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Effect of continuous combined therapy with vitamin K_2 and vitamin D_3 on bone mineral density and coagulofibrinolysis function in postmenopausal women

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Abstract

Objectives: To investigate the therapeutic effect of combined use of vitamin K_2 and D_3 on vertebral bone mineral density in postmenopausal women with osteopenia and osteoporosis. *Subjects and methods:* We enrolled 172 women with vertebral bone mineral density < 0.98 g/cm^2 (osteopenia and osteoporosis) as measured by dual-energy X-ray absorptiometry. In this study, we employed the criteria for diagnosis of osteopenia and osteoporosis using dual energy X-ray absorptiometry proposed by the Japan Society of Bone Metabolism in 1996. Subjects were randomized into four groups (each having 43 subjects in vitamin K_2 therapy group, vitamin D_3 therapy group, vitamin K_2 and D_3 combined therapy group, or a control group receiving dietary therapy alone) and treated with respective agents for 2 years, with bone mineral density was measured prior to therapy and after 6, 12, 18, and 24 months of treatment. The bone metabolism markers analyzed were serum type 1 collagen carboxyterminal propeptide (P1CP), serum intact osteocalcin, and urinary pyridinoline. Tests of blood coagulation function consisted of measurement of activated partial thromboplastin time (APTT) and analysis of concentrations of antithrombin III (AT III), fibrinogen, and plasminogen. *Results:* Combined therapy with vitamin K_2 and D_3 for 24 months markedly increased bone mineral density ($4.92 \pm 7.89\%$), while vitamin K_2 alone increased it only $0.135 \pm 5.44\%$. The bone markers measured, revealed stimulation of both bone formation and resorption activity. We observed an increase in coagulation and fibrinolytic activity that was within the normal range, suggesting that balance was maintained in the fibrinolysis–coagulation system. *Conclusions:* Continuous combination therapy with vitamin K_2 and D_3 may be useful for increasing vertebral bone mass in postmenopausal women. Furthermore, the increase in coagulation function observed during this therapy was within the physiological range, and no adverse reactions were observed.

Keywords: Vitamin K_2; Vitamin D_3; Bone mineral density; Postmenopause; Coagulofibrinolysis

1. Introduction

Postmenopausal women experience accelerated bone loss, which leads to osteoporosis and in-

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creased risk of development of fractures in the spine (vertebral crush fractures), forearm (Colles' fractures) and hip. Osteoporotic fractures of the spine and forearm are associated with significant morbidity, but the most serious consequences of bone loss arise in patients with hip fracture, which is associated with a significant increase in mortality (15–20%), particularly in the elderly [1]. Several approaches are currently available to prevent and treat osteoporosis, including dietary modifications and drugs which inhibit bone resorption. There are now a variety of treatment approaches available for the management of osteoporosis. Inhibitors of bone resorption, including calcium, the vitamin Ds, ipriflavone, bisphosphonates, calcitonins and gonadal steroids have been variously shown to prevent bone loss or to reduce fractures [2–4]. The effect of vitamin supplements on bone metabolism indices in patients with osteopenia and osteoporosis has received scant attention in the literature. However, some authors have reported that vitamin D and K may prevent bone loss and fractures [5–11]. There is evidence that calcium is important not only for peak bone mass development but also in reducing bone loss in postmenoapusal women. It has been believed that Vitamin D and calcium (and possibly vitamin K) are vital to prevention of bone loss and fracture. Matsunaga found a synergistic effect of vitamin D and K in reducing bone loss in ovariectomized rats [10]. Furthermore, it was observed that efficacy of vitamin K was stronger in high bone resorption and high plasma vitamin D level [12]. Thus, in conditions of high turnover in bone metabolism such as early postmenopausal period, it is conceivable that the clinical effect of vitamin K may increase when the plasma vitamin D level obtained by vitamin D administration is sufficient.

The causes of osteoporosis have not yet been fully elucidated, but recently published reports suggest a possible involvement of vitamin K deficiency [13–15]. It has been suggested that γ-carboxyglutamic acid (Gla)-containing protein (osteocalcin, BGP), a bone matrix protein, plays a key role in bone metabolism [16]. The glutamic acid residues of osteocalcin are γ-carboxylated by the enzyme carboxylase, and vitamin K is essential to this process. Vitamin K_2, the human-activated form of vitamin K, is said to promote the healing of bone fractures, to have a therapeutic effect on osteoporosis, and to inhibit bone resorption [17,18]. It may also play a role in bone metabolism other than the γ-carboxylation of osteocalcin. The addition of vitamin K_2 and activated vitamin D_3 to a system of cultured osteoblasts has been shown to promote calcification [19]. In Japan, the incidence of fractures in the eastern part of the country is lower than that in the west, a phenomenon that may be associated with differences in food culture between the two regions, specifically, in the consumption of natto (fermented soybeans). Natto is rich in the MK-7 (menaquinone-7) form of vitamin K_2, which has the same effect as the MK-4 form of vitamin K_2, one of the medications currently used to treat osteoporosis. Estrogen is an agent of choice for both prevention and treatment of postmenopausal osteoporosis; however, once estrogen treatment is discontinued, bone mass density drop fairly quickly, and relatively high incidence of side effects have been observed [6]. Long-term adherence to hormone replacement therapy is considered to be low compliance. Effective alternatives for prevention of bone loss in recently postmenopausal women include vitamin K and vitamin D. Although, single therapies with these are effective in inhibiting bone loss, prevention of bone loss in postmenopausal women is more difficult than hormone replacement therapy [7,20,21], and the clinical results of combined therapy have not been fully examined [9,22,23].

In this communication, we present the results of our study of the clinical effects of combined use of vitamin K_2 and D_3 on postmenopausal osteopenia and osteoporosis, and of its effects on bone metabolism and the coagulofibrinolysis system.

2. Subjects and methods

We enrolled 172 women with a bone mineral density of the lumbar spine < 0.98 g/cm^2, as measured by dual-energy X-ray absorptiometry (DEXA, Lunar DPX-L), in the present randomized study. All subjects were attending the De-

partment of Obstetrics and Gynecology at Osaka Medical College for outpatient consultations related to climacteric/menopausal conditions. We randomly assigned them to one of the following four groups by month of birth (January–March, April–June, July–September, October–December) after sufficient informed consent: vitamin K_2 therapy group (43 subjects; menaquinone-4: Glakay 45 mg per day), vitamin D_3 therapy group (43 subjects; 1-a hydroxycholecalciferol: Onealfa 1 μg per day), vitamin K_2 and D_3 combined therapy group (43 subjects), or a control group receiving dietary therapy alone (43 subjects). This study started with 172 subjects, with 43 patients in each of the four groups, while 12, 26, 38 and 46 patients dropped out within 6, 12, 18 and 24 months of treatment, respectively, (overall dropout rate 26.7%). We enrolled 126 patients who have finished this study of 24 months and analyzed their data. Table 1 shows the characteristics of 126 patients at the start of the study. The profiles for age, postmenopausal duration, percentage of patients within 3 years after menopause, serum calcium, serum gonadotropin and estradiol values, and physique did not differ significantly among the four groups. There were no significant differences on one-way factorial analysis of variance (ANOVA) in basal levels of bone mineral density among the four groups.

The patients were not given specific instructions regarding adequate dietary calcium intake and did not take part in a program of exercise. However, use of daily products including milk in meals was instructed when questions about calcium intake were asked. Patients in each group were treated for 2 years, during which periodic measurements of bone mineral density and evaluations of bone metabolism markers and coagulofibrinolysis function were performed. Vertebral bone mineral density was measured at baseline and after 6, 12, 18

Table 1
Initial values for the subjects

Groups	Vitamin K_2	Vitamin D_3	Combination	Controls	Significant differences
Number of patients	30	32	31	33	
Age (S.D.) years	54.1(7.4)	52.8 (5.6)	53.3 (4.5)	53.5 (6.0)	n.s.
Number of years (S.D.) from menopause	2.59 (4.8)	3.02 (2.0)	2.41 (3.5)	3.60 (3.8)	n.s.
Age within 3 years of menopause(%)	11/30 (36.7%)	10/32 (31.3%)	13/31 (41.9%)	12/33 (36.4%)	n.s.
Mean (S.D.) body mass index[a]	22.2 (2.6)	22.7 (3.4)	21.7 (2.9)	22.9 (3.5)	n.s.
Mean (S.D.) percent fat[b]	19.6 (2.9)	20.1 (2.4)	19.9 (2.3)	20.9 (3.2)	n.s.
Mean (S.D.) serum calcium level (mg/dl)	9.7 (0.99)	10.4 (0.92)	10.7 (0.94)	9.9 (0.96)	n.s.
Mean (S.D.) serum phosphate level (mg/dl)	4.07 (0.22)	3.96 (0.22)	4.15 (0.28)	4.11 (0.25)	n.s.
Mean (S.D.) serum creatinine level (mg/dl)	0.98 (0.053)	0.93 (0.049)	1.01 (0.049)	1.05 (0.047)	n.s.
Mean (S.D.) plasma hormone levels					
FSH (mIU/ml)	63.8 (21.4)	56.9 (30.2)	65.1 (29.0)	59.7 (24.4)	n.s.
LH (mIU/ml)	30.1 (15.3)	28.5 (12.8)	29.1 (17.3)	24.2 (12.1)	n.s.
Estradiol (pg/ml)	10.5 (5.9)	13.1 (6.3)	10.7 (6.2)	11.3 (7.8)	n.s.

[a] Body mass index expressed as weight (kg)/height $(m)^2$.
[b] Percent fat was measured by DPH (Lunar Hadiation, Madison, WI, USA).
FSH and LH were greater than 30 and 15 IU/ml, respectively, in all women. Figures in parenthesis indicate 1 standard deviation. *P*-value assessed using ANOVA. No significant differences were found in any of the parameters among the groups.

and 24 months of therapy by dual energy X-ray absorptiometry (model DPX, Lunar Radiation Corp, Madison, WI, USA) which uses a constant potential X-ray source at 12.5 fJ and a K-edge filter (cecium) to achieve a congruent beam of stable dual energy radiation. The effective energies were 6.4 and 11.2 fJ. Bone mineral density was assessed for the L2-4 region and expressed as the average areal density (BMD = g/cm^2). We defined osteopenia as a BMD value below 0.98 g/cm^2 and osteoporosis as a BMD below 0.83 g/cm^2. In this study, we employed the criteria for diagnosis of osteopenia and osteoporosis using dual energy X-ray absorptiometry which was proposed by the Japan Society of Bone Metabolism in 1996 [24]. The in vivo and in vitro coefficients of variation were 0.73 and 0.41%, respectively. The bone metabolism markers analyzed were serum type 1 collagen carboxyterminal propeptide (P1CP), serum intact osteocalcin, and urinary pyridinoline. Serum type 1 collagen carboxyterminal propeptide (P1CP) was measured by radioimmunoassay using a kit from Orion Diagnostica (Espoo, Finland). Serum intact osteocalcin concentrations were measured by radioimmuassay using a kit from Yamasa Corp (Chiba, Japan). Urinary excretions of pyridinoline were measured by HPLC (Sumitomo Metal Bioscience Lab.) according to the methods of Eyre et al. [25], and values were corrected using the urinary excretion of creatinine. To confirm effects of vitamin K, we monitored the time course of changes in urinary concentration of γ-carboxyglutamate (Gla)/Cr. The urinary concentration of γ-carboxyglutamate was measured by HPLC (Sumitomo Metal Bioscience Lab) according to the methods of Kuwada and Katayama [26]and values were corrected using the urinary excretion of creatinine. Tests of blood coagulation function consisted of measurement of activated partial thromboplastin time (APTT) and analysis of concentrations of antithrombin III (AT III), fibrinogen, and plasminogen. These measurements were performed using standard laboratory methods.

Data were statistically analyzed by ANOVA or the Wilcoxon signed-rank test, and the level of significance was set at $P < 0.05$. Results are presented as mean and standard deviation (mean ± S.D.).

3. Results

There were no significant differences on one-way factorial ANOVA in basal levels of bone mineral density among the four groups consisting of the 172 patients who started the study ($P = 0.23$) and the 126 patients who continued this clinical study for 2 years ($P = 0.057$). However, there were significant differences in this parameter between the combined group and vitamin K_2 ($P = 0.0101$) and vitamin D_3 ($P = 0.028$) groups for the latter 126 patients on multiple comparison by ANOVA. Since 26.7% of the patients dropped out over 2 years, although the present study was begun with 172 subjects and dropouts include patients with relatively high BMD in the combined therapy group, basal level of BMD was significantly lower than those in the vitamin K_2 or D_3 alone therapy group in the evaluation including only those subjects who completed the 2-year study. The following appear to be biased data given the above considerations. In the vitamin K_2 alone group, mean bone mineral density remained lower than the baseline level (0.876 ± 0.091 g/cm^2) up to 18 months after the start of treatment, but was slightly higher (not significant) at 24 months (0.888 ± 0.112 g/cm^2). In contrast in the vitamin K_2 and D_3 combined therapy group bone mineral density was 0.052 g/cm^2 higher than the baseline level (0.820 ± 0.097 g/cm^2) at 6 months ($P < 0.001$, significant), and higher BMD levels were sustained up to 24 months (Table 2). The time course of percentage changes in BMD in the 126 patients who continued this study for 24 months is shown in Fig. 1. There were no significant differences from control for the subjects receiving vitamin K_2 alone therapy at 6 months ($1.31 \pm 6.94\%$) or 12 months ($0.736 \pm 6.09\%$), whereas the percentage change in BMD was significantly higher than control at 18 months ($0.278 \pm 6.55\%$, $P < 0.05$) and 24 months ($0.135 \pm 5.44\%$, $P < 0.05$). On the other hand, the percentage increases in BMD in the vitamin K_2 and D_3 combined therapy group were significantly higher ($4.10 \pm 5.88\%$ at 6 months; $P < 0.001$, $5.86 \pm 6.85\%$ at 12 months; $P < 0.001$, $5.01 \pm 8.11\%$ at 18 months; $P < 0.001$, and $4.92 \pm 7.89\%$ at 24 months; $P < 0.001$) than those in the control group. Compared

Table 2
Vertebral bone mass before and during treatment

	N	0 Month	6 Months	12 Months	18 Months	24 Months
Vitamin K	30	0.876 (0.091)	0.880 (0.084)	0.857 (0.071)	0.853 (0.053)	0.888 (0.112)
Vitamin D	32	0.872 (0.102)	0.873 (0.099)	0.876 (0.076)	0.874 (0.071)	0.877 (0.094)
Combination	31	0.820 (0.097)	0.872 (0.101)**	0.865 (0.106)**	0.863 (0.119)	0.845 (0.120)*
Controls	33	0.864 (0.089)	0.857 (0.089)	0.853 (0.101)	0.841 (0.088)**	0.829 (0.089)**

*$P<0.05$; **$P<0.001$, significance was determined using Wilcoxon's signed-rank test, and P-values refer to differences in bone mass levels in the treated groups compared with their levels at the start of the study. Mean (S.D.) values are expressed as g/cm^2.

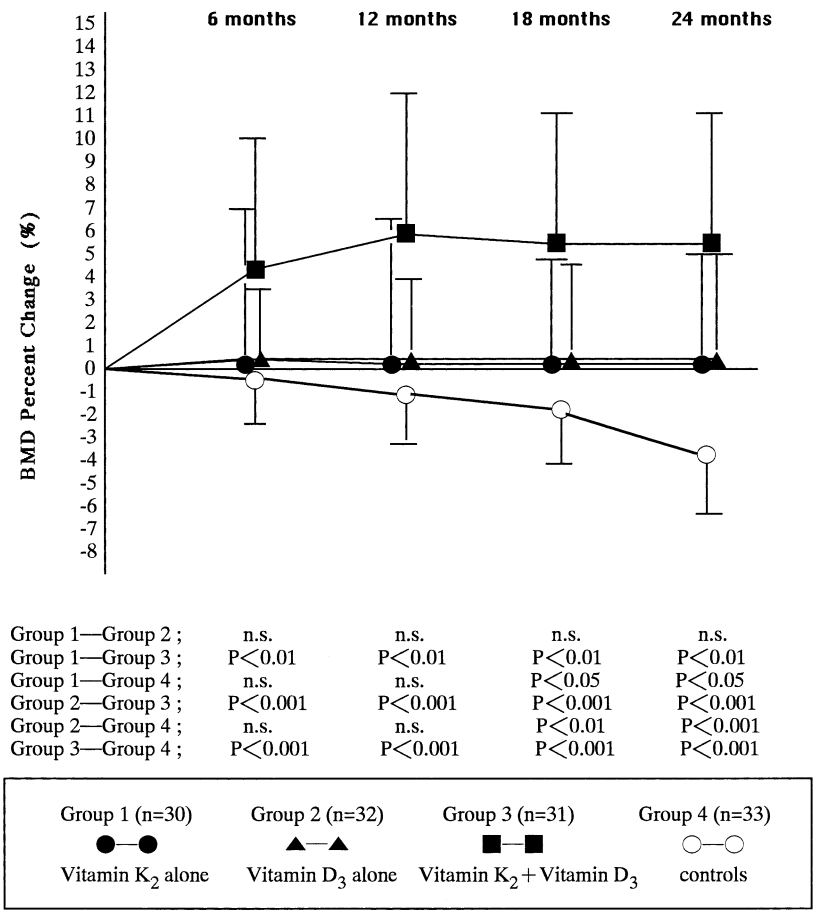

Fig. 1. Percentage changes (mean ± S.D.) from baseline in bone mineral density in all four groups during the 24-month study. P-value assessed using Wilcoxon signed-rank test.

with the vitamin K_2 or D_3 alone therapy group, BMD was significantly increased in the combined therapy group from 6 to 24 months after the start of treatment (vitamin D_3—combined therapy; $P<0.001$, vitamin K_2—combined therapy; $P<0.01$). Furthermore, it was found that there were more responders to treat in the combined therapy group than in the vitamin K_2 and D_3 alone group.

In the 24-month treatment, BMD increase of 5% or higher was exhibited by 9.4 and 23.3% of the subjects in the vitamin K_2 and D_3 alone groups, respectively, but it was exhibited by 45.2% of the subjects in the combined therapy group (significantly higher than in the vitamin D_3 alone group, $P = 0.014$). In the combined therapy group, moreover, 67.8% of responders had BMD increase of 2% or higher. Although non-responders with BMD decrease up to 22.6% were found, the percentage of such patients was significantly lower than that in the vitamin D_3 alone group (71.9%) ($P = 0.025$) (Table 3).

There were no significant differences among any of the groups in any of the background parameters, bone parameters or coagulofibrinolysis parameters (Table 4). Measurement of urinary γ-carboxyglutamate (Gla)/Cr concentration, which was used to confirm the absorption and physiological activity of vitamin K_2, revealed that the concentration increased significantly with treatment in both the vitamin K_2 alone therapy group (6 M; $53.0 \pm 38.2\%$, 18 M; $82.5 \pm 56.3\%$, 24 M; $56.8 \pm 38.8\%$, $P < 0.01$) and the vitamin K_2 and D_3 combined therapy group (6 M; $43.9 \pm 43.6\%$, 12 M; $44.9 \pm 72.1\%$, 18 M; $49.9 \pm 49.5\%$, $P < 0.01$). Serum intact osteocalcin and urinary pyridinoline levels tended to increase with duration of treatment (12 and 18 months) in the combined therapy group. Serum intact osteocalcin level increased significantly by $36.0 \pm 44.8\%$ at 18 months ($P < 0.05$) in the combined therapy group. Urinary pyridinoline level was significantly increased at 18 months ($89.6 \pm 112.3\%$, $P < 0.01$)

Table 3
Percentages of responders and non-responders to 24-months treatment

	Vitamin K_2	Vitamin D_3	Combination	Significant differences
Increase in BMD				
5% and more	23.3% (7/30)	9.4% (3/32)	45.2% (14/31)	D3-Comb.: $P = 0.014$, others: n.s.
2–5%	23.3% (7/30)	6.3% (2/32)	22.6% (7/31)	n.s.
0–2%	10.0% (3/30)	2.5% (4/32)	9.7%(3/31)	n.s.
Decrease in BMD				
0–2%	23.3% (7/30)	34.4% (11/32)	6.5% (2/31)	D3-Comb.: $P = 0.025$, others: n.s.
2% and more	20.0% (6/30)	37.5% (12/32)	16.1% (5/31)	n.s.

Table 4
Baseline values of bone metabolism and coagulofibrinolysis parameters

Groups	Vitamin K_2	Vitamin D_3	Combination	Controls	Significance of differences
Mean (S.D.) intact osteocalcin (ng/ml)	6.5 (2.8)	5.9 (3.1)	5.8 (2.2)	6.0 (2.9)	n.s.
Mean (S.D.) P1 CP (ng/ml)	107.7 (34.6)	111.1 (39.9)	117.9 (46.1)	113.2 (45.8)	n.s.
Mean (S.D.) urinary pyridinoline/creatinine (pmol/MCM. Cr.)	41.1 (17.2)	40.1 (10.6)	40.6 (20.6)	38.9 (23.0)	n.s.
Mean (S.D.) γ-carboxyglutaminate (Gla)/creatinine (nmol/MCM. Cr.)	40.6 (18.3)	42.5 (20.6)	43.8 (17.4)	41.4 (19.1)	n.s.
Mean (S.D.) APTT (s)	31.0 (5.7)	30.7 (5.4)	29.3 (5.2)	29.8 (5.6)	n.s.
Mean (S.D.) antithrombin III activity (%)	112.6 (13.1)	111.5 (12.5)	107.5 (13.1)	109.4 (12.8)	n.s.
Mean (S.D.) fibrinogen (mg/dl)	292.0 (48.5)	295.1(51.5)	281.2 (59.4)	288.7 (50.8)	n.s.
Mean (S.D.) plasminogen (%)	110.8 (12.4)	108.6 (11.1)	109.4 (10.9)	109.3 (12.2)	n.s.

Figures in parenthesis indicate 1 standard deviation. *P*-value assessed using ANOVA. No significant differences were found in any of the parameters among the groups. P I CP, type I procollagen C-terminal propeptide; APTT, active partial thromboplastin time.

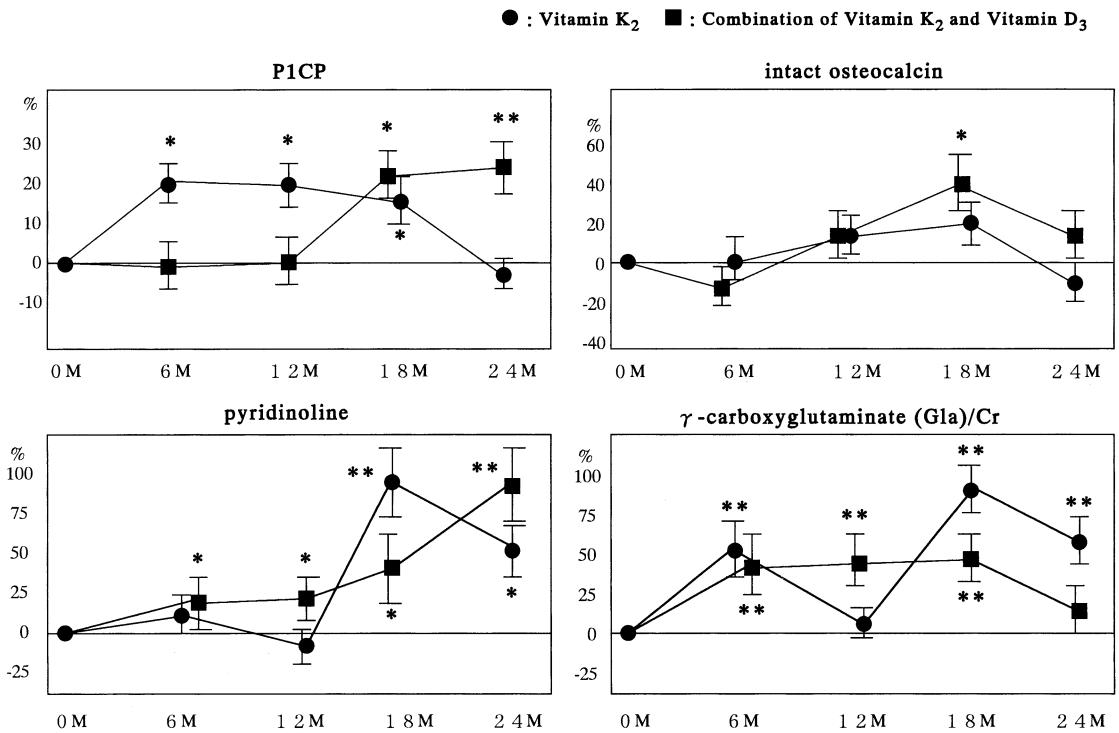

Fig. 2. Percentage changes from baseline in bone metabolism markers in all four groups during the 24-month study. Values are expressed as mean ± S.E.M. *P*-value assessed using Wilcoxon signed-rank test. *: $P < 0.05$, **: $P < 0.01$ compared with the baseline.

and 24 months ($53.4 \pm 55.7\%$, $P < 0.05$) in the vitamin K_2 alone therapy group. In the combined therapy group, urinary pyridinoline level was significantly increased at 6 ($17.5 \pm 36.4\%$, $P < 0.05$), 12 ($27.5 \pm 18.7\%$, $P < 0.05$), 18 months ($29.4 \pm 31.7\%$, $P < 0.05$) and 24 months ($84.5 \pm 51.9\%$, $P < 0.01$) of treatment.

Serum P1CP was approximately 20% higher at 6 months ($19.8 \pm 27.5\%$, $P < 0.05$) and 12 months ($18.7 \pm 37.2\%$, $P < 0.05$) in the vitamin K_2 alone therapy group and returned to baseline levels at 24 months. In contrast, in the combined therapy group baseline levels were more or less maintained up to 12 months, but increases of 21.8 (± 22.5)% and 24.2 (± 23.1)% were then recorded at 18 months ($P < 0.05$) and 24 months ($P < 0.01$), respectively (Fig. 2). Table 5 shows the correlation between BMD change and bone metabolic marker changes during treatment with vitamin K_2 or combined therapy. There was a significant positive correlation between BMD change and changes in serum P1CP levels at 12 months in the vitamin K_2 treatment group ($P = 0.0012$). A significant positive correlations were also observed between BMD change and changes in serum P1CP level ($P = 0.034$) and intact osteocalcin level ($P = 0.035$) after 12 months of combined therapy. At 24 months of combined therapy, we also observed a positive correlation between BMD change and intact osteocalcin level ($P = 0.002$). On the other hand, we observed a significant negative correlation between BMD change and change in urine pyridinolin level at 12 ($P = 0.001$) and 24 months (0.004) of combined therapy.

Tests of coagulation function revealed a gradual decline in APTT in both the vitamin K_2 alone group and combined therapy with vitamin D_3 group, which fell significantly to 15.0 ± 8.4 and $14.2 \pm 11.5\%$ below baseline at 6 months ($P < 0.01$) and 18 months ($P < 0.05$), respectively, in the vitamin K_2 alone therapy, and fell signifi-

cantly to 19.1 ± 10.4 and $15.2 \pm 9.5\%$ below baseline at 18 months ($P < 0.01$) and 24 months ($P < 0.05$) in the combined therapy with vitamin D_3 group. Serum AT III activity gradually increased over time, and increased significantly to 8.2 ± 6.4 and $8.3 \pm 7.5\%$ above baseline at 18 months ($P < 0.05$) and 24 months ($P < 0.05$), respectively, in the vitamin K_2 alone therapy, and increased significantly to 11.1 ± 7.4 and $15.2 \pm 7.5\%$ above baseline at 18 months ($P < 0.01$) and 24 months ($P < 0.01$), in the combined therapy with vitamin D_3 group. Serum fibrinogen and plasminogen levels also tended to increase gradually over time. Significant increases in serum fibrinogen levels were observed at 6 months ($26.0 \pm 41.5\%$, $P < 0.05$) from baseline in the vitamin K_2 alone therapy group, and observed at 18 months ($28.1 \pm 35.5\%$, $P < 0.05$) and 24 months ($30.2 \pm 33.5\%$, $P < 0.05$) from baseline in the combined therapy group. Significant increases in serum plasminogen levels were observed at 18 months ($14.2 \pm 12.2\%$, $P < 0.05$ and $11.9 \pm 8.5\%$, $P < 0.01$) and 24 months ($19.6 \pm 12.6\%$, $P < 0.01$ and $19.2 \pm 13.5\%$, $P < 0.05$) from baseline in both the vitamin K_2 alone therapy group and combined therapy group (Fig. 3).

4. Discussion

Vitamin K occurs naturally in two forms, K_1 and K_2. The K_2 congener, menaquinone-4, which has the most potent γ-carboxylation activity, reportedly improves bone mass in patients with involutional osteoporosis [17]. In the present study, we attempted to determine whether combination therapy with vitamin K_2 and D_3 yields a synergistic effect in maintaining or increasing bone mineral density due to promotion of calcification in postmenopausal women with decreased bone mass. Treatment with menaquinone-4 alone for 24 months produced an increase of $0.135 \pm 5.44\%$ in BMD, confirming a maintenance effect on BMD. For combination therapy consisting of vitamin K_2 and D_3, however, marked increases in BMD of 5.86 ± 6.85 and $4.92 \pm 7.89\%$ were observed at 12 and 24 months, respectively. Combined therapy appears to have an effect on vertebral BMD in the first 6–12 months, while thereafter mean rate of increase in BMD decline. The rate of increase appeared to decrease slightly because the bone metabolic profile was almost stabilized by sufficient supplementation of vitamin K_2 and D_3 after 12 months, followed by the effects of physiological aging. However, since the

Table 5
Correlations between percent changes in vertebral BMD and bone markers

	Correlation value (R)	Regression coefficient	P-value
Vitamin K_2 alone 12 months therapy			
P1CP	0.299	19.29	0.187
Pyridinoline	0.226	−10.35	0.326
Intact osteocalcin	0.100	23.98	0.703
Vitamin K_2 alone 24 months therapy			
P1CP	0.684	8.31	0.001
Pyridinoline	0.019	−4.51	0.938
Intact osteocalcin	0.140	31.53	0.605
Combined therapy with vitamin K_2 and D_3 12 months			
P1CP	0.410	17.90	0.034
Pyridinoline	0.603	4.48	0.001
Intact osteocalcin	0.540	19.36	0.035
Combined therapy with vitamin K_2 and D_3 24 months			
P1CP	0.317	26.86	0.107
Pyridinoline	0.540	26.60	0.004
Intact osteocalcin	0.588	25.60	0.002

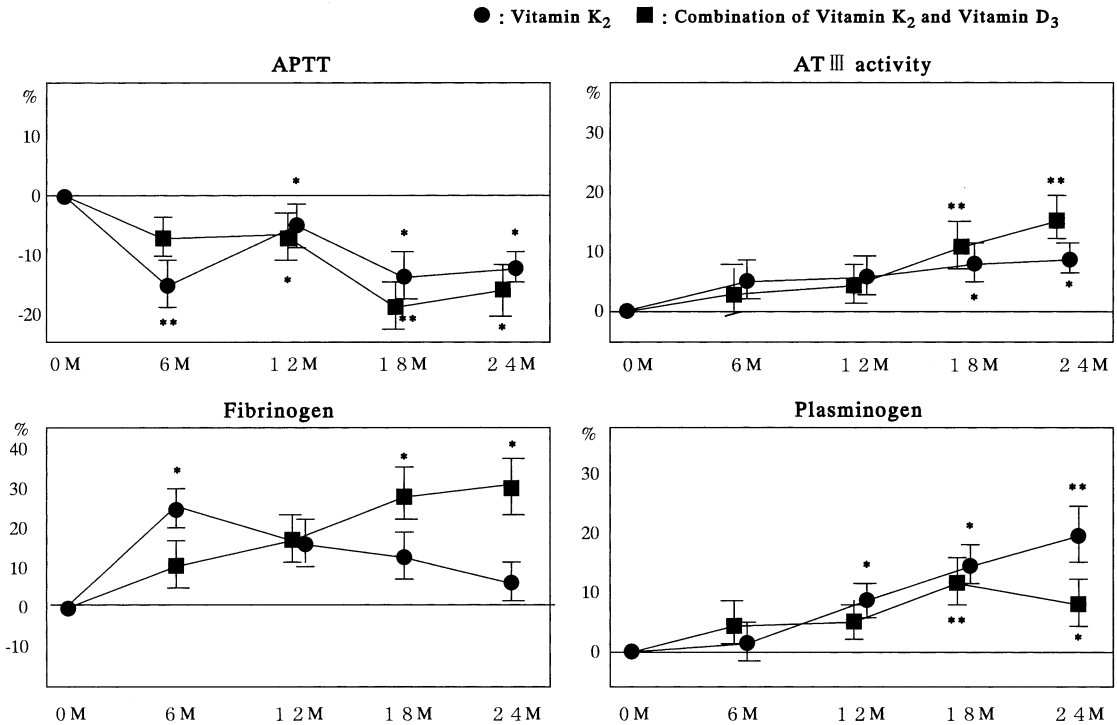

Fig. 3. Percentage changes from baseline in coagulation function in all four groups during the 24-month study. Values are expressed as mean ± S.E.M. *P*-value assessed using Wilcoxon signed-rank test. *: $P < 0.05$, **: $P < 0.01$ compared with the baseline.

rate of decrease was smaller than the 1.9% per year exhibited by the control group, it appeared that decrease in BMD could be suppressed by combined therapy with vitamin K_2 and D_3 for a period from several years to more than 10 years after menopause, leading to prevention of bone fracture. Koshihara and colleagues described a synergistic effect in an in vitro system containing cultured osteoblasts, in which this process of calcification was greatly promoted by the presence of vitamin D_3 [27]. Iwamoto et al. reported that vitamin K_2 suppressed decrease in spinal BMD, compared with vitamin D_3 treatment [28]. In another recent study, vitamin K_2 partially prevented bone loss caused by estrogen deficiency, when combined with vitamin D_3 [29].

There was a tendency toward higher concentrations of intact osteocalcin at times up to 18 months of treatment in the groups receiving vitamin K_2 alone and combined therapy, reflecting the fact that bone turnover had increased. In osteoporosis, there is negative imbalance between bone resorption and bone formation, resulting in bone loss. We observed significant BMD change at 6 months of combined therapy, while mean levels of bone formation markers did not increase and bone resorption marker increased significantly. Although clear explanation of this contradiction is difficult, there appeared to be more cases in which osteogenesis became dominant in the balance of formation and resorption in bone metabolism, since there were many responders in the combined therapy group. Moreover, the mean values of bone resorption markers did not decrease compared with the previous value due to very strong bone resorption in the postmenopausal period, but the change in such markers was sufficient to suppress bone resorption. The significant positive correlation between individual BMD change and serum P1CP level in the 24-month vitamin K_2 alone therapy and the significant positive correlation between individual BMD

change and serum P1CP level and intact osteocalcin level in the group with combined therapy with vitamin D_3 suggested that vitamin K_2 can accelerate bone formation, and that this formation can be potentiated by combination with vitamin D_3. In recent in vitro studies, menaquinone-4 modulated proliferation and function of mouse cultured osteoblastic cells in vitro [30].

In the coagulation system, plasma concentrations of the vitamin K-dependent and contact factors have important effects. Coagulation disturbances are due to impaired vitamin K metabolism, defective synthesis of coagulation factors and regulatory proteins, impaired clearance of activated coagulation factors and increased fibrinolysis [31,32]. It has been shown that vitamin K_2 (menaquinone-4) improves fibrinolysis. Coagulation function was evidently promoted by continuous, long-term administration given the observation of increased concentrations of AT III and fibrinogen. However, these changes, which occurred in response to the promotion of coagulation function, remained within the normal range, whereas plasminogen values tended to increase gradually. Thus, promotion of fibrinolytic function was also observed, suggesting that coagulofibrinolytic balance may have been maintained in the physiological fibrinolytic system. Ronden et al. reported that very high doses of vitamin K_2 affected neither blood coagulation characteristics nor the blood platelet aggregation rate [33].

In conclusion, the results of this in vivo study involving patients with postmenopausal decreased bone mass suggest that combined therapy with vitamin K_2 and D_3 increases bone turnover and promotes bone formation and calcification, resulting in marked increases in bone mineral density and improvement in bone quality. Given that the increases in coagulation function observed were variations within the physiological range, and no adverse drug reactions were observed, this mode of combined therapy may be very effective in preventing postmenopausal bone fracture.

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