Decreased Circulating Levels of Vitamin K and 25-Hydroxyvitamin D in Osteopenic Elderly Men

Michio Tamatani, Shigeto Morimoto, Masayoshi Nakajima, Keisuke Fukuo, Toshio Onishi, Shoichi Kitano, Tadaaki Niinobu, and Toshio Ogihara

Changes in the circulating factors participating in involutional osteoporosis have been intensively investigated in women, but little is known about this in men. We investigated the possible participation of circulating factors including testosterone, vitamin D metabolites, and vitamins K_1 and K_2 in osteopenia in elderly men. In a group of 27 ambulatory men aged 74 ± 10 years (mean ± SD; range, 60 to 90), the bone mineral density (BMD) of the second to fourth lumbar vertebrae was measured by dual-energy x-ray absorptiometry (DXA) and expressed as a Z score, the age-adjusted BMD value for the Japanese population (mean ± SD, 0 ± 1). Although the plasma level of total testosterone significantly decreased with age in the group, it did not significantly correlate with the Z score. However, the plasma levels of 25-hydroxyvitamin D (25-OHD), phylloquinone, menaquinone-7 (MK-7), and albumin were significantly positively correlated with the Z score. Moreover, plasma 25-OHD and both phylloquinone and MK-7 were significantly positively correlated in the subjects. These observations suggest that depressed circulating levels of 25-OHD and vitamin K concomitantly and cooperatively participate in osteopenia in elderly men, which may reflect the etiology of the type II moiety of involutional osteoporosis.

OSTEOPENIA including involutional osteoporosis is a medically and socially important disease in the elderly, since it is the main causal factor for bone fractures and resulting disability.[1] The loss of bone mass in both sexes is accompanied by an increased risk of fractures,[2] although the incidence of osteopenic fracture is lower in elderly men than in elderly women.[3] Whereas the causes and importance of age-related changes in bone metabolism and resulting bone loss have been extensively studied in women, there is little information in men. In women, several potentially important factors have been identified, including estrogen deficiency and changes in vitamin D metabolism.[4]

Vitamin K is an important factor for γ-carboxylation of specific glutamyl residues in clotting factors and in extrahepatic proteins, including the noncollagenous γ-carboxyglutamic acid (Gla) containing proteins of bone matrix such as osteocalcin.[5] Since osteocalcin was discovered in bone matrix,[6] much attention has been paid to the role of vitamin K in bone metabolism. Although the precise biological function of osteocalcin is still not clear, it is possible that vitamin K may play an important role in bone metabolism through γ-carboxylation of this substance. In nature, there are two types of vitamin K: vitamin K_1 (phylloquinone) and vitamin K_2 (menaquinones). Recently, it has been reported that circulating levels of vitamin K_1[7,8] and K_2[8,9] are decreased in osteopenic elderly subjects with fractures, although most of the subjects studied have been women.

Cross-sectional[10] and longitudinal[11] studies have demonstrated that men also lose bone mass with increasing age. However, the pathophysiological basis for this age-dependent bone loss is not clear. In the present study, we measured circulating levels of testosterone, vitamin D metabolites including 25-hydroxyvitamin D (25-OHD) and 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], and vitamin K compounds in elderly men, and evaluated the possible participation of these circulating factors in the determination of vertebral bone mineral density (BMD).

SUBJECTS AND METHODS

Subjects

The subjects were 27 ambulatory men aged 74 ± 10 years (mean ± SD; range, 60 to 90). All of these elderly subjects were residents of a nursing home. Patients who had diseases affecting bone metabolism such as Cushing's syndrome, diabetes mellitus, hyperthyroidism, and hyperparathyroidism or who had been treated with corticosteroids or warfarin were excluded from the study. None of the subjects had been treated with antibiotics or a vitamin K regimen or any treatment that might affect calcium (Ca) metabolism. All subjects received an ordinary nursing home diet containing about 600 mg/d Ca and 1,000 mg/d inorganic phosphate (Pi) for at least 1 month before the study. The body mass index (BMI) was assessed as reported elsewhere.[12]

Biochemical Assays

Blood samples were obtained from subjects early in the morning after an overnight fast. The plasma was separated from the blood sample, which was drawn into a lithium-heparin tube after a minimal delay, the serum was separated by centrifugation (3,000 × *g* for 30 minutes) of the blood sample after 1 hour at room temperature, and both were stored at −20°C until assayed.

Plasma total testosterone was determined by a radioimmunoassay using a commercial kit (Diagnostic Products, Los Angeles, CA); the detection limit was 0.35 nmol/L. Serum 25-OHD was determined by a competitive-binding assay using normal rat serum as a binding protein,[13] and 1,25-$(OH)_2$D was determined by a radioreceptor assay.[14] Plasma levels of vitamins K_1 and K_2 were measured by high-performance liquid chromatography according to the method of Langenberg and Tjaden.[15] The detection limits for plasma phylloquinone and menaquinone-7 (MK-7) were 0.25 and 0.79 nmol/L, respectively. The plasma level of protein induced by vitamin K absence or antagonist-II (PIVKA-II), an incompletely carboxylated prothrombin, as a possible biochemical marker for vitamin K deficiency[16] was analyzed by an enzyme immunoassay kit (Eisai Pharmaceutical, Tokyo, Japan) to exclude the possibility that the subjects had been vitamin K–deficient for a long period. Serum levels of Ca, Pi, albumin, and creatinine (Cr) were measured with an automated multichannel analyzer (Hitachi,

From the Department of Geriatric Medicine, Osaka University Medical School, Osaka; and Department of Internal Medicine, Hanwa-Senboku Hospital, Osaka, Japan.

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Address reprint requests to Shigeto Morimoto, MD, Department of Geriatric Medicine, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565, Japan.

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Tokyo, Japan). The serum corrected Ca level was determined according to the method of Payne et al.[17]

Measurement of BMD

Measurement of BMD of the second to fourth lumbar vertebrae (L2-L4) was performed by dual-energy x-ray absorptiometry (DXA) using a Lunar DPX (Lunar Radiation, Madison, WI). The variation in BMD measurements in each vertebra after five repositionings was less than 0.8%. BMD was expressed either as the value obtained by DXA (grams per square centimeter) or as a Z score (mean value for the patient's age group, 0 ± 1 SD) in the Japanese population.[18] The BMD of each subject in this study was obtained using L2-L4 BMD except in cases with a previous compression fracture, osteophytes, or severe scoliosis, where only the values from the other two vertebrae were used for calculation. The subjects were divided into two groups: normal BMD (n = 15), with BMD of at least peak bone density (PBD) − 2.5 SD, and decreased BMD (n = 12), with BMD less than PBD − 2.5 SD, calculated using values from a Japanese study.[18]

Statistical Analysis

All data are presented as the mean ± SD. Comparisons between the two groups for any parameter were performed by the Mann-Whitney test. A correlation of two parameters was determined either by a nonparametric method using Spearman's rank correlation analysis, or by a parametric method using Pearson's correlation analysis after appropriate transformation of the data to a normal distribution. Scatter plots of the data were visually inspected to assess the appropriateness of the linear model and the need to evaluate and include higher-order polynominal terms in the model. The relative importance of various determinants of the Z score was assessed by stepwise multiple regression analysis. Where the estimate was less than the detection limit, an arbitrary value midway between zero and the detection limit was assigned. P values less than .05 were regarded as significant.

RESULTS

In all of the subjects studied, Spearman's rank correlation analysis disclosed that age significantly negatively correlated with weight ($r_s = -.50$, $P < .05$), BMI ($r_s = -.39$, $P < .05$), and plasma total testosterone ($r_s = -.42$, $P < .05$), but not with other parameters, including BMD.

Table 1 summarizes clinical and biochemical characteristics of elderly male subjects in the normal and decreased BMD groups. The number of subjects with phylloquinone or MK-7 levels less than the detection limit was five and 12 of 27, respectively. Plasma levels of PIVKA-II were less than the detection limit (0.063 arbitrary U/mL) in all subjects, excluding the possibility that the subjects had been vitamin K–deficient for a long period. None of the subjects had values below the detection limit for other circulating parameters, including testosterone, 25-OHD, and 1,25-$(OH)_2$D. Although there was no significant difference in the clinical parameters, the decreased BMD group showed significantly lower circulating levels of albumin, 25-OHD, phylloquinone, and MK-7 than the normal BMD group. However, all serum values of Ca corrected for albumin, Pi, Cr, and 1,25-$(OH)_2$D, respectively, were similar in the two groups. Circulating levels of albumin, phylloquinone, MK-7, and 25-OHD were significantly positively correlated with the Z score by Spearman's rank correlation analysis (albumin, $r_s = .53$, $P < .01$; phylloquinone, $r_s = .52$, $P < .01$; MK-7, $r_s = .59$, $P < .005$; and 25-OHD, $r_s = .56$, $P < .005$). However, no other clinical or circulating parameters significantly correlated with the Z score. When circulating levels of phylloquinone, MK-7, and 25-OHD were tested for the distribution pattern, the cumulative frequency ratios against each of the circulating levels were plotted linearly on a log-normal probability graph (Fig 1), showing that the circulating levels can be normalized by power transformation into a logarithm. Using Pearson's correlation analysis, log(phylloquinone), log(MK-7), and log(25-OHD) all again significantly positively correlated with the Z score (phylloquinone, $r = .46$, $P < .05$; MK-7, $r = .50$, $P < .01$; 25-OHD, $r = .55$, $P < .01$; Fig 2).

Table 1. Clinical and Laboratory Characteristics of the Subjects (N = 27)

Characteristic	Normal Range	Group: Normal BMD (n = 15)	Group: Decreased BMD (n = 12)
Age (yr)		73.9 ± 9.7	73.8 ± 11.2
Height (m)		1.58 ± 0.07	1.57 ± 0.07
Weight (kg)		51.1 ± 8.8	50.5 ± 6.3
BMI (kg/m²)		20.4 ± 2.8	20.6 ± 3.6
BMD (g/cm²)		0.97 ± 0.11	0.72 ± 0.10‡
Z score		−0.32 ± 0.55	−1.76 ± 0.60§
Albumin (g/L)	35-55	39.1 ± 2.8	36.5 ± 2.0*
Cr (mmol/L)	<133	99.0 ± 27.4	91.0 ± 18.6
Ca (mmol/L)	2.2-2.6	2.22 ± 0.09	2.32 ± 0.14
Pi (mmol/L)	1.0-1.4	1.01 ± 0.10	1.10 ± 0.26
Testosterone (nmol/L)	10-35	14.6 ± 6.9	13.5 ± 5.2
25-OHD (nmol/L)	20-100	35.4 ± 15.7	21.7 ± 9.5†
1,25-$(OH)_2$D (nmol/L)	5-14	5.3 ± 0.5	4.7 ± 2.4
Phylloquinone (nmol/L)		0.85 ± 0.73	0.60 ± 0.73*
MK-7 (nmol/L)		1.44 ± 0.85	0.71 ± 0.35*

NOTE. Data are the mean ± SD.

*$P < .05$, †$P < .01$, ‡$P < .001$, §$P < .0001$: v normal BMD group by Mann-Whitney test.

Spearman's rank correlation analysis also disclosed a significant positive correlation between circulating levels of 25-OHD and both phylloquinone ($r_s = .39$, $P < .05$) and MK-7 ($r_s = .38$, $P < .05$), and between plasma levels of phylloquinone and MK-7 ($r_s = .54$, $P < .01$). The serum albumin level was not significantly correlated with either 25-OHD, phylloquinone, or MK-7.

The results of stepwise multiple regression analysis of the Z score are summarized in Table 2. The circulating level of phylloquinone was the most important, and that of 25-OHD the second most important, determinant of the Z score. Using these two circulating factors, approximately 40% of the Z score could be explained.

DISCUSSION

The causative factors for involutional osteoporosis have been extensively investigated in women: estrogen deficiency is the main cause of postmenopausal osteoporosis.[3] Low BMI is also known as one of the risk factors for senile osteoporosis, probably because estrogen is mainly synthesized by adipose tissue in postmenopausal women.[19] Although a causative role for decreased circulating levels of testosterone in osteopenia has been claimed in male hypogonadal patients,[20] no significant correlation between the plasma level of total testosterone and BMD was observed in elderly men in the present study, despite the apparent decrease in plasma total testosterone in these subjects with age, consistent with a previous report.[21] However, the role of low circulating free testosterone levels in the

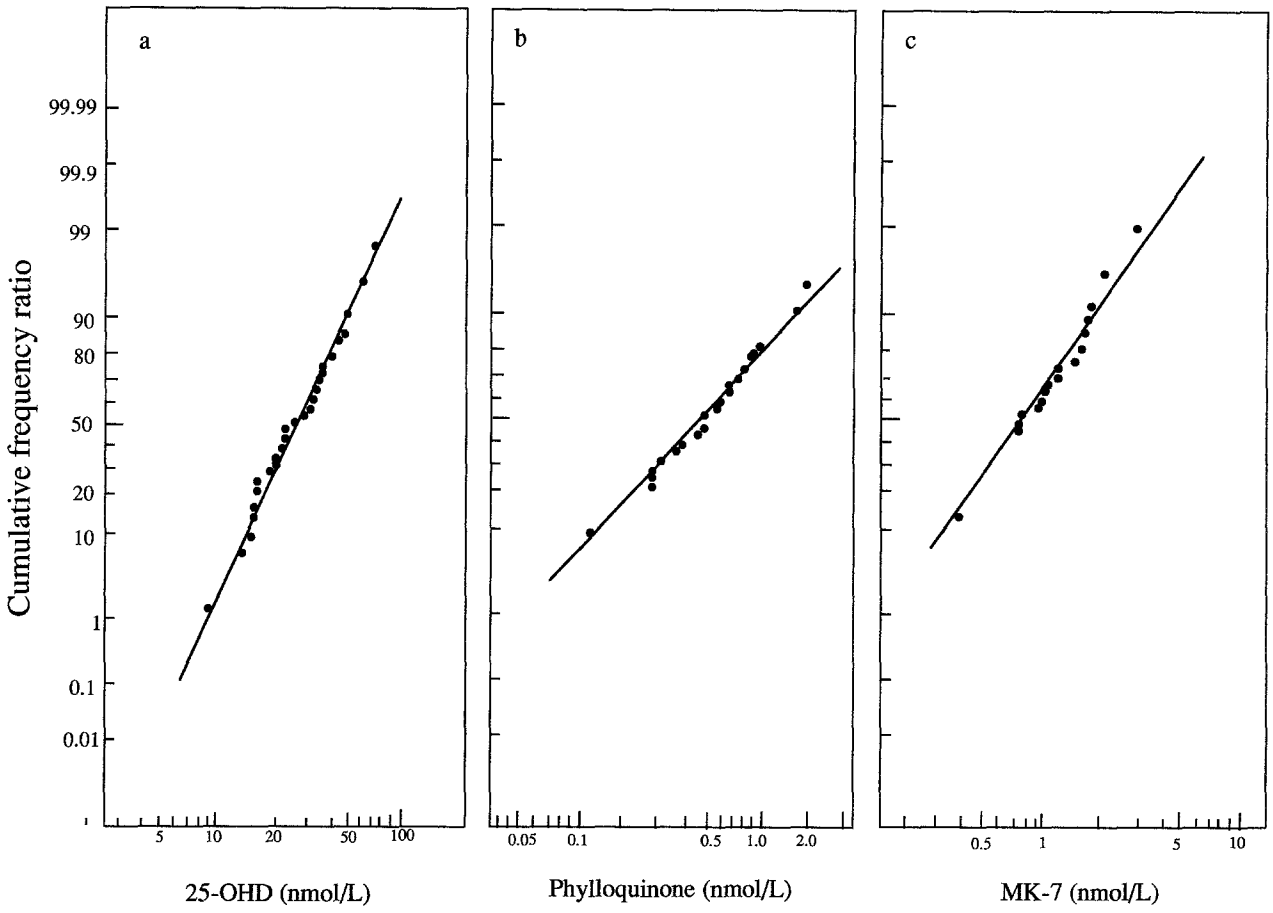

Fig 1. Relationship between the cumulative frequency ratio and plasma level of (a) 25-OHD, (b) phylloquinone, and (c) MK-7 on log-normal probability graph with linear regression line.

pathogenesis of age-related bone loss in elderly men remains to be further elucidated, since circulating levels of hormone-binding globulin are increased with age, resulting in a further decrease in circulating testosterone.[22] Age itself was not a significant determining factor for BMD in our subjects, probably due to the narrow age range.

On the other hand, elderly men with decreased BMD in the present study showed significant decrease in the circulating

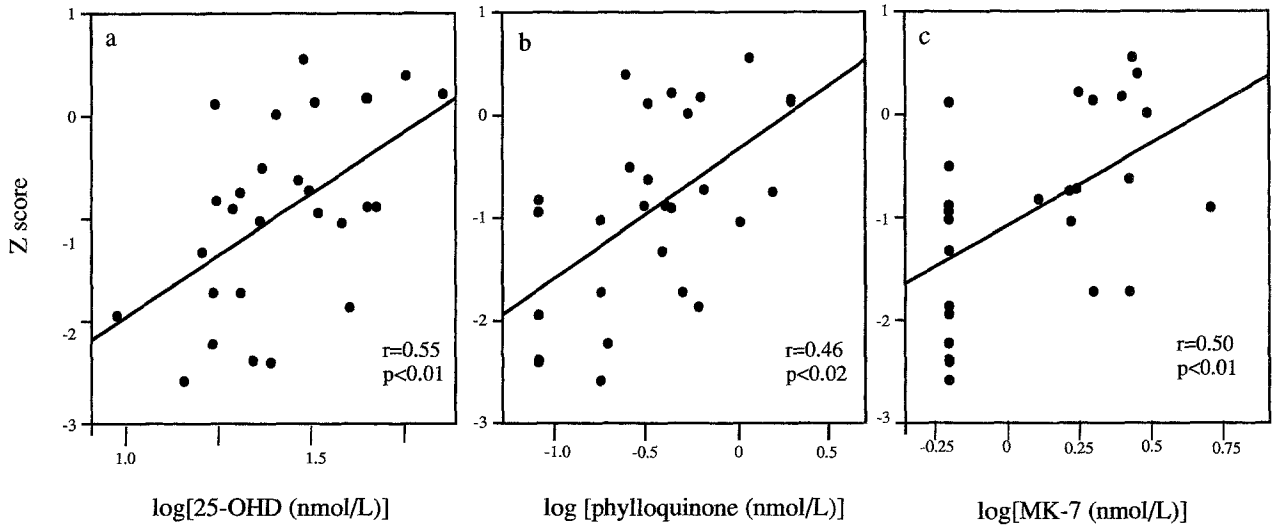

Fig 2. Correlation between the age-adjusted vertebral BMD Z score and plasma level of (a) 25-OHD ($r = .55$, $P < .01$), (b) phylloquinone ($r = .46$, $P < .05$), and (c) MK-7 ($r = .50$, $P < .01$) with linear regression line. Correlation analysis was performed after transformation of the levels of circulating substances to logarithmic values.

Table 2. Stepwise Multiple Regression Analysis of the Z Score

	Simple R	β-Weight	Multiple R	Multiple R^2
Phylloquinone	.54	.39	.54	.29
25-OHD	.53	.39	.64	.41

NOTE. Stepwise multiple regression analysis was performed after transformation of the plasma levels of phylloquinone and 25-OHD to logarithmic values.

levels of 25-OHD, phylloquinone, and MK-7, the major components of circulating vitamin K,[23] and albumin compared with the elderly men with normal BMD. In all subjects, these parameters significantly positively correlated with the Z score by the nonparametric method. Moreover, circulating levels of phylloquinone, MK-7, and 25-OHD showed a log-normal distribution, partly compatible with previous reports,[24,25] and after logarithmic transformation, these factors again significantly positively correlated with the Z score by the parametric method. Of these factors in the osteopenia of our elderly male subjects, a decreased serum albumin level was observed in a previous study,[26] and poor nutrition and low calorie intake are common features in elderly people with hip fractures.[27,28] Decreased serum 25-OHD, but not 1,25-$(OH)_2$D, was also reported to be a factor in osteopenia in men,[29] although conflicting results are also reported.[11,30] Diminished dermal synthesis of vitamin D occurs because of age-related decreases in the synthesis of 7-dehydrocholesterol, and is a contributing factor to the nutritional disturbance of vitamin D in aged subjects.[31] A suboptimal vitamin D status has been reported in elderly women,[32] and has been associated with osteopenia[33] and an increased risk of hip fracture.[34] Besides the well-known Ca-regulating actions of 1,25-$(OH)_2$D,[35] the role of 25-OHD in bone metabolism is also reported.[36] Since our elderly male subjects with decreased BMD did not show definite biochemical features of vitamin D–deficient osteomalacia characterized by decreased serum levels of Pi and Ca,[37] the present observations suggest that mild vitamin D deficiency could contribute to the pathogenesis of osteopenia in elderly men, although a different opinion is also reported.[11]

The decreased plasma levels of vitamins K_1 and K_2 in the osteopenic elderly males deserve special mention. 1,25-$(OH)_2$D directly induces the synthesis of osteocalcin, the main noncollagenous protein of bone matrix, by promoting transcription of its gene,[38] and vitamin K is a key factor for γ-carboxylation of specific glutamyl residues in osteocalcin.[32] Since noncarboxylated osteocalcin cannot bind to hydroxyapatite,[5] vitamin K deficiency has been thought to be one of the causative factors in bone loss. Modulation of bone cellular functions by vitamin K has been reported in vitro.[39] Clinical studies also showed that phylloquinone supplementation in postmenopausal women induced a decrease in biochemical markers for bone resorption such as urinary excretion of Ca and hydroxyproline.[40] Moreover, vitamin K deficiency may participate in the increase of the noncarboxylated fraction of osteocalcin in the circulation[41,42] and bone[42] in elderly subjects. Vitamin K has been postulated to be sequestered from the circulation for use at the fracture site, since circulating levels of vitamins K_1 and K_2 were depressed in elderly women who sustained hip fractures.[8] However, no direct evidence for the participation of decreased plasma vitamin K in osteopenia in the elderly has been reported. The present study offers the first evidence that the decreased circulating level of vitamin K has a direct consequence for osteopenia in the elderly. Moreover, stepwise multiple regression analysis suggested that decreases in the circulating level of both vitamin K and 25-OHD were cooperative participants in osteopenia in elderly men.

The cause of vitamin K deficiency in osteopenic elderly men is not clear. Green leafy vegetables, beans, and fermented foods are rich in phylloquinone and menaquinones, respectively.[43] However, microfloral synthesis of menaquinones is likely to contribute significantly to vitamin K stores in the liver.[44] Moreover, significant decreases in plasma levels of some menaquinones have been reported even in healthy elderly subjects.[27] Therefore, it is possible that an age-related alteration in menaquinone-producing bacteria of the gut flora and/or some unidentified impaired metabolism of menaquinones also participate in the decrease of plasma vitamin K in osteopenic elderly subjects.

Riggs and Melton[3] postulated that there are at least two distinct syndromes of involutional osteoporosis: type I osteoporosis, mainly occurring in postmenopausal women, and type II osteoporosis, appearing in both elderly men and women aged 70 years and older. Whereas the causative role of estrogen deficiency has been confirmed in type I osteoporosis, the present observations indicate the importance of subclinical deficiencies of both vitamin D and vitamin K in type II osteoporosis, at least in the elderly men of the present study. However, since this study is cross-sectional, the present observations provide only presumptive evidence for the etiological importance of these vitamin deficiencies in osteopenia. More elaborate investigations are therefore needed to establish the role of vitamins K and D in type II osteoporosis.

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