes of ionized calcium, phosphorus, and PTH [35]. Chow et al. have obtained similar results with a significant improvement of the spine and femoral neck BMD in subjects affected by PHPT after treatment with alendronate [34].

Our data have demonstrated the ability of alendronate to increase BMD in osteoporotic patients with NPHPT. As far as osteoporosis representing an indication for PTX, these findings might raise some doubts about the real need of parathyroidectomy in this group of patients. Moreover, our data seem to confirm the results of the meta-analysis by Sankaran et al. on skeletal effects of interventions in mild primary hyperparathyroidism. The authors have shown that surgical treatment and anti-resorptive therapies increase BMD in mild PHPT to a similar degree and that medical treatment could represent a reasonable option in a patient with mild hyperparathyroidism and low BMD [23]. According to other observations in primary hyperparathyroidism [38, 35], our results confirmed the effects of alendronate in decreasing both bone resorption and formation markers also in patients with NPHPT. The anti-

Table 3 BMD measurements in treated and control groups of patients at baseline and after 12 months of observation. Differences between values at 12th month and baseline are expressed both as absolute difference (abs) and as percentage change (%)

BMD	Treated group ($n=15$) mean±SD [abs; (%)]			Control group ($n=15$) mean±SD [abs; (%)]		
	Baseline	12 months	p	Baseline	12 months	p
Lumbar L1–L4 (g/cm ²)	0.781±0.071	0.819±0.074 [0.038; (4.7)]	0.001	0.772±0.074	0.759±0.072 [0.013; (−1.6)]	0.001
T-score	−2.3±0.2	−1.9±0.2		−2.3±0.2	−2.5±0.2	
Femoral neck (g/cm ²)	0.623±0.095	0.640±0.092 [0.017; (2.6)]	0.001	0.643±0.081	0.632±0.083 [0.011; (−1.7)]	0.001
T-score	−2.0±0.3	−1.8±0.2		−1.8±0.2	−2.0±0.3	
Total femur (g/cm ²)	0.701±0.112	0.732±0.111 [0.031; (4.0)]	0.001	0.710±0.083	0.700±0.080 [0.010; (−1.4)]	0.001
T-score	−2.0±0.3	−1.7±0.2		−1.9±0.2	−2.0±0.2	

resorptive effects of alendronate were rapidly seen within the first trimester of the study and they were sustained up to the 6th month, confirming the well-known data that are available in postmenopausal osteoporosis [39, 40].

Although treatment with vitamin D₃ [25–28] and vitamin D₂ [29, 30] slightly decreases PTH levels in patients with vitamin D deficiency and primary hyperparathyroidism, it is not clear whether they can be considered a valuable and safe tool to manage NPHPT due to the lack of published data in this particular disease. Only a randomized placebo controlled [28] and an uncontrolled trial [31] have demonstrated a significant reduction of bone resorption markers and an increase of lumbar spine BMD in patients with primary hyperparathyroidism treated with vitamin D. Some doubts were raised on the safety of this treatment: in fact, other investigators have reported increased plasma and urine calcium levels after treatment with vitamin D [26, 29]. In our population, the administration of vitamin D was safe and it did not induce any cases of hypercalcemia or hypercalciuria. As shown in Table 2, both plasma and urinary calcium did not significantly increase during the study and PTH was unaffected by the treatments. In contrast with patients treated with alendronate, the weekly treatment with 2800 IU of cholecalciferol in the control group was not able to arrest bone loss or to decrease BMT.

Although an alendronate alone group was not included in our study, it may be presumed that the beneficial effect seen on the BMD was entirely due to alendronate. Indeed, Macdonald HM et al. have demonstrated that, in postmenopausal women with vitamin D deficiency (at baseline), treatment with daily oral vitamin D₃ at 400 IU (1 year) did not change markers of bone metabolism (CTX included) and was not able to significantly arrest the bone loss at hip compared to the treatment with 1000 IU/day [41]. Moreover, Recker et al. showed that a once-weekly tablet containing both cholecalciferol (2800 IU) and alendronate (70 mg) did not provide any further anti-resorptive improvement (evaluated by changes in bone bio-markers) compared to the single alendronate (70 mg) treatment [42].

To limit the potential difference in terms of bioavailability, all the subjects were instructed to take Fosavance® and cholecalciferol once a week, at the same time (in the morning, before breakfast) in a fasting state. Indeed, as vitamin D is liposoluble, its oral absorption could increase if ingested with a fat-rich meal [43] although there are contrasting evidences.

We found only a slight, not clinically significant change in 25-OH vitamin D levels after supplementation with cholecalciferol in the treated group. As we administered 2800 IU/week (400 IU/day) of cholecalciferol to our patients, it is reasonable to believe that they should have raised their plasma vitamin D of a small amount that would have been difficult to detect in our small groups. Indeed, it is described that 40 IU/day of vitamin D for 6 months can induce a rise in plasma 25(OH) D₃ of about 0.7 ng/ml [44]. Holick et al. demonstrated that

ergocalciferol can produce a significant rise in plasma 25(OH) D₃ only in patients with low basal vitamin D. Our patients had normal plasma vitamin D before the study, and it is highly probable that their plasma 25(OH) D₃ did not change over the study period [45]. It might be that in the control group, the compliance to the therapy was lower compared to the treated group. However, at each visit, we asked each subject to confirm they had taken the drugs as requested. Moreover, the small sample size makes further interpretation difficult.

Our study has some limitations. It was not designed as a double-blind trial, the sample size was small (even if the prevalence and incidence of NPHPT are low), and we did not measure ionized calcium nor the radius BMD. Moreover, we did not evaluate BMT at the end of the study as these measurements were only programmed after 3 and 6 months, respectively. This decision was taken in advance, as we were interested in measuring early changes of bone turnover. After having seen the unexpected increase of BMD, we realized that an evaluation of BMT at the end of the study could have added more power to our results.

In conclusion, for the first time, the results of this study indicate that in postmenopausal women with NPHPT, treatment with alendronate together with vitamin D significantly increases BMD at the most clinically relevant skeletal sites. Moreover, treatment with alendronate and/or vitamin D does not affect calcium, phosphate, and 25-OH vitamin D serum levels as well as urinary calcium excretion.

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Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of interest None.

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