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Vitamin K_2 (Menatetrenone) Effectively Prevents Fractures and Sustains Lumbar Bone Mineral Density in Osteoporosis

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ABSTRACT

We attempted to investigate whether vitamin K_2 (menatetrenone) treatment effectively prevents the incidence of new fractures in osteoporosis. A total of 241 osteoporotic patients were enrolled in a 24-month randomized open label study. The control group (without treatment; $n = 121$) and the vitamin K_2–treated group ($n = 120$), which received 45 mg/day orally vitamin K_2, were followed for lumbar bone mineral density (LBMD; measured by dual-energy X-ray absorptiometry [DXA]) and occurrence of new clinical fractures. Serum level of Glu-osteocalcin (Glu-OC) and menaquinone-4 levels were measured at the end of the follow-up period. Serum level of OC and urinary excretion of deoxypyridinoline (DPD) were measured before and after the treatment. The background data of these two groups were identical. The incidence of clinical fractures during the 2 years of treatment in the control was higher than the vitamin K_2–treated group ($\chi^2 = 10.935$; $p = 0.0273$). The percentages of change from the initial value of LBMD at 6, 12, and 24 months after the initiation of the study were $-1.8 \pm 0.6\%$, $-2.4 \pm 0.7\%$, and $-3.3 \pm 0.8\%$ for the control group, and $1.4 \pm 0.7\%$, $-0.1 \pm 0.6\%$, and $-0.5 \pm 1.0\%$ for the vitamin K_2–treated group, respectively. The changes in LBMD at each time point were significantly different between the control and the treated group ($p = 0.0010$ for 6 months, $p = 0.0153$ for 12 months, and $p = 0.0339$ for 24 months). The serum levels of Glu-OC at the end of the observation period in the control and the treated group were 3.0 ± 0.3 ng/ml and 1.6 ± 0.1 ng/ml, respectively ($p < 0.0001$), while the serum level of OC measured by the conventional radioimmunoassay (RIA) showed a significant rise ($42.4 \pm 6.9\%$ from the basal value) in the treated group at 24 months ($18.2 \pm 6.1\%$ for the controls; $p = 0.0081$). There was no significant change in urinary DPD excretion in the treated group. These findings suggest that vitamin K_2 treatment effectively prevents the occurrence of new fractures, although the vitamin K_2–treated group failed to increase in LBMD. Furthermore, vitamin K_2 treatment enhances γ-carboxylation of the OC molecule. (J Bone Miner Res 2000;15:515–521)

Key words: vitamin K_2 (Menatetrenone), bone mineral density, fracture, osteoporosis, osteocalcin

INTRODUCTION

VITAMIN K IS KNOWN to activate blood coagulation factors through post-translational modification of the protein molecule.[1–3] Recent studies indicate that vitamin K also plays a role in bone metabolism. Price et al. and Haushka et al. first described a vitamin K–dependent bone-specific protein called bone Gla protein or osteocalcin (OC).[4,5] This molecule is considered to be the most abundant noncollagenous protein in the bone. OC has glutamic residues in its molecule and these are converted to γ-carboxyglutamic acid (Gla) through post-translational modification mediated by a vitamin K–dependent carboxylase. Gla residue(s) can bind to hydroxyapatite crystals and can regulate the growth of these crystal.[6] Furthermore, both in vivo and in vitro studies show that vitamin K or its analogues can act directly on bone metabolism. Hara et al. reported that vitamin K_2 inhibits bone resorption partly through the inhibition of prostaglandin E_2 synthesis in organ culture.[7] Akiyama et al. showed that vitamin K_2 inhibits

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osteoclast-like cell formation in vitro, and Koshihara et al. reported that vitamin K_2 enhances human osteoblast–induced mineralization with or without coincubation with 1,25-hydroxyvitamin D_3 [$1,25(OH)_2D_3$].[8,9] In steroid-treated and ovariectomized rat, vitamin K_2 inhibits bone loss[10,11] In addition, Hart et al. and Hodges et al. reported low serum phylloquinone levels in the patients with femoral neck fractures.[12,13] Plantalech et al., Szulc et al., and Vergnaud et al. measured serum undercarboxylated OC, which may reflect low activity of vitamin K, and higher incidence of femoral neck fracture was observed in the patients with high levels of undercarboxylated OC.[14–16] We reported that metacarpal bone mineral density (BMD) is increased in osteoporosis through the administration of vitamin K_2.[17] These reports led us to expect that the administration of vitamin K may prevent osteoporotic fractures.

In the present study, we attempted to investigate whether vitamin K_2 prevents trabecular bone loss and the occurrence of bone fractures in osteoporosis in a randomized open label trial.

SUBJECTS AND METHODS

Patient selection and evaluation of osteoporosis

The female subjects were selected from the 746 patients with osteoporosis registered to the Research Institute and Practice for Involutional Diseases, Nagano, Japan. Criteria for selection were (1) patients without treatment for osteoporosis for more than 3 months before the present study and (2) agreement to participate in the present study after the informed consent.

A total of 241 osteoporotic patients were enrolled in the present study. The diagnosis of osteoporosis was made according to the criteria proposed by the Japanese Society of Bone Metabolism in 1996.[18] Briefly, patients with low lumbar BMD (LBMD) (<70% of young adult mean) or with one or more nontraumatic vertebral fractures and lumbar BMD less than 80% of young adult mean were diagnosed as having osteoporosis.

The patients were randomly allocated to two groups: group 1 was treated with 150 mg/day elemental calcium (control group; $n = 121$) and group 2 was treated with 45 mg/day menatetrenone orally together with the same dose of elemental calcium as the controls (vitamin K_2 group; $n = 120$) and monitored for 24 months. The patients were prohibited from taking any other drugs that could affect bone and calcium metabolisms. The patients were given no specific instructions regarding daily calcium, vitamins D and K intake, or a program of exercise. The means of daily calcium intake in the present study were 483 mg/day for the controls and 536 mg/day for the K_2 group. All patients were ambulatory. Vitamin K_2 (menatetrenone) capsules were purchased from Eizai Co., Ltd., Tokyo, Japan (Gla-kay®).

Bone mineral measurement

BMD at lumbar vertebrae (L2–L4 BMD) was measured by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L (Lunar Rad. Wisconsin, WI, U.S.A.) at anteroposterior (AP) view. The inter-assay variance of this method in our laboratory was 0.5 ± 0.5% (CV ± SD).[19] To guard against machine drift, a quality assurance test was carried out every day and densitometer performance remained constant during the test period (1995 ~ 1998).

Fracture assessment

AP and lateral radiographs of the thoracic and lumbar spine were taken before and after the 12-month and 24-month period of the trial. Vertebral fractures were diagnosed with visual semiquantitative assessment according to the following criteria without any information of the patients:

1. Twenty percent or greater reduction of vertebral height (anterior height, middle height, and posterior height) than the neighboring vertebrae
2. When the vertebral height of anterior or middle portion of the body was 80% or less than the height of posterior margin, we diagnosed fractured vertebrae.
3. A new vertebral fracture was defined as a 20% or greater decrease in any of three heights of a vertebral body between baseline and as seen on follow-up films.

The X-ray films were evaluated by two physicians (M.S. and Y.S.), independently, and if the diagnosis of vertebral fracture was not unanimous, the measurement of vertebral height using a caliper was performed.

When the vertebral radiograph at baseline contained vertebral fracture(s) in the L2–L4 region, the patient was excluded from the enrollment. When the vertebral radiograph at 24 months contained new vertebral fracture(s) in the L2–L4 region, the L2–L4 BMD data of all measurements were excluded from the BMD analysis. However, we included patients with new vertebral fracture(s) into the fracture incidence analysis. Fracture frequencies in the two groups were compared by the χ^2-test. Other sites of new fractures were identified by X-ray pictures.

Measurements of serum and urinary parameters

Serum levels of intact parathyroid hormone (PTH, immunoradiometric assay [IRMA]; Nichols Institute Diagnostics, CA, U.S.A.), 25-hydroxyvitamin D (25-OHD; competitive protein binding assay), and $1,25(OH)_2D_3$, (radioreceptor assay) were measured before the treatment in order to exclude the patients with metabolic bone diseases.[20,21] Serum levels of OC (RIA, Cis, France) and urinary excretion of deoxypyridinoline (DPD; high performance liquid chromatography [HPLC] method; Teijin Bio-Laboratories, Tokyo, Japan) were measured before the treatment and 12 months and 24 months after the treatment.[22,23] Glu-OC (Takara Shuzo Co., Shiga, Japan) also was measured 24 months after the treatment, because a Glu-OC ELISA system was not available at the beginning of the study.[16] Reactivity of the monoclonal antibody of this ELISA system raised to bovine OC was 100% to $Glu^{17,21,23}$-OC and was 6%

to Gla[17,21,23]-OC, respectively. The intra-assay coefficient of variation assessed by 10 measurements was less than 7.3%. The interassay CV evaluated by repeat measurements on 8 separate days over 10 weeks was less than 8.5%.

Serum levels of vitamin K such as phylloquinone, menaquinone-4, and menaquinone-7 were measured at the end of the treatment by electrochemical measurement after the purification using an HPLC system.[12]

Statistical analyses

The baseline characteristics of the patients in the control group and the vitamin K_2 treatment group were compared by Student's *t*-test (two-tailed). The significance of changes from baseline in L2–L4 BMD between the control and the vitamin K_2–treated groups were examined by the percentage change at each evaluation point by Fisher's PSLD in the protocol-compatible cases (PC analysis). For the dropout cases in the control and the treated groups, an intent-to-treat (ITT) analysis also was performed. In this analysis, the last observation of L2–L4 BMD was considered as the final data and the difference between the final data of the two groups was determined. The frequency of new vertebral fractures was compared between the groups by the χ^2-test in the cases who obtained the complete data set of X-ray set of both initial and 24 months later. The significance level was set at 5% (two-tailed). The data are expressed as the mean ± SE.

RESULTS

Breakdown of the cases

A total of 241 cases (control, 121; vitamin K_2, 120) were enrolled. Among these subjects, 51 cases (21.2%; control, 22 [18.2%]; vitamin K_2, 29 [24.2%]) were excluded from the fracture analysis and PC analysis of LBMD, because these subjects failed to obtain the X-ray films and BMD data at 24 months. The remaining 190 subjects were evaluated for fracture incidence. Of the 190 subjects, 10 cases (control, 5; vitamin K_2, 5) were excluded from the PC analysis of BMD, because these patients showed new vertebral fractures in the L2–L4 region. In the ITT analysis, a total of 26 cases (16 cases for the control and 10 cases for the K_2 group) was excluded because these patients had no follow-up data of LBMD. Furthermore, the 10 cases that had new vertebral fractures in L2–L4 region also were excluded from the ITT analysis of LBMD. As a result, a total of 205 cases and a total of 180 cases were applied to the ITT and PC analysis of LBMD, respectively. The breakdown of the cases is shown in Fig. 1.

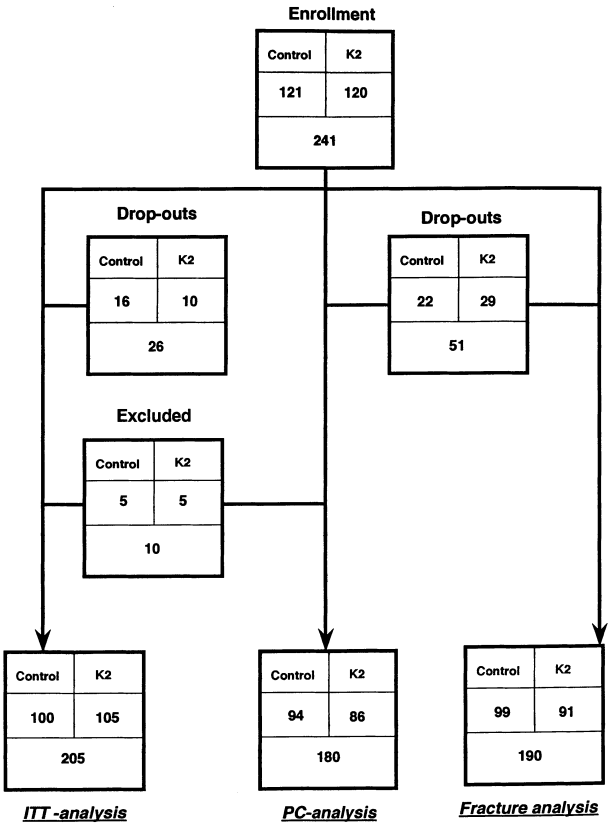

FIG. 1. Breakdown of the cases. A total of 241 patients with osteoporosis were enrolled in the present study. Dropout means discontinuation of the study and the data at the observation period could not be obtained. The dropout rates of the control and the vitamin K_2–treated group are not significantly different. A total of 10 patients (control goup, 5; vitamin K_2 group, 5) was excluded from the analysis of the change in LBMD, because new vertebral fracture(s) in the L2–L4 region were observed during the observation period.

Baseline characteristics

Table 1 shows the baseline characteristics of the two groups. There were no significant differences. The baseline prevalences of vertebral fracture(s) otherwise in the L2–L4 region in the two groups were 35.5% (43 of 121 cases) for the control and 38.3% (46 of 120 cases) for the vitamin K_2–treated group. The background data of the dropout cases of both groups were identical, suggesting that the presence of dropout cases did not result in significant data biases (data not shown).

LBMD

Figure 2A shows the percent changes in L2–L4 BMD during 24 months of treatment compared with the baseline values in the control and vitamin K_2–treated group. The L2–L4 BMD at 6, 12, and 24 months after the initiation of observation for the vitamin K_2–treated group was 1.4 ± 0.7% (0.755 ± 0.011g/cm^2), −0.1 ± 0.6% (0.744 ± 0.013 g/cm^2), and − 0.5±1 .0% (0.735 ± 0.016 g/cm^2), respectively, whereas the corresponding values in the control group were −1.8 ± 0.6% (0.746 ± 0.013 g/cm^2), −2.4 ± 0.7% (0.740 ± 0.013 g/cm^2), and −3.3 ± 0.8% (0.736 ±0.016 g/cm^2), respectively. There was a significant difference between the two groups at each of the observed time points ($p = 0.0010$ at 6 months, $p = 0.0153$ at 12 months, and $p = 0.0339$ at 24 months). In the case of ITT analysis, the final change in L2–L4 BMD for the treated and the control group

TABLE 1. BACKGROUND DATA OF THE SUBJECTS

Item	*Control*	*Vitamin K_2*
Number of cases	121	120
Age in years	68.0 ± 0.8	66.4 ± 0.8
Weight in kg	47.3 ± 0.7	48.1 ± 0.7
Height in cm	148.7 ± 0.6	149.2 ± 0.6
YSM	19.1 ± 0.9	17.5 ± 1.0
Ca (mg/dl)	9.1 ± 0.04	9.1 ± 0.04
P (mg/dl)	3.5 ± 0.04	3.5 ± 0.04
U-Ca/Cr ratio	0.231 ± 0.012	0.220 ± 0.011
Intact PTH (ng/ml)	36.8 ± 1.4	38.5 ± 1.4
25-OHD (ng/ml)	20.9 ± 0.8	20.6 ± 0.6
$1,25(OH)_2$ D(pg/ml)	38.0 ± 1.2	36.5 ± 1.2
Al-P (IU)	186.1 ± 5.6	186.8 ± 4.8
OC (ng/ml)	12.6 ± 0.4	13.0 ± 0.3
DPD (pmole/μmol Cr)	7.8 ± 0.3	7.8 ± 0.2
Initial LBMD (g/cm^2)	0.756 ± 0.010	0.747 ± 0.010
Patients with baseline vertebral fracture (%)	43/121 (35.8%)	46/120 (38.7%)

All the data are expressed mean ± SE. There was no significant difference between the control and vitamin K_2–treated group on the basis of their background data.

YSM, years since menopause.

was −0.4 ± 0.7% and −2.6 ± .6%, respectively (p = 0.0191; Fig. 2B).

Fracture incidence

Analysis of new clinical fractures was performed in 190 patients (Table 2). Thirty new vertebral fractures (30.3%), two forearm fractures, two femoral neck fractures, and one metacarpal bone fracture in the foot were observed in the control group during the observation period, while in vitamin K_2–treated group, 13 new vertebral fractures (10.9%) and one forearm fracture occurred. The fracture incidence in the vitamin K_2–treated group was significantly (χ^2 = 10.935; p = 0.0273) lower than the control group.

Bone turnover markers

Bone turnover markers such as serum OC level and urinary excretion of DPD were measured before the observation and 12 months and 24 months after the observation. There were no significant changes in the urinary excretion of DPD (Table 3) while the serum level of OC measured by the conventional RIA[22] (Cis, France) in the vitamin K_2–treated group showed a significant rise from the baseline value (35.7 ± 7.8% at 12 months and 42.4 ± 6.9% at 24 months). In the control group, those also increased by 9.3 ± 5.4% at 12 months and 18.2 ± 6.1% at 24 months, but these values were significantly lower than those in the vitamin K_2–treated group (p = 0.0144 and 0.0081, respectively). The serum level of Glu-OC was measured at the final day of observation. A significantly lower serum level of Glu-OC was observed in the vitamin K_2–treated group (1.6 ± 0.1 ng/ml)

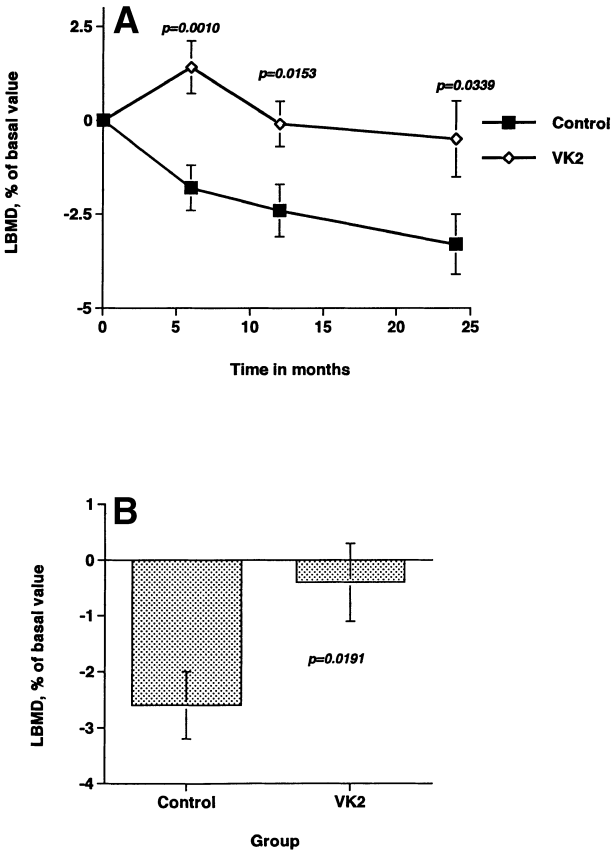

FIG. 2. Effect of vitamin K_2 on LBMD. Data are means ± SE. The comparison between the groups by analysis of variance. (A and B) Indicate the results of PC analysis and ITT analysis, respectively. Closed squares and open diamonds indicate the control group and the vitamin K_2–treated group, respectively.

TABLE 2. FRACTURE INCIDENCE

Group	*No*	*Vertebral*	*Forearm*	*Femoral neck*	*Other site*	*Total*
Control	64	30	2	2	1	99
K_2	77	13	1	0	0	91

Analysis of the incidence of new clinical fractures was performed in 190 subjects (control, 99; vitamin K_2, 91). The difference between the two groups was significant by the χ^2-test (χ^2 = 10.935; p = 0.0273).

than that in the control group (3.0 ± 0.3 ng/ml; $p < 0.0001$; Table 3).

Serum levels of vitamin K

Serum levels of phylloquinone and menaquinone-4 were measured on the final day of observation. The serum samples were obtained exactly 2 h after intake of vitamin K_2 orally. The serum levels of menaquinone-4 in the vit-

TABLE 3. EFFECT OF VITAMIN K_2 ON BONE TURNOVER MARKERS AND SERUM LEVEL OF MENAQUINONE-4

Markers	*Control* *12 months* *24 months*	*Vitamin K_2* *12 months* *24 months*	*Significance* *12 months* *24 months*
OC (% of the	9.4 ± 5.4	35.7 ± 7.8	0.0144
basal value)	18.2 ± 6.1	42.4 ± 6.9	0.0081
Glu-OC (ng/ml)	—	—	—
	3.0 ± 0.3	1.6 ± 0.1	<0.0001
DPD (% of the	4.1 ± 8.6	1.0 ± 7.2	ns
basal value)	−0.1 ± 4.7	1.9 ± 6.2	ns
MK-4 (ng/ml)	—	—	—
	0.3 ± 0.2	65.2 ± 9.9	<0.0001
Phylloquinone	—	—	—
(ng/ml)	1.2 ± 0.1	1.1 ± 0.1	ns

The serum level of OC and the urinary excretion of DPD were measured before initiation of trial and at 12 months and 24 months after the initiation of trial, and the values represented in the table are the percentage change from the basal value. The serum levels of Glu-OC and menaquinone-4 were measured only at the end of the observation. Although the serum level of OC was increased after vitamin K_2 treatment, the serum Glu-OC was lower in the treated group than the controls, suggesting that Gla-OC is increased by the treatment. Urinary excretion of DPD did not change after the treatment. Thus, vitamin K_2 treatment did not inhibit bone resorption, at least in the dose used in the present study.

—, not determined.

amin K_2–treated group (65.2 ± 9.9 ng/ml) were significantly higher than those of the control group (0.3 ±0.2 ng/ml; $p <$ 0.0001). The serum levels of phylloquinone in the treated and the control groups were 1.2 ± 0.1 ng/ml and 1.1 ±0.1 ng/ml, respectively; these values were not significantly different, suggesting that the vitamin K_1 intake from food is not different between the groups (Table 3).

DISCUSSION

Vitamin K activates blood coagulation factors by converting glutaminic residues (Glu) to γ-carboxy glutaminic residues (Gla). The bone-specific protein OC is processed by vitamin K in the same way. The role of OC in the calcification of bone is not clear, but it may regulate the growth of hydroxyapatite crystals.[6] Both OC knockout mice and warfarin treatment during pregnancy result in hyperostosis, which indicates that Gla-containing OC promotes normal calcification of bone.[24–27] Furthermore, vitamin K_2 (menatetrenone) has been reported to inhibit bone resorption through inhibition of prostaglandin synthesis and of osteoclast formation.[7,8] These observations prompted us to investigate the effect of vitamin K_2 on bone metabolism in osteoporosis.

In the present study, vitamin K_2 clearly maintained lumbar BMD for 2 years and apparently inhibited the occurrence of new bone fracture in osteoporosis.

The cause of osteoporotic fracture is believed to be multifactorial. Among these contributing factors, BMD is the most reliable predictor.[28] Thus, the increase in BMD after intervention has been considered to be effective in reducing the risk of fracture. According to this concept, the measurement of BMD in clinical trials for the treatment of osteoporosis was a secondary endpoint to assess the efficacy of a drug for osteoporosis. However, there has been no direct evidence showing a relationship between increase of BMD and a decrease in the occurrence of bone fractures in various modes of therapy for osteoporosis. In fact, active vitamin D_3, 1α-hydroxyvitamin D_3 (1α-OHD_3) has been reported to decrease the occurrence of new vertebral fracture by about half to one-third of that in the controls, despite showing only about a 1% increase of BMD from the baseline value after 1 or 2 years of treatment.[29,30] On the other hand, estrogen replacement therapy or alendronate treatment increased BMD by about 3–5% after 2 years of treatment.[31–33] Although these latter two treatments apparently were more potent than the 1α-OHD_3 treatment, judging from the increment in BMD, the effect of these treatments on prevention of fractures seemed to be equal. These data indicate that the stronger effect on BMD does not guarantee a more efficient prevention of new fractures.[34] The present results support this concept.

The exact mechanism(s) of that reduction is still unclear, although the occurrence of new fractures in the vitamin K_2–treated group was lower than in the controls. The effects of vitamin K_2 on bone turnover markers were quite different from those seen in the potent inhibitors of bone resorption such as estrogen and bisphosphonates.[35–37] The serum level of OC measured by the conventional RIA system showed a significant increase by about 40% from the baseline indicating that bone formation may be accelerated by this mode of therapy. In addition, serum levels of Glu-OC at 24 months after the treatment with vitamin K_2 were significantly lower than those in the controls. This possibly means that vitamin K_2 treatment enhanced both γ-carboxylation of Glu residues and secretion of the OC molecule. Undercarboxylated OC has been reported to be a cause of bone fracture in osteoporosis.[14–16] A connection between OC carboxylation and incidence of clinical fractures also is suggested by the present study. Vitamin K_2 reduced the occurrence of fractures and increased carboxylation of OC. These data are correlative, and further research is required to establish a causal link between OC carboxylation and bone fractures. In the present study, there were no significant changes in bone resorption markers such as urinary pyridinium excretion. Therefore, the prevention of bone fractures by vitamin K_2 may not be caused by inhibition of bone resorption entirely, despite the fact that vitamin K_2 had been reported to inhibit bone resorption in vitro.[7,8]

The serum level of menaquinone -4 (vitamin K_2) was markedly increased after vitamin K_2 treatment, and this means that vitamin K_2 as used in the present study is considered to be a pharmacologic treatment but not a replacement therapy.

We did not use placebo capsules for vitamin K_2 in the control group. We cannot exclude the possibility that this

may have differentially affected the behavior of study participants in the control and treatment groups, although we consider this possibility unlikely. A nationwide placebo-controlled study on the effectiveness of vitamin K_2 in the prevention of bone fracture is currently underway in Japan.

In summary, vitamin K_2 treatment in osteoporosis successfully inhibited the occurrence of new bone fractures and maintained LBMD. The role of vitamin K_2 in the prevention of bone fractures is not fully understood, but accelerated γ-carboxylation of OC or bone formation may constitute significant factors. This is the first report showing the effects of vitamin K_2 on trabecular BMD and on bone fracture prevention in osteoporosis.

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