Effect of combined administration of vitamin D_3 and vitamin K_2 on bone mineral density of the lumbar spine in postmenopausal women with osteoporosis

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Abstract The effect of the combined administration of vitamin D_3 and vitamin K_2 on bone mineral density (BMD) of the lumbar spine was examined in postmenopausal women with osteoporosis. Ninety-two osteoporotic women who were more than 5 years after menopause, aged 55–81 years, were randomly divided into four administration groups: vitamin D_3 (1α hydroxyvitamin D_3, $0.75\mu g/day$) (D group; $n = 29$), vitamin K_2 (menatetrenone, $45mg/day$) (K group; $n = 22$), vitamin D_3 plus vitamin K_2 (DK group, $n = 21$), and calcium (calcium lactate, $2g/day$) (C group; $n = 20$). BMD of the lumbar spine (L2–L4) was measured by dual energy X-ray absorptiometry at 0, 1, and 2 years after the treatment started. There were no significant differences in age, body mass index, years since menopause, and initial BMD among the four groups. One-way analysis of variance (ANOVA) with repeated measurements showed a significant decrease in BMD in the C group ($P < 0.001$). Two-way ANOVA with repeated measurements showed a significant increase in BMD in the D and K groups compared with that in the C group ($P < 0.05$ and $P < 0.001$, respectively), and a significant increase in BMD in the DK group compared with that in the C, D, and K groups ($P < 0.0001$, $P < 0.05$ and $P < 0.01$, respectively). These findings indicate that combined administration of vitamin D_3 and vitamin K_2, compared with calcium administration, appears to be useful in increasing the BMD of the lumbar spine in postmenopausal women with osteoporosis.

Key words Vitamin D_3 · Vitamin K_2 · Postmenopausal women · Osteoporosis · Bone mineral density (BMD)

Introduction

Osteoporosis is a major public health problem, and is characterized by low bone mass and increased risk of fractures. Marked bone loss is observed, with accelerated bone remodeling after menopause in women. The rate of bone loss in untreated postmenopausal women 3–5 years after menopause is 4.5% per year of spinal trabecular mineral density,[7] and bone loss declines thereafter. Because of the event of menopause, osteoporosis primarily affects postmenopausal women.

When bone mineral density (BMD) decreases below the level of the fracture threshold, osteoporotic fractures, such as vertebral fractures, can actually occur without evidence of a fall.[5] Multiple vertebral fractures not only cause back pain but also produce a round back, which decreases the volume in the thoracic and abdominal cavities, potentially resulting in multiple organ dysfunction. Therefore, even vertebral fractures can be one of the factors in the reduction of life span. Thus, it is important not only to prevent falls but also to prevent loss of vertebral BMD, or to increase vertebral BMD to a level above the fracture threshold to reduce vertebral fractures in people with osteoporosis. One therapeutic approach should focus on preventing loss of vertebral BMD.

In Japan, it is generally recognized that vitamin D_3 or vitamin K_2 treatment can prevent BMD loss of the lumbar spine in postmenopausal women with osteoporosis.[21–23] However, clinically, the effect of combined administration of vitamin D_3 and vitamin K_2 on the BMD of the lumbar spine has not been established. The purpose of the present study was to examine the effect of the combined administration of vitamin D_3 and vitamin K_2 on BMD of the lumbar spine in postmenopausal women with osteoporosis.

Subjects and methods

Subjects

One hundred and eighty-seven women who were more than 5 years after menopause were diagnosed with oste-

oporosis,[24] between July 1993 and April 1998 in our clinic. All of them had randomly received some therapeutic interventions, such as vitamin D_3, vitamin K_2, calcitonin, etidronate, and/or calcium administration, and/or exercise, and 92 women, aged 55–81 years, completed the present trial for 2 years. They were randomly divided into four administration groups: vitamin D_3 (1α hydroxyvitamin D_3, 0.75 µg/day) administration (D group; $n = 29$), vitamin K_2 (menatetrenone, 45 mg/day) administration (K group; $n = 22$), vitamin D_3 plus vitamin K_2 administration (DK group; $n = 21$), and calcium (calcium lactate, 2 g/day) administration (C group; $n = 20$). Preliminary screening included a medical history, physical examination, blood biochemical studies, plain X-ray examination of the lumbar spine, and BMD measurement of the lumbar spine. Biochemical studies were performed by standard automated laboratory techniques. BMD of the lumbar spine was measured as described below. None of the subjects had a history of hormone (estrogen) replacement therapy or had ever taken medication that affects bone metabolism prior to the present trial. None of the subjects had engaged in sporting activity for at least the most recent 5 years before the trial or during the present trial. Plain X-ray examinations of the lumbar spine in these women did not find any evidence of vertebral fractures or marked deformation. Their serum calcium, phosphorus, and alkaline phosphatase levels were all within normal limits. Informed consent was obtained from all participants.

Diet evaluation, and calcium and vitamin D supplementation

All subjects completed 7-day food records during the initial screening, according to the instructions of a registered dietitian. After the initial dietary assessment, all subjects were encouraged to have 1000 mg of calcium and 400 IU of vitamin D daily through food, and encouragement was given by the dietitian every 3 months.

Measurement of BMD of lumbar spine

BMD of the lumbar spine (L2–L4) in the anteroposterior view was measured by dual energy X-ray absorptiometry (DXA). An XR-26 instrument (Norland, Fort Atkinson, WI, USA) was used between July 1993 and January 1996, and an XR-36 instrument (Norland) was used between February 1996 and April 1998. The coefficient of variation (100 × SD/mean) of five measurements each time with repositioning within 72 h for both instruments was less than 1.2% in three persons. The coefficient of variation of two measurements using the two instruments (Norland XR-26 and XR-36) in five persons was less than 1.1%. BMD was assessed at 0, 1, and 2 years after the treatment started.

Statistical analysis

Data values are expressed as means ± SE in the Table and Figures. Analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) test was used to compare differences in baseline characteristics and percent changes in BMD at 1 and 2 years among the four groups. One-way ANOVA with repeated measurements was used to examine the significance of longitudinal changes in BMD in the C group. Two-way ANOVA with repeated measurements was used to compare longitudinal changes in BMD among the four groups. A significance level of $P < 0.05$ was used for all comparisons.

Results

Characteristics of subjects

Table 1 shows the baseline characteristics of the study subjects in the D, K, DK, and C groups. Their respective mean ages were 63.4, 65.8, 63.6, and 63.5 years. There were no significant differences in mean age; height; body weight; body mass index; years since menopause; serum calcium, phosphorus and alkaline phosphatase levels; and daily calcium intake among the four groups. The respective mean initial BMD of the lumbar spine was 0.697, 0.682, 0.677, and 0.691 g/cm.[2] There were no significant differences in initial BMD of the lumbar spine among the four groups.

Changes in BMD of lumbar spine

Figure 1 shows the percent changes in BMD of the lumbar spine at 1 and 2 years in the D, K, DK, and C groups. Figure 2 shows the longitudinal percent changes in BMD of the lumbar spine in the D, K, DK, and C groups. In the C group, the mean percent changes in BMD were −0.53% at 1 year and −0.79% at 2 years compared with the baseline, and these longitudinal changes were significant ($P < 0.001$; one-way ANOVA). The corresponding changes were −0.44% and +0.38% in the D group, and −0.20% and +0.90% in the K group, and the changes at 2 years in both these groups were significant compared with those in the C group (both $P < 0.05$, ANOVA Fisher's PLSD). Two-way ANOVA with repeated measurements showed that the longitudinal changes in BMD in the D and K groups were significant compared with those in the C group ($P < 0.05$ and $P < 0.001$, respectively). In the DK group,

Table 1. Characteristics of subjects

	D (n = 29)	K (n = 22)	DK (n = 21)	C (n = 20)
Age (years)	63.4 ± 0.8	65.8 ± 1.2	63.6 ± 1.0	63.5 ± 1.5
Height (m)	1.53 ± 0.01	1.52 ± 0.01	1.53 ± 0.01	1.54 ± 0.02
Body weight (kg)	48.8 ± 0.9	49.4 ± 1.8	51.4 ± 1.1	50.3 ± 1.9
Body mass index (kg/m^2)	2.08 ± 0.3	21.5 ± 0.8	22.1 ± 0.4	21.0 ± 0.6
Years since menopause	14.8 ± 0.9	16.0 ± 1.4	15.0 ± 1.2	14.7 ± 1.4
Serum calcium (mg/dl)	9.3 ± 0.1	9.4 ± 0.1	9.3 ± 0.1	9.2 ± 0.1
Serum phosphorus (mg/dl)	3.4 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.4 ± 0.1
Serum ALP (IU/I)	224.3 ± 12.4	221.5 ± 10.3	232.5 ± 8.9	215.4 ± 9.4
Calcium intake (mg/day)	505.0 ± 20.1	495.1 ± 26.0	476.6 ± 31.1	495.3 ± 32.0
BMD of lumbar spine (g/cm^2)	0.697 ± 0.009	0.682 ± 0.018	0.677 ± 0.028	0.691 ± 0.018

Data values are expressed as means ± SE. Analysis of variance (ANOVA) with Fisher's PLSD test was used to compare differences in baseline characteristics among the D, K, DK, and C groups. There were no significant differences in mean age; height; body weight; body mass index; years since menopause; serum calcium, phosphorus, and alkaline phosphatase (ALP) levels; and initial bone mineral density (BMD) of the lumbar spine among the four groups

D, Vitamin D_3 (1α-hydroxyvitamin D_3, 0.75 μg/day) administration; K, vitamin K_2 (menatetrenone, 45 mg/day) administration; DK, vitamin D_3 plus vitamin K_2 administration; C, calcium (calcium lactate, 2 g/day) administration
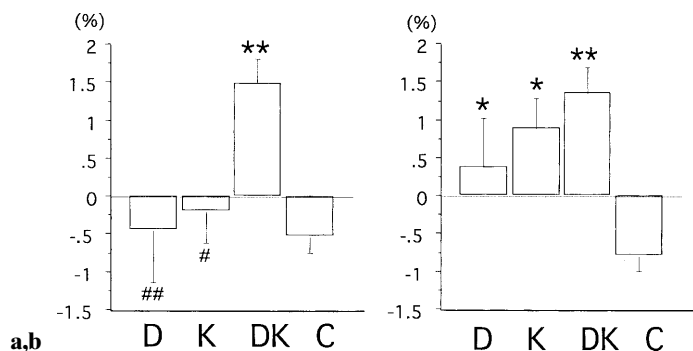

Fig. 1a,b. Percent changes in bone mineral density (BMD) of lumbar spine **a** at 1 year and **b** at 2 years. Data values are expressed as means ± SE. Analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) test was used to compare the percent changes in BMD at 1 and 2 years among the four groups (D, K, DK, and C). In the C group, the mean percent changes in BMD were −0.53% at 1 year and −0.79% at 2 years compared with the baseline. The corresponding changes were −0.44% and +0.38% in the D group, and −0.20% and +0.90% in the K group, and the changes at 2 years in both these groups were significant compared with those in the C group (both $P < 0.05$). In the DK group, on the other hand, the corresponding changes were +1.49% and +1.35%, and the changes at 1 year were significant compared with those in the C, D, and DK groups ($P < 0.01$; $P < 0.01$ and $P < 0.05$, respectively); the changes at 2 years were significant compared with those in the C group ($P < 0.01$). D, Vitamin D_3 (1α-hydroxyvitamin D_3, 0.75 μg/day) administration; K, vitamin K_2 (menatetrenone, 45 mg/day) administration; DK, vitamin D_3 plus K_2 administration; C, calcium (calcium lactate, 2 g/day) administration. * $P < 0.05$ vs. C group; **$P < 0.01$ vs. C group; #$P < 0.05$ vs. DK group; ##$P < 0.01$ vs. DK group
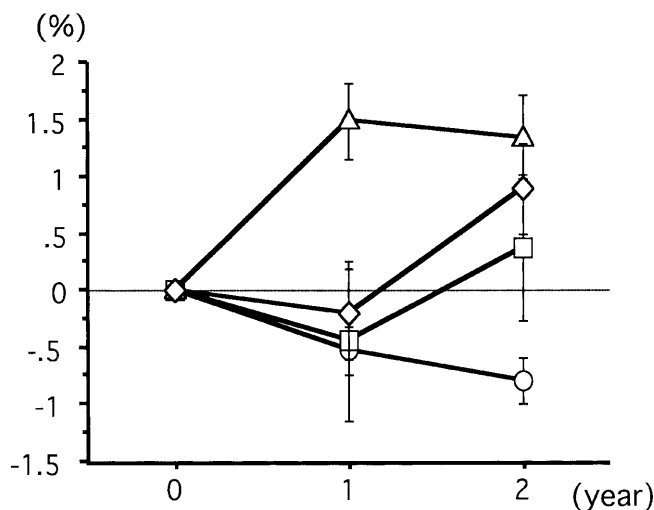

Fig. 2. Longitudinal percent changes in BMD of lumbar spine. Data values are expressed as means ± SE. One-way ANOVA with repeated measurements was used to examine the significance of longitudinal changes in BMD of the lumbar spine in the C group (*circles*). Two-way ANOVA with repeated measurements was used to compare longitudinal changes in BMD of the lumbar spine among the four groups. In the C group, longitudinal changes in BMD were significant ($P < 0.001$, one-way ANOVA). In the D group (*squares*) and K group (*diamonds*), longitudinal changes in BMD were significant compared with those in the C group ($P < 0.05$ and $P < 0.001$, respectively, two-way ANOVA). In the DK group (*triangles*), on the other hand, the longitudinal changes in BMD were significant compared with those in the C, D, and K groups ($P < 0.0001$; $P < 0.05$; and $P < 0.01$, respectively, two-way ANOVA)

on the other hand, the corresponding changes were +1.49% and +1.35%, and the changes at 1 year were significant compared with those in the C, D, and DK groups ($P < 0.01$; $P < 0.01$ and $P < 0.05$, respectively, ANOVA with Fisher's PLSD); the changes at 2 years were significant compared with those in the C group ($P < 0.01$, ANOVA with Fisher's PLSD). Two-way ANOVA with repeated measurements showed that the longitudinal changes in BMD in the DK group were significant compared with those in the C, D, and K groups ($P < 0.0001$; $P < 0.05$, and $P < 0.01$, respectively).

Discussion

The present study demonstrated that either vitamin D_3 or vitamin K_2 administration for 2 years significantly increased BMD of the lumbar spine in postmenopausal women with osteoporosis, while calcium administration significantly decreased it. Combined administration of vitamin D_3 and vitamin K_2 significantly increased BMD of the lumbar spine, and the increase was greater than that with the single administration of vitamin D_3 or vitamin K_2 (indicated by two-way ANOVA with repeated measurements). With regard to the effects of calcium administration on the bone mass of the lumbar spine in postmenopausal women, Cumming[4] reviewed published trials using the technique of meta-analysis, and reported that calcium supplementation had a consistently positive effect in postmenopausal women at all sites except the vertebrae, and that this effect was greatest in the studies in which the baseline calcium intake was low, the mean age of the subjects was high, and/or the subjects had clinical evidence of osteoporosis. On the other hand, Dawson-Hughes[6] reported that, while the spine in early postmenopausal women was unresponsive to calcium supplementation even at higher doses, bone loss from the spine could be retarded in late postmenopausal women with low-calcium diets by increasing their calcium intake. The results in these review studies suggest that calcium administration in late postmenopausal women with osteoporosis cannot prevent loss of BMD of the lumbar spine. Similarly, in the calcium administration group in the present study, mainly consisting of late postmenopausal women with osteoporosis, a significant decrease in BMD of the lumbar spine was observed.

1α Hydroxyvitamin D_3 is a pro-drug of 1,25 dihydroxyvitamin D_3. Activated vitamin D_3, i.e., 1α hydroxyvitamin D_3 or 1,25 dihydroxyvitamin D_3, is known to facilitate calcification by promoting calcium absorption by the intestine and modulating the secretion of parathyroid hormone.[2,28] In addition, activated vitamin D_3 has been demonstrated to activate the synthesis of osteocalcin, a calcified tissue protein containing γ-carboxyglutamic acids, and to promote its production by stimulating osteoblasts.[2,18] Thus, administration of activated vitamin D_3 has been recognized as a useful treatment for osteoporosis. On the other hand, vitamin K_2 is known to be essential for the carboxylation of osteocalcin.[13,26] Because noncarboxylated osteocalcin cannot bind to hydroxyapapite in mineralized tissues until the γ-carboxylation of osteocalcin,[14,26] vitamin K_2 may play a role in the mineralization of bone. In addition, the inhibitory effect of vitamin K_2 on bone resorption has also been reported.[11] Thus, vitamin K_2 has attracted attention as a potential drug for the treatment for osteoporosis.

There are numerous studies of the effects of 1,25 dihydroxyvitamin D_3.[1,3,8–10,15,20,25,27] Nevertheless, only a few well controlled prospective studies of the effect of either 1α hydroxyvitamin D_3 or vitamin K_2 on BMD of the lumbar spine in postmenopausal women have been reported. Available evidence suggested that 1α hydroxyvitamin D_3 was effective for preventing loss of BMD of the lumbar spine without any serious adverse effects in postmenopausal Japanese women,[23] and that vitamin K_2 increased metacarpal BMD and prevented loss of BMD of the lumbar spine in Japanese patients with osteoporosis.[22] Thus, a single administration of vitamin D_3 or vitamin K_2 has been shown to prevent loss of BMD of the lumbar spine in postmenopausal women with osteoporosis. Similarly, loss of BMD of the lumbar spine was prevented and BMD of the lumbar spine was increased by either vitamin D_3 or vitamin K_2 administration in the present study, although its mechanism remains unclear.

Very few clinical studies have reported the effect of the combined administration of vitamin D_3 and vitamin K_2 in subjects with osteoporosis, and the effect of the combined administration of vitamin D_3 and vitamin K_2 on BMD of the lumbar spine in postmenopausal women with osteoporosis has not been established. In an experimental study, Matsunaga et al.[19] demonstrated that concomitant use of vitamin D_3 and vitamin K_2 in ovariectomized rats suppressed cancellous bone loss caused by ovariectomy to a level similar to that in controls, although the single administration of vitamin D_3 or vitamin K_2 did not significantly this bone loss. However, no evidence has been reported on the effect of the combined administration of vitamin D_3 and vitamin K_2 on bone mass in animals with established osteoporosis induced by ovariectomy. The present clinical study demonstrated that the single administration of vitamin D_3 or vitamin K_2 for 2 years slightly increased BMD of the lumbar spine in postmenopausal women with osteoporosis, and their combined administration enhanced this BMD increase. Based on these findings in the present clinical and the findings in

previous experimental studies, it appears possible that the effects of vitamin D_3 and vitamin K_2 administration on bone mass are interactive and additive, and their combined administration could prevent bone loss in early postmenopausal women and increase bone mass in late postmenopausal women with osteoporosis.

The mechanism for the positive action of the combined administration of vitamin D_3 and vitamin K_2 on BMD of the lumbar spine in the present study remains unclear. It has been shown that vitamin D_3 is necessary prior to the carboxylation of osteocalcin by vitamin K_2, because osteocalcin is induced by the action of activated vitamin D_3. Actually, Orimo et al.[21] showed that the effect of vitamin K_2 on BMD was greater in osteoporotic patients with higher levels of serum 1,25 dihydroxyvitamin D_3. Koshihara et al.[16,17] showed that the effect of vitamin K_2 on human osteoblast-induced mineralization was enhanced in the presence of vitamin D_3. Moreover, Hara et al.[12] showed that the effect of vitamin K_2 on ovariectomy-induced bone loss was affected in rats with vitamin D_3-containing diet, but not in the rats with a vitamin D_3-free diet. These lines of evidence suggest that vitamin K_2 distinctively enhances activated vitamin D_3-induced mineralization by osteoblasts when there is administration of activated vitamin D_3. We speculate that vitamin K_2-dependent carboxylation of activated vitamin D_3-induced osteocalcin is probably an important factor in the effectiveness of the combined administration of vitamin D_3 and vitamin K_2 on bone mass. That is, vitamin K_2 administration enhances the carboxylation of highly produced osteocalcin by vitamin D_3 administration, resulting in a vigorous increase in mineralization in bone tissue. Thus, the combined administration of vitamin D_3 and vitamin K_2 enhanced the increase in BMD of the lumbar spine that resulted from the single administration of vitamin D_3 or vitamin K_2 in the present study. We could not measure bone formation and resorption markers in serum and urine in the present trial. Therefore, further study is needed to clarify the mechanism by which either vitamin D_3 or vitamin K_2 administration increased BMD of the lumbar spine and their combined administration enhanced this BMD increase in postmenopausal women with osteoporosis.

The endpoint of treatment for osteoporosis is to prevent osteoporotic fractures. In the present study, we could not assess the incidence of vertebral fractures. Further study with a large number of subjects is also needed to clarify the effect of the combined administration of vitamin D_3 and vitamin K_2 on the incidence of osteoporotic fractures.

In conclusion, the findings in the present study indicate that either vitamin D_3 or vitamin K_2 administration appears to increase BMD of the lumbar spine in postmenopausal women with osteoporosis, and that the combined administration of vitamin D_3 and vitamin K_2 appears to enhance this increase. The combined administration of vitamin D_3 and vitamin K_2 may be a useful treatment for postmenopausal women with osteoporosis.

References

1. Aloia JF, Vaswani A, Yeh JK, et al. Calcitriol in the treatment of postmenopausal osteoporosis. Am J Med 1989;84:401–8.
2. Beresford JN, Gallagher JA, Poser JW, et al. Production of osteocalcin by human bone cells in vitro. Effects of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$, parathyroid hormone, and glucocorticoids. Met Bone Dis Rel Res 1984;5:229–34.
3. Christiansen C, Christiansen MS, Rodbro P, et al. Effect of 1,25-dihydroxy-vitamin D_3 in itself or combined with hormone treatment in preventing postmenopausal osteoporosis. Eur J Clin Invest 1981;11:305–9.
4. Cumming RG. Calcium intake and bone mass: a quantitative review of the evidence. Calcif Tissue Int 1990;47:194–201.
5. Dalsky GP, Stocke KS, Ehsani AA, et al. Weight-bearing exercise training and lumbar bone mineral content in postmenopausal women. Ann Intern Med 1988;108:824–8.
6. Dawson-Hughes B. Calcium supplementation and bone loss: a review of controlled clinical trials. Am J Clin Nutr 1991;54:274S–80S.
7. Ettinger B, Genant HK, Cann CE. Postmenopausal bone loss is prevented by treatment with low-dosage estrogen with calcium. Ann Intern Med 1987;106:40–5.
8. Falch JA, Odegaard OR, Finnager AM, et al. Postmenopausal osteoporosis: no effect of 3 years treatment with 1,25-dihydroxycholecalciferol. Acta Med Scand 1987;221:199–204.
9. Gallagher JC, Riggs BL, Recker RR, et al. The effect of calcitriol on patients with postmenopausal osteoporosis with special reference to fracture frequency. Proc Soc Exp Biol Med 1989;191:287–92.
10. Gallagher JC, Goldgar D. Treatment of postmenopausal osteoporosis with high dose of synthetic calcitriol. Ann Intern Med 1990;113:649–55.
11. Hara K, Akiyama Y, Shiraki M, et al. Menatetrenone inhibits bone resorption partly through inhibition of PG E_2 synthesis in vitro. J Bone Miner Res 1993;8:535–42.
12. Hara K, Akiyama Y, Tomiuga T, et al. Influence of vitamin D_3 on inhibitory effect of vitamin K_2 on bone loss in ovariectomized rats. Folia Pharmacol Jpn 1994;104:101–9 (in Japanese).
13. Hauschka PV, Lian JB, Gallop PM. Direct identification of the calcium-binding amino acid, γ-carboxyglutamate, in mineralized tissue. Proc Natl Acad Sci USA 1975;72:3925–9.
14. Haushka PV, Carr SA. Calcium-dependent α-helical structure in osteocalcin. Biochemistry 1982;21:2538–47.
15. Jensen GF, Meinecke B, Boesen J, et al. Does $1,25(OH)_2D_3$ accelerate spinal bone loss? A controlled therapeutic trial in 70-year-old women. Clin Orthop Rel Res 1985;192:215–21.
16. Koshihara Y, Hoshi K, Shiraki M. Enhancement of mineralization of human osteoblasts by vitamin K_2 (menaquinone 4). Igakunoayumi 1992;161:439–40 (in Japanese).
17. Koshihara Y, Hoshi K. Vitamin K_2 enhances osteocalcin accumulation in the extracellular matrix of human osteoblasts in vitro. J Bone Miner Res 1997;12:431–8.
18. Lian J, Stewart C, Puchacz E, et al. Structure of the rat osteocalcin gene and regulation of vitamin D-dependent expression. Proc Natl Acad Sci USA 1989;86:1143–7.
19. Matsunaga S, Ito H, Sakou T. The effect of vitamin K and D supplementation on ovariectomy-induced bone loss. Calcif Tissue Int 1999;65:285–9.

20. Nordin BEC, Moris HA. Osteoporosis and vitamin D. J Cell Biochem 1992;49:19–25.
21. Orimo H, Fujita T, Onomura T, et al. Clinical evaluation of Ea-0167 (menatetrenone) in the treatment of osteoporosis. Phase III double-blind multicenter comparative study with alfacalcidol. Clin Eval 1992;20:45–100 (in Japanese).
22. Orimo H, Shiraki M, Fujita T, et al. Clinical evaluation of menatetrenone in the treatment of involutional osteoporosis. A double-blind multicenter comparative study with 1α-hydroxyvitamin D_3. J Bone Miner Res 1992;(Suppl 1):S122.
23. Orimo H, Shiraki M, Hayashi Y, et al. Effects of 1α-hydroxyvitamin D_3 on lumbar bone mineral density and vertebral fractures in patients with postmenopausal osteoporosis. Calcif Tissue Int 1994;54:370–6.
24. Orimo H, Sugioka Y, Fukunaga M, et al. Diagnostic criteria of primary osteoporosis. J Bone Miner Metab 1998;16:139–50.
25. Ott SM, Chesnut III CH. Calcitriol treatment is not effective in postmenopausal osteoporosis. Ann Intern Med 1989;110:267–74.
26. Prince PA. Vitamin K-dependent formation of bone gla protein (osteocalcin) and its function. Vitam Horm 1985;42:65–108.
27. Tilyard MW, Spears GFS, Thomson J, et al. Treatment of postmenopausal osteoporosis with calcitriol or calcium. N Engl J Med 1992;326:357–62.
28. Weinstein RS, Underwood JL, Huston MS, et al. Bone histomorphometry in vitamin D-deficient rats infused with calcium and phosphorus. Am J Physiol 1984;246:499–505.