Vitamin K status of healthy Japanese women: age-related vitamin K requirement for γ-carboxylation of osteocalcin[1–3]

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ABSTRACT

Background: Vitamin K deficiency is associated with low bone mineral density and increased risk of bone fracture. Phylloquinone (K_1) and menaquinone 4 (MK-4) and 7 (MK-7) are generally observed in human plasma; however, data are limited on their circulating concentrations and their associations with bone metabolism or with γ-carboxylation of the osteocalcin molecule.

Objectives: The objectives were to measure the circulating concentrations of K_1, MK-4, and MK-7 in women and to ascertain whether each form of vitamin K is significantly associated with bone metabolism.

Design: Plasma concentrations of K_1, MK-4, MK-7, undercarboxylated osteocalcin (ucOC; measured by using the new electrochemiluminescence immunoassay), intact osteocalcin (iOC), calcium, and phosphorus; bone-derived alkaline phosphatase activity; and concentrations of urinary creatinine, *N*-terminal telopeptide, and deoxypyridinoline were measured in healthy women ($n = 396$).

Results: On average, MK-7 and MK-4 were the highest and lowest, respectively, of the 3 vitamers in all age groups. K_1 and MK-7 correlated inversely with ucOC, but associations between nutritional basal concentration of MK-4 and ucOC were not observed. Multiple regression analysis indicated that not only K_1 and MK-7 concentrations but also age were independently correlated with ucOC concentration and the ratio of ucOC to iOC. The plasma K_1 or MK-7 concentration required to minimize the ucOC concentration was highest in the group aged ≥70 y, and it decreased progressively for each of the younger age groups.

Conclusions: The definite role of ucOC remains unclear. However, if submaximal γ-carboxylation is related to the prevention of fracture or bone mineral loss, circulating vitamin K concentrations in elderly people should be kept higher than those in young people. *Am J Clin Nutr* 2006;83:380–6.

KEY WORDS Phylloquinone, menaquinone 4, menaquinone 7, vitamin K, plasma concentrations, osteocalcin, bone turnover makers, healthy elderly women

INTRODUCTION

Vitamin K is a cofactor for the enzyme responsible for the conversion of specific glutamyl residues to γ-carboxyglutamyl residues in a limited number of blood coagulation factors (1) and bone-related proteins (2, 3). Vitamin K is thought to maintain bone strength via the γ-carboxylation of osteocalcin (4) and to suppress arterial calcification via γ-carboxylation of matrix glutamic acid residues of protein (5). In vitamin K insufficiency or deficiency, a small amount of undercarboxylated osteocalcin (ucOC) is released from the osteoblast into the circulation. Thus, the serum concentration of ucOC has been considered a sensitive marker of vitamin K status in bone (6, 7). High concentrations of circulating ucOC have been associated with low bone mineral density (8, 9) and a greater risk of hip fracture evening those subjects without any abnormalities in the blood coagulation system (10, 11).

Vitamin K naturally exists in 2 forms, namely phylloquinone (K_1) and a group called vitamin K_2, also called menaquinones. K_1 is widely distributed in green and leafy vegetables, whereas menaquinones exist preferentially in meats [menaquinone (MK)-4], eggs (MK-4), curd (MK-7), cheese (MK-7), and fermented soybeans (MK-7). The predominant dietary and circulating form of vitamin K for people in the United States, Europe, and most other parts of the world is K_1 (12, 13). However, the other major component of dietary vitamin K for the Japanese, especially those living in eastern Japan, is MK-7, which is mainly derived from soybeans fermented by *Bacillus natto* (referred to as natto). The small amount of available data on circulating vitamin K concentrations of healthy women has limited precise examination of the relation between vitamin K status and bone health. In the current study, using our new liquid chromatography–atmospheric pressure chemical ionization–tandem mass–mass spectrometry (LC-APCI-MS/MS) method for the measurement of K_1, MK-4, and MK-7, we investigated the relations between the plasma concentrations of K_1, MK-4, or MK-7, as biochemical measures of vitamin K status, and bone turnover markers, including ucOC, in women stratified by age. As far as we know, there are no previous studies including the precise

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measurement of plasma MK concentrations and the detailed analyses of their associations with bone turnover markers and the status of γ-carboxylation of the osteocalcin molecule in premenopausal or postmenopausal healthy women. The objectives of the current study were to examine the associations between the biochemical measures of vitamin K status and bone metabolic markers in healthy Japanese women and to evaluate age-related differences in the requirement for vitamin K in bone in terms of the γ-carboxylation of osteocalcin.

SUBJECTS AND METHODS

Subjects

Women aged 30–88 y were recruited from the rural area of Nagano prefecture, Japan, where most people frequently eat natto. Women with a metabolic bone disease other than primary osteoporosis and women who were taking medicine related to bone metabolism—eg, active vitamin D, vitamin K, a vitamin K antagonist, estrogen, bisphosphonate, and steroids—were excluded. A total of 396 women with a $\bar{x} \pm$ SD age of 63.0 ± 10.9 y met the inclusion criteria for this study. The subjects were stratified into 3 groups by age: 30–49, 50–69, and ≥70 y old. All of the subjects in the group aged 30–49 y were premenopausal, and all of the subjects in the other 2 groups were postmenopausal.

Measurements

After a 12-h overnight fast, plasma and urine samples were collected from the patients in the morning and stored immediately at −30 °C until measurement. The urine sample was a second morning void per kit instructions. Plasma K_1, MK-4, and MK-7 concentrations were measured by using an LC-APCI-MS/MS method (14). Plasma ucOC, as a sensitive marker of vitamin K deficiency or insufficiency, was measured with the new electrochemiluminescence immunoassay (Sanko Junyaku Co Ltd, Ibaragi, Japan). The specific antibody to ucOC was purchased from Takara Shuzo Co Ltd (Kyoto, Japan). In brief, the measurement is conducted as follows: after the ucOC in the sample is bound to anti-human ucOC prepared on the solid phase by using magnetic beads, antiosteocalcin labeled with a ruthenium complex emitting luminescence in response to an electrochemical change is bound, and then electricity is applied onto the electrode. The ruthenium complex emits luminescence according to the amount of the ruthenium-labeled antibody that is bound. We measured iOC with an immunoradiometric assay (Mitsubishi Kagaku BioClinical Laboratories Inc, Tokyo, Japan). We measured bone resorption markers, urinary excretion of *N*-telopeptide [(NTx) as measured by using an enzyme-linked immunosorbent assay (Osteomark; Ostex International, Seattle, WA), total deoxypyridinoline (DPD; as measured by HPLC after hydrolysis of the urine sample; Teijin Bio-Lab, Tokyo, Japan), and a bone formation marker, bone-derived alkaline phosphatase [(BAP) measured by using an enzyme immunoassay and a microplate (Sumitomo Seiyaku Biomedical Co Ltd, Osaka, Japan). For the evaluation of calcium metabolism, the serum concentrations of calcium, phosphorus, and the ratio of urinary calcium to creatinine were measured. Height and weight were measured, and body mass index (BMI) was calculated as the weight (in kg) divided by the height (in m^2).

Statistical analysis

All statistical analyses were performed by using JMP statistical software (version 5.0.1 J; SAS Institute Inc, Cary, NC). For cross-sectional analyses, simple regression analysis was performed to assess the associations between plasma K_1, MK-4, or MK-7 and ucOC, the ratio of ucOC to intact osteocalcin (ucOC:iOC), or plasma or urinary bone metabolic variables. Pearson's correlation coefficient (r) and the corresponding P values were used to evaluate the relation of plasma vitamin K concentrations to each variable. In secondary analyses, multiple linear regression analyses were performed to explore determinants of ucOC, ucOC:iOC, and DPD, which simple regression analysis showed to be significantly related to vitamin K concentration.

To evaluate the age-related difference in the amount of vitamin K required to minimize ucOC:iOC, nonlinear logarithmic regression analysis was performed by using the plasma K_1 or MK-7 concentration as an independent variable and ucOC:iOC as a dependent variable after subjects were classified into the 3 age groups. Significant differences in intercepts and slopes of regression curves between the 3 age groups were evaluated by analysis of covariance.

Written informed consent was obtained from all participants. The study protocol was approved by the ethic committee of the Research Institute and Practice for Involutional Diseases.

RESULTS

Subjects

The characteristics of the study samples are shown in **Table 1**. Weight and height were negatively correlated with age, whereas BMI and plasma triacylglycerol and total cholesterol concentrations did not significantly differ between the 3 age groups. Bone turnover markers, including ucOC and BAP activity in the serum and NTx and DPD in the urine, were significantly correlated with age, whereas the plasma iOC concentration was not. Moreover, MK-7 was the major contributor to plasma vitamin K concentration in this Japanese population, unlike in Americans and Europeans. Plasma concentrations of K_1 and MK-7 were highest in the group aged 50–69 y and lowest in the group aged ≥70 y. Plasma concentrations of MK-4 did not significantly differ between the 3 age groups. Plasma concentrations of MK-7 were ≈3–4 times higher than those of K_1 in any of the age groups. Plasma phosphorus concentrations differed significantly between the 3 age groups, although the actual differences were small and within the normal laboratory range and did not appear to be of practical importance.

Distribution of plasma concentrations of phylloquinone and menaquinones 4 and 7

As shown in **Figure 1**, K_1 was detected in all subjects at a range of 0.13 to 8.83 ng/mL, and the mean (±SD) and median values in the groups aged 30–49, 50–69, and ≥70 y were 1.52 ± 1.02 and 1.16 ng/mL, 1.74 ± 1.29 and 1.31 ng/mL, and 1.29 ± 1.09 and 0.94 ng/mL, respectively. MK-4 was detected in 56.8% of the subjects, and the mean and median values were 0.07 ± 0.14 and 0.01 ng/mL, 0.10 ± 0.19 and 0.03 ng/mL, and 0.09 ± 0.15 and 0.02 ng/mL, respectively. MK-7 was detected in 97.8% of the subjects, and the mean and median values were 4.96 ± 6.93 and 2.65 ng/mL, 8.42 ± 11.44 and 3.92 ng/mL, and 4.21 ± 6.81 and 2.14 ng/mL, respectively. Similar distribution patterns for

Table 1
Subject characteristics[1]

	Age group			
	30–49 y (n = 52)	50–69 y (n = 208)	≥70 y (n = 136)	P
Age (y)	45.4 ± 4.3	59.6 ± 5.3	74.9 ± 4.1	<0.001
Time since menopause (y)	0.0 ± 0.0	9.9 ± 6.2	25.2 ± 5.1	<0.001
Body weight (g)	55.3 ± 8.4	53.2 ± 7.5	48.7 ± 7.5	<0.001
Body height (cm)	155.7 ± 5.4	152.8 ± 5.3	148.0 ± 5.5	<0.001
BMI (kg/m^2)	22.8 ± 3.1	22.8 ± 3.0	22.2 ± 3.0	0.170
Triacylglycerol (mg/dL)	134.0 ± 95.1	138.3 ± 77.4	132.8 ± 62.6	0.793
Total cholesterol (mg/dL)	192.1 ± 39.7	202.4 ± 31.2	199.1 ± 31.8	0.126
K_1 (ng/mL)	1.52 ± 1.02	1.74 ± 1.29	1.29 ± 1.09	0.002
MK-4 (ng/mL)	0.07 ± 0.14	0.10 ± 0.19	0.09 ± 0.15	0.563
MK-7 (ng/mL)	4.96 ± 6.93	8.42 ± 11.44	4.21 ± 6.81	<0.001
ucOC (ng/mL)	3.59 ± 2.17	4.39 ± 2.79	5.51 ± 3.82	<0.001
iOC (ng/mL)	6.91 ± 3.02	9.11 ± 8.92	8.82 ± 4.30	0.149
ucOC:iOC	0.54 ± 0.28	0.55 ± 0.35	0.73 ± 0.68	0.003
Calcium (mg/dL)	9.18 ± 0.39	9.21 ± 0.37	9.22 ± 0.45	0.820
Phosphorus (mg/dL)	3.38 ± 0.47	3.53 ± 0.47	3.41 ± 0.46	0.025
BAP (U/L)	25.69 ± 9.85	32.81 ± 11.56	32.28 ± 12.30	<0.001
NTx (pmol BCE/μmol ceatinine)	45.16 ± 18.94	57.49 ± 31.15	63.95 ± 27.81	<0.001
DPD (nmol/mmol creatinine)	5.50 ± 1.86	5.97 ± 2.49	7.39 ± 3.15	<0.001

[1] All values are $\bar{x}$ ± SD. K_1, phylloquinone; MK, menaquinone; ucOC, undercarboxylated osteocalcin; iOC, intact osteocalcin; BAP, bone-derived alkaline phosphatase; NTx, *N*-terminal telopeptide; BCE, bone collagen equivalents; DPD, deoxypyridinoline. Parametric comparisons used ANOVA. All women in the group aged 30–49 y were premenopausal, and all women in the other 2 groups were postmenopausal.

each plasma concentration of K_1, MK-4, and MK-7 were observed in the 3 age groups.

Plasma concentrations of phylloquinone and menaquinone 4 or 7 and anthropometric, nutritional, or calcium metabolic variables

As shown in **Table 2**, plasma concentrations of K_1 correlated significantly and positively with BMI and cholesterol, triacylglycerol, MK-4, and MK-7 concentrations and correlated negatively with age, ucOC:iOC, and ucOC and urinary DPD concentrations. In contrast, plasma MK-7 concentrations also significantly correlated negatively with age, ucOC concentrations, and ucOC:iOC but not with urinary DPD concentrations. No correlations were observed between plasma concentrations of MK-4 and ucOC, ucOC:iOC, or concentrations of NTx, DPD, or BAP.

Plasma concentrations of phylloquinone or menaquinone 7 and plasma concentrations of undercarboxylated osteocalcin or the ratio of undercarboxylated osteocalcin to intact osteocalcin

Stepwise multiple linear regression analyses were performed to explore determinants of ucOC, ucOC:iOC, and urinary DPD. Plausible predictors (ie, age, BMI, cholesterol, triacylglycerol, K_1, MK-4, and MK-7) were included in the original model. Forward stepwise regression was performed, and a P value > 0.25 was used to exclude some of the predictors that had been in the original model. As a result, not only K_1 and MK-7 concentrations but also age correlated independently with ucOC concentrations and ucOC:iOC (**Table 3**). In contrast, only age and K_1 concentrations correlated independently with urinary DPD concentrations.

The age-related differences in the regression curves obtained from nonlinear logarithmic regression analysis with the use of plasma K_1 or MK-7 concentrations as an independent variable and ucOC:iOC as a dependent variable are shown in **Figure 2**. In the relation between plasma K_1 concentrations and ucOC:iOC, it was confirmed that the intercepts in the 3 age groups differed significantly ($P < 0.001$). In contrast, in the relation between plasma MK-7 concentrations and ucOC:iOC, significant differences were observed between the intercepts ($P < 0.001$) and slopes ($P < 0.022$) in the 3 age groups. In both analyses, with the use of the plasma K_1 and MK-7 concentrations, values of the intercept and slope of the curves were highest in the group aged ≥70 y and decrease progressively in the 2 younger age groups. An age-related difference clearly observed at a low vitamin K concentration. When the groups aged 50–69 and ≥70 y were compared, the vitamin K concentrations that had reached a plateau in the former group was significantly lower than that in the latter group.

DISCUSSION

Several reports on the circulating concentrations of vitamin K in Japanese women have been published. Kawana et al (15) reported circulating concentrations of K_1, MK-4, and MK-7 in healthy elderly women and women with vertebral fracture or hip fracture. In their study, a large proportion of the subjects had undetectable concentrations of K_1 (35% and 38% of healthy control subjects and patients with fractures, respectively) and MK-7 (52% and 24% of healthy control subjects and patients with fractures, respectively). Kaneki et al (16) reported that the concentrations of K_1, MK-4, and MK-7 were undetectable in 0%, 90%, and 24%, respectively, of 49 postmenopausal women in Japan. In another study, the concentrations of K_1 and MK-7 were

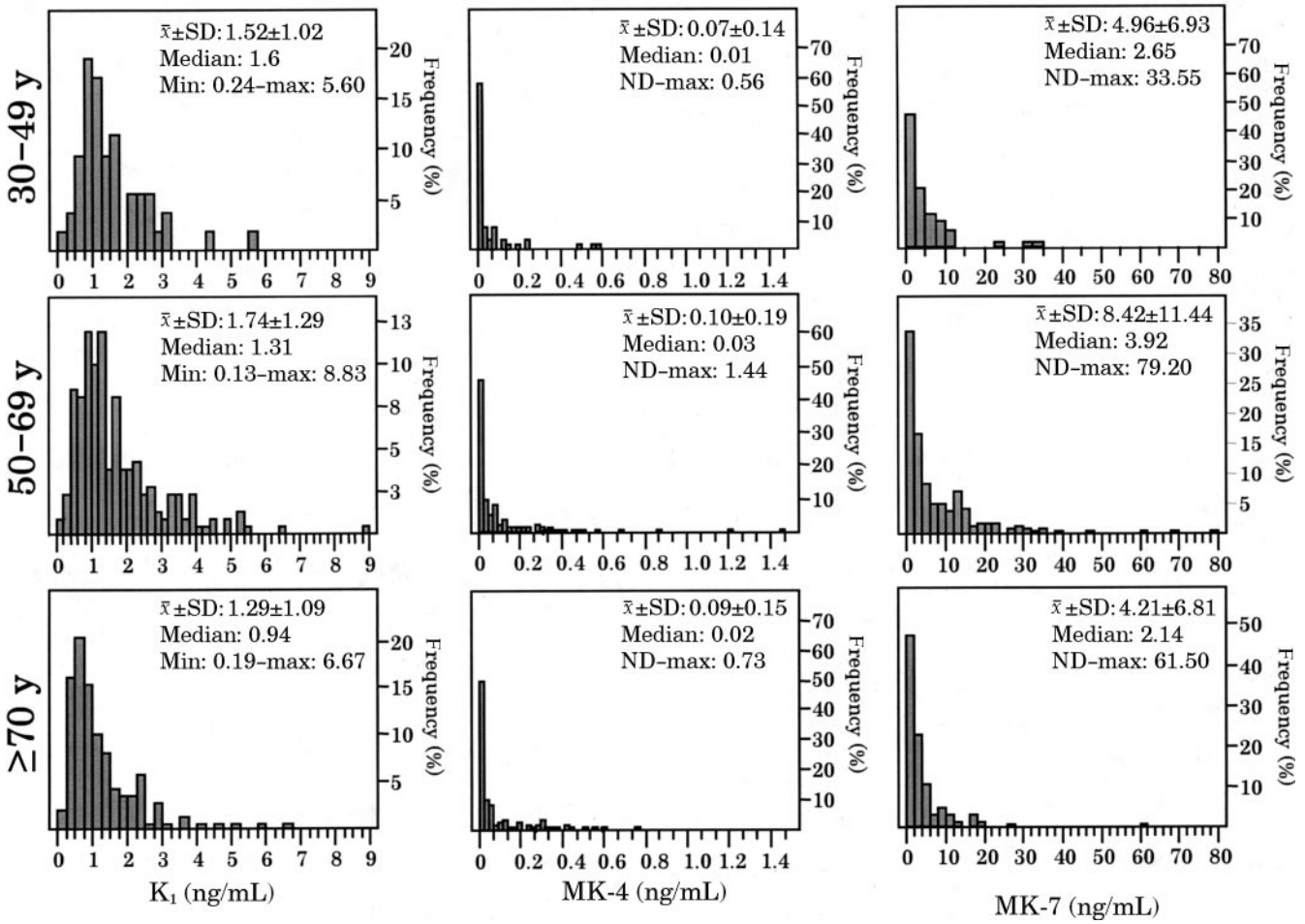

FIGURE 1. Distribution of plasma phylloquinone (K_1) and menaquinone (MK) 4 and MK-7 concentrations in 3 age groups. K_1 was detected in all subjects. MK-4 and MK-7 were detected in 56.8% and 97.8% of subjects, respectively. Min, minimum; max, maximum; ND, not detected. $n = 52$, 208, and 136 in the groups aged 30-49, 50–69, and ≥70 y, respectively.

undetectable in < 5% and 15%, respectively, of groups of European whites (13) and Japanese (16, 17). The existence of large interlaboratory differences in the measurement of MK-4 and MK-7 has been suggested (15). Therefore, we recently established a precise and sensitive method for simultaneously measuring plasma K_1, MK-4, and MK-7 by using the LC-APCI-

Table 2
Relation between plasma vitamin K concentrations and bone metabolism variables[1]

	K_1		MK-4		MK-7	
	r	*P*	*r*	*P*	*r*	*P*
Age	−0.146	0.004	−0.006	0.916	−0.125	0.013
BMI	0.147	0.004	0.093	0.092	0.086	0.087
Cholesterol	0.122	0.016	−0.017	0.759	0.156	0.002
Triacylglycerol	0.342	<0.001	0.047	0.398	0.062	0.221
K_1	—	—	—	—	—	—
MK-4	0.153	0.005	—	—	—	—
MK-7	0.173	0.001	0.009	0.875	—	—
ucOC	−0.216	<0.001	0.020	0.720	−0.227	<0.001
ucOC:iOC	−0.203	0.001	−0.005	0.927	−0.181	0.001
NTx	−0.071	0.189	−0.066	0.249	−0.015	0.781
DPD	−0.223	<0.001	−0.065	0.254	−0.091	0.101
BAP	0.013	0.815	0.001	0.988	0.004	0.937

[1] K_1, phylloquinone; MK, menaquinone; ucOC, undercarboxylated osteocalcin; iOC, intact osteocalcin; NTx, *N*-terminal telopeptide; DPD, deoxypyridinoline; BAP, bone-derived alkaline phosphatase. Pearson's correlation coefficient (*r*) and the corresponding *P* values were used to evaluate the relation of the plasma vitamer K concentration with each variable.

Table 3
Relation between undercarboxylated osteocalcin (ucOC), the ratio of ucOC to intact osteocalcin (iOC), or deoxypyridinoline (DPD) and age[1]

	ucOC		ucOC:iOC		DPD	
	r^2	P	r^2	P	r^2	P
Age	0.054	<0.001	0.030	0.008	0.095	<0.001
K_1	0.047	0.001	0.041	0.002	0.050	<0.001
MK-7	0.052	<0.001	0.033	0.009	NR	

[1] K_1, phylloquinone; MK, menaquinone; NR, no relation. Stepwise multiple linear regression analyses were performed to explore determinants of ucOC, ucOC:iOC, or DPD. Plausible predictors [age, BMI, cholesterol, triacylglycerol, K_1, MK-4, and MK-7] were included in the original model. Forward stepwise regression was performed, and $P > 0.25$ was used for variable removal.

MS/MS technique (14). With this method, we detected K_1 in the plasma of all subjects who participated in this study, and the proportion of subjects with undetectable concentrations of MK-4 and MK-7 was 47.8% and 1.9%, 42.0% and 2.3%, 45.3% and 2.3%, respectively, in the groups aged 30–49, 50–69, and ≥70 y, respectively. Thus, we believe the current system of assay for serum vitamin K has achieved a significant improvement in terms of the minimum detectable sensitivity.

The mean plasma K_1 concentrations of the groups aged 30–49, 50–69, and ≥70 y were 1.52, 1.75, and 1.29 ng/mL, respectively, which were almost the same as or slightly higher than those of previous reports (13, 18). As we expected, the plasma MK-4 concentration was below the limit of detection in nearly half of the women, and the concentrations were extremely low. There were no significant differences between the plasma concentrations of MK-4 and ucOC or the plasma ucOC:iOC in any of the age groups. This lack of difference does not indicate the failure of MK-4 to reduce the concentration of plasma ucOC. In fact, pharmacologic doses of MK-4 significantly decreased the serum ucOC concentration (19). It has been suggested that K_1 is converted in part into MK-4 in various tissues of animals after K_1 ingestion, and thus the plasma concentrations of K_1 may reflect the plasma MK-4 concentrations (20). In our study, however, no correlation was observed between the plasma concentrations of K_1 and MK-4 in any of the age groups. Differences in the circulating concentrations of MK-7maybe attributable to differences in eating habits, especially in the intake of fermented soybean (natto). Kaneki et al (16) reported that the average plasma MK-7 concentrations in Japanese women in Tokyo were higher than those in Japanese women in Hiroshima (Tokyo: 5.26 ng/mL; Hiroshima: 1.22 ng/mL) because natto is eaten frequently in Tokyo but seldom in Hiroshima. The customary natto intake in the population in the current study is almost same as that in the Tokyo area. When the plasma K_1 and MK-7 concentrations in the 3 age groups were compared, both K_1 and MK-7 were highest in the group aged 50–69 y. Triacylglycerol-rich lipoproteins are known to serve as carriers of K_1 in the circulation (21). Consistent with the previous reports (22), we found a significant positive correlation between the plasma concentrations of K_1 or MK-7 and the plasma triacylglycerol concentrations. In this regard, the subjects aged 50–69 y seemed to have a higher vitamin K status, as well as a faster lipoprotein metabolism, than did the other 2 age groups.

Plasma K_1 concentrations correlated negatively and independently with urinary DPD concentrations. It was reported that 15 d of dietary vitamin K depletion led to increased bone

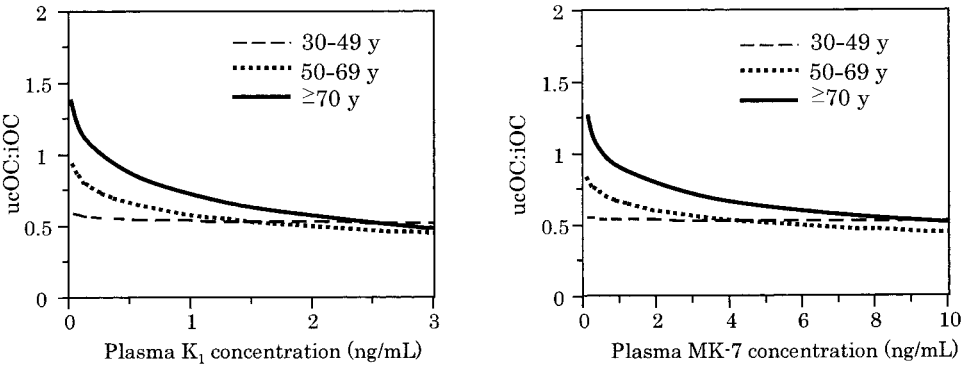

FIGURE 2. Association between the plasma phylloquinone (K_1) or menaquinone (MK) 7 concentration and the ratio of undercarboxylated osteocalcin (ucOC) to intact osteocalcin (iOC) in 3 age groups. Regression curves and P values were obtained from nonlinear logarithmic regression analysis by using the plasma K_1 or MK-7 concentrations and ucOC:iOC. $n = 52$, 208, and 136 in the groups aged 30–49, 50–69, and ≥70 y, respectively. The equations of regression curves using the plasma K_1 concentration and ucOC:iOC were $y = 0.546 - 0.015 \log x$ ($P = 0.8151$) for the group aged 30–49 y, $y = 0.589 - 0.117 \log x$ ($P = 0.0020$) for the group aged 50–69 y, and $y = 0.748 - 0.224 \log x$ ($P = 0.0101$) for the group aged ≥70 y, where y = mean ucOC:iOC, and x = mean plasma concentration of K_1 or MK-7. Intercepts of the 3 age groups were significantly different ($P < 0.001$, analysis of covariance). The equations of regression curves that used the plasma MK-7 concentrations and ucOC:iOC were $y = 0.550 - 0.006 \log x$ ($P = 0.8401$) for the group aged 30–49 y, $y = 0.677 - 0.094 \log x$ ($P < 0.0001$) for the group aged 50–69 y, and $y = 0.870 - 0.172 \log x$ ($P = 0.0005$) for the group aged ≥70 y, where y = mean ucOC:iOC, and x = mean plasma concentration of K_1 or MK-7. Significant differences were detected in intercepts ($P < 0.001$, analysis of covariance) and slopes ($P < 0.022$, analysis of covariance) of the 3 age groups.

turnover, as measured by the serum osteocalcin and urinary NTx concentrations; these markers were subsequently normalized by K_1 repletion for 10 d (200 μg/d) (23). In other studies, these bone turnover markers either were not significantly changed (7, 18) or were increased (24, 25) by K_1 supplementation. In our study, plasma K_1 concentrations correlated significantly only with urinary DPD concentrations, not with urinary NTx and plasma BAP concentrations, which suggested that vitamin K may be a weak regulatory factor for bone turnover in women.

In this cross-sectional observational study of healthy Japanese women, we observed a significant negative correlation between the plasma concentrations of K_1 or MK-7 and the plasma ucOC concentration or ucOC:iOC in the subjects aged $>$ 50 y; however, these correlations were not observed in the subjects aged 30–49 y. It is conceivable that the plasma concentrations of vitamin K required for γ-carboxylation of osteocalcin in younger subjects may be lower than those required in older subjects, and, thus, even at a concentration below the median, the plasma concentrations of vitamin K in the groups aged 30–49 y may be almost sufficient for the subjects to sustain normal γ-carboxylation of ucOC in the bone. In previous reports (26, 27), the circulating ucOC concentrations in elderly women were higher than those in young women. We also confirmed that the plasma ucOC concentration and ucOC:iOC were highest in the oldest group (Table 1), which suggests an age-related difference in osteocalcin γ-carboxylation efficiency. Figure 2 clearly indicates that requirements for vitamin K increase with age. According to the minimum ucOC:iOC (0.546 for K_1 and 0.550 for MK-7) presumed by the logarithmic regression analysis of the group aged 30–49 y, minimum ucOC:iOC in the groups aged 50–69 and $\geq$70 y would be achieved by the consumption of 1.4 and 2.5 ng K_1/mL, respectively, and of 3.9 and 6.4 ng MK-7/mL, respectively. However, because almost all subjects had both K_1 and MK-7 in their plasma, the effect of K_1 and MK-7 to reduce ucOC:iOC could not be compared. Binkley et al (28) reported that, when 1000 μg K_1/d was supplemented for 1 wk, the K_1 concentrations rose to 2.9 ng/mL, and the ucOC percentage reached a minimum in subjects aged 19–36 y. In other studies, younger or elderly subjects who supplemented their diets with 1000 μg K_1/d for 1 wk had 6.5 or 12 nmol K_1/L (2.9 or 5.4 ng K_1/mL), respectively, at the end of the week, and the ucOC percentage decreased maximally in both age groups (18). The mechanisms of the increase in the vitamin K requirement with aging are not clear. It may be that a decrease in the number of osteoblasts—target cells of vitamin K—or in the enzymatic activity of γ-carboxylase in osteoblasts with aging may be related to the mechanism.

This is the first report showing the difference between the requirements for K_1 and MK-7 to minimize ucOC concentrations in healthy young adult and elderly women. Several reports indicated that the increase in serum ucOC concentrations is a risk factor for fractures (29–32), and a high ucOC concentration correlates with low bone mineral density (33). The precise role of ucOC remains unclear. However, if the subminimum ucOC concentration is related to the prevention of bone fracture or bone mineral loss, circulating vitamin K concentrations in elderly people should probably be kept higher than those in younger people.

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