Nutritional Sciences 9(1), February, 2006, pp.42-47

Effects of Calcium and Vitamin D Supplementation on Bone Mineral Density and Biochemical Markers in Osteoporotic Postmenopausal Women*

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It has been reported that taking a proper amount of calcium and vitamin D helps to increase bone mineral density (BMD) and is effective in decreasing the risk of osteoporosis. This study investigated the supplementary effects of calcium and vitamin D on postmenopausal women who had osteoporosis and used calcium and vitamin D supplements. The study subjects consisted of osteoporotic postmenopausal women who were recruited from the Department of Orthopedics in a university-affiliated hospital. Sixty-seven study subjects were orally administrated 1,000 mg of calcium (calcium carbonate) and 2.5 mg of active vitamin D (1-α hydroxyvitamin D) (cholecalciferol 250 IU) twice a day for a year and a half. BMD and biochemical markers were evaluated and repeated every six months. One year after the intervention test, the bone mineral density of the lumbar spine was significantly increased as compared to the baseline. Six months after supplement administration, the level of serum alkaline phosphatase began to decrease, and afterwards a significant difference was maintained. Concentration of 1, 25-dihydroxy-vitamin D at 1.5 years was higher than that of the baseline. In comparison with that of the baseline, the level of urinary hydroxyproline in the study subjects over six months was significantly decreased. This study confirmed that effects such as BMD improvement and changes in biochemical markers appeared at least one year after administration of supplements.

Key words: Calcium, Vitamin D, Supplementation, Bone mineral density, Osteoporosis, Postmenopausal women

Received December 12, 2005; Revised January 15, 2006; Accepted January 25, 2006

INTRODUCTION

Osteoporosis is a disease of the skeleton in which the bones gradually lose mineral density and the fine structures of bone tissues are destroyed. The bones are hence gradually prone to fractures.[1)] It can cause serious health problems for potentially hundreds of millions people worldwide. It would, therefore, be very beneficial to investigate how to diagnose and prevent the disease. It has been reported that taking a proper amount of calcium and vitamin D helps to increase bone mineral density (BMD) and is effective in decreasing the risk of osteoporosis.[2)]

The results of studies demonstrating the effects of supplementation of calcium and vitamin D on femoral BMD have been controversial.[3,4)] Shea *et al.*[5)] investigated in their meta-analysis the effects of calcium supplementation on bone mineral density and fractures in postmenopausal women using controlled trials. A total of 1,806 subjects had taken 15 trials where diets containing calcium or vitamin D supplementation were given to postmenopausal women for a year. The results showed that the treated group was more effective than a placebo group relating to BMD.

Calcium supplementation resulted in a prophylactic effect whereby there was a decrease of BMD in postmenopausal women, especially in women at five years after menopause.[6)] An intervention study with elderly people showed that the supplementation of calcium only or in combination with vitamin D resulted in the prevention of BMD reductions as well as of fractures, the curing of secondary hyperparathyroidism, and the acceleration of bone turnover.

This study aims to find out the effects on bone metabolism including BMD of the lumbar spine when calcium and vitamin D supplements are administrated to postmenopausal women for one and half years.

* This work was supported by the Korea Research Foundation Grant funded by the Korean Government (R04-2003-000-10088-0).
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MATERIALS AND METHODS

1. Study Subjects

The study subjects were recruited from the Department of Orthopedics at a university-affiliated hospital. They consisted of postmenopausal women who had confirmed diagnoses of osteoporosis prior to this study. The subjects were limited to those who were not provided with medicines or supplements for treating bone metabolic diseases prior to the clinical intervention study. In addition, those treated with any medicine, either beneficial or harmful on bone turnover, were excluded.

All of the study subjects agreed to participate in this clinical intervention study pursuant to the recommendations of their doctors. The general procedure of this study was approved by the University Ethics Committee.

Sixty-seven study subjects were orally administrated 1,000 mg of calcium (calcium carbonate) and 2.5 mg of active vitamin D (1-α hydroxyvitamin D) (cholecalciferol 250 IU) twice a day (morning and evening) for one and half years from March 2004. Before oral administration and every six months thereafter, the onset bone mineral density was assessed, and blood and urine relating to bone metabolism were collected and analyzed. The differences between the baseline and intervention periods were determined accordingly.

Estimation of assistance and compliance of the subjects were performed by researchers and clinical staffs every other month. The subjects' compliance scores were recorded when they visited the medical center every other month. Whenever the subjects came to the medical center to take blood and urine tests and to have their BMD assessed, we double-checked any by-effect caused by calcium and vitamin D supplements.

2. Dietary Assessment

Measurement of diet consumption was examined twice before (baseline) and right after the complete intervention study using a food frequency questionnaire. The reliability and validity of food and nutrient intake evaluations drawn from the food frequency questionnaire has been published in precedent papers.[7,8] The average daily nutrient intake of each subject was obtained by summing up all the intakes of 100 food items after calculating each value in consideration of food intakes by item and nutrient contents per 100 g of food. This value was converted into a daily nutrient intake on the basis of food intake frequency (9 categories: never or rare, once a month, twice or three times a month, once or twice a week, three or four times a week, five or six times a week, once a day, twice a day, three times a day) and the portion of the food (three categories: small, medium, large) presented in the food intake frequency questionnaire. In this intervention, we evaluated the per capita daily nutrient intake of calories, protein, fat, carbohydrates, calcium, phosphorus, iron, potassium, zinc, vitamins A, B_1, B_2, niacin, vitamin C, B_6, E, folate, and cholesterol.

3. Analysis of Biochemical Markers Relating to Bone Metabolism

BMD was assessed at lumbar spine (L2-L4) with Lunar instrument (Madison, Wisconsin, USA) using Dual-energy X-ray absorptiometry. Only one technician measured the BMD of all participants using identical equipment throughout the study. T-score was calculated by the following equation: (subject's BMD - young adult's BMD) / standard deviation of young adult's BMD.

The biochemical analyses were performed by the university -affiliated hospital reference laboratory. Blood and urine from the subjects were collected after overnight fasting. Serum samples were obtained by centrifugation and stored at -20 ℃. Urine samples were collected from a second voiding at the same time as serum extraction and stored at -20 ℃. They were coded in such a way that those performing the assay did not know which groups were being assayed. Serum and urine biochemical markers were analyzed using automatic analyzer (Hitachi 7020, Japan). Serum 25-hydroxy-vitamin D, 1,25 $(OH)_2D$, serum alkaline phosphatase (ALP) isoenzyme, and urinary hydorxyproline were determined by radioimmunoassay using kits (Boehringer Mannheim Co., Germany).

4. Statistical Analysis

Using the SPSS software package, we confirmed whether independent sampling, homogeneity of diversity and normal distribution were satisfied for model assumption. Data were analyzed using paired *t*-test when they showed normal distribution while data showing non-normal distribution were analyzed using Mann-Whitney rank sum test. Diet consumption of the subjects who completed the study for eighteen months and biochemical analytic values were compared and analyzed between the baseline and the intervention period. Data were expressed as mean±standard deviation with $p<0.05$ accepted as representing statistical significance

RESULTS

1. Study Subjects

At the beginning of the study, a total of 67 postmenopausal

women with osteoporosis participated. Six months later, there were 64 subjects; one year later, 62 subjects; and at the end of the study (one and half years later) there were 58 study subjects. The chronological age, age at menarche, age at menopause, years since menopause, age at the 1st full-term pregnancy, and age at the last delivery of the participants was 63.4±6.8 (mean±standard deviation), 16.8±2.1, 48.9±3.2, 14.7±8.0, 23.9±4.8, and 36.5±5.1 years old, respectively.

2. Nutrient Intakes

The nutrient intake estimation was performed twice, and the result is shown in Table 1. There were statistical differences at $p<0.05$ in carbohydrate, phosphorus, potassium and niacin between baseline and intervention period. The mean of daily dietary calcium intakes was less than 450 mg, which was pretty low when compared to dietary reference intakes.[9]

Table 1. Nutrient intakes of the study subjects at the baseline and 18-month intervention period (n=58)

Variables	Baseline	18-month intervention
Energy (kcal)	1645.17±489.09[1)]	1616.47±476.50
Protein (g)	63.05± 25.23	62.45± 24.38
Fat (g)	39.53± 13.50	36.43± 14.51
Carbohydrate (g)	263.38± 68.12	262.17± 64.59*
Ca (mg)	439.43±289.08	442.43±279.01
P (mg)	880.90±301.24	930.49±312.45*
Fe	11.16± 5.26	11.78± 4.98
K (mg)	2166.71±980.67	2396.69±964.32*
Vit. A (RE)	575.76±468.10	523.85±465.13
Vit. B_1 (mg)	1.02± 0.57	0.92± 0.49
Vit. B_2 (mg)	0.93± 0.68	0.95± 0.59
Niacin (mg)	14.79± 6.03	17.11± 5.98*
Vit. C (mg)	68.73± 51.27	64.58± 50.29
Zn (mg)	7.81± 3.12	8.28± 2.90
Vit. B_6 (mg)	20.09± 9.04	21.62± 8.39
Folate (mg)	206.83±176.80	204.52±169.45
Vit. E (mg)	11.63± 9.12	11.32± 8.86
Cholesterol (mg)	251.57±149.01	278.71± 137.8

1) mean±SD
* statistically different at $p<0.05$

3. Assessment of Bone Mineral Bensity

One year after the intervention test, bone mineral density of the lumbar spine was significantly increased compared to the baseline (Fig. 1). The mean T-scores of BMD were -2.54 and -2.04 at 1 year and 1.5 year, respectively, compared to -2.88 at the baseline, which were statistically different either at $p<0.05$ (1 year) or at $p<0.01$ (1.5 year).

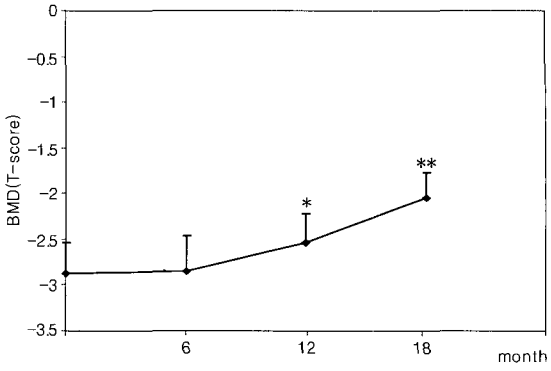

Fig. 1 Changes in BMD (T-score)
Values are mean±SD.
* ($p<0.05$) and ** ($p<0.01$) indicate a difference between the baseline and intervention period.

4. Analysis of Biochemical Markers

Concentration of serum 25-hydroxy-vitamin D during one and half years of supplement administration was not significantly different between baseline and intervention period (Fig. 2). However, concentration of 1, 25-dihydroxy-vitamin D (107 nmol/L) at 1.5 years was higher than that of the baseline (101 nmol/L) (Fig. 3).

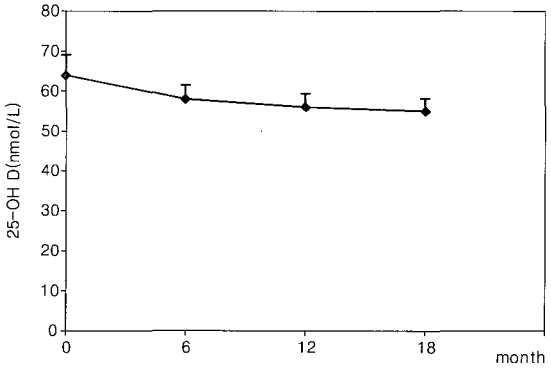

Fig. 2 Changes in serum 25-OH D (nmol/L)
Values are mean±SD

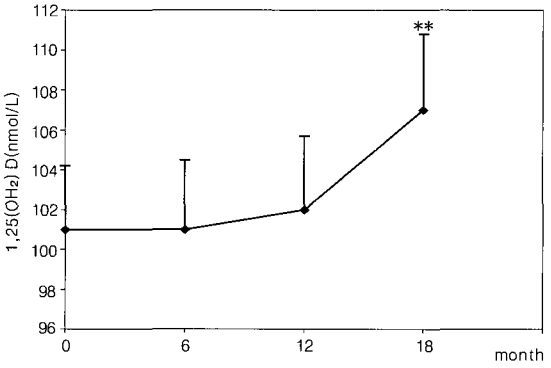

Fig. 3 Changes in 1,25 $(OH)_2$ D (nmol/L)
Values are mean±SD.
** ($p<0.01$) indicates a difference between the baseline and intervention period

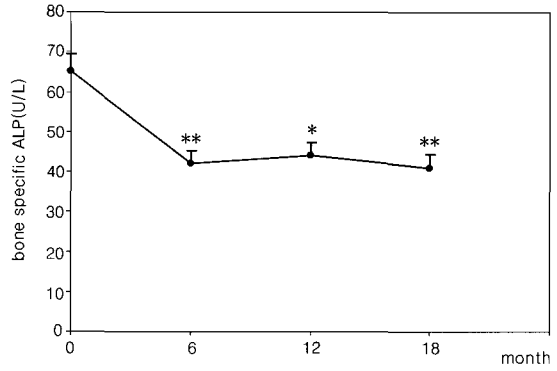

Fig. 4 Changes in bone-specific ALP (U/L)
Values are mean±SD.
* ($p<0.05$) and ** ($p<0.01$) indicate a difference between the baseline and intervention period.

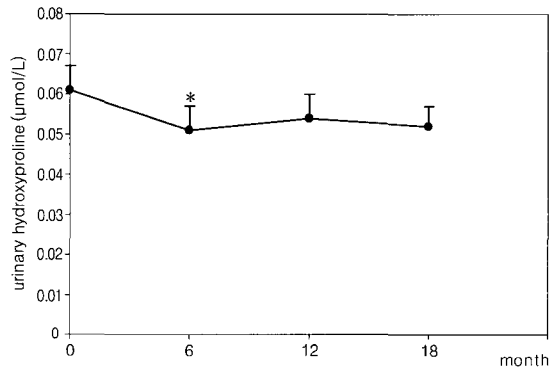

Fig. 5 Changes in urinary hydroxyproline (μmol/L).
Values are mean±SD.
* ($p < 0.05$) indicates a difference between the baseline and intervention period.

Six months after the supplement administration, the level of serum alkaline phosphatase began to decrease, and a significant difference had maintained (Fig. 4). The values resulted in 42 (35% decrease), 44 (32% decrease), and 41 nmol/L (37% decrease) at 6 months, 1 year and 1.5 years compared to 65 at the baseline.

In comparison with that of the baseline (0.061 μmol/L), the level of urinary hydroxyproline in the study subjects over six months (0.051 μmol/L) was significantly decreased; however, the levels of the subjects at one year and over one-and-half years were not significantly different from that of the baseline (Fig. 5).

DISCUSSION

The effects of calcium and/or vitamin D supplementation on bone mineral density may turn out to have different results depending on the characteristics of the study subjects (ages, health status, onset of menopause, presence /absence of fracture or osteoporosis, body mass index, and so on), dose and type of supplementation, duration of study, type of bone biochemical markers, etc. The findings of this study support the hypothesis that postmenopausal women whose usual dietary calcium intake is low are more likely to benefit from calcium and vitamin D supplements as shown in other controlled studies.[10,11] The present study confirms improvements in BMD, observed in subjects who were given supplements with calcium and vitamin D, in accordance with a three-year randomized, placebo controlled trial by Dawson-Hughes.[12] 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.[10]

Previous interventional studies showed that continuous supplementation with either calcium alone or in combination with vitamin D_3 prevents bone loss[13,14] or osteoporotic fractures.[15,16] Also, Christiansen *et al.*[17] suggested supplementation with low-dose vitamin D has only limited effects on BMD in postmenopausal vitamin D-replete women. In contrast, elderly subjects with vitamin D deficiency and secondary hyperparathyroidism usually experience significant gains in bone mass and substantial reductions in fracture risk during and after supplementation with vitamin D. In one study[18] with 55 healthy subjects for a two-year intervention supplemented with 500 mg calcium and 500 IU vitamin D, the mean semiannual increase in BMD after supplementation with calcium and vitamin D_3 was 0.8% at the lumbar spine and 0.1% at the femoral neck.

In a prospective randomized trial showing the effects of calcitonin on acute bone loss after pertrochanteric fractures among 55 women aged between 70 and 80 years,[19] bone alkaline phosphatase was found to be significantly higher in the calcitonin-treated group on the 15th post-operative day and remained high throughout the three-month observation period. Values for total bone alkaline phosphatase were also significantly increased in the calcitonin-treated group at three months. Urinary hydroxyproline values were significantly lower in the calcitonin-treated group at both the 15th and 45th days after surgery.

In addition, in a study of elderly postmenopausal women,[20] treatment for four years with 0.5 μg calcitriol increased BMD at the femoral neck and the lumbar spine and enhanced both the intestinal absorption and the urinary excretion of calcium, and decreased PTH secretion. Also, the serum bone-specific alkaline phosphatase was decreased after one year compared to control, although there was

no difference in the urinary hydroxyproline level.

In a three-year investigation, treatment with supplemental 0.25 μg calcitriol and 1 g calcium reduced the rate of new vertebral fractures,[21] but no study is available on whether hip fractures could also be prevented by the treatment. Similarly, in another study, the most bone gain at the lumbar spine occurred during the first three months of the treatment with 0.75 μg 1-α $(OH)D_3$ and 150 mg calcium among fifty Japanese postmenopausal women.[22] These findings can be explained by the phenomenon known as the "bone remodeling transient."[23] In healthy adults, the transient lasts about nine months, which is the time needed to reach a new equilibrium state. If the balance between bone formation and resorption within one remodeling unit is not appreciably changed by the treatment used, no further gain in bone mass occurs after the transient and bone loss may begin again.

In terms of calcium supplementation, calcium-reduced bone loss, secondary hyperparathyroidism, and bone turnover have already been described by Bertoli *et al.*[24] Peacock *et al.*[25] also confirmed 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 25 OH vitamin D_3 is less effective, and because its effects are seen only when calcium intake is low suggests that its beneficial effect is to reverse calcium insufficiency.

The effect of calcium and vitamin D supplements on bone mineral density of calcium has been demonstrated in this study. As concluded by Dawson-Hughes,[12] it is recommended that postmenopausal women with osteoporosis meet current calcium and vitamin D intake requirements continuously, with dietary reference intakes for instance (DRI).[9]

According to the criteria given by WHO, the principal entry criterion of osteoporotic patients should be a bone density (T-score) of not more than -2.5 SD.[26] In this study, with a one year supplementation of calcium and vitamin D, osteoporotic postmenopausal women were able to overcome T-scores of BMD of over almost -2.5 SD. In conclusion, calcium and vitamin D supplements for at least one year may be useful in postmenopausal women with osteoporosis taking inadequate calcium intakes.

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