Vitamin K Content of Foods and Dietary Vitamin K Intake in Japanese Young Women

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Summary Several reports indicate an important role for vitamin K in bone health as well as blood coagulation. However, the current Adequate Intakes (AI) might not be sufficient for the maintenance of bone health. To obtain a closer estimate of dietary intake of phylloquinone (PK) and menaquinones (MKs), PK, MK-4 and MK-7 contents in food samples (58 food items) were determined by an improved high-performance liquid chromatography method. Next, we assessed dietary vitamin K intake in young women living in eastern Japan using vitamin K contents measured here and the Standard Tables of Food Composition in Japan. PK was widely distributed in green vegetables and algae, and high amounts were found in spinach and broccoli (raw, 498 and 307 μg/100 g wet weight, respectively). Although MK-4 was widely distributed in animal products, overall MK-4 content was lower than PK. MK-7 was observed characteristically in fermented soybean products such as natto (939 μg/100 g). The mean total vitamin K intake of all subjects (using data from this study and Japanese food composition tables) was about 230 μg/d and 94% of participants met the AI of vitamin K for women aged 18–29 y in Japan, 60 μg/d. The contributions of PK, MK-4 and MK-7 to total vitamin K intake were 67.7, 7.3 and 24.9%, respectively. PK from vegetables and algae and MK-7 from pulses (including fermented soybean foods) were the major contributors to the total vitamin K intake of young women living in eastern Japan.

Key Words vitamin K, phylloquinone, menaquinone, content of foods, dietary intake

In nature, there are two major forms of vitamin K. Vitamin K1 (phylloquinone, PK) is produced by plants and algae, and the vitamin K2 series (menaquinones, MKs) are derived from bacteria and animals. MKs comprise a family of molecules distinguished from PK by unsaturated side-chains of isoprenoid units varying in length from 1 to 14 repeats (1). PK is widely distributed in green leafy vegetables and vegetable oils. In contrast, MKs are found in animal products, including chicken egg yolk, butter and cheeses (2, 3). Fermented soybean products also contain substantial amounts of MK-7 and may be of nutritional importance for populations consuming this class of foods, such as Japanese (4).

The role of vitamin K is a cofactor for an enzyme that converts specific glutamyl residues in several proteins such as plasma clotting factors II (prothrombin), VII, IX and X to γ-carboxyl glutamyl (Gla) residues. These vitamin K-dependent proteins play crucial roles in blood coagulation. In addition, several reports indicate an important role for vitamin K in bone health. Three vitamin K-dependent proteins, osteocalcin, matrix Gla protein and protein-S have been identified as bone-matrix protein and protein-S have been identified as bone-matrix components produced by osteoblasts (5–7). The administration of vitamin K results in increased bone-mineral density (BMD) and reduced bone resorption in humans (8, 9). In epidemiological studies, low dietary vitamin K intake was associated with an increased incidence of hip fracture (10, 11).

The available data estimated by diet records in Europe and the US suggest that the PK intake of many individuals is falling to meet the current guideline in the UK, 1 μg/kg body weight/d and Adequate Intakes (AI) in the US, 120 μg/d for adult men and 90 μg/d for adult women. It was reported that average values for dietary vitamin K intake was around 60–70 μg/d in British and American studies (12, 13). In Japan, AI of vitamin K is set at 75 μg for adult men, 60 μg for women aged 18–29 y, and 65 μg for women 30 y and over as a probable sufficient quantity for the maintenance of normal blood clotting. However, recent epidemiological studies indicate that the current guideline based on the maintenance of plasma prothrombin concentration might not be sufficient for the maintenance of bone health (14). In addition, the assessment of dietary intake of both PK and MKs is incomplete. In par-

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ticular, current available data on MK content of foods are thought to be insufficient to estimate dietary vitamin K intake in regions where people habitually eat fermented foods. Recently, we have developed an improved assay method for vitamin K in human plasma using two kinds of high-performance liquid chromatography (HPLC) systems optimized for the determination of MK-4 and less polar derivatives, PK and MK-7 (15). We applied this technique to measure PK and MKs in food samples. Dietary intakes of PK, MK-4 and MK-7 in Japanese young women were also estimated.

**MATERIALS AND METHODS**

**Chemicals and reagents.** PK, MK-4, MK-5, MK-6, MK-7, MK-8, MK-9 and MK-10 were kindly provided by Elsai Co., Ltd. (Tokyo, Japan). The vitamin K analogs, 2-methyl-3-hexadecyl-1,4-naphthoquinone (I.S.-C16) and 2-methyl-3-nona decyl-1,4-naphthoquinone (I.S.-C19) were synthesized in our laboratory as described previously (15). HPLC-grade solvents and reagents for chemical synthesis were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

**Measurement of vitamin K content of foods.** Several examples of cereals, pulses, vegetables, algae, fish and shellfish, meat, eggs, milk and dairy products, fats and oils, beverages, seasonings, spices and dressings which are eaten on a daily basis in Japan (Table 1) were selected for analysis of vitamin K. They were purchased from retail stores representing the three major food chains in the Kobe area in 2004 and 2005. There were six subsamples weighting 0.1–0.5 kg of each food item, and three of them were prepared to present analysis. For general food items, homogenized edible samples (1–5 g) with internal standard solution (I.S.-C16 and I.S.-C19, 0.5 μg/100 μL ethanol each) and 1 g of sea sand were pestled in 10 mL of acetone three times, filtered, and extracted with 40 mL of diethyl ether twice. After dehydration with Na2SO4, the ether layer was evaporated under reduced pressure. The residue was dissolved with 5 mL of hexane, and passed through a Sep-Pak Vac silica cartridge (6 cc, 1 g, Waters, Milford, MA, USA) washed with 20 mL of hexane. Vitamin K was eluted with 10 mL of hexane-diethyl ether (97:3). The eluate was evaporated under reduced pressure, and the residue was dissolved with 1.0–2.5 mL of ethanol. Aliquots (40 μL) were subjected to two kinds of HPLC. For oil and fat products, samples (1.0–2.5 g) with internal standard solution were dissolved with 5 mL of hexane. The mixture was shaken for 5 min before centrifuging at 3,000 rpm for 5 min, and 4.5 mL of the hexane layer was purified by silica-gel cartridge and then subjected to HPLC.

The HPLC system consisted of an LC-10ADVP pump (Shimadzu, Kyoto, Japan), an SII-10ADVP auto injector (Shimadzu), a CTO-10ADVP column oven (Shimadzu) set to 35°C, and an RF-10AXL, fluorescence detector (Shimadzu) set to an excitation wavelength of 320 nm (System 1) or 240 nm (System 2) and an emission wavelength of 430 nm. The data acquired by a C-R8A chromatopac (Shimadzu) were processed by CLASS-
PR10 software (Shimadzu). Separations were performed on a CAPCELL PAK C18 UG120 (4.6×250 mm, 5 μm, Shisuido Co., Ltd., Tokyo, Japan). An RC-10 platinum-reduction column (4.0×15 mm, Itica, Kyoto, Japan) was located between the HPLC column and the fluorescence detector. For determination of MK-4, the mobile phase was a 95:5 (v/v) mixture of methanol and water (System 1). For determination of PK and MK-7, the mobile phase was a 95:5 (v/v) mixture of methanol and ethanol (System 2). The flow-rate was 1.0 mL/min in both systems.

Dietary vitamin K intake assessment. Healthy Japanese young women aged 20–23 y (mean 21.2 y, n = 125) who were enrolled at Kagawa Nutrition University in Saitama Prefecture, eastern Japan, voluntarily participated in this study during 2003. Informed consent was obtained from each subject. The subjects consumed self-selected foods, and food intake over 3 d including a weekend and two weekdays was recorded by weight. The vitamin K intake was calculated from the vitamin K content of foods measured in this study (Table 1). For the vitamin K content of foods which were not measured objects in this study, the fifth revised and enlarged edition of the Standard Tables of Food Composition in Japan was used (16). In such cases, vitamin K content of foods of plant origin and animal products was calculated as PK and MK-4, respectively. MK-4-equivalent content of MK-7 was used to calculate MK-7 intake in consideration of the difference of molecular weight:

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\text{MK-4-equivalent content of MK-7} = \frac{C \times 444.7}{649.0}
\]

where C is the content of MK-7, 444.7 is the molecular weight of MK-4, and 649.0 is the molecular weight of MK-7.

RESULTS

HPLC analysis of extracts from food samples

First, the extracts from food samples without internal standards were subjected to HPLC. PK and MK-4 were detected in many food items. MK-7 was detected in fermented soybean products mainly. MK-5, MK-6 and MK-8 were detected in several kinds of fermented soybeans, natto, in the range of 2 to 34 μg/100 g. MK-6, MK-8 and MK-9 were observed in processed cheese in the range of 0.5 to 7 μg/100 g. MK-10 was not detected in the food items tested here. Thus, it was decided to measure PK, MK-4 and MK-7 in this study. Figure 1 shows the chromatographic profiles of authentic standards (Fig. 1A and D), extract from egg yolk with or without post-column reduction (Fig. 1B and C) and extract from fermented soybeans, natto, with or without post-column reduction (Fig. 1E and F). MK-4, I.S-C16, PK, I.S-C19 and MK-7 were successfully isolated from other substances in foods, and were not detected without post-column reduction. The values obtained by HPLC with fluorescence detection agreed with those obtained by the LC-APCI/MS/MS method using some of the same food samples (17) (data not shown).

PK content of foods

Table 1 shows the PK, MK-4 and MK-7 content of various food items. We subdivided common Japanese foods into 11 categories based on the Standard Tables of Food Composition in Japan. High amounts of PK were found in vegetables such as komatsuna (cruciferous vegetables, Brassica campestris var. peruviridis, raw, 319 μg/100 g), perilla (raw, 1.007 μg/100 g), garlic chrysanthemum (raw, 230 μg/100 g), broccoli (raw, 307 μg/100 g) and spinach (raw, 498 μg/100 g). Boiled cabbage, komatsuna, garlic chrysanthemum and spinach contained more PK per 100 g than raw ones. High amounts of PK were also found in algae such as roasted and seasoned liver (dried, 413 μg/100 g), hijiki (Hizikia fusiform, dried, 175 μg/100 g) and wakame, seaweed (dried, 1.293 μg/100 g), vegetable fats and oils such as soybean oil (234 μg/100 g) and mixed vegetable oil (164 μg/100 g), and teas such as green powdered tea (3.049 μg/100 g). Although tea leaves of natural leaf tea and black tea contained high amounts of PK (1.876 and 1.036 μg/100 g, respectively), little PK was found in the brew (0.1 μg/100 g). Considerable amounts of PK were also detected in seasonings, spices and dressings such as mayonnaise (whole egg type, 197 μg/100 g).
Vitamin K Content of Foods and Dietary Vitamin K Intake

**MK content of foods**

MK-4 was widely distributed in fish, meat, eggs, milk and dairy products, and seasonings, spices and dressings; however, overall MK-4 content was lower than PK. Relatively high amounts of MK-4 were found in chicken meat (raw, 27 \( \mu g/100\) g), the egg yolk of hen’s eggs (raw, 64 \( \mu g/100\) g), and mayonnaise (egg yolk type, 38 \( \mu g/100\) g). MK-7 was observed characteristically in fermented soybean foods such as natto (939 \( \mu g/100\) g), Hikiwari natto (chopped natto, 827 \( \mu g/100\) g), and black soybean natto (796 \( \mu g/100\) g). The food items measured here had little MK-7 except for natto.

**Dietary vitamin K intake assessment**

First, we assessed dietary vitamin K intake in young women living in eastern Japan aged 20–23 y (n=125). Mean intake of total vitamin K was estimated at 230.2±143.3 \( \mu g/d \) and 94% of participants met the AI of vitamin K for women aged 18–29 in Japan. 60 \( \mu g/d \) (Fig. 2A). Mean daily intakes of PK, MK-4 and MK-4-equivalent MK-7 were estimated at 155.9±91.1, 16.9±10.4 and 57.4±83.7 \( \mu g/d \), respectively (Fig. 2B, C and D). The contributions of PK, MK-4 and MK-7 to total vitamin K intake were 67.7, 7.3 and 24.9%, respectively. The percentage contribution of each food group to total vitamin K intake is shown in Fig. 3A. Vegetables and pulses including fermented soybean products were the main sources of vitamin K in young women living in eastern Japan and accounted for 78.3% of total vitamin K intake. The percentage contribution of each food group to PK, MK-4 and MK-7 intakes are shown in Fig.

**Fig. 2. Distribution of daily intakes of vitamin K and of their derivatives in Japanese young women (n=125).** (A) Total vitamin K; (B) PK; (C) MK-4; (D) MK-4-equivalent MK-7.

**Fig. 3. Percentage contribution of each food group to vitamin K intake in young Japanese women (n=125).** (A) Total vitamin K; (B) PK; (C) MK-4; (D) MK-7. Food items were categorized based on the 5th revised and enlarged edition of the Standard Tables of Food Composition in Japan.
3B, C and D. Vegetables, algae and fats and oils accounted for 86.5% of PK intake, while the major sources of MK-4 intake were meat (43.8%), milk and dairy products (17.0%) and eggs (15.9%). Almost all of MK-7 intake was derived from pulses including fermented soybeans, natto.

From the results shown in Fig. 2D, it was expected that about half of the participants did not consume fermented soybean, natto. Therefore, we also assessed dietary vitamin K intake separately divided into natto eaters (n = 53) and non-natto eaters (n = 72) based on 3-d diet records. Mean total vitamin K intakes of natto eaters and non-natto eaters were estimated at 333.6 ± 138.2 and 154.1 ± 87.8 μg/d, respectively. In natto eaters, the contribution of MK-7 to total vitamin K intake reached 40.6%. The percentage contribution of each food group to total vitamin K intake in natto eaters and non-natto eaters are shown in Fig. 4A and B. In natto eaters, the largest contributor to the total vitamin K intake was pulses including fermented soybean products and accounted for 45.1%. Meanwhile, in non-natto eaters, the largest contributor to the total vitamin K was vegetables.

**DISCUSSION**

In this paper, we measured the vitamin K content of food items habitually eaten in Japan. It was confirmed that PK was widely distributed in green vegetables. PK was found in traditional Japanese green leafy vegetables such as komatsuna and garland chrysanthemum as well as spinach and broccoli. High amounts of PK were also found in algae and green powdered tea, which are also frequently consumed in Japan. In a review article, Booth and Suttle (18) reported the PK content of common foods as the median value obtained from five reports published in 1993–1997. The PK content of spinach and broccoli was reported as 380 μg/100 g and 180 μg/100 g, respectively. In this report, the PK content of these foods was higher than these values (spinach, 498 μg/100 g; broccoli, 307 μg/100 g). The PK content of cabbages and olive oil was similar to the previous report. These results raise the possibility that the PK content in some vegetables might be different between regions. In addition, extraction of vitamin K derivatives from vegetables might be difficult to achieve with high efficiency. In the case of spinach and broccoli, the recovery rates of internal standards were relatively low, ranging from 25 to 35%. Therefore, careful examinations regarding the PK content of vegetables are needed.

We also measured MK-4 and MK-7 in the same food samples. Due to the low concentration of MKs in food samples, their detection has had practical difficulties; therefore, there are few reliable data on MKs of various food items. On the basis of previous studies, it is assumed that MK contents are low except in some fermented soybean products and cheeses (4, 19). We presented here that many animal products contained a relatively small amount of MK-4 and several forms of fermented soybean foods, natto, contained a high amount of MK-7. Limited food items such as processed cheese and natto contained only a small quantity of MK-6, MK-8 and MK-9. These findings are in agreement with the results reported previously. In Japan, natto made using a strain of *Bacillus subtilis* with high productivity of MK-7 has received approval as food for specified health use; thus, further studies on MK-7 content of natto will be needed. Compared with PK, the food items measured here had less MK except for MK-7 in natto; however, the absorption of PK from green vegetables is poor and only 10–15% of PK is bioavailable (20, 21). In addition, circulating MK-7 concentration after the consumption of natto was about 10 times higher than PK after eating spinach (19). Moreover, MK-4 activates the transcription of extracellular matrix-related genes through a steroid xenobiotic receptor (22). Thus, daily intake of MKs might be important and considerable, even if it is less than PK. There are several reports suggesting the conversion of PK into MK-4 in animal experiments (23, 24). PK supplementation resulted in higher MK-4 levels in tissues when compared with vitamin K-deficient values. Recently, it was demonstrated that menadione is a catalytic product of PK, MK-4 and MK-7 in humans (25). These observations suggest that some MK-4 in tissue results from the uptake and prenylation of menadione.

Next, we assessed dietary vitamin K intake in young women living in Saitama Prefecture, eastern Japan. The dietary total vitamin K intake calculated based on the vitamin K content of foods measured in this study and the fifth revised and enlarged edition of Standard Tables of Food Composition in Japan was about 230 μg/d. We also demonstrated that vitamin K intake from vegetables and pulses including fermented soybeans accounted for about 80% of total vitamin K intake. The Standard Tables of Food Composition in Japan contains information on all vitamins except biotin. The vitamin K content of each food is listed as the total content of PK and MK-4 except MK-7 in natto. Kimura et al. (26) also carried out a survey of vitamin intake in Japanese young women and reported that daily intake of vitamin K calculated by food records and the fifth revised edition of the Standard Tables of Food Composition in Japan was 191 ± 156 μg/d. This result is similar to ours and both values met the AI of vitamin K for women aged 18–29 y in Japan, 60 μg/d. By vitamin K derivatives, PK, which accounted for 67.7% of the total vitamin K intake, is considered to be the primary dietary source of vitamin K, as in Europe and the US. The PK intake calculated in this study (155 ± 91.1 μg/d) was higher than the average level in previous reports. The mean dietary PK intake estimated for healthy young and middle aged people range from 68 to 111 μg/d in Europe and the US (13, 27, 28). The MK-4 intake calculated in this study (16.9 ± 10.4 μg/d) was also higher than in the previous report. Geleijnse et al. (29) reported that the mean MK-4 intakes estimated for older Dutch men and women were 7.7 ± 3.4 and 6.3 ± 2.8 μg/d, respectively. MK-4-equivalent MK-7 intake derived from natto consumption was comparable to the AI value, 57.4 ±
83.7 μg/d. However, there are great differences between individuals in natto consumption. In this study, 42.4% of participants consumed natto and mean total vitamin K intake for them was about twofold higher than that of non-natto eaters. It was also demonstrated that the largest contributor to the total vitamin K intake of natto eaters was pulses including fermented soybean products unlike for non-natto eaters. Thus, natto consumption can influence total vitamin K intake. Kaneki et al. (30) reported that serum MK-7 levels were significantly higher in frequent natto eaters and a statistically significant inverse correlation was observed between the incidence of hip fractures in women and natto consumption in Japan. Ikeda et al. (31) also showed significant positive associations between natto intake and the rates of changes in BMD in postmenopausal women. Moreover, we demonstrated that plasma concentration of MK-7 correlated inversely with under carboxylated osteocalcin concentration as well as PK in Japanese (32). Thus, PK derived from vegetables and oils and MK-7 derived from natto may have a nutritionally important role in maintaining the bone health in Japanese. Further epidemiological study on vitamin K intake, including MKs and bone, and reconsideration of the dietary habit-based vitamin K requirement are thought to be required.

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