

Original Article

Effect of calcium and vitamin D supplementation with and without collagen peptides on bone turnover in postmenopausal women with osteopenia

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Abstract

Objectives: Collagen peptides (CPs) seem to exert beneficial effects on bone and may have a role as a treatment option. In the present randomized prospective study, we aimed to examine the efficacy, as expressed by changes in P1NP and CTX, and the tolerability of 3-month supplementation of calcium, vitamin D with or without bioactive CPs in postmenopausal women with osteopenia. **Methods:** Fifty-one female, postmenopausal women with osteopenia were allocated to two groups: Group A received a sachet containing 5 g CPs, 3.6 g calcium lactate (equivalent to 500 mg of elemental calcium) and 400 IU vitamin D3 and group B received a chewable tablet containing 1.25 g calcium carbonate (equivalent to 500 mg of elemental calcium) and 400 IU vitamin D3 daily. **Results:** In group A, the P1NP levels significantly decreased by 13.1% ($p < 0.001$) and CTX levels decreased by 11.4% ($p = 0.058$) within 3 months of supplementation. In group B, P1NP and CTX did not change. Group A presented better compliance in comparison to group B and no adverse events contrary to group B. **Conclusions:** These findings may reflect the reduction of the increased bone turnover in postmenopausal women with the use of calcium, vitamin D and CPs supplements. The addition of CPs in a calcium and vitamin D supplement may enhance its already known positive effect on bone metabolism. Clinical Trial ID: NCT03999775.

Keywords: Collagen Peptides, Bone Turnover Markers, Calcium Supplement, Osteopenia, Postmenopausal Women

Introduction

Osteoporosis is undoubtedly one of the most common diseases affecting older individuals with debilitating consequences¹. Osteopenia, defined as T-score between

-1 and -2.5, has also been associated with increased risk of osteoporotic fractures and the associated morbidity and mortality². Prompt diagnosis, prevention and treatment of both osteopenia and osteoporosis are essential in order to minimize future fracture risk. The mainstay of treatment of osteopenia and osteoporosis includes dietary changes, regular weight-bearing exercises, calcium and vitamin D supplementation and pharmacologic treatment mainly with antiresorptive or anabolic agents². Collagen peptides (CPs), also called collagen hydrolysates produced by hydrolysis of collagen, have also been shown to have high oral bioavailability and could have a place as a treatment option³⁻⁹.

Type I collagen comprises approximately 95% of the entire collagen content of bone. Bone matrix, unlike other connective tissues, possesses the unique ability to become calcified. Spindle or plate-shaped crystals of hydroxyapatite are found between and around collagen fibers, oriented in the same direction as collagen fibers are¹⁰⁻¹². Nowadays, it is well-documented that type I collagen molecules are involved in the mechanical properties of bone^{12,13}. Collagen

The study was supported by Vlvapharm SA. The necessary amount of Colabone® sachets, containing 5 g bioactive collagen peptides (Fortibone®), 3.6 g calcium lactate (equivalent to 500 mg of elemental calcium) and 400 IU vitamin D3 for the conduction of the study was also provided by Vivapharm SA. The authors have nothing to declare.

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peptide compounds seem to exert their beneficial effect on bone by affecting bone remodeling and mineralization of the bone matrix, promoting the proliferation and differentiation of pre-osteoblasts while reducing the maturation of osteoclasts¹⁴⁻¹⁶. Several preclinical studies performed in mice and rats support this notion and also suggested that orally administrated CPs increased bone mineral density (BMD), as well as the compositional and the biodynamic characteristics of vertebrae¹⁷⁻¹⁹. Human studies in postmenopausal women have also yielded positive results with increased BMD and blood biomarkers after 6 months and 1 year of oral administration^{6,7}.

In the present randomized prospective study, we aimed to examine and compare the efficacy, as represented by the changes in bone biomarkers procollagen type I N-terminal propeptide (P1NP) and C-terminal telopeptide of collagen I (CTX), and tolerability of 3-month supplementation of calcium, vitamin D with and without bioactive CPs in postmenopausal women with osteopenia.

Materials and methods

The study protocol was approved by the ethic committee of the KAT General Hospital of Athens and written informed consent was obtained from all participants. The study is registered at ClinicalTrials.gov (NCT03999775). Subjects could withdraw from the study at any time, either by their personal decision or at the discretion of the investigator; withdrawn subjects were not replaced.

Female, postmenopausal women with T-score in the osteopenic range ($-1.0 > T\text{-score} > -2.5$) at either the lumbar spine (LS) or femur as measured by dual energy X-ray absorptiometry (DXA), were included in our study. Patients with T-score in the osteoporotic range ($T\text{-score} < -2.5$) at any site, patients receiving supplements of calcium and/or vitamin D at that time or during the last year, or medications known to positively or negatively affect bone turnover or BMD at that time or during the last 3 years (e.g. antiresorptive agents, oestrogens, systemic corticosteroids), or having a known secondary cause of osteoporosis (e.g. alcohol abuse, thyrotoxicosis etc) were excluded from the study. Patients who did not attend to their follow-up appointment and consequently had only the baseline measurements were also excluded from the analysis.

On the basis of these criteria, 51 postmenopausal women were recruited after carrying measurements of BMD at the LS and the femur using DXA. All participants were assessed at baseline, provided information on their demographics and medical history and they completed a brief questionnaire assessing their dietary calcium intake. The subjects received the study's supplementation and instructions at baseline and the follow-up visit was scheduled. Participants were reviewed after 3 months, when they were asked about their self-reported compliance and persistence, any health issues or side effects experienced and other medications. Compliance was also estimated, at the follow-up appointment, when the

supplement or a prescription was given to the patients and they were asked if they had any remnant of the supplement. Patients were classified as non-compliant with 0-49% drug intake, partially compliant with 50-74% drug intake and compliant with 75-100% drug intake. A gentle reminder was also performed for the continuous receipt of the medicine.

Participants were randomized into two groups. Group A consisted of 24 subjects who received a sachet containing 5 g bioactive collagen peptides (Fortibone®), 3.6 g calcium lactate (equivalent to 500 mg of elemental calcium) and 400 IU vitamin D3 (Colabone®, Vivapharm SA) per day, provided by us. Group B (control) consisted of 27 subjects who received a chewable tablet containing 1.25 g calcium carbonate (equivalent to 500 mg of elemental calcium) and 400 IU vitamin D3 per day, which is one of the commonest supplement combinations given in everyday clinical practice, prescribed from us.

The primary endpoint of the study was the change of P1NP and CTX levels following the 3-month calcium, vitamin D and CPs supplementation. The secondary endpoints were the comparison of %-changes of P1NP and CTX levels between the group of calcium, vitamin D and CPs and the group of calcium and vitamin D supplementation, the comparison of adverse effects (tolerability), and/or the adherence to treatment between the two groups.

Biochemical measurements

All blood samples were collected at baseline and after 3 months after overnight fasting for the measurement of common biochemical blood exams to exclude secondary causes of osteoporosis and more specific blood exams including total calcium, intact-parathyroid hormone (iPTH), 25-hydroxy-vitamin D [25(OH)D], N-MID-osteocalcin (N-MID-OC), total procollagen type I N-terminal propeptide (total-P1NP) and C-terminal telopeptide of collagen I (β -CTX). All samples were centrifuged within one hour from collection (at 3000 rpm for 10 min), aliquoted and stored at -80°C until tested.

Total serum calcium levels were measured with a colorimetric assay on Architect-8000 Chemistry analyzer (Abbott, Chicago, IL, USA). The measurement range of this assay is 2.0-24.0 mg/dL (or 0.50-6.00 mmol/L) the total analytical imprecision of this assay in our laboratory is $<1.0\%$.

Serum iPTH levels were measured by a second-generation electrochemiluminescence immunoassay (ECLIA) on Cobas e411 automated analyzer (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. The measurement range of this assay is 1.20-5000 pg/mL or 0.127-530 pmol/L. The total analytical imprecision of this assays in our laboratory is $<4.0\%$.

Serum levels of total-P1NP (reference range 16.27-73.87 ng/mL) were determined with an ECLIA immunoassay on Cobas e411. This method measures the total-P1NP. The measurement range of this assay is 5-1200 ng/mL. The total analytical imprecision of this assays in our laboratory is $<4.5\%$.

Serum levels of the β -CTX (reference range <1.008 ng/

Table 1. Demographics and baseline characteristics of the two groups.

	Group A (n=21)	Group B (n=22)	p-value
Age (years)	62.1 ± 6.3	62 ± 7.6	0.964
BMI (kg/cm ²)	26.8 ± 5.1	26.5 ± 4.6	0.842
Dietary calcium intake (mg/day)	799 ± 427	671 ± 358	0.3
Calcium (mmol/L)	2.35 ± 0.08	2.39 ± 0.12	0.289
25(OH)vitD3 (nmol/L)	66.55 ± 24.07	73.05 ± 27.17	0.412
PTH (pmol/L)	5.67 ± 1.54	5.57 ± 1.76	0.849
OC (ng/mL)	24.9 ± 7.1	26.4 ± 9.3	0.578
LS BMD (g/cm ²)	1.024 ± 0.083	0.986 ± 0.057	0.094
LS T-score	-1.28 ± 0.757	-1.61 ± 0.485	0.084
TH BMD (g/cm ²)	0.833 ± 0.081	0.824 ± 0.059	0.678
TH T-score	-1.4 ± 0.679	-1.4 ± 0.494	0.767

BMI, body mass index; PTH, parathyroid hormone; OC, osteocalcin; LS, lumbar spine; TH, total hip; BMD, bone mineral density.

Table 2. Mean values of bone turnover markers of the two groups at baseline and 3 months of supplementation and comparisons between and within the groups.

	Group A	Group B	Between groups p-value
P1NP baseline (mean ± SD)	60.2 ± 15.6	59.1 ± 25.2	0.201
P1NP at 3 months (mean ± SD)	52 ± 15.1	57.1 ± 24.1	0.981
Within group p-value	<0.001	0.210	
P1NP % change from baseline to 3 rd month (mean ± SD)	-13.1 ± 12.3	-2.1 ± 12.6	0.011
CTX baseline (mean ± SD)	384 ± 107	418 ± 189	0.773
CTX at 3 months (mean ± SD)	333 ± 112	421 ± 210	0.382
Within group p-value	0.058	0.922	
CTX % change from baseline to 3 rd month (mean ± SD)	-11.4 ± 24	3.5 ± 29.9	0.079

P1NP, procollagen type I N-terminal propeptide; CTX, C-terminal telopeptide of collagen I. P1NP in ng/ml, CTX in pg/ml.

mL) were determined with an ECLIA immunoassay on Cobas e411. The measurement range of this assay is 0.010-6.00 ng/mL or 10-6000 ng/L. The total analytical imprecision of this assays in our laboratory is <3.5%.

Serum levels of total 25(OH)D levels were determined with an ECLIA immunoassay on Cobas e411. The measurement range of this assay is 3-70 ng/mL or 7.5-175 nmol/L. The total analytical imprecision of this assays in our laboratory is <4.7%.

Serum levels of N-MID-OC were determined with an ECLIA immunoassay on Cobas e411. This assay detects both the N-MID fragment as well as the intact molecule of OC. The measurement range of this assay is 0.5-300 ng/mL. The total analytical imprecision of this assay in our lab is <3.5%.

Statistical analysis

Intention-to-treat (ITT) analysis was performed. The Pearson's chi-square test was used to evaluate differences

between categorical values, the Mann-Whitney U test to evaluate differences between continuous and ordinal data of the two groups, and the Wilcoxon signed-rank test to evaluate differences between the not normally distributed P1NP and CTX levels within the groups. All the continuous data were presented as mean ± standard deviation (SD) for the homogeneity of the presentation. All the statistical tests were performed using SPSS 20 statistical software (SPSS Inc, Chicago, IL). A value of p<0.05 was selected to indicate statistical significance.

Results

After 3 months, 21 patients of group A and 22 patients of group B were analysed. Demographics and baseline characteristics were comparable between the two groups (Table 1). All the patients had creatinine levels within the normal range, with a mean glomerular filtration rate by the

MDRD Equation 83 ± 16.7 mL/min/1.73m². One patient of group A withdrew her consent because of unrelated illness (she was diagnosed with fibromyalgia). Two patients of group B withdrew their consent because of unrelated illness (the one was diagnosed with breast cancer and the second with ankylosing spondylitis), and one patient because she had attributed biliary colic to the supplement. Furthermore, 2 patients from group A and 2 from group B did not attend the scheduled follow-up.

In group A, P1NP levels significantly decreased by 13.1% ($p < 0.001$) and CTX levels decreased by 11.4% ($p = 0.058$) within 3 months of supplementation while in group B, P1NP levels decreased by 2.1 % and CTX levels increased by 3.5% (both $p > 0.05$). Post-hoc power analysis indicated that our study had 64% and 53% power of demonstrating a decrease of 8 ng/ml and 50 pg/ml, for P1NP and CTX, respectively. The % decrease of P1NP was significantly lower in group A as compared with group B (-13.1 ± 12.3 % vs. -2.1 ± 12.6 %, $p = 0.011$), while % change of CTX tended to be lower, albeit not significantly, in group A as compared with group B (-11.4 ± 24 % vs. 3.5 ± 29.9 %, $p = 0.079$). Post-hoc power analysis indicated that our study had 81% power of demonstrating a between groups difference of $>10\%$ in % change from baseline for P1NP (Table 2).

In group A, all patients (100%) were compliant to the supplement. In group B, 17 patients (77%) were compliant, 2 (9%) partially compliant, while 3 (14%) were non-compliant ($p = 0.027$). There were no reported serious adverse events. Three patients (14%) reported minor adverse events (constipation, indigestion and biliary colic) in group B ($p = 0.083$). The first patient remained in group B with a change to a similar supplement, the second patient was allocated to group A because she declined to change her supplement to a similar one as she had the same experience in the past, and the patient who attributed the biliary colic to the initiation of the supplement withdrew her consent.

Discussion

As part of osteopenia prevention and treatment, the use of supplemental calcium and vitamin D therapy has been shown to suppress bone turnover, increase bone mass, and even decrease fracture incidence²⁰⁻²². Interestingly, even in young adults, calcium and vitamin D supplementation improves bone health²³. The favorable effects of calcium and vitamin D supplements on blood biomarkers have been demonstrated by several studies²⁴⁻²⁶. Our results were consistent with these studies. Rajatanavin et al found a significant decrease of P1NP by 22.7% and of CTX by 32.5% after 1 year of 500 mg calcium supplementation²⁴. Kruger et al presented a decrease of P1NP by 18% and of CTX by 28% after 3 months of fortified milk containing 900 mg calcium and 6.4 µg vitamin D²⁵ and Aloia et al found decrease of P1NP by 7.2% and of CTX by 6.3% at 15 weeks of supplementation with 1200 mg calcium and 100 µg vitamin D²⁶.

Despite the extensive research on calcium and vitamin D

supplementation, only a few clinical studies have evaluated the efficacy of CPs so far. In the randomized, double-blind placebo-controlled study, conducted by Elam et al, it was shown that after a 6-month administration of calcium, vitamin D and calcium-collagen chelate dietary supplement in osteopenic postmenopausal women, biomarkers of bone turnover were improved. Specifically, TRAP5b (tartrate-resistant acid phosphatase isoform-5b) and sclerostin were reduced and the bone specific alkaline phosphatase/TRAP5b ratio was increased⁷. Significant increase of BMD was also observed with smaller corresponding changes of bone biomarkers in their 3-month preliminary study, which is closer to the timeframe of the present study²⁷. Recently, another randomized, double-blinded, placebo-controlled study performed by König et al also yielded similar results⁶. This study evaluated the effects of CPs compared to placebo (receiving maltodextrin) on BMD and biomarkers after 1 year. The biomarkers used were P1NP and CTX showing a significant increase in P1NP by 11.6% in CPs group compared to placebo and a significant increase in CTX by 17.6% in placebo group compared to CPs group. However, unlike Elam's et al.^{7,27} and the present study, calcium or vitamin D was not provided in either group albeit encouraged according to patients' needs.

On the other hand, no significant effect of dietary supplementation with CPs on biochemical bone markers was proved by Cuneo et al over a period of 6 months²⁸. They conducted a randomized double-blind clinical assay in postmenopausal osteopenic women. A comparison of levels of bone markers (CTX, osteocalcin and bone specific alkaline phosphatase) between collagen hydrolysates and placebo group demonstrated no differences. However, the authors highlighted that the majority of the patients exhibited poor calcium intake and increased body weight, parameters that may have influenced their results²⁸. In this study collagen hydrolysates supplement was used without calcium and vitamin D supplementation, as in the study of König et al.⁶. This fact could be the reason why we cannot compare our study's results with these of the above studies.

In the present study, in comparisons within the groups, there was a statistically significant decrease of P1NP by 13.1% and a trend of decrease of CTX levels by 11.4% within 3 months of calcium, vitamin D and bioactive CPs supplementation, but there was no significant change of these bone biomarkers within 3 months of calcium and vitamin D without CPs supplementation in osteopenic postmenopausal women. In comparisons between the groups, the supplementation of calcium, vitamin D and bioactive CPs for 3 months had a statistically significant decrease of P1NP levels in comparison with the change observed with the supplementation of calcium, vitamin D without bioactive CPs. Respectively, the supplementation of calcium, vitamin D and bioactive CPs for 3 months showed a trend of decrease of CTX levels in comparison with the change observed with the supplementation of calcium, vitamin D without bioactive CPs. The decrease of P1NP and CTX after CPs supplementation was in line with previous experimental studies^{14,15} and may

reflect the reduction of the already increased bone turnover in postmenopausal women¹⁵. However, this study did not achieve to show any positive effect on bone biomarkers of calcium and vitamin D supplementation as similar clinical studies did²⁴⁻²⁶. Thus, we could conclude that the addition of CPs in a calcium and vitamin D supplement may enhance its already known positive effect on bone metabolism.

As already mentioned, CPs are products of collagen hydrolysis with high oral bioavailability^{4,5}. To better document the bioavailability of hydroxyprolyl-glycine (Hyp-Gly), Sugihara et al quantified the ratio of Hyp-Gly and prolyl-hydroxyproline (Pro-Hyp) in the peripheral blood after oral administration, which was found to be increased²⁹. The results were in accord with those of Shigemura et al, which showed that serum hydroxyproline peptide levels, especially hydroxyproline-glycine, increased in a dose-dependent manner and reached their peak after an hour of oral administration of collagen hydrolysates³.

The mechanism leading to the beneficial effects of CPs remain unclear. Kim et al observed that collagen hydrolysate enhanced osteoblastic differentiation in human cells via the expression of the COL1A1 gene and involved the ERK/MAPK signaling pathway¹⁵. Moreover, Liu J et al. used MC3T3-E1 pre-osteoblasts and concluded that bovine CPs increased osteoblast proliferation, and played valuable role in osteoblast differentiation and mineralization of the bone matrix³⁰. However, our results and previous experimental studies measuring P1NP as a biomarker of bone formation presented reduction of P1NP^{14,15} similarly with that observed with the use of calcium and vitamin D²⁴⁻²⁶.

A limitation of this study may be considered the small number of patients and thus of moderate power to detect significant effects. Nonetheless, to our knowledge there are only three published studies examining the effect of CPs supplementation on bone metabolism and this is the first study that shows positive effects on the two reference markers of bone turnover, CTX and P1NP³¹. Another limitation could be the fact that although the decrease of P1NP was statistically significant, it did not exceed the least significant change of 30-40% to prove a therapeutic effect²⁸. However, this change of P1NP with the use of calcium and vitamin D with or without CP supplements was expected³¹ and was in accordance with the relative clinical studies²⁴⁻²⁶. Furthermore, the different form of calcium provided in the two study groups may lead to the difference in the minor adverse events (14% vs 0%) with the calcium lactate being better tolerated than the calcium carbonate³². However, we decided to approach the everyday clinical practice and compare supplements that already exist in the market. A third limitation is related to the compliance of the patients. The supplement with CPs was provided by the investigators directly to the patients without additional cost, whereas the supplement of calcium, vitamin D without CPs was prescribed and then the patient had to purchase it from the pharmacy.

The present study shows the decrease of P1NP and CTX levels within 3 months of supplementation of calcium, vitamin D with bioactive CPs and no alteration

of bone markers after supplementation of calcium, vitamin D without CPs for the same period in osteopenic postmenopausal women. This finding may reflect the decrease of bone turnover with the use of calcium, vitamin D and CPs supplements and that the addition of CPs in a calcium and vitamin D supplement may enhance its already known positive effect on bone metabolism. Nevertheless, the elucidation of the appropriate dosage and the effects of the long-term treatment that is usually required in the setting of osteopenia and osteoporosis are of utmost importance and should be addressed in future studies.

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