

# Role of Vitamin K2 in the Treatment of Postmenopausal Osteoporosis

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**Abstract:** Vitamin K2, raloxifene, and bisphosphonates, such as etidronate, alendronate, and risedronate, are widely used in the treatment of postmenopausal osteoporosis in Japan. A meta-analysis study has demonstrated the efficacy of anti-resorptive agents: raloxifene and etidronate have been shown to reduce the incidence of vertebral fractures, and alendronate and risedronate have been shown to reduce the incidence of both vertebral and hip fractures. Furthermore, a report of the World Health Organization (WHO) has provided evidence from a randomized controlled trial suggesting that vitamin K2, which may stimulate bone formation *via*  $\gamma$ -carboxylation of osteocalcin and/or steroid and xenobiotic receptors (SXR), reduces the incidence of vertebral fractures, despite having only modest effects on the bone mineral density (BMD). Based on the weight of the currently available evidence, it is recommended that alendronate and risedronate, rather than vitamin K2, should be chosen initially for the treatment of postmenopausal osteoporosis, because these agents have been shown to be the most efficacious for reducing the incidence of both vertebral and hip fractures among the current range of commercially available agents. However, the more potent anti-fracture efficacy of combined treatment with the anti-resorptive and commercially available anabolic agents may need to be established. Some studies have shown that combined treatment with a bisphosphonate and vitamin K2 may be more effective than treatment with a bisphosphonate alone in preventing vertebral fractures. On the other hand, the results of a preclinical study do suggest the possible efficacy of combined treatment with vitamin K2 and raloxifene in the prevention of vertebral and hip fractures in postmenopausal women, although no clinical studies have reported on the effects of combined treatment with vitamin K2 and raloxifene in postmenopausal women with osteoporosis. Vitamin K deficiency, as indicated by high serum levels of undercarboxylated osteocalcin, has been shown to contribute to the occurrence of hip fractures in elderly women. Thus, we propose that the important role of vitamin K2 used in combination with bisphosphonates or raloxifene should not be underestimated in the prevention of fractures in postmenopausal women with osteoporosis with vitamin K deficiency.

**Keywords:** Vitamin K2, undercarboxylated osteocalcin, steroid and xenobiotic receptor (SXR), bone formation, postmenopausal osteoporosis.

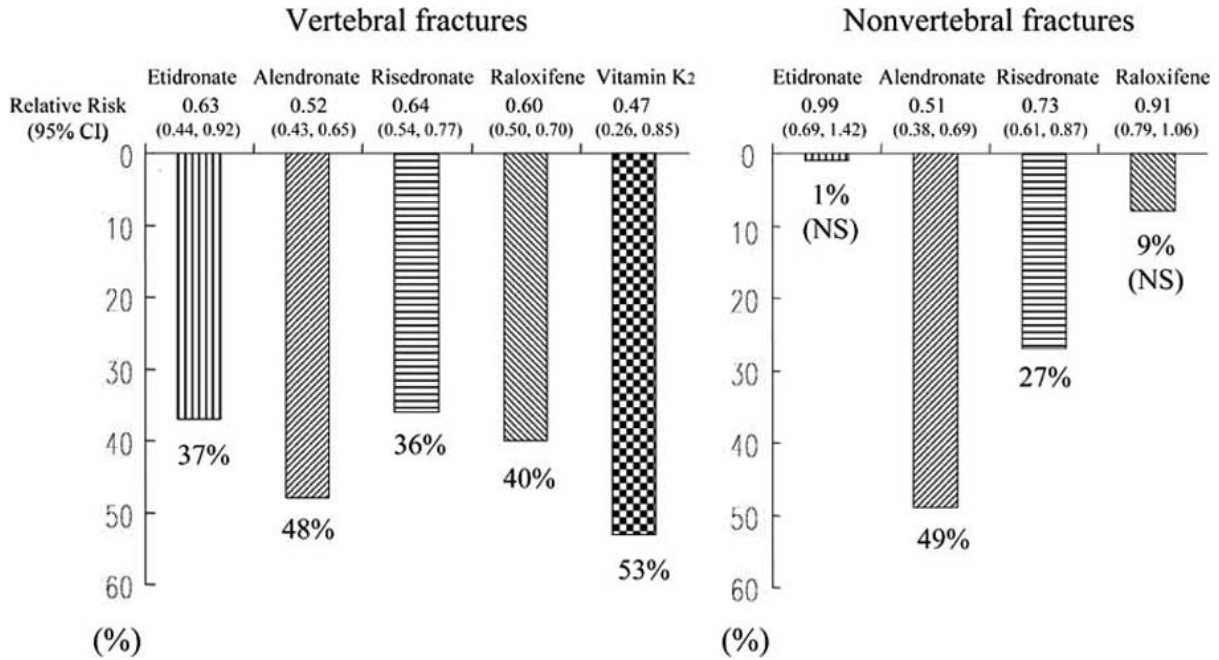
## INTRODUCTION

Osteoporosis commonly affects postmenopausal women, placing them at a significantly increased risk for fractures – especially, fractures of the vertebrae and hips. Such fractures often lead to disability and increased mortality [1,2]. Thus, management of osteoporosis is of great clinical significance.

Treatment of osteoporosis is usually conducted in accordance with the principles of evidence-based medicine (EBM). EBM incorporates information derived from the highest-quality investigations with clinical judgment and patient values, to allow optimal clinical management. Available clinical evidence has been classified hierarchically into various levels, with meta-analysis representing the highest level, followed by randomized controlled trials (RCTs), which have long been considered as the “gold standard” in the context of clinical investigations. In the context of osteoporosis treatment, meta-analysis studies have suggested alendronate, risedronate, etidronate, and raloxifene are efficacious agents for preventing vertebral fractures in

postmenopausal women with osteoporosis [3,4], with a reported reduction in the incidence of vertebral fractures of 48 %, 36 %, 37 %, and 40 %, respectively, with the use of the four agents (Fig. 1). Alendronate and risedronate have been shown to be especially efficacious for the prevention of nonvertebral fractures [3,4], with a reported reduction in the incidence of nonvertebral fractures of 49 % and 27 %, respectively (Fig. 1). On the other hand, while no significant differences in the efficacy of risk reduction of vertebral fractures have been reported among the four anti-resorptive agents, alendronate is probably more efficacious than etidronate, risedronate, or raloxifene for the prevention of nonvertebral fractures [4]. With respect to hip fractures, RCTs have shown that alendronate and risedronate reduce their incidence by 50 % and 40 %, respectively, in postmenopausal women with osteoporosis [5,6]. Although the results of RCTs also showed the anti-fracture efficacy of ibandronate, parathyroid hormone (PTH), and strontium ranelate, which are not available in Japan, in postmenopausal women with osteoporosis, these agents is likely not to prevent hip fractures (Table 1) [7-11]. A recent meta-analysis study has also shown that in patients with a bone mineral density (BMD) T-score of less than or equal to  $-2.0$ , or a vertebral fracture, a consistent reduction in hip fracture risk was observed in patients receiving alendronate therapy, with

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**Fig. (1).** Reduction of the risk of vertebral and nonvertebral fractures by five interventions in postmenopausal women with osteoporosis [3,16,17].

Meta-analysis studies have suggested that alendronate, risedronate, etidronate, and raloxifene are the most efficacious agents for the prevention of vertebral fractures in postmenopausal women with osteoporosis; with a reduction in the incidence of vertebral fractures of 48 %, 36 %, 37 %, and 40 %, respectively, following treatment with these drugs [3]. Alendronate and risedronate have also been shown to be efficacious for the prevention of nonvertebral fractures, with a reduction in the incidence of nonvertebral fractures of 49 % and 27 %, respectively, following treatment with these drugs [3]. A report of the World Health Organization (WHO) Scientific Group presented evidence for the efficacy of various therapies in osteoporosis [16]. The evidence level for the usefulness of vitamin K2 (menatetrenone) in reducing the incidence of vertebral fractures is B, which corresponds to positive evidence from smaller non-definitive randomized controlled trials (RCTs), on the basis of a RCT from Japan. Vitamin K2 (menatetrenone) has been reported to reduce the incidence of vertebral fractures by 53% in patients with postmenopausal or age-related osteoporosis [17]; however, it has not yet been established as an efficacious agent for preventing nonvertebral fractures. CI: confidence interval. NS: not significant.

an overall reduction in the risk of 45%; for patients who met the criteria of osteoporosis, as defined by the World Health Organization (WHO), the overall risk reduction was 55% [12]. Therefore, alendronate, risedronate, etidronate, or raloxifene would be the first drugs of choice in younger postmenopausal women with osteoporosis who have an increased risk of vertebral fractures in countries where PTH, strontium ranelate, and ibandronate are not available, while alendronate, or risedronate where alendronate is not available, would be the first drug of choice in elderly women with osteoporosis, who carry an increased risk of both vertebral and hip fractures. It has been reported that alendronate exerts greater beneficial effects than raloxifene on the BMD and bone turnover in postmenopausal women with a low BMD [13]. The long-term safety and efficacy of alendronate and risedronate therapy (ten and seven years, respectively), have been shown in postmenopausal women with osteoporosis [14,15].

Vitamin K2 (menatetrenone) is widely used for the treatment of osteoporosis in Japan. A report of the WHO Scientific Group has provided evidence for the efficacy of the

**Table 1. Efficacy for Vertebral, Nonvertebral and Hip Fractures by Six Interventions in Postmenopausal Women with Osteoporosis [7-11]**

	Vertebra	Nonvertebra	Hip
Raloxifene	○	×	×
Ibandronate	○	×	×
Alendronate	○	○	○
Risedronate	○	○	○
PTH	○	○	×
Strontium ranelate	○	○	×

PTH: parathyroid hormone

various therapies for osteoporosis (Table 2) [16]; the evidence level for the efficacy of vitamin K2 (menatetrenone) in reducing the incidence of vertebral fractures is B, which corresponds to positive evidence from smaller non-definitive

**Table 2. Evidence for the Efficacy of Therapies in Osteoporosis [16]**

Intervention	BMD	Vertebral fracture	Nonvertebral fracture	Hip fracture
Calcium	A	B	B	D
Calcium + Vitamin D	A	–	A	A
Estrogens	A	A	A	A
Tibolone	A	–	–	–
Alendronate	A	A	A	A
Etidronate	A	B	D	D
Risedronate	A	A	A	A
Ibandronate	A	–	–	–
Calcitonin	A	C	C	D
Fluoride	A	C	–	–
Anabolic steroids	A	–	–	D
Calcitriol	C	C	C	–
Alfacalcidol	C	C	–	D
Raloxifene	A	A	–	–
Ipriflavone	B	–	–	–
Vitamin K <sub>2</sub> (Manatetrenone)	B	B	–	–

Evidence A, positive evidence from one or more, adequately powered, randomized controlled trials; B, positive evidence from smaller non-definitive randomized controlled trials; C, inconsistent results from Randomized controlled trials; D, positive results from observational studies; –, efficacy not established or not tested.

RCTs. On the other hand, the evidence level for the efficacy of alendronate and risedronate in reducing the incidence of both vertebral and nonvertebral fractures is A, which corresponds to positive evidence from one or more, adequately powered RCTs, and the evidence level of the efficacy of raloxifene in reducing the incidence of vertebral fractures is A. A RCT from Japan suggests that vitamin K<sub>2</sub> sustains the lumbar BMD and reduces the incidence of vertebral fractures, with a reported reduction in the incidence of vertebral fractures of 53% in patients with postmenopausal osteoporosis; this rate is similar to that reported for treatment with any of the four anti-resorptive agents (Fig. 1) [17]. However, the level of evidence qualifying the skeletal efficacy was higher for alendronate, risedronate, and raloxifene than for vitamin K<sub>2</sub>.

The question then arises, “What is the place of vitamin K<sub>2</sub> in the treatment of osteoporosis?” This paper discusses the role of vitamin K<sub>2</sub> in the treatment of postmenopausal osteoporosis.

**EFFECTS OF VITAMIN K<sub>2</sub> ON BONE METABOLISM**

Vitamin K<sub>2</sub> is known to be a cofactor of  $\gamma$ -carboxylase, which converts the glutamic acid (Glu) residues in the osteocalcin (OC) molecule to  $\gamma$ -carboxyglutamic acid (Gla) and is, therefore, essential for the  $\gamma$ -carboxylation of OC [18-21]. Thus, vitamin K<sub>2</sub> is thought to be involved in bone formation as an essential cofactor for the  $\gamma$ -carboxylation of OC. However, recent evidence suggests that vitamin K<sub>2</sub> also has a transcriptional regulatory function [22]; it was shown to be a transcriptional regulator of bone-specific genes that

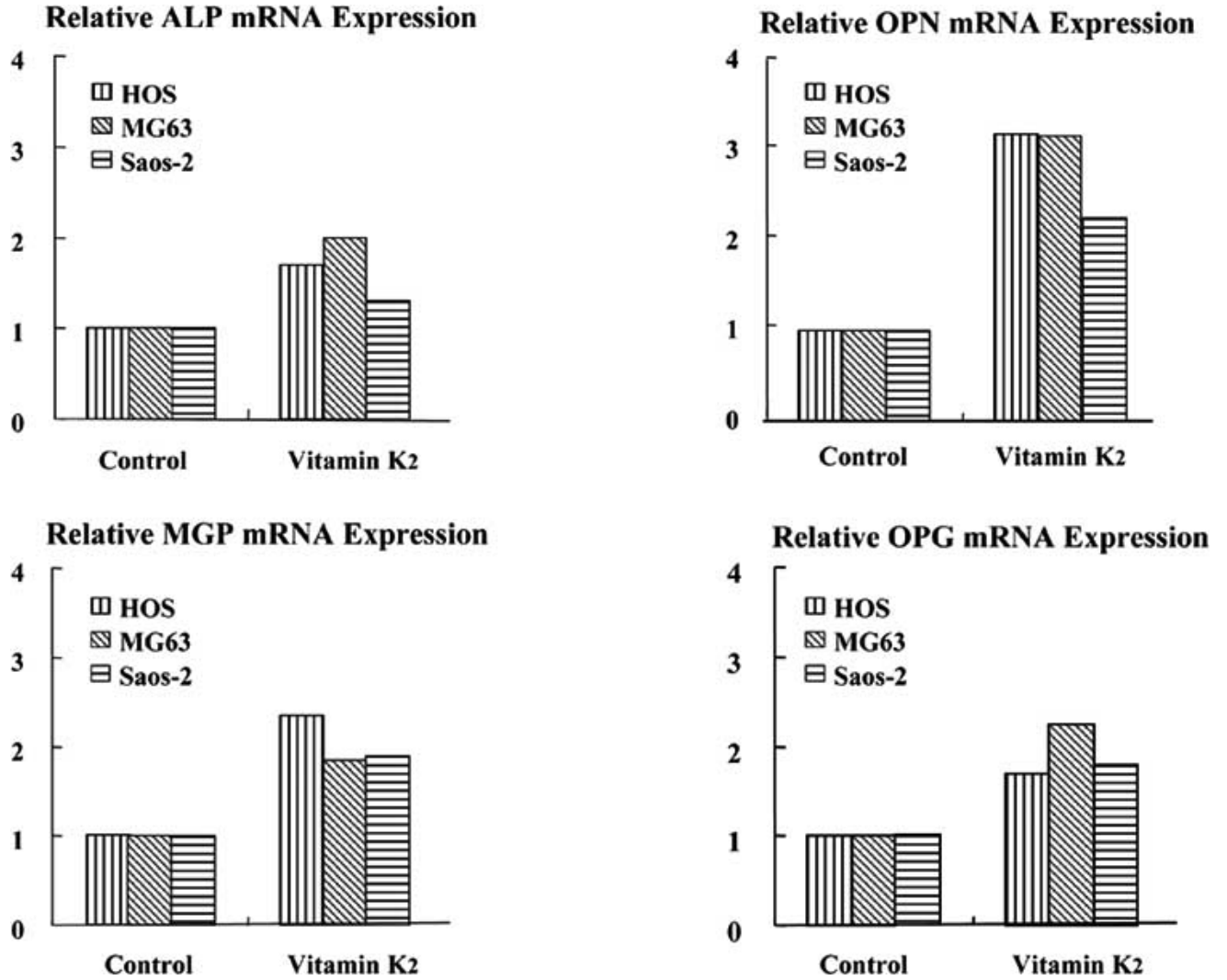
acts through steroid and xenobiotic receptors (SXR) to induce the expression of osteoblastic markers, such as alkaline phosphatase (ALP), osteoprotegerin, osteopontin, and matrix Gla protein (Fig. 2). Thus, the role of vitamin K<sub>2</sub> in the regulation of bone formation may involve both  $\gamma$ -carboxylation of OC and transcriptional regulation mediated by the SXR. The effect of vitamin K<sub>2</sub> on bone formation has been considered to be of interest in the treatment of osteoporosis.

**VITAMIN K DEFICIENCY, BMD AND FRACTURE RISK**

The associations among vitamin K deficiency, the risk of hip fractures, and the BMD in elderly people are summarized in Table 3 [23-30]. Vitamin K deficiency, as indicated by a low serum ucOC level or possibly a low ratio of serum carboxylated OC to serum total OC, may contribute to the risk of hip fractures in the elderly, and possibly to BMD loss in selected cohorts of elderly people (Fig. 3 and Table 3).

**EFFECTS OF VITAMIN K<sub>2</sub> TREATMENT ALONE ON POSTMENOPAUSAL OSTEOPOROSIS**

The effects of vitamin K<sub>2</sub> (menatetrenone) on the BMD and the incidence of vertebral fractures in postmenopausal or age-related osteoporosis (mainly postmenopausal osteoporosis) are summarized in Tables 4 and 5 [17,31-36]. Vitamin K<sub>2</sub> (menatetrenone) markedly increases the serum level of OC, sustains the lumbar BMD (Fig. 4) [17], and effectively prevents the occurrence of osteoporotic fractures (mainly vertebral fractures) in postmenopausal women with



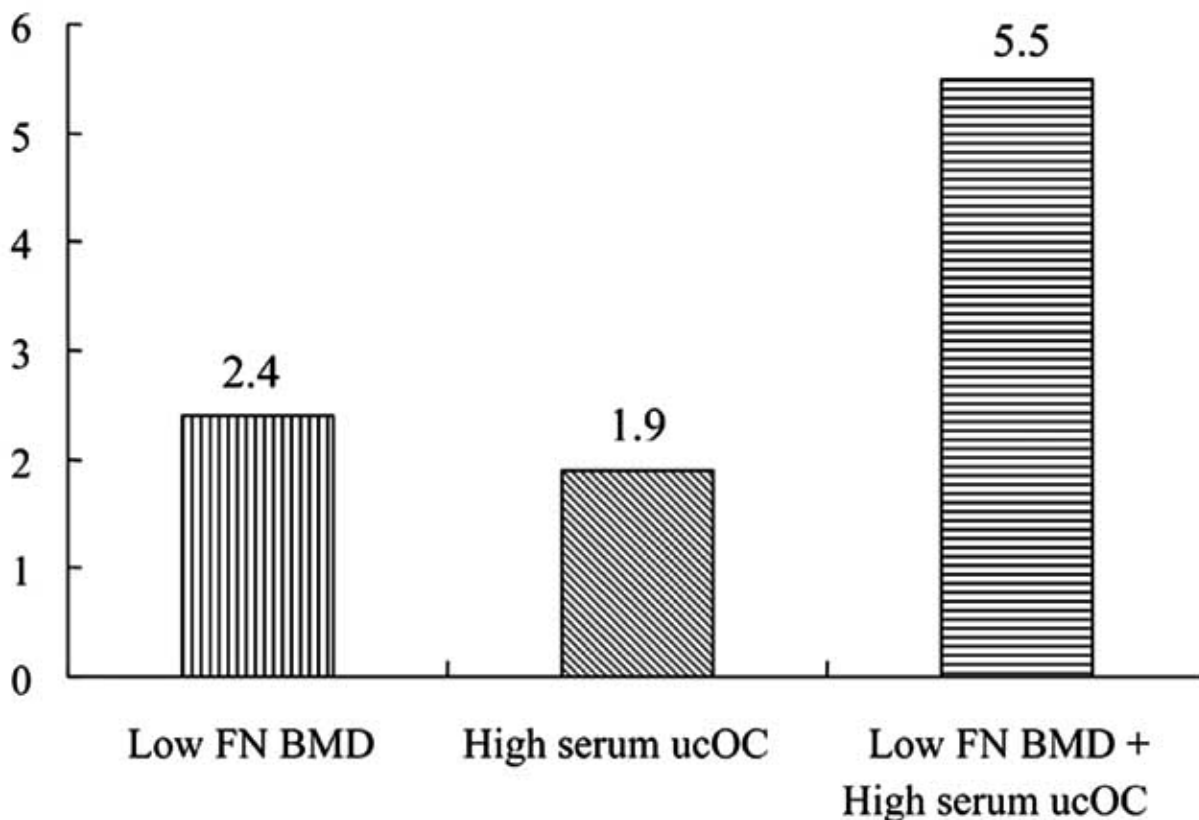
**Fig. (2).** Effects of vitamin K2 on osteoblastic marker genes in osteosarcoma cell lines [22].

Steroid and xenobiotic receptor (SXR) mRNA is expressed in osteosarcoma cell lines, and vitamin K2 induces the expression of the prototypical SXR target gene *CYP3A4* in these cells. Human osteosarcoma cell lines HOS, MG63, and Saos-2 were cultured in phenol red-free DMEM supplemented with 10% resin charcoal-stripped FBS in the presence or absence of ligands for 48 hours. Bone marker gene expression was then determined by quantitative real time RT-PCR. Data from quantitative real-time RT-PCR are shown as the mRNA expression levels of the marker genes normalized to the expression level of glyceraldehyde-3-phosphate dehydrogenase. Values represent the average of triplicates. Data from the control and 10- $\mu$ M vitamin K2-treatment are shown. Vitamin K2 is a transcriptional regulator of bone-specific genes that acts through SXR to induce the expression of osteoblastic markers, such as ALP, OPN, MGP, and OPG. ALP: alkaline phosphatase, OPN: osteopontin, MGP: matrix Gla protein, OPG: osteoprotegerin.

osteoporosis (Tables 4 and 5). Rapid conversion of ucOC to carboxylated OC following menatetrenone treatment was confirmed in elderly osteoporotic women with vertebral fractures (Fig. 5) [37]. While vitamin K2 may have only a

modest effect on BMD loss (Table 4), it may have the potential to prevent osteoporotic fractures (mainly vertebral fractures) (Table 5).

### Risk of hip fracture (Odd's ratio)



**Fig. (3).** Assessment of femoral neck BMD and serum ucOC level to predict the risk of hip fractures in elderly women [28].

Elderly women with both low femoral neck BMD and high serum ucOC level were at a higher risk for hip fractures (odd's ratio, 5.5) than elderly women with only a low femoral neck BMD (odd's ratio, 2.4) or a high serum ucOC level (odd's ratio, 1.9).

FN: femoral neck, BMD: bone mineral density. OC: osteocalcin, ucOC: undercarboxylated OC.

### EFFECTS OF VITAMIN K<sub>2</sub> TREATMENT IN COMBINATION WITH OTHER AGENTS ON POSTMENOPAUSAL OSTEOPOROSIS

#### Why does Combined Treatment Need to be Considered?

The efficacies of alendronate, risedronate, etidronate, raloxifene, and vitamin K<sub>2</sub> for postmenopausal osteoporosis have been demonstrated. However, except for PTH and strontium ranelate, all of the other currently available treatment options for the prevention of osteoporotic fractures are limited in scope, efficacy, and acceptability to patients [38]. The reduction in the risk of vertebral and hip fractures following treatment with the above agents is no more than 55%. Thus, considerable effort is being expended to develop newer and more effective treatments for postmenopausal osteoporosis and to refine/optimize existing treatments [38].

The novel drugs include an expanding array, including those that primarily inhibit osteoclastic bone resorption; osteogenic compounds, bisphosphonates, inhibitors of receptor activator of nuclear factor- $\kappa$ B ligand signaling, cathepsin K inhibitors, c-src kinase inhibitors, integrin inhibitors, and chloride channel inhibitors [38]. Intermittent

PTH treatment effectively prevents osteoporotic fractures [39]. Other osteoblast-targeted (anabolic) agents are also likely to be introduced; orally active PTH analogues, antagonists of the calcium sensing receptor, PTH-related peptide analogues, and/or agents that induce osteoblast anabolism *via* pathways involving key, recently identified, molecular targets (wnt low-density lipoprotein receptor-related protein-5 signaling, sclerostin and matrix extracellular phosphoglycoprotein) [38]. However, the anti-fracture efficacy of these novel treatments remains to be established through RCTs and/or meta-analysis studies.

Additive effects of the anti-resorptive agents, alendronate plus raloxifene have been reported. A one-year RCT assessed the effects of combined treatment with alendronate plus raloxifene in 331 postmenopausal women with osteoporosis (mean age: 63–64 years) [40]. Alendronate and raloxifene increased the lumbar and femoral neck BMD, and decreased the serum levels of OC and urinary levels of cross-linked C-terminal telopeptides of type I collagen in an additive and independent manner. That is, alendronate and raloxifene reduced the bone turnover more effectively than either drug alone, resulting in a greater BMD increment. However,

**Table 3. Vitamin K Deficiency, BMD and Fracture Risk**

Investigator (year reported)	Subjects of the study	Outcome
Booth (2000) [23]	335 elderly men and 553 elderly women (mean age: 75.2 years)	Individuals in the highest quartile of vitamin K intake (median: 254 µg/day) had a lower fully adjusted relative risk (0.35) of hip fractures than did those in the lower quartile of intake (median: 56 µg/day). However, no associations were found between the daily vitamin K intake and the BMD, or the apo E4 allele and the BMD.
Sato (2005) [24]	100 women (mean age: 79.8 years) with Alzheimer's disease	The body mass index, metacarpal BMD, and serum vitamin K1 levels were lower, and the serum undercarboxylated (uc) OC levels were higher, in the patients with severe dementia than in those with mild dementia. The serum vitamin K1 levels were negatively correlated with the serum uc OC levels.
Kaneki (2001) [25]	49 women (age range: 50–84 years) living in Tokyo (eastern Japan) and 25 women (age range: 51–66 years) living in Hiroshima (western Japan)	The serum MK-7 levels were higher in the frequent consumers of "natto" than in the infrequent consumers, and large "natto" intakes were associated with a marked, sustained increase in the serum MK-7 levels. Furthermore, the incidence of hip fractures in women was also correlated with the "natto" consumption level.
Szulc (1993) [26]	195 elderly women (age range: 70–101 years)	23% of the subject (45 women) had the serum ucOC levels above the upper limit of the normal range for young women. During an 18-month follow-up, 15 of these women sustained a hip fracture, and the baseline serum ucOC levels in these women were found to be higher than those in women who did not develop any hip fractures. The risk of hip fracture was increased in the women with the elevated serum ucOC levels (relative ratio: 5.9).
Szulc (1996) [27]	183 women (age range: 70–97 years)	Above findings were confirmed on a larger number of cases of hip fractures that occurred over 3 years in 183 women.
Vergnaud (1997) [28]	7598 healthy women over 75 years of age living independently	Elderly women with both a femoral neck BMD in the lowest quartile and the serum ucOC levels measured by enzyme-linked immunosorbent assay (ELISA) in the highest quartile were at a higher risk of hip fractures, with an odd's ratio of 5.5, as compared to those with only one of these two independent risk factors; <i>i.e.</i> those with only a low BMD had an odd's ratio of hip fractures of 2.4, and those with only a high serum ucOC level had an odd's ratio of hip fractures of 1.9 (Fig. 4).
Luukinen (2000) [29]	792 home-dwelling persons over 70 years of age	The adjusted relative risk of fractures was elevated in subjects with a low serum level of carboxylated OC (hazard ratio: 2.00) and a low carboxylated OC/total OC ratio (hazard ratio: 5.32). The multivariable-adjusted relative risk of hip fractures (n=26) in relation to a low carboxylated OC/total OC ratio was 3.49, as compared to persons without any hip fracture.
Feskanich (1999) [30]	72327 women (age range: 38–63 years)	During a 10-year follow-up, 270 hip fractures were reported; women in the 2nd to 5th quintiles for vitamin K intake had a significantly lower age-adjusted relative risk (relative risk: 0.70) of hip fractures than the women in the lowest quintile.

BMD: bone mineral density, OC: osteocalcin, ucOC: undercarboxylated osteocalcin.

whether this difference would also translate into a better fracture risk reduction remains uncertain, and over suppression of bone turnover by combined use of potent anti-resorptive agents may be a concern. Because anti-resorptive agents, such as alendronate, risendronate, etidronate, and raloxifene and anabolic agents such as vitamin K2 have different actions on bone metabolism, combined treatment with vitamin K2 and anti-resorptive agents may be expected to be potentially more effective for preventing osteoporotic fractures. Although the concurrent use of alendronate may reduce the anabolic effects of PTH in postmenopausal women with osteoporosis [41], there is some evidence to suggest the efficacy of combined treatment with vitamin K2 and anti-resorptive agents in the treatment of postmenopausal osteoporosis.

### Combined Treatment with Vitamin K2 and Vitamin D3

No studies have shown the effect of vitamin K2 and native vitamin D/calcium on postmenopausal osteoporosis.

However, a few studies have demonstrated the effect of combined treatment with vitamin K2 (menatetrenone) and active vitamin D3 (alfacalcidol) on postmenopausal osteoporosis [32,35,42]. This combined treatment seems to have an additive effect on the lumbar BMD in early postmenopausal women with osteopenia/osteoporosis (mean age: 52.8–54.1 years) [35] or relatively late postmenopausal women with osteoporosis (mean age: 64.0 years) [32], but may increase bone resorption, resulting in a decrease in the lumbar BMD and an increase in the incidence of vertebral fractures in elderly patients with osteoporosis [42]. Thus, the efficacy of combined treatment with menatetrenone and alfacalcidol for osteoporosis is controversial. However, it is likely that the effect on the lumbar BMD is most pronounced in studies in which the mean age and years since menopause of the subjects were low and the degree of osteoporosis was mild. That is, combined treatment with vitamin K2 and vitamin D3 may be effective for mild osteoporosis in early postmenopausal women. Further

**Table 4.** Effect of Vitamin K<sub>2</sub> (Menatetrenone) on BMD in Postmenopausal or Age-Related Osteoporosis

Investigator (year reported)	Intervention	Groups	Duration	BMD
Orimo (1992) [31]	Menatetrenon (45 mg/day) (n=272) Alfacalcidol (1 µg/day) (n=274)	48 weeks	Metacarpus	-0.6% -1.5%
Shiraki (2000) [17]	Menatetrenon (45 mg/day) (n=86) Control (calcium 150 mg/day) (n=94)	24 months	Lumbar spine	-0.5% -3.3%
Iwamoto (2000) [32]	Menatetrenone (45 mg/day) (n=22) Control (calcium lactate 2 g/day) (n=20)	24 months	Lumbar spine	-0.5% -3.3%
Iwamoto (2001) [33]	Menatetrenon (45 mg/day) (n=23) Control (calcium lactate 2 g/day) (n=24)	24 months	Forearm (ultradistal)	+0.9% -0.8%
Ozuru (2002) [34]	Menatetrenon (45 mg/day) (n=34)	48 weeks	Lumbar spine	No change
Ushiroyama* (2002) [35]	Menatetrenon (45 mg/day) (n=30) Control (diet therapy) (n=33)	24 months	Lumbar spine	+1.4% -4.1%
Ishida (2004) [36]	Menatetrenon (45 mg/day) (n=63) Control (no treatment) (n=60)	24 months	Forearm (distal third)	-1.9% -3.3%

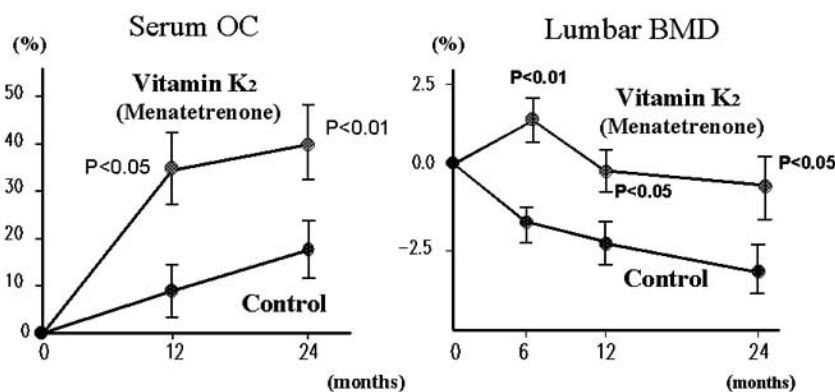
\*Subjects with both osteoporosis and osteopenia were included. BMD: bone mineral density.

studies are needed to establish the efficacy of the combination in the treatment of postmenopausal osteoporosis.

#### Combined Treatment with Vitamin K<sub>2</sub> and Bisphosphonates

Iwamoto *et al.* [43] examined the effects of combined treatment with vitamin K<sub>2</sub> (menatetrenone) and the bisphosphonate, etidronate, in 100 postmenopausal women with osteoporosis, to investigate the additive beneficial effects of two classes of drugs in postmenopausal women with osteoporosis (mean age: 65.5 years; range: 53–78 years). The forearm (ultradistal) BMD decreased from the baseline after 24 months of treatment with calcium ( $P < 0.05$  by one-way ANOVA with repeated measurements), was sustained by treatment with menatetrenone, and increased to a similar

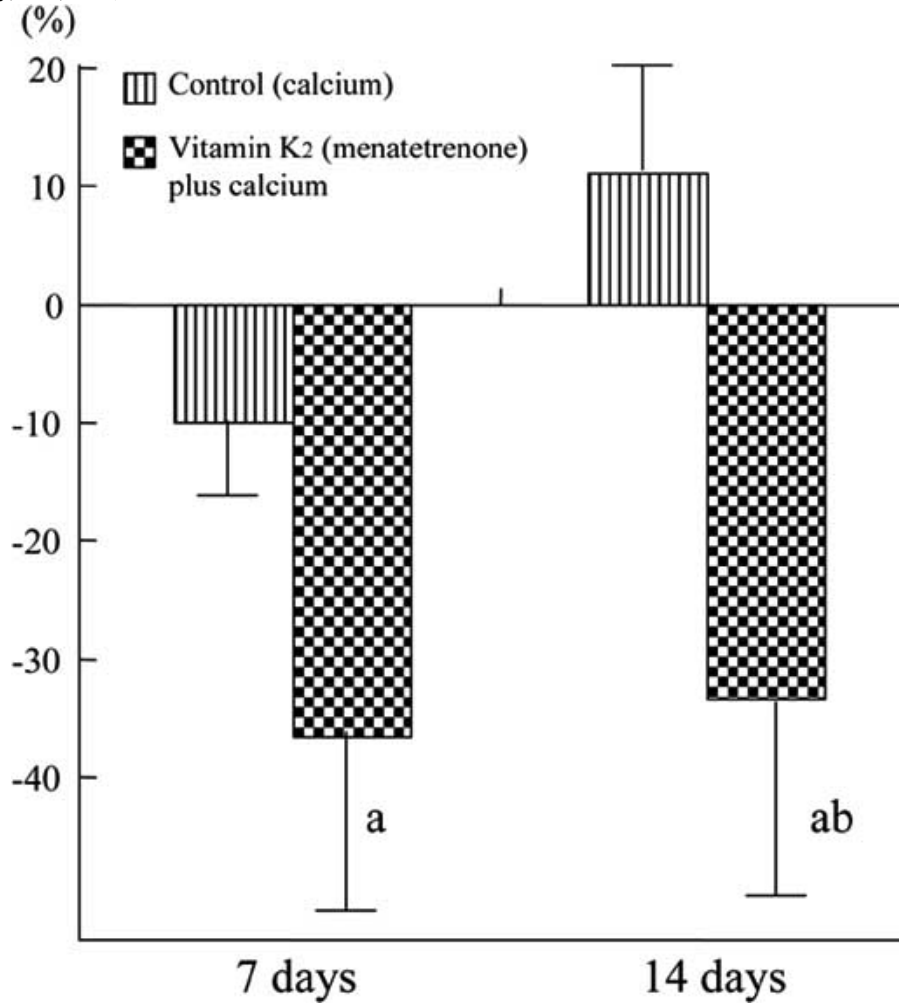
extent following treatment with menatetrenone plus etidronate or etidronate alone (one-way ANOVA with repeated measurements). After 24 months of treatment, the incidence of vertebral fractures was reduced in patients receiving menatetrenone or etidronate as compared with that in patients receiving calcium, while it was even more markedly reduced in patients receiving menatetrenone plus etidronate (Fig. 6). Although further studies on larger numbers of subjects are needed, the results of our study suggest that cyclical etidronate combined with menatetrenone might be more effective than cyclical etidronate alone for preventing vertebral fractures in postmenopausal women with osteoporosis. These results are also supported by the results of an experimental study; Ito [44] clearly demonstrated that while risedronate prevented the deterioration in the connectivity of the trabeculae in the proximal tibial



**Fig. (4).** Effect of vitamin K<sub>2</sub> (menatetrenone) on the lumbar BMD and serum OC level in postmenopausal women with osteoporosis [17].

The serum OC level markedly increased in the vitamin K<sub>2</sub>-treated group as compared with that in the control group. The lumbar BMD was sustained in the vitamin K<sub>2</sub> (menatetrenone)-treated group and decreased in the control group. The mean percent changes in the lumbar BMD from the baseline at 6, 12, and 24 months after the start of treatment were -1.8 %, -2.4 %, and -3.3 %, respectively in the control group (calcium, 150 mg/day), and +1.4 %, -0.1 %, and -0.5 %, respectively in the menatetrenone group (45 mg/day). The changes in the serum OC level and lumbar BMD at each time-point were significantly different between the control group and the menatetrenone group (serum OC:  $P < 0.05$  at 12 months and  $P < 0.01$  at 24 months; lumbar BMD:  $P < 0.01$  at 6 months and  $P < 0.05$  at 12 and 24 months, by analysis of variance [ANOVA] with Fisher's PLSD test).

BMD: bone mineral density, OC: osteocalcin.



**Fig. (5).** Percentage changes in the ucOC level from the baseline in elderly osteoporotic women treated with vitamin K2 (menatetrenone) plus calcium or calcium alone [37].

Twenty osteoporotic women with vertebral fractures were randomly divided into two groups: the menatetrenone plus calcium treatment group (n=10) and the calcium alone (control) group (n=10). The duration of the treatment was 14 days. Data are presented as the means and SE. a: P<0.05 vs. baseline, b: P<0.01 vs. control by the paired or unpaired t-test. A significant reduction in the serum ucOC level from the baseline was observed 7 and 14 days after the start of treatment in the menatetrenone-treated group, and a significant reduction in the serum ucOC level as compared with the control group was observed 14 days after the start of the treatment in the menatetrenone-treated group.

ucOC: undercarboxylated osteocalcin.

metaphysis in ovariectomized rats, and vitamin K2 increased the trabecular thickness, combined treatment with risedronate and vitamin K2 had an additive effect in preventing the deterioration of the trabecular bone architecture in ovariectomized rats. Thus, combined treatment with vitamin K2 and bisphosphonates may be useful to prevent vertebral fractures in postmenopausal women with osteoporosis.

**Combined Treatment with Vitamin K2 and Raloxifene**

No clinical studies have yet been reported on the effects of combined treatment with vitamin K2 and raloxifene in postmenopausal women with osteoporosis, with the exception of only one preclinical study which demonstrated the efficacy of this combination therapy. Iwamoto *et al.* [45] demonstrated the skeletal effects of vitamin K2 plus

raloxifene in ovariectomized rats. Vitamin K2 increased bone formation, whereas raloxifene reduced the bone turnover, as did vitamin K2 plus raloxifene. Raloxifene, but not vitamin K2, prevented ovariectomy-induced bone loss in the distal femoral and proximal tibial metaphyses, as did the vitamin K2 plus raloxifene combination. No significant beneficial effect of either raloxifene or vitamin K2 was observed on the femoral neck bone strength; however, the femoral neck strength was greater in the vitamin K2 plus raloxifene group than in the sham-operated control group. Raloxifene and vitamin K2 had complementary effects on bone resorption and formation activities, respectively, resulting in a significant improvement of the femoral neck bone strength. These rat data suggest interesting therapeutic possibilities that would require clinical verification though RCTs.

**Table 5. Effect of Vitamin K<sub>2</sub> (Menatetrenone) on the Incidence of Vertebral Fractures in Postmenopausal Women with Osteoporosis**

Investigator (year)	Groups	Duration	Incidence of vertebral fractures	Relative risk (95% CI)
Shiraki (2000) [17]	Menatetrenon (45 mg/day) (n=91) Control (calcium 150 mg/day) (n=99)	24 months	14.3% 30.3%	0.47 (0.26-0.85)*
Iwamoto (2001) [32]	Menatetrenon (45 mg/day) (n=23) Control (calcium 2 g/day) (n=24)	24 months	8.7% 25.0%	0.35 (0.08-1.55)*
Ishida (2004) [36]	Menatetrenon (45 mg/day) (n=63) Control (no treatment) (n=60)	24 months	14.3% 28.3%	0.53 (0.25-1.10)

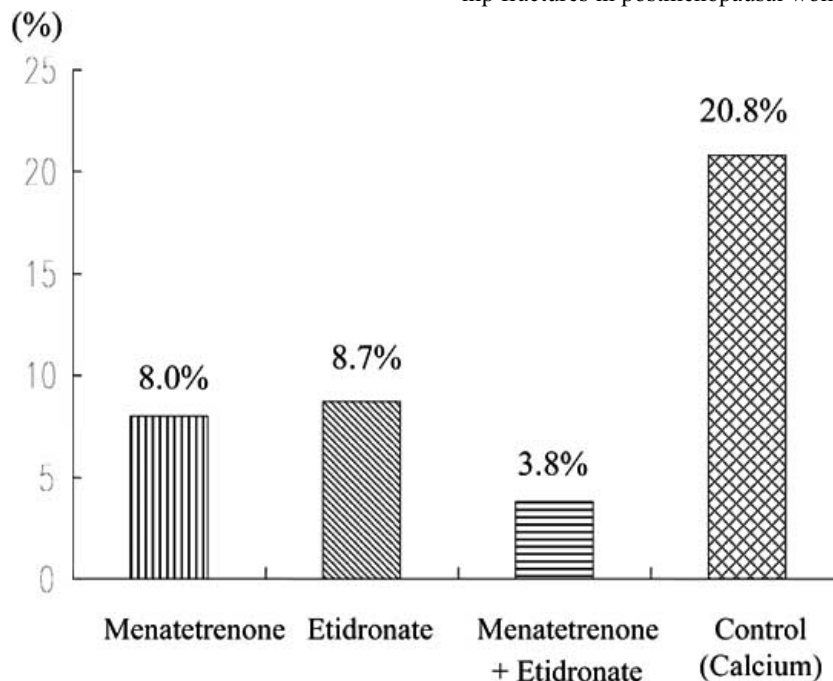
\*Statistically not significant.

**LIMITATIONS**

We have demonstrated the efficacy of vitamin K<sub>2</sub> alone or in combination with bisphosphonates or raloxifene for postmenopausal osteoporosis. However, most of the studies have been conducted on small and selected cohorts, and the results may not hold out for other populations, in particular vitamin K-replete populations. Furthermore, the efficacy of vitamin K<sub>2</sub> in reducing the incidence of vertebral and nonvertebral fractures has not necessarily been established. Thus, further studies are needed to confirm not only the effect of vitamin K<sub>2</sub> alone, but also the combined effect of vitamin K<sub>2</sub> and bisphosphonates or raloxifene on vertebral, nonvertebral, and hip fractures in postmenopausal women with osteoporosis.

**CONCLUSIONS**

The role of vitamin K<sub>2</sub> in the treatment of postmenopausal osteoporosis has been discussed. Alendronate and risedronate, rather than vitamin K<sub>2</sub>, should be initial drugs of choice for the treatment of postmenopausal osteoporosis, because they have been shown to be the most efficacious among the current range of commercially available agents at reducing the incidence of both vertebral and hip fractures. Combined treatment with a bisphosphonate and vitamin K<sub>2</sub> appears to be more effective than single-agent treatment with a bisphosphonate for the prevention of vertebral fractures. On the other hand, the results of a preclinical study do suggest the possible efficacy of this drug combination for the prevention of vertebral and hip fractures in postmenopausal women, although no clinical



**Fig. (6).** Incidence of vertebral fractures in postmenopausal women with osteoporosis treated with vitamin K<sub>2</sub> (menatetrenone), etidronate, menatetrenone plus etidronate, or calcium for 24 months [42].

During a 24-month period, a vertebral fracture occurred in two patients treated with menatetrenone (n=23) or etidronate (n=25), one patient treated with menatetrenone plus etidronate (n=26), and six patients treated with calcium (n=24). The incidence of vertebral fractures was 8.0 % in patient treated with menatetrenone, 8.7 % in patients treated with etidronate, 3.8 % in patients treated with menatetrenone plus etidronate, and 20.8 % in patients treated with calcium. The incidence of vertebral fractures was similarly reduced in patients treated with menatetrenone or etidronate as compared with that in patients treated with calcium, and markedly reduced by treatment with menatetrenone plus etidronate. Further studies on large numbers of patients are needed to confirm these results.

studies have reported on the effects of combined treatment with vitamin K<sub>2</sub> and raloxifene in postmenopausal women with osteoporosis. Furthermore, vitamin K deficiency has been reported to contribute to the occurrence of hip fractures in elderly women. Thus, we propose that the important role of vitamin K<sub>2</sub> used in combination with bisphosphonates or raloxifene should not be underestimated in the prevention of fractures in postmenopausal women with osteoporosis especially with vitamin K deficiency.

## REFERENCES

- [1] Ensrud KE, Thompson DE, Cauley JA, *et al.* Prevalent vertebral deformities predict mortality and hospitalization in older women with low bone mass. *J Am Geriatr Soc* 2000; 48: 241-9.
- [2] Kanis JA, Oden A, Johnell O, DeLaet C, Jonsson B, Oglesby AK. The components of excess mortality after hip fracture. *Bone* 2003; 32: 468-73.
- [3] Cranney A, Guyatt G, Griffith L, Wells G, Tugwell P, Rosen C. Meta-analyses of therapies for postmenopausal osteoporosis. IX: Summary of meta-analyses of therapies for postmenopausal osteoporosis. *Endocr Rev* 2002; 23: 570-8.
- [4] Wehren LE, Hosking D, Hochberg MC. Putting evidence-based medicine into clinical practice: comparing anti-resorptive agents for the treatment of osteoporosis. *Curr Med Res Opin* 2004; 20: 525-31.
- [5] McClung MR, Geusens P, Miller PD, *et al.* Hip Intervention Program Study Group: Effect of risedronate on the risk of hip fracture in elderly women. Hip Intervention Program Study Group. *N Engl J Med* 2001; 344: 333-40.
- [6] Black DM, Cummings SR, Karpf DB, *et al.* Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. *Lancet* 1996; 348: 1535-41.
- [7] Neer RM, Arnaud CD, Zanchetta JR, *et al.* Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* 2001; 344: 1434-41.
- [8] Meunier PJ, Roux C, Seeman E, *et al.* The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N Engl J Med* 2004; 350: 459-68.
- [9] Reginster JY, Seeman E, De Vernejoul MC, *et al.* Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. *J Clin Endocrinol Metab* 2005; 90: 2816-22.
- [10] Chesnut III CH, Skag A, Christiansen C, *et al.* Oral Ibandronate Osteoporosis Vertebral Fracture Trial in North America and Europe (BONE). Effects of oral ibandronate administered daily or intermittently on fracture risk in postmenopausal osteoporosis. *J Bone Miner Res* 2004; 19: 1241-9.
- [11] Delmas PD, Recker RR, Chesnut CH 3rd, *et al.* Daily and intermittent oral ibandronate normalize bone turnover and provide significant reduction in vertebral fracture risk: results from the BONE study. *Osteoporos Int* 2004; 15: 792-8.
- [12] Papapoulos SE, Quandt SA, Liberman UA, Hochberg MC, Thompson DE. Meta-analysis of the efficacy of alendronate for the prevention of hip fractures in postmenopausal women. *Osteoporos Int* 2005; 16: 468-74.
- [13] Sambrook PN, Geusens P, Ribot C, *et al.* Alendronate produces greater effects than raloxifene on bone density and bone turnover in postmenopausal women with low bone density: results of EFFECT (Efficacy of FOSAMAX versus EVISTA Comparison Trial) International. *J Intern Med* 2004; 255: 503-11.
- [14] Bone HG, Hosking D, Devogelaer JP, *et al.* Alendronate Phase III Osteoporosis Treatment Study Group. Ten years' experience with alendronate for osteoporosis in postmenopausal women. *N Engl J Med* 2004; 350: 1189-99.
- [15] Mellstrom DD, Sorensen OH, Goemaere S, Roux C, Johnson TD, Chines AA. Seven years of treatment with risedronate in women with postmenopausal osteoporosis. *Calcif Tissue Int* 2004; 75: 462-8.
- [16] The WHO Scientific Group: Prevention and management of osteoporosis. *World Health Organ Tech Rep Ser* 2003; 921: 86-109.
- [17] Shiraki M, Shiraki Y, Aoki C, Miura M. Vitamin K<sub>2</sub> (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. *J Bone Miner Res* 2000; 15: 515-21.
- [18] Hauschka PV, Lian JB, Cole DEC, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiol Rev* 1989; 69: 990-1047.
- [19] Koshihara Y, Hoshi K. Vitamin K<sub>2</sub> enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts *in vitro*. *J Bone Miner Res* 1997; 12: 431-8.
- [20] Shearer MJ. Vitamin K. *Lancet* 1995; 345: 229-34.
- [21] Vermeer C, Jie KSG, Knapen MHJ. Role of vitamin K in bone metabolism. *Annu Rev Nutr* 1995; 15: 1-22.
- [22] Tabb MM, Sun A, Zhou C, *et al.* Vitamin K<sub>2</sub> regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *J Biol Chem* 2003; 278: 43919-27.
- [23] Booth SL, Tucker KL, Chen H, *et al.* Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr* 2000; 71: 1201-8.
- [24] Sato Y, Honda Y, Hayashida N, Iwamoto J, Kanoko T, Satoh K. Vitamin K deficiency and osteopenia in elderly women with Alzheimer's disease. *Arch Phys Med Rehabil* 2005; 86: 576-81.
- [25] Kaneki M, Hedges SJ, Hosoi T, *et al.* Japanese fermented soybean food as the major determinant of the large geographic difference in circulating levels of vitamin K<sub>2</sub>: possible implications for hip-fracture risk. *Nutrition* 2001; 17: 315-21.
- [26] Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J Clin Invest* 1993; 91: 1769-74.
- [27] Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture: a three year follow-up study. *Bone* 1996; 18: 487-8.
- [28] Vergnaud P, Garnero P, Meunier PJ, Breart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. *J Clin Endocrinol Metab* 1997; 82: 719-24.
- [29] Luukinen H, Kakonen SM, Pettersson K, *et al.* Strong prediction of fractures among older adults by the ratio of carboxylated to total serum osteocalcin. *J Bone Miner Res* 2000; 15: 2473-8.
- [30] Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr* 1999; 69: 74-9.
- [31] Orimo H, Fujita T, Onomura T, Inoue T, Kushida K, Shiraki M. Clinical evaluation of Ea-0167 (menatetrenone) in the treatment of osteoporosis. *Clin Eval* 1992; 20: 45-100 (in Japanese).
- [32] Iwamoto J, Takeda T, Ichimura S. Effect of combined administration of vitamin D<sub>3</sub> and vitamin K<sub>2</sub> on bone mineral density of the lumbar spine in postmenopausal women with osteoporosis. *J Orthop Sci* 2000; 5: 546-51.
- [33] Iwamoto J, Takeda T, Ichimura S. Effect of menatetrenone on bone mineral density and incidence of vertebral fractures in postmenopausal women with osteoporosis: a comparison with the effect of etidronate. *J Orthop Sci* 2001; 6: 487-92.
- [34] Ozuru R, Sugimoto T, Yamaguchi T, Chihara K. Time-dependent effects of vitamin K<sub>2</sub> (menatetrenone) on bone metabolism in postmenopausal women. *Endocr J* 2002; 49: 363-70.
- [35] Ushiroyama T, Ikeda A, Ueki M. Effect of continuous combined therapy with vitamin K<sub>2</sub> and vitamin D<sub>3</sub> on bone mineral density and coagulofibrinolysis function in postmenopausal women. *Maturitas* 2002; 41: 211-21.
- [36] Ishida Y, Kawai S. Comparative efficacy of hormone replacement therapy, etidronate, calcitonin, alfacalcidol, and vitamin K in postmenopausal women with osteoporosis: The Yamaguchi Osteoporosis Prevention Study. *Am J Med* 2004; 117: 549-55.
- [37] Miki T, Nakatsuka K, Naka H, *et al.* Vitamin K<sub>2</sub> (menaquinone 4) reduces serum undercarboxylated osteocalcin level as early as 2 weeks in elderly women with established osteoporosis. *J Bone Miner Metab* 2003; 21: 161-5.
- [38] Grey A, Reid IR. Emerging and potential therapies for osteoporosis. *Expert Opin Investig Drugs* 2005; 14: 265-78.

- [39] Neer RM, Arnaud CD, Zanchetta JR, *et al.* Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med* 2001; 344: 1434-41.
- [40] Johnell O, Scheele WH, Lu Y, Reginster JY, Need AG, Seeman E. Additive effects of raloxifene and alendronate on bone density and biochemical markers of bone remodeling in postmenopausal women with osteoporosis. *J Clin Endocrinol Metab* 2002; 87: 985-92.
- [41] Black DM, Greenspan SL, Ensrud KE, *et al.*; PaTH Study Investigators. The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. *N Engl J Med* 2003; 349: 1207-15.
- [42] Kobayashi S, Takaoka K, Shiraki M. Therapy. EHDP+vitamin D<sub>3</sub> or vitamin K<sub>2</sub>. *Clinical calcium* 2002; 12: 950-4 (in Japanese).
- [43] Iwamoto J, Takeda T, Ichimura S. Combined treatment with vitamin K<sub>2</sub> and bisphosphonate in postmenopausal women with osteoporosis. *Yonsei Med J* 2003; 44: 751-6.
- [44] Ito M. Bone mass, microstructure, and quality related with bone strength. – The effects of antiresorptive agents – *J Jpn Soc Bone Morphom* 2000; 12: 51-4 (in Japanese).
- [45] Iwamoto J, Yeh JK, Schmidt A, *et al.* Raloxifene and vitamin K<sub>2</sub> combine to improve the femoral neck strength of ovariectomized rats. *Calcif Tissue Int* 2005 (in press).

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