Effect of supplementation of calcium and Vitamin D on bone mineral density and bone mineral content in peri- and post-menopause women
A double-blind, randomized, controlled trial
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Abstract

Background: Osteoporosis is a serious global health problem for the future, that is why improving diagnostic methods and prevention of this disease could be helpful. Objectives: To assess the effects of calcium supplementations combined with Vitamin D on bone mineral density (BMD) and bone mineral content (BMC) in a representative sample of peri- and post-menopausal women in a double-blind, randomized, controlled trial was undertaken. Design: A total of 120 women aged over 45 were included in a randomised placebo-controlled, double-blind trial on the effect of a daily dietary supplementation of calcium and Vitamin D on bone mineral density and bone mineral content, over a 30-month period. Methods: Dietary intake assessment; dual-energy X-ray absorptiometry to measure total body and segmental bone mineral density and bone mineral content at beginning of the study and every 15 months were undertaken. Results: There was no significant change in dietary calcium or Vitamin D intakes in either of the treatment groups during the 30-month intervention period. The change in total BMD in the calcium group was significantly different from that in the placebo group (P < 0.005). The placebo group lost a total BMD at a rate of about 0.4% per year. There was an inverse correlation between BMD and age. Conclusions: The effect of calcium and Vitamin D supplementation on bone mineral density of calcium has been demonstrated in this group of young adult women. Our results showed the positive effect of calcium and Vitamin D supplementation in women both peri- and post-menopausal status; for this reason a supplementation of calcium and Vitamin D should be recommended as a strategic option in helping to prevent early postmenopausal bone loss.

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Keywords: Calcium; Vitamin D; Bone mineral density; Bone mineral content; Peri-menopause; Post-menopause

1. Introduction

Osteoporosis is a disease affecting many millions of people around the world. It is characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to bone fragility and a consequent increase in risk of fracture [1,2].

Osteoporosis is a significant public health problem associated with increased mortality and morbidity, Wallace 2003 [3] shows that adequate calcium intake may help to enhance bone mass, thus decreasing the risk of osteoporotic fracture later in life. It is now widely accepted that prescribing appropriate calcium and Vitamin D intake is an important clinical strategy, whether or not other medications are also recommended.

One of the major problems to consider in the risk of osteoporosis is lactose malabsorption. However, if the subjects maintain a reasonably high calcium intake an adverse effect on bone mineral density may not occur [4].

Diet appears is linked to osteoporosis, and calcium and Vitamin D are both important, at least in the older population. A number of studies of Vitamin D and calcium supplementation, alone or combination, have shown positive effects on femoral bone mineral density (BMD) on the contrary others studies did not demonstrate this effect [5-12].

Shea et al. [4] summarize controlled trials examining the effect of calcium on bone density and fractures in postmenopausal women. In 15 trials (1806 patients) where post-menopausal women, assuming calcium supplementation or usual calcium intake in the diet, were studied; and
segmental and total bone mineral density were measured and followed patients for at least 1 year, they found calcium to be more effective than a placebo in reducing rates of bone loss after two or more years of treatment [4].

Calcium supplementation, aiming at a total calcium intake of at least 1500 mg/day, has a partial protective effect on postmenopausal bone loss, this effect being documented mainly in women more than 5 years after menopause [12].

It is now possible to measure bone mass with highly precise, safe and noninvasive technology. Dual energy X-ray absorptiometry (DXA) can detect bone loss well before it becomes evident by conventional X-rays or by fracture [13].

Because measurement of bone density is the single most important predictor of fracture risk, it is a critically important tool to apply to the population at risk, which includes women who have definable risk factors for osteoporosis, such as menopause, as well as those with a family history of osteoporosis, life-long low calcium intake, smoking, extremes in thinness, anorexia, certain diseases and medications.

Preventing bone loss associated with menopause and aging and maintaining bone mineral content provide important opportunities for the prevention of osteoporosis and fractures.

The aim of the study is to evaluate the effects calcium supplements combined with Vitamin D on bone mineral density (BMD) and bone mineral content (BMC) in a representative sample of Italian peri- and post-menopausal women (age 45–55 years) compared with a placebo in a double-blind, randomized, controlled trial.

2. Materials and methods

2.1. Subjects

A hundred and twenty women aged over 45 were included in a randomized placebo-controlled, double-blind trial on the effect of a daily dietary supplementation of calcium and Vitamin D on bone mineral density and bone mineral content.

A total of 120 Italian women (age range: 45–55 years) who had experienced perimenopause and recently post-menopause (6–36 months before study entry). Total of 120 women, 60 in the peri-menopausal (age range: 45–50 years); 60 in post-menopausal (age range: 50–55 years).

Women were excluded if their spine bone mineral density was more than 2S.D.s or below the normal peak bone mineral density or if they had a history of nontraumatic spine or hip fracture. Women with disorders of bone mineral metabolism were also excluded, as well as those with recent (within 1 year of study entry) major gastrointestinal disease (such as peptic ulcer, esophageal disease, and malabsorption. Other exclusion criteria were previous treatment with bisphosphonates or fluoride (>1 mg/day) or treatment within 12 months of enrolment with estrogen, progestin, calcitonin, glucocorticoids, anticonvulsant agents, phosphate-binding antacids, or excessive Vitamin A or Vitamin B.

Women who regularly used (>four times per week) any treatment that might cause gastrointestinal irritation (such as aspirin), women who smoked more than 20 cigarettes per day, or drank three or more alcoholic beverages per day were also excluded. All the subjects gave informed consent for the experimental procedures which had been approved by ethical committee of the University of Rome “Tor Vergata”.

2.2. Study design

The study was designed as a double-blind, controlled-trial comparing a calcium supplement with appropriate placebos. After a baseline study, at the Human Nutrition Unit in an outpatient department, volunteers were randomly assigned to the Oral Placebo Group or to the Oral Treatment Group (OPG or OTG) and they were studied over a 30-month period. The women had participated in various studies of body composition as volunteers, at the Human Nutrition Unit.

At baseline physical examination; dietary intake assessment; total body bone mineral density and bone mineral content were assessed. The same measurements were repeated at 15 months and at 30 months.

At each visit, vital signs were measured and any new worsening symptom was recorded. Physical examination was performed at the baseline, and by normal visits. Standard laboratory safety evaluation (including evaluation of haematological, renal, and liver function) was done at every visit. Investigators reported any unfavourable or unintended clinical or laboratory events as adverse experiences.

At each visit a diary of fracture was recorded, and food frequency questionnaires entered into the dietary database, and prescription and non-prescription treatments recorded.

2.3. Study setting

Fondazione Nova Salus Trasacco Aquila, University of Rome “Tor Vergata”, Human Physiology.

2.4. Treatment

Calcium and Vitamin D supplementation was given in one Tablet containing 500 mg calcium and 200 IU Vitamin D (Wyeth, Italia); or a placebo. Placebos were of the same shape, colour, and consistency as the active supplements.

Participants were randomly assigned to one of two regimens: Oral Placebo, Oral Treatment.

3. Methods

3.1. Dietary intake assessment

For the non-intervention studies, 7-day food diaries were assessed by registered dieticians using a food frequency questionnaire validated for the Italian population were assessed at beginning and end of the study [14].
Table 1
Characteristics of the subjects at the beginning of the follow-up study

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n = 120)</th>
<th>Placebo group (n = 60)</th>
<th>Treatment group (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.86 ± 3.80</td>
<td>49.9 ± 3.73</td>
<td>49.8 ± 3.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.22 ± 6.85</td>
<td>158.35 ± 7.69</td>
<td>158.08 ± 5.95</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.88 ± 14.00</td>
<td>73.11 ± 14.9</td>
<td>66.64 ± 12.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.92 ± 5.40</td>
<td>29.2 ± 5.5</td>
<td>26.6 ± 4.8*</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>28.2 ± 10.5</td>
<td>30.6 ± 11.2</td>
<td>25.7 ± 9.2*</td>
</tr>
<tr>
<td>Lean (kg)</td>
<td>39.2 ± 4.7</td>
<td>39.5 ± 5.2</td>
<td>38.9 ± 4.3</td>
</tr>
</tbody>
</table>

* P < 0.01 significantly different from placebo group.

3.2. Dual-energy X-ray absorptiometry measurement

Dual-energy X-ray absorptiometry (DXA) measurements were performed using a Lunar (Madison, Wisconsin, USA) DXA instrument (software version 3.6). Total body and segmental (spine, arms, legs, trunk) bone mineral density and bone mineral content was measured by dual energy X-ray absorptiometry (DXA), scans were performed, providing data on fat tissue (kg), lean tissue (kg), and bone mineral content (kg). The fat-free mass from DXA was calculated as the sum of lean tissue and bone mineral content. The body fat percentage from DXA was calculated as fat tissue/body weight. DXA measurements were performed with a total body scanner (Model DPK, Lunar, Madison, WI, software version 3.6) that uses a constant potential X-ray source at 12.5 kV and a K-edge filter to achieve a congruent beam of stable dual-energy content (40 and 70 kV). DXA measures an Rst value, which is theoretically related to soft tissue composition. The coefficient of variation for bone measurements is less than 1%; CVs on this instrument for five subjects scanned six times over a 9-months period were 2.2 for fat mass and 1.1 for fat free mass [15].

3.3. Statistics and analysis

Descriptive statistics for all variables were calculated. The two groups were compared at baseline to evaluate the success of the randomisation. The change in BMD was compared using ANOVA. The differences from baseline were used as the outcomes for this analysis, allowing for treatment and visit effects and their interactions. The data were also analysed using repeated measurements ANOVA, which accounted for the correlation over time within a subject. In this analysis, missing data were assumed to occur randomly.

4. Results

The characteristics of the 120 subjects at the beginning of the follow-up study are listed in Table 1. There were no significant differences in age, height, however body weight and fat were significantly different among the groups and BMI was significantly higher in the placebo group. The number of subjects who dropped out of the calcium group (eight) were higher than the placebo group but not in a significant way (six). Gastrointestinal symptoms, mainly constipation, caused six subjects to drop out. Nine subjects who failed to complete the 30-month period did so for time reasons.

There was no significant change in dietary calcium or Vitamin D intakes in any of the treated groups during the 30-month intervention period. During follow-up, however, mean daily dietary calcium and Vitamin D intake slightly increased but did not reach a statistically significant level. Physical activity scores did not differ between groups and during the follow-up study.

The changes in total BMD are given in Fig. 1, the change in total BMD in the treatment group was significantly different from that in the placebo group (P < 0.005). BMD of OP was 1.102; 1.098 and 1.098 g/cm² at baseline at 15 months and at 30 months respectively. BMD of OT was 1.101; 1.111 and 1.111 g/cm² at baseline at 15 months and at 30 months, respectively. There were significant differences between groups in BMD at 15 months and at 30 months (P < 0.005).

The placebo group lost total BMD at a rate of about 0.4% per year. There was an inverse correlation between BMD and age (r = −0.35); furthermore, BMD was significantly correlated with body weight and body mass index (r = 0.5 and 0.35, respectively) for both groups.

![Fig. 1. Bone mineral density measured by DXA in treatment and placebo groups during the study.](image-url)
5. Discussion

The findings of this study support the hypothesis that healthy women who are most likely to benefit from calcium supplements are those whose usual dietary calcium intake is low. Our results agree with those of other controlled studies [16,17]. Although dietary supplements contribute significantly to total calcium and Vitamin D intakes, use of these supplements is known to be sporadic.

The present study confirm improvements in BMD, observed in subjects who were given supplements with calcium and Vitamin D, in accord with data by Dawson-Hughes [18]. Although less effective than estrogen-progesterone-calcium, calcium augmentation alone significantly retards bone loss from the femoral neck and improves calcium balance in recently postmenopausal women. Dietary calcium augmentation should be recommended as a strategic option in helping to prevent early postmenopausal bone loss [19].

As previously described by Jensen et al. [20] in our study significant relationship between body weight and bone mineral density was observed. Jensen et al. showed that initially during a weight loss program, calcium supplementation seemed to protect against bone loss and whereas continued diet-induced weight loss was accompanied by generalized bone loss. Shapses et al. [21] studied the effect of calcium supplementation (1 g/day) on bone mass and remodelling during weight loss in obese pre-menopausal women. Our study agrees with the results of Shapses et al. that calcium supplementation slightly increases bone mineral density in obese pre-menopausal women. However, recently Shapses et al. [22] suggest that calcium supplementation did not significantly affect the amount of weight or fat lost by women counselled to follow a moderately restricted diet for 25 weeks [22].

Calcium reduced bone loss, secondary hyperparathyroidism, and bone turnover has already been described by Bertoli et al. [23]. Peacock also confirms that a calcium supplement of 750 mg/day prevents loss of BMD, reduces femoral medullary expansion, secondary hyperparathyroidism, and high bone turnover. A supplement of 15 μg/day 25OH Vitamin D3 is less effective, and because its effects are seen only when calcium intake is low suggesting its beneficial effect is to reverse calcium insufficiency [24]. To obtain adequate calcium intake throughout life it is auspicious to follow the “Dietary Reference Intakes” (DRIs) that are a reference value that can be used for planning and assessing diets for the healthy population and for many other purposes [25]. The DRIs encompass the Estimated Average Requirement (EAR), the Recommended Dietary Allowance (RDA), The Adequate Intake (AI), and the Tolerable Upper Intake Level (UL) [22]. When inadequate food calcium intake occurs, or in particular conditions, such as menopause or pregnancy, calcium supplement may be necessary.

The effect of calcium and Vitamin D supplements on bone mineral density of calcium has been demonstrated in this study. The difference observed between two groups are relevant in the clinical setting because they highlight the need to supply calcium and Vitamin D in peri- and post-menopausal women to prevent osteoporosis especially in women who do not reach the daily intake of calcium and Vitamin D. Furthermore, as concluded by Dawson-Hughes [18], it is recommended that women meet current calcium and Vitamin D intake requirements continuously. Physical activity is also recommended as indicated by Andreoli [26] who demonstrated the positive effects of different sports on bone density and muscle mass in highly trained athletes. Furthermore, as suggested by WHO/FAO Expert Consultation 2003 [1] on “diet nutrition and the prevention of chronic diseases” dietary and some lifestyle recommendations developed in respect of other chronic diseases may prove helpful in terms of reducing fracture risk such as increased physical activity; reduced sodium intake; increased consumption of fruits and vegetables; maintenance of a healthy body weight; avoidance of smoking; limitation of alcohol.

Finally, calcium and Vitamin D supplements may be useful in subjects with an inadequate diet as suggested by DRI and it is safe to take less than 2.500 mg/die of Calcium and 20 μg/die of Vitamin D [25].

References


