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a bleeding tendency is the classic symptom of vitamin K deficiency. There are also proteins that are vitamin K-dependently γ-carboxylated in tissues other than the liver, and many of these may have functional significance. Recently, vitamin K deficiency in extra-hepatic tissues has also been reported to be associated with health problems.[4,5]

Osteocalcin is a vitamin K-dependent Gla protein, and is the most abundant non-collagenous bone matrix protein produced by osteoblasts. Through γ-carboxylation, osteocalcin gains the ability to bind hydroxyapatite, and regulates bone mineralization.[4] Recent clinical evidences strongly suggest that skeletal vitamin K deficiency increases the risk of fractures.[6] Matrix Gla protein (MGP); another vitamin K-dependent protein, is an inhibitor of vascular calcification.[7,8] Therefore, vitamin K deficiency is associated with health problems in a tissue-specific manner, and thus leads to a bleeding tendency, osteoporosis, and vascular calcification due to vitamin K deficiency in the liver, skeleton, and vasculature, respectively.

Previous studies have indicated that the bleeding tendency is a serious clinical problem in SMID patients.[9] Additionally, subjects with neurodevelopmental disabilities are reported to be at a high risk of fractures.[10,11] Nevertheless, reports on the incidence of vitamin K deficiency in these patients are scarce.[12] These considerations led us to examine the vitamin K status in patients with SMID.

2. Subjects and methods

2.1. Subjects

The study subjects were 82 SMID patients (41 males and 41 females) living in the residential hospital, the Biwako Gakuen Kusatsu Medical and Welfare Center for Children and Persons with SMID. Detailed information about this study was provided to the subjects or their proxy, and written consent was obtained for their participation in this study. The study protocol was approved by the ethics committee of the institution described above. The exclusion criteria were treatment with vitamin K, warfarin, or multivitamin supplementation. Patients with pre-existing liver or bone disease were also excluded.

Of the 102 subjects residing in the above facility, written informed consent was obtained from 85 cases. Three subjects were excluded because they were taking vitamin K or warfarin. None of the subjects had experienced overt signs or symptoms attributable to vitamin K deficiency. In addition, none of the subjects were being treated with any drugs that may have interfered with the vitamin K metabolism. Mechanical ventilation support and tracheostomy were required in 7 and 18 subjects, respectively.

2.2. Biochemical measurements

Blood was obtained from each patient after an overnight fasting. After centrifugation, the serum was maintained at −30 °C until the analysis. The serum levels of protein induced by vitamin K absence-II (PIVKA-II) and undercarboxylated osteocalcin (ucOC) were measured by an electro chemiluminescence immunoassay (ECLIA) (EIDIA Co., Ltd, Tokyo, Japan), which are vitamin K-dependent hepatic and bone markers, respectively. In the current study, the reference range for PIVKA-II was defined to be less than 28 mAU/mL, which is 2SD above the mean value of 14.2 mAU/mL.[13] Similarly, the reference range for ucOC was 5.37 ng/mL for women, and 5.47 ng/mL for men, respectively, which are 2SD above the corresponding mean values (2.51 ng/mL for men and 3.01 ng/mL for women).[14] The platelet count and international normalized ratio (INR), were also measured. The laboratory reference range was 0.90–1.14.

2.3. Assessment of the nutrients intake

The 7-day nutrient intake was assessed by the food record method. The intake of vitamin K was calculated by multiplying the amount of vitamin K supplied from the institution by the average percentage intake. Based on these records, the intake of vitamin K by the patients was calculated using a software program (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan). The vitamin K intake/kg body weight was also calculated.[15]

2.4. Statistical analyses

Statistical analyses were performed using the SPSS 18.0 J software program for Windows (SPSS, Japan Inc., Tokyo, Japan). The associations between the variables were analyzed by the Spearman's correlation coefficient. The multiple regression analyses were performed to identify the independent variables that affect the vitamin K status. The differences between four independent groups were analyzed by the Mann–Whitney *U* test with the Bonferroni correction. The breakpoint for vitamin K intake was decided as follows. Subjects were divided into two groups; those with their vitamin K intake (μg/BW kg/day) above the predetermined cut-off value and those below it. Various cut-off values were screened starting from 1.0 μg/BW kg/day to 6.0 μg/BW kg/day with 0.5 μg/BW kg/day intervals. Then, comparison was made between the two groups on the serum concentrations of PIVKA-II and ucOC. The breakpoint was so determined as the cut-off level of vitamin K intake with the most significant difference, which was defined as the minimum *p* value or maximum absolute *t* value in Student's *t*-test.[16]

3. Results

3.1. The baseline patient characteristics and data from blood examinations

The baseline characteristics of the study subjects and the data from blood examination are shown in Table 1. Because of their

Table 1
Characteristics of subjects.

	Number/data	Duration (y)	Reference range
Bedridden state	63 (77)	38 ± 12 (37)	
Enteral feeding	36 (44)	16 ± 11 (14.5)	
Long-term antibiotic treatment	19 (23)	6.3 ± 3.9 (7.0)	
AED administration	71 (87)	36 ± 14 (35)	
Monotherapy	21 (26)	41 ± 14 (43)	
Combined therapy	50 (61)	34 ± 13 (33)	
Age (y)	39.4 ± 12.6 (39.0)		
Male/female	41/41		
Height (cm)	147.6 ± 10.5 (148.5)		
Weight (kg)	34.4 ± 5.6 (34.4)		
BMI (kg/m^2)	15.8 ± 2.2 (15.7)		
INR	1.02 ± 0.10 (1.01)		0.90–1.14
Serum albumin (g/dL)	4.0 ± 0.4 (4.0)		3.7–5.3
Serum total cholesterol (mg/dL)	166 ± 29 (164)		130–219
Serum triglyceride (mg/dL)	80 ± 39 (71)		35–149
Cholinesterase	307 (256, 360)		200–465
GOT (U/L)	22 (18, 28)		10–40
GPT (U/L)	19 (14, 24)		6–40
γGTP (U/L)	57 (32, 94)		<48
Creatinine	0.40 ± 0.13 (0.42)		M0.6–1.15/ F0.45–0.85
C-reactive protein	0.29 (0.13, 1.00)		<0.30

Mean ± SD (Med), Median (Q1–Q3).

Please cite this article in press as: Nagae A, et al., Enteral nutrition and antibiotic use increase the risk for vitamin K deficiency in patients with severe motor and intellectual disabilities, e-SPEN Journal (2013), http://dx.doi.org/10.1016/j.clnme.2012.12.002

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neurological deficits, 63 and 19 subjects were bedridden or remained in a sitting position, respectively. The underlying diseases were cerebral palsy (CP) coupled with mental retardation in 66 patients, degenerative diseases in 11, anoxic encephalopathy in four, and sequelae of encephalitis in one patient. Forty-six subjects were able to receive nutrition by oral intake (OI). The remaining 36 subjects were enterally fed via the intragastric and jejunal routes in 27 and 9 subjects, respectively. All subjects with enteral nutrition (EN) received a polymeric diet.

Nineteen and 71 subjects were under long-term treatment with antibiotics and anti-epileptic drugs (AEDs), respectively. The mean duration of antibiotic treatment was 7.0 years. Although the subjects' BMIs were apparently low (average; 16 kg/m^2), they were within the reference range for those with SMID (15–18 kg/m^2).[17] The INR was within the reference range for all except two subjects.

3.2. Distribution of PIVKA-II and ucOC

The serum concentrations of PIVKA-II and ucOC were 60.9 ± 106.5 mAU/mL (median: 29.0 min–max; 10–632 mAU/mL) and 5.44 ± 5.70 ng/mL (median: 3.49; min–max; 0.39–32.56), respectively (Fig. 1).

In 52% of the subjects, the serum PIVKA-II level was above the upper normal range of 28 mAU/mL. In 30% of the subjects, the serum level of ucOC was above the upper reference range.

3.3. Comparison of the vitamin K status between subjects based on their type of feeding or drug treatment

The multiple regression analyses were performed to identify the determinants of the vitamin K status. For PIVKA-II, the R^2 was 0.229, and EN and antibiotic use were the independent predictors of the PIVKA-II level, the β-coefficient being 0.221 for EN and 0.327 for antibiotic use, respectively. In contrast, antibiotic treatment was the only significant determinant for the ucOC concentration with the R^2 of 0.270 and the β-coefficient of 0.428.

As shown in Table 2, the percentage of energy intake from protein was higher and that from fat was lower in subjects receiving EN than in those with OI. The energy intake from carbohydrates was not significantly different between these two groups. The average vitamin K intake, including that from EN, was 4.5 μg/BW/day in the whole subjects. The intake was significantly lower (2.0 μg/BW/day) in those receiving EN than in those with OI (5.7 μg/BW/day).

The serum levels of PIVKA-II and ucOC in subjects receiving EN were significantly higher than those in subjects with OI. The serum levels of PIVKA-II and ucOC were both significantly higher in subjects undergoing antibiotic treatment than in those without. Of interest, the subjects who were receiving antibiotics had a significantly lower vitamin K intake compared to those without antibiotic treatment. There were no differences in the markers for a bleeding tendency between subjects based on their type of feeding or drug treatment.

3.4. The combined effects of antibiotic treatment and EN on the vitamin K status

Then, subjects were divided into four groups based on the presence or absence of EN and antibiotic treatment (Fig. 2). In those with both factors (EN/AB+ group), the serum PIVKA-II and ucOC concentrations were higher than those in the other three groups. There were significant differences between the EN/AB+ group and the OI/AB− group in all parameters. In the EN group, the serum ucOC concentration was significantly different between the patients receiving antibiotics and those who were not.

We next examined the interaction of antibiotics with EN. The effects of antibiotics on the levels of PIVKA-II and ucOC were significantly different between patients receiving EN and who had OI (interaction p value of PIVKA-II; $p = 0.06$, ucOC; $p = 0.02$).

3.5. Determination of breakpoints of vitamin K for serum levels of PIVKA-II and ucOC

Next, we studied the breakpoint of vitamin K intake for PIVKA-II and ucOC. Considering the significant interaction between antibiotic use and vitamin K intake, only the data from the subjects without antibiotic treatment were used for analyses. In Fig. 3, vitamin K intake was significantly and inversely correlated with the serum PIVKA-II ($r = -0.448$, $p < 0.001$). The negative correlation

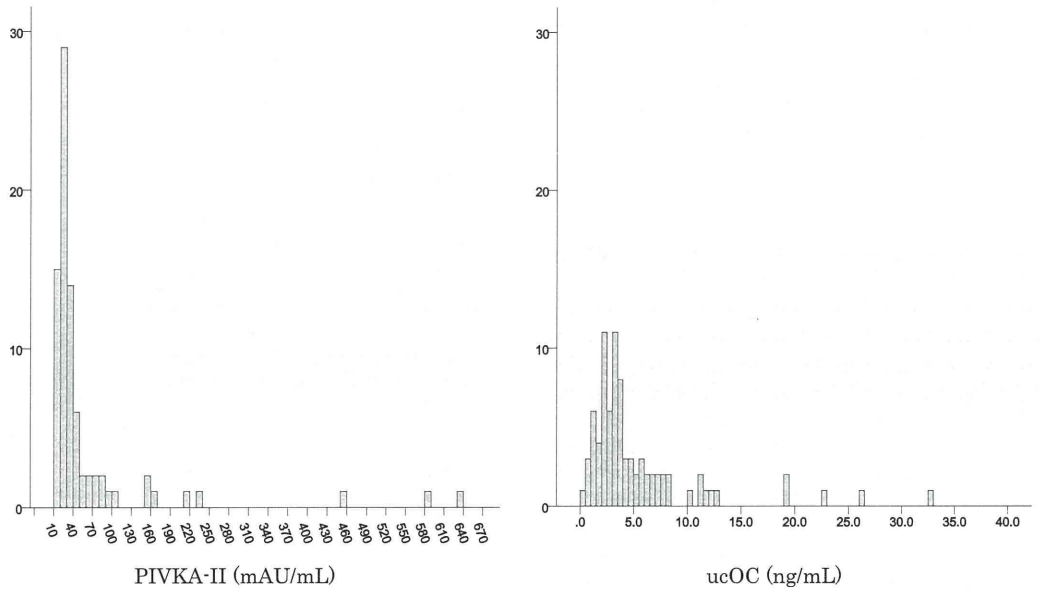

Fig. 1. Distribution of PIVKA-II and ucOC. The left and right panels show the distribution of the serum PIVKA-II and ucOC levels, respectively, in the SMID patients. The min–max value was 10–632 mAU/mL for PIVKA-II and was 0.4–32.6 ng/mL for ucOC.

Please cite this article in press as: Nagae A, et al., Enteral nutrition and antibiotic use increase the risk for vitamin K deficiency in patients with severe motor and intellectual disabilities, e-SPEN Journal (2013), http://dx.doi.org/10.1016/j.clnme.2012.12.002

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Table 2
Comparison of the vitamin K status between subjects based on their type of feeding or drug treatment.

	All ($n = 82$)	OI ($n = 46$)	EN ($n = 36$)	*P*-value	−Antibiotics ($n = 63$)	+Antibiotics ($n = 19$)	*P*-value
OI/EN	46/36	–	–	–	44/19	2/17	0.001
Antibiotics (+/−)	19/63	2/44	17/19	0.001	–	–	–
AEDs (+/−)	71/11	39/7	32/4	0.590	54/9	17/2	0.675
Energy intake (kcal)	1219 ± 270 (1213)	1379 ± 192 (1372)	1015 ± 207 (1000)	0.000	1274 ± 252 (1290)	1036 ± 242 (1000)	0.001
Protein intake (g)	54 ± 10 (56)	58 ± 6.5 (58)	48 ± 12.1 (49)	–	55 ± 10 (56)	51 ± 11 (53)	–
Protein intake (E%)	17.9 ± 2.5 (17.5)	17.0 ± 1.0 (17.1)	19.1 ± 3.2 (20.2)	0.000	17.3 ± 2.3 (17.4)	19.8 ± 2.1 (20.7)	0.000
Fat intake (g)	32 ± 9.8 (35)	39 ± 4.9 (39)	25 ± 9.2 (23)	–	35.4 ± 8.5 (37)	24.0 ± 9.0 (22.1)	–
Fat intake (E%)	23.9 ± 4.1 (24)	25.4 ± 1.8 (25.2)	22.0 ± 5.2 (21.1)	0.000	24.9 ± 3.3 (24.6)	20.5 ± 4.5 (20.7)	0.000
Carbohydrates intake (g)	175 ± 40 (169)	196 ± 34 (192)	147 ± 28 (143)	–	181 ± 37 (177)	154 ± 40 (153)	–
Carbohydrates (E%)	58.2 ± 3.5 (57.0)	57.6 ± 2.5 (56.9)	58.9 ± 4.4 (57.1)	0.150	56.9 ± 2.8 (57.0)	59.3 ± 6.2 (57.1)	0.151
V.K intake (μg/day)	161 (74, 214)	208 (177, 222)	70 (54, 82)	–	180 (90, 218)	67 (54, 82)	–
V.K intake/BW (μg/BW kg/day)	4.5 (2.0, 6.1)	5.7 (5.0, 6.3)	2.0 (1.6, 2.4)	0.000	5.3 (2.5, 6.2)	2.2 (1.4, 2.5)	0.000
PIVKA-II (mAU/mL)	29 (22, 44)	24 (20,29)	51 (33, 98)	0.000	25 (21, 35)	60 (24, 156)	0.001
ucOC (ng/mL)	3.4 (2.3, 6.1)	3.3 (2.1, 4.0)	4.8 (2.9, 10.3)	0.005	3.3 (2.2, 4.7)	7.2 (2.9, 19.3)	0.007
INR	1.02 ± 0.10 (1.01)	1.00 ± 0.06	1.05 ± 0.14	0.118	1.01 ± 0.06	1.07 ± 0.19	0.175

Mean ± SD (Med) or median (Q1, Q3). Unpaired *t*-test or Mann–Whitney *U* test depending on normality.

between serum ucOC level and vitamin K intake had borderline significance ($r = -0.247$, $p = 0.051$). INR was not significantly correlated with vitamin K intake in SMID subjects. The breakpoints of vitamin K intake for PIVKA-II and ucOC were 2.5 μg/BW/day and 5.5 μg/BW/day, respectively.

4. Discussion

Few studies have previously examined the prevalence of vitamin K deficiency in SMID patients. Yoshikawa et al. reported a high prevalence of vitamin K deficiency in SMID children based on their serum PIVKA-II level.[12] In the current study, we have studied both serum levels of PIVKA-II and ucOC. As a result, serum concentrations of PIVKA-II and ucOC were above mean + 2SD of the healthy subjects in 52% and 30% of the patients, respectively. Thus, we have shown that SMID patients had high prevalence of vitamin K deficiency based on both hepatic and bone markers.

In order to identify the determinants of the vitamin K status, the multiple regression analyses were performed, which revealed that the route of energy and nutrient administration and antibiotic use contributed to the blood levels of PIVKA-II and ucOC. On the basis of this result, the subjects were divided into four groups based on the presence or absence of EN and antibiotic use (Fig. 2). Those receiving both EN and antibiotic therapy had the poorest vitamin K status.

The association of EN with a poorer vitamin K status may be explained by the much lower vitamin K intake in these subjects. With regard to antibiotic use, two underlying mechanisms might be considered. One is the possible interference with the vitamin K cycle by the antibiotics. When acting as the co-factor for γ-carboxylase, vitamin K is oxidized to the epoxiside form, which is then re-utilized through its reduction to the hydroquinone form by reductases. Some antibiotics are known to interfere with this cycle, and may decrease the efficacy of vitamin K utilization. For example, cepharosporins with N-methylthiotetrazole (nMTT), thiadiazolethiol, or the methylthiadiazolethiol chemical structure, directly inhibit vitamin K epoxide reductase activity.[18] Another possible mechanism is their effect on the intestinal flora.[19] Intestinal bacteria are known to produce the long-chain forms of menaquinones. The bacterial contribution to the overall vitamin K status is not considered to be large when sufficient vitamin K is supplied from the diet. However, in situations where the food-derived vitamin K

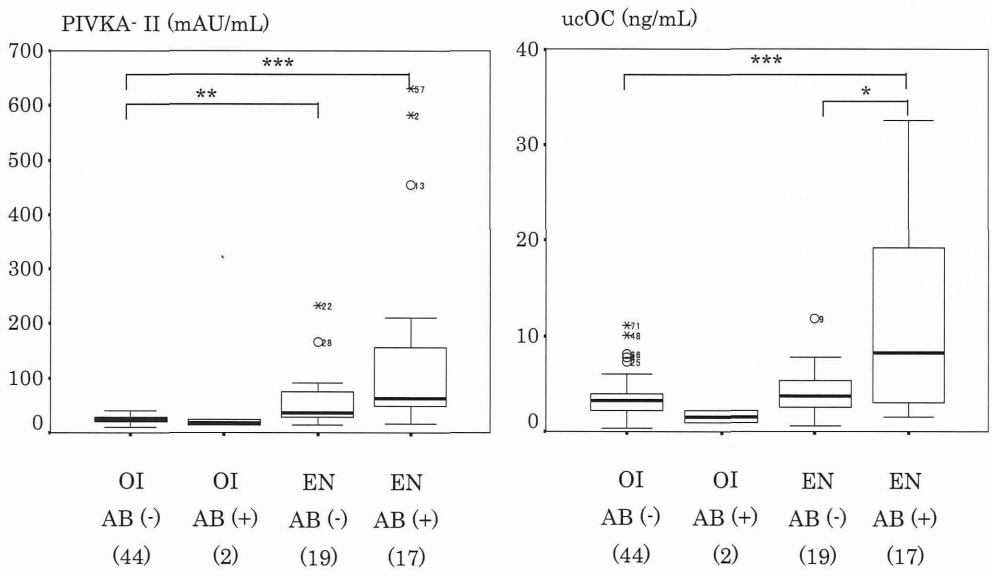

Fig. 2. The effects of antibiotic use and enteral nutrition on the vitamin K status. The abbreviations used are as follows; OI/AB(−); the subjects with oral intake who were antibiotic free, OI/AB(+); the subjects with oral intake and antibiotic use, EN/AB(−); the subjects with enteral nutrition who were antibiotic free, and EN/AB(+); the subjects with enteral nutrition and antibiotic use. The number of patients is shown below each group. The differences between the four independent groups were analyzed by the Mann–Whitney *U* test with a Bonferroni correction.

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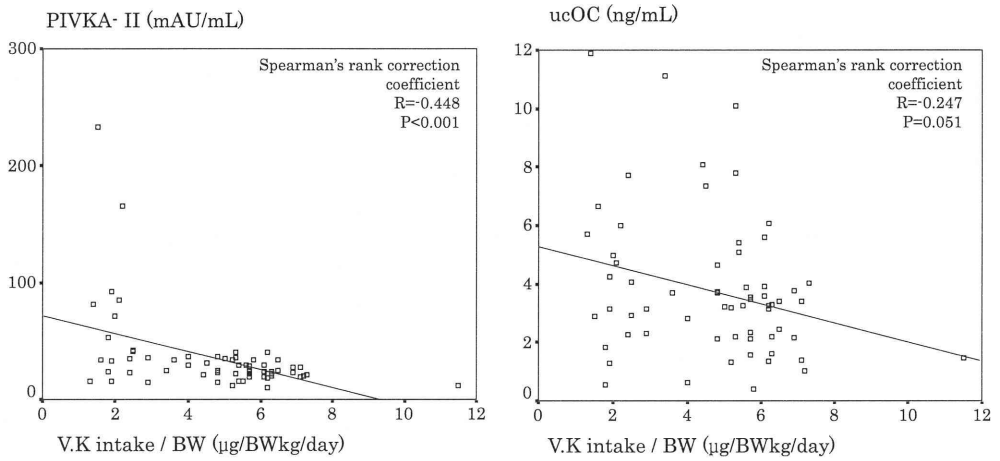

Fig. 3. Correlations between vitamin K intake and the PIVKA-II and ucOC levels. The correlations between vitamin K intake and biomarkers for vitamin K deficiency in subjects without antibiotic treatment were analyzed by Spearman's correlation.

intake is limited, intestinal bacteria can make a significant contribution to the overall vitamin K status. Although the relative contribution of these two factors is still to be decided in our study, antibiotic use increases the risk for vitamin K deficiency, and it is likely that subjects having both EN and antibiotic use are at the highest risk for vitamin K deficiency.

Next, we examined the breakpoint of vitamin K intake for PIVKA-II and ucOC using data from subjects without antibiotic treatment. It was 2.5 μg/BW kg/day for PIVKA-II. In contrast, the breakpoint for ucOC was 5.5 μg/BW/day, which is markedly higher than that for PIVKA-II. This difference is likely to be due to the "first pass effect", wherein vitamin K, absorbed from the intestine, is first utilized in the liver, and then becomes available in the extra-hepatic tissues such as bone. For this reason, bone is considered to be more susceptible to vitamin K deficiency.[20,21] As shown in Fig. 2 analysis of data from the subjects without long-term antibiotic use has shown that the serum PIVKA-II level was significantly higher in subjects with EN than those with OI. In contrast, there was no significant difference in serum ucOC level between these two groups. Such difference would be accounted for by the difference in the percentage of subjects with vitamin K intake below the breakpoint. Median vitamin K intake (μg/BW/day) with Q1 and Q3 in the parentheses was 2.0 (1.6, 2.4) and 5.7 (5.0, 6.3) in those with EN and OI, respectively. Thus, vitamin K intake was below the breakpoint of 2.5 μg/BW/day for PIVKA-II in most patients with EN, whereas it was far above it in practically all subjects with OI. In contrast, vitamin K intake was far below the breakpoint of 5.5 μg/BW/day for ucOC in most subjects with EN, whereas the median intake was just around the breakpoint in those with OI.

We have to mention some limitations of this study. First, this study was devoid of the control group. Thus, the reference ranges for serum PIVKA-II and ucOC levels were arbitrarily decided to be 2SD above the mean level. Second, circulating vitamin K concentration was not measured in the present study. Recently, a novel measurement procedure with LC/MS/MS technology has been developed.[22] It is, however, highly time-consuming and requires special instruments, and is not easily applicable to epidemiological or clinical use. Additionally, there are multiple circulating forms of vitamin K such as phylloquinones (vitamin K_1) and various menaquinones (vitamin K_2). Thus, we believe that lack of data on the blood levels of vitamin K is not a serious drawback of our study.

Vitamin K deficiency in patients with neurodevelopmental disabilities, to which little attention has been paid, is likely to be of clinical relevance including coagulation problems or increased fracture risk. Although various therapeutic drugs are available with robust evidence for fracture prevention, most of them are not easily applicable to SMID patients. For example, bisphosphonates are not indicated to those with dysphagia, and selective estrogen receptor modifier (SERM) is not recommended to bedridden subjects considering the risk of venous thromboembolism. Thus, vitamin K could be a reasonable candidate to be given to the SMID subjects for the prevention of fracture. Further studies including the cohort and intervention ones are advocated to clarify the clinical significance of vitamin K in SMID subjects.

In summary, SMID patients showed a high prevalence of vitamin K deficiency as judged by the markers for the vitamin K status both in the liver and bone, to which EN and antibiotic use were significant contributing factors. Those with the co-existence of both conditions were at an even higher risk for vitamin K deficiency.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgment

We thank Drs. Naoko Tsugawa, Toshio Okano, Masakazu Miura and Dr. Hiroaki Kohno for their valuable advice. The authors thank the entire staff of the Biwako Gakuen Kusatsu Medical and Welfare Center for Children and Persons with SMID. The authors also thank all of the SMID patients that were enrolled in this study.

The authors have signed the declaration of originality and personal contribution: AN carried out the studies and data analyses and drafted the manuscript. AK contributed to the conception and design of the study with KiT. AN, AK, and MK conducted the research. KuT measured the serum concentrations of PIVKA-II and ucOC. YT supervised the research. AN, AK, and KiT were responsible for the statistical analyses and critical revision of the manuscript for intellectual content and for final approval of the manuscript. All authors read and approved the final manuscript.

This study was supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS) and Health Labour Sciences Research Grant.

References

1. Haak P, Lenski M, Hidecker MJ, Li M, Paneth N. Cerebral palsy and aging. *Dev Med Child Neurol* 2009;**51**(Suppl. 4):16–23.
2. Sleigh G, Sullivan PB, Thomas AG. Gastrostomy feeding versus oral feeding alone for children with cerebral palsy (Review). *Cochrane Database Syst Rev* 2004.

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3. Vermeer C. Gamma-carboxyglutamate-containing proteins and the vitamin K-dependent carboxylase. *Biochem J* 1990;**266**:625–36.
4. Booth SL. Skeletal functions of vitamin K-dependent proteins: not just for clotting anymore. *Nutr Rev* 1997;**55**:282–4.
5. Vermeer C, Shearer MJ, Zittermann A, Bolton-Smith C, Szulc P, Hodges S, et al. Beyond deficiency: potential benefits of increased intakes of vitamin K for bone and vascular health. *Eur J Nutr* 2004;**43**:325–35.
6. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr* 2000;**71**: 1201–8.
7. Danziger J. Vitamin K-dependent proteins, warfarin, and vascular calcification. *Clin J Am Soc Nephrol* 2008;**3**:1504–10.
8. Schurgers LJ, Cranenburg EC, Vermeer C. Matrix Gla-protein: the calcification inhibitor in need of vitamin K. *Thromb Haemost* 2008;**100**:593–603.
9. Kumode M, Mekata Y, Hujita Y, Shimada M. Problem of long-term enteral nutrition on the severely disabled. *J Severe Motor Intellect Disabil* 1994;**19**:53–7 [in Japanese].
10. Sheridan KJ. Osteoporosis in adults with cerebral palsy. *Dev Med Child Neurol* 2009;**51**(Suppl. 4):38–51.
11. Ward KA, Caulton JM, Adams JE, Mughal MZ. Perspective: cerebral palsy as a model of bone development in the absence of postnatal mechanical factors. *J Musculoskelet Neuronal Interact* 2006;**6**:154–9.
12. Yoshikawa H, Yamazaki S, Watanabe T, Abe T. Vitamin K deficiency in severely disabled children. *J Child Neurol* 2003;**18**:93–7.
13. Kozu K, Nakanishi T, Okuda H, Watanabe K, Saito S, Tanaka M, et al. Development of PIVKA-II measuring reagent (ED038) by ECL technology and its performance characteristics. *Rinsho to Kenkyu* 1996;**73**:2656–64 [in Japanese].
14. Nishimura J, Arai N, Fujimatsu J. Measurement of undercarboxylated osteocalcin by electrochemiluminescence immunoassay with the "Picolumi ucOC" kit. *Igaku to Yakugaku* 2007;**57**:523–5 [in Japanese].
15. Ministry of Health, Labour, and Welfare, Japan. *Dietary reference intakes for Japanese 2010*. Tokyo: Daiichi-Shuppan; 2010 [in Japanese].
16. Tsugawa N, Uenishi K, Ishida H, Minekami T, Doi A, Koike S, et al. A novel method based on curvature analysis for estimating the dietary vitamin K requirement in adolescents. *Clin Nutr* 2012;**31**(2):255–60.
17. Pawlyn Jillian, Carnaby Steven. *Profound intellectual and multiple disabilities: nursing complex needs*. Wiley-Brackwell; 2009. p. 262–3.
18. Matsubara T, Touchi A, Harauchi T, Takano K, Yoshizaki T. Depression of liver microsomal vitamin K epoxide reductase activity associated with antibiotic-induced coagulopathy. *Biochem Pharmacol* 1989;**38**:2693–701.
19. Brody T. *Nutritional biochemistry*. 2nd ed. Academic Press; 1999. p. 537–8.
20. Booth SL, Martin L, Peterson JW, Saltzman E, Dallal GE, Wood RJ. Dietary phylloquinone depletion and repletion in older women. *J Nutr* 2003;**133**:2565–9.
21. Schurgers LJ, Shearer MJ, Hamulyák K, Stöcklin E, Vermeer C. Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects. *Blood* 2004;**104**:2682–9.
22. Suhara Y, Kamao M, Tsugawa N, Okano T. Methods for the determination of vitamin K homologues in human plasma using high-performance liquid chromatography-tandem mass spectrometry. *Anal Chem* 2005;**77**:757–63.

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J Nutr Sci Vitaminol, 59, S57–S66, 2013

Dietary Reference Intakes for Japanese 2010: Fat-Soluble Vitamins

Kiyoshi TANAKA[1], Junji TERAO[2], Yoshihiro SHIDOJI[3], Hiroshi TAMAI[4], Eri IMAI[5] and Toshio OKANO[6]

[1] *Department of Food and Nutrition, Kyoto Women's University, Kyoto 605–8501, Japan*
[2] *Department of Food Science, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770–8503, Japan*
[3] *Department of Nutrition and Health Science, Graduate School of Human Health Science, University of Nagasaki, Nagasaki 851–2195, Japan*
[4] *Department of Pediatrics, Osaka Medical College, Osaka 569–8686, Japan*
[5] *Section of the Dietary Reference Intakes, Department of Nutritional Epidemiology, National Institute of Health and Nutrition, Tokyo 162–8636, Japan*
[6] *Department of Hygienic Sciences, Kobe Pharmaceutical University, Kobe, 658–8558, Japan*

(Received October 26, 2012)

Summary We have determined the Dietary Reference Intakes for fat-soluble vitamins (vitamin A, vitamin D, vitamin E, and vitamin K) for the Japanese. Regarding vitamin A, the estimated average requirement (EAR) and the recommended dietary allowance (RDA) were defined for those aged 1 y old and over. For vitamin D, vitamin E, and vitamin K, the EAR or RDA was not adopted, because of the insufficient data available. Thus, the adequate intake (AI) was determined for those vitamins based on the food surveillance data and biomarkers for each vitamin. The AI for vitamin D was decided as the median intake of vitamin D in the population with a circulating 25-hydroxy vitamin D level which was high enough for bone health. The basis for the AI for vitamin E was the median intake of α-tocopherol in the healthy population considering the lack of unfavorable health consequences attributable to its deficiency. The AI for vitamin K was determined as the vitamin K intake, required to avoid blood coagulation abnormalities. The tolerable upper intake level (UL) was determined for vitamin A, vitamin D and vitamin E, but not for vitamin K, since no adverse effects have been reported even with its high dosage.

Key Words vitamin A, vitamin D, vitamin E, vitamin K

Vitamin A

Background information

Compounds with potent vitamin A activity in vivo after oral intake include retinol; retinal; carotenoids; and 50 different types of provitamin A carotenoids, including β-carotene, α-carotene, and β-cryptoxanthin. The retinol equivalent (RE) is the vitamin A unit used in Dietary Reference Intakes for Japanese (DRIs-J) 2010, the most current Dietary Reference Intakes (DRIs) for the Japanese. Retinoic acid, a hormone binding to the nuclear receptor, is responsible for the majority of vitamin A activity in vivo, but is not converted to retinal or retinol in vivo, and its content in food is relatively low. Retinylester provitamin A carotenoids are the main forms of vitamin A contained in animal and plant foods, respectively. Retinylester hydrolase in the intestinal brush border catalyzes the hydrolysis of retinylester to retinol, which is then absorbed at a rate that ranges from 70% to 90% (*1*, *2*). Cleavage of carotenoids yields 2 molecules of vitamin A (retinal) from β-carotene (*3*) and 1 molecule from other provitamin A carotenoids.

In the DRIs-J 2010, the absorption rate of β-carotene is 1/6 of its total value, which is in accordance with rate in the DRIs for the United States and Canada (*4*). Assuming that the conversion rate of β-carotene to retinol is 50%, the bioavailability of β-carotene as vitamin A is 1/12 (1/6×1/2), such that 12 μg of food-derived β-carotene would correspond to 1 μg in RE units. Thus, the following formula can be used to convert the value of food-derived vitamin A-related compounds into RE units:

Retinol equivalent (μg RE)
=retinol (μg)+β-carotene (μg)×1/12
+α-carotene (μg)×1/24+β-cryptoxantin (μg)
×1/24+other provitamin A carotenoids (μg)
×1/24.

A word of caution is indicated when calculating the value for oil-solubilized β-carotene, as its bioavailability as a form of vitamin A is 1/2 of its total value, such that 2 μg of fat-solubilized β-carotene would correspond to 1 μg of retinol.

Determining DRIs

Classical vitamin A deficiency leads to corneal xerosis in infants and possibly to blindness and to night blindness in adults. Other deficiency signs include growth retardation; skeletal and neurological development defects; disturbed growth and differentiation of epi-

E-mail: tanakak@kyoto-wu.ac.jp

thelial cells; dryness, thickening, and keratinization of the skin; immunodeficiency; and susceptibility to infection (5). Due to the abundant storage of vitamin A in the liver, inadequate intake does not lead to decreased plasma retinol concentration unless hepatic vitamin A storage is below 20 μg/g (6, 7). Thus, plasma retinol concentration cannot be used as an index of vitamin A status. Theoretically, hepatic vitamin A storage is the best index, but its measurement is highly invasive and not applicable to humans. Thus, the vitamin A intake required to maintain minimal hepatic vitamin A storage has been used for estimating the Estimated Average Requirement (EAR) for vitamin A.

Compartment analysis assuming the existence of 3 compartments—serum, liver, and other tissues—has shown that the daily disposal rate of vitamin A is approximately 2% (8, 9). Using this percentage, the daily disposal amount (DDA), daily disposal rate (DDR), body storage (BS) according to body weight (BW), and hepatic storage (HS) of vitamin A can be calculated as follows:

DDA (μg/d)=BS (μg)×DDR (2%/d (10)).

BS/BW (μg/kg BW)

=HS (≥20 μg/g)×liver weight/BW (21 g/kg BW) ×10/9,

where 90% of the body storage of vitamin A is in the liver (10, 11).

DDA/BW (μg/[kg BW·d])

=BS (≥20 μg/g×21 g/kg×10/9)×DDR (2/100)

=9.3 μg/kg BW.

Thus, the amount of vitamin A intake required to compensate for its daily elimination, thereby ensuring that hepatic storage of vitamin A is maintained and vitamin A deficiency is avoided, is estimated to be 9.3 μg RE/kg BW/d.

EAR and Recommended Dietary Allowance (RDA) for adults

The EAR for vitamin A for those aged 18 y and above, as calculated by multiplication of the reference value of 9.3 μg RE/kg BW/d and the reference BW, is 550 to 600 μg RE/d for males and 450 to 500 μg RE/d for females. Assuming the inter-individual variability in vitamin A requirement to be 20% (4), multiplication of these EAR values by 1.4 yields an RDA of 800 to 850 μg RE/d for males and 650 to 700 μg RE/d for females.

EAR and RDA for children

The RDA for children aged 6 to 17 y was determined by extrapolation from the EAR for adults aged 18 to 29 y by the 0.75th power of the BW ratio, which represents the ratio of body surface area (4). Extrapolation of the adult EAR to preschool children based on BW ratio may yield values that maintain plasma retinol levels below 20 μg/100 mL, and thus render children susceptible to corneal xerosis (12). Therefore, the RDA for children aged less than 5 y must be at least 200 μg RE/d to avoid this unfavorable outcome; therefore, for children aged less than 5 y, the DDA was calculated as follows, assuming the ratio of liver weight/BW to be 42 g/kg BW (10):

DDA/BW (μg/kg BW/d)

=BS (≥20 μg/g×42 g/kg×10/9)×DDR (2/100)

=18.7 μg/kg BW.

Using the value obtained, the EAR for children aged 1 to 5 y was calculated as follows:

EAR=18.7 μg/kg BW/d×reference BW×(1+growth factor)

=EAR×1.4.

Adequate Intake of infants aged 0 to 5 mo

Vitamin A concentration in breast milk is highest during the first 10 d after delivery, after which it gradually decreases (13, 14). Based on the values for average vitamin A concentration (411 μg RE/L) (14) and daily milk intake (0.78 L/d) (15, 16), vitamin A intake in breast milk-fed infants aged 0 to 5 mo was estimated at 320 μg RE/d. Thus, adequate intake (AI) for this age group was determined to be 300 μg/d. The level of provitamin A carotenoids was not taken into account because its availability is unknown.

AI of infants 6 to 11 mo

Based on extrapolation from the AI for infants aged 0 to 5 mo, the AI for infants aged 6 to 11 mo was determined to be 400 μg RE/d. The level of provitamin A carotenoids was not taken into account because its availability is unknown.

Amount to be added during pregnancy

The amount of vitamin A transported to the fetus through the placenta must be taken into account when estimating the vitamin A requirement for pregnant women. At the late-stage of a fetus, the amount of vitamin A deposited in the fetal liver was 1,800 μg (17, 18) so that the total amount of vitamin A transported to the fetus during pregnancy is estimated at 3,600 μg. Using this value, the EAR value for the additional amount of vitamin A required during the late stage was determined to be 60 μg RE/d, which, assuming an inter-individual variability of 20% (4), yielded an RDA value of 80 μg RE/d during the late-stage. The additional amount required during the early- and mid-stage was not determined.

Amount to be added during lactation

Based on measurement of the amount of vitamin A secreted in breast milk, the EAR value for the additional amount of vitamin A required during lactation was estimated at 300 μg RE/d, which, assuming an inter-individual variability of 20%, yielded an RDA value of 450 μg RE/d (4).

Tolerable upper intake level

An elevated plasma level of retinoic acid is considered responsible for most clinical signs (19) and symptoms of vitamin A intoxication, such as headache. Based on reported fetal abnormalities due to excessive intake of vitamin A, (20, 21) the no observable adverse effect level (NOAEL) during pregnancy was estimated at 4,500 μg RE/d, which, assuming an uncertainty factor of 1.5 and taking the additional amount into account, yielded an upper level (UL) of 3,000 μg RE/d.

Based on research into hepatotoxicity caused by the excessive vitamin A deposition (22), the NOAEL in adults was estimated at 13,500 μg RE/d, which, assuming an uncertainty factor of 5, yielded a UL of 2,700 μg RE/d. Based on clinical observation of increased intracranial pressure in infants caused by excessive vitamin

Table 1. DRIs for vitamin A (μg RE/d).[1]

Sex	Males				Females			
Age	EAR[2]	RDA[2]	AI[3]	UL[3]	EAR[2]	RDA[2]	AI[3]	UL[3]
0–5 mo	—	—	300	600	—	—	300	600
6–11 mo	—	—	400	600	—	—	400	600
1–2 y	300	400	—	600	250	350	—	600
3–5 y	300	450	—	700	300	450	—	700
6–7 y	300	450	—	900	300	400	—	900
8–9 y	350	500	—	1,200	350	500	—	1,200
10–11 y	450	600	—	1,500	400	550	—	1,500
12–14 y	550	750	—	2,000	500	700	—	2,000
15–17 y	650	900	—	2,500	450	650	—	2,500
18–29 y	600	850	—	2,700	450	650	—	2,700
30–49 y	600	850	—	2,700	500	700	—	2,700
50–69 y	600	850	—	2,700	500	700	—	2,700
≥70 y	550	800	—	2,700	450	650	—	2,700
Pregnant women (amount to be added)								
Early-stage					+0	+0	—	—
Mid-stage					+0	+0	—	—
Late-stage					+60	+80	—	—
Lactating women (amount to be added)					+300	+450	—	—

DRIs, Dietary Reference Intakes; RE, retinol equivalents; EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.

[1] Retinol equivalent (μg RE)=retinol (μg)+β-carotene (μg)×1/12+α-carotene (μg)×1/24+β-cryptoxanthin (μg)×1/24+other provitamin A carotenoids (μg)×1/24.

[2] Including provitamin A carotenoids.

[3] Excluding provitamin A carotenoids.

A intake (*23*), the NOAEL in infants was estimated at 6,000 μg RE/d, which, assuming an uncertainty factor of 10, yielded a UL of 600 μg RE/d.

The UL for children aged 1 to 17 y was determined by extrapolation from the UL for adults based on the ratio of body surface area. For safety reasons, the values for men were applied to women. Extrapolation to infants aged 1 to 2 y old yielded a UL of 500 μg RE/d, which is lower than that for infants aged 6 to 11 mo (600 μg RE/d). Thus, the UL for infants aged 1 to 2 y old was revised to 600 μg RE/d. Although a recent study found that ingesting approximately 1,500 μg RE/d of retinol for 30 y doubled the fracture risk in the elderly (*24*), data from other studies contradicted this finding. Thus, determination of a separate UL for vitamin A for the elderly was not considered in developing the most recent DRIs. Moreover, as excessive intake of β-carotene has not been reported to be associated with the unfavorable consequences of vitamin A intoxication described above, the level of provitamin A carotenoids was also not included in the estimation of UL.

Remarks regarding carotenoids

Due to the strict regulation of their conversion into vitamin A, provitamin A carotenoids, when ingested orally, cannot cause vitamin A intoxication. Unconverted provitamin A carotenoids, as well as carotenoids that are not metabolized to vitamin A are stored in vivo as they are. Beneficial actions have been reported with ingestion of these carotenoids, including anti-oxidant activity and immune potentiation and photoprotection of skin by anti-oxidation. Regarding the benefits of specific carotenoids, prevention of prostate cancer by lycopene, improvement in age-related macular degeneration by lutein and zeaxanthin, and the maintenance of retinal pigment by lutein and zeaxanthin have also been reported. Although the results of cohort studies suggest that higher intake of carotenoids is associated with lower incidence of lung cancer (*25*), supplementary intervention has been reported to be ineffective or even harmful in the prevention of cancer, especially lung cancer (*26–29*). Thus, further research into the efficacy and safety of carotenoids is required. In developing the current DRIs, the carotenoids were not separately considered because their deficiency has not been reported.

DRI values for vitamin A are listed in Table 1.

Vitamin D

Background information

Vitamin D_2 and vitamin D_3 are naturally occurring compounds with potent vitamin D activity. The indices for the DRI of vitamin D is based on the summation of the values of these 2 compounds. The human body obtains vitamin D from 2 sources. One is exposure to ultraviolet irradiation, which converts pro-vitamin D_3

(7-dehydrocholesterol) in the skin to pre-vitamin D_3, which in turn is converted into vitamin D_3 by thermal isomerization. The other is dietary intake of vitamin D_2 and vitamin D_3 from such sources as mushrooms and fish; good sources for vitamin D_2 and vitamin D_3, respectively. The current DRIs do not discriminate between vitamin D_2 and D_3 intake because the compounds have similar characteristics and a similar molecular weight and exert an almost equal level of biological activity.

Vitamin D is first metabolized to 25-hydroxy vitamin D (25OHD) before being metabolized to 1α,25-dihydroxy vitamin D (1α,25$(OH)_2$D), its active form. Major actions of vitamin D include enhancing the absorption of calcium and phosphate in the intestine and kidneys and stimulating bone formation and growth. Circulating 25OHD level is the best index of vitamin D status. As vitamin D deficiency and resultant hypocalcemia cause elevated levels of serum parathyroid hormone (PTH), serum concentration of PTH can also be a good index of vitamin D deficiency (*30*).

Adequate intake

Evidence for determining AI

Vitamin D deficiency impairs calcium absorption from the intestine and kidney, thus decreases calcium availability, resulting in rickets in children and osteomalacia in adults. In adults, especially the elderly, even so-called "vitamin D insufficiency," which is milder than vitamin D deficiency, can result in increased secretion of PTH, increased bone resorption, and decreased bone mineral density. Therefore, the basis for determining the vitamin D requirement is maintenance of a serum 25OHD level sufficiently high to maintain normal calcium availability and avoid elevation of serum PTH level. Due to limitations on the data available, AI was determined as the median intake of vitamin D in a population in which the required circulating 25OHD level is maintained.

AI for adults

In a study conducted in the northern United States, an area in which residents receive limited sunshine exposure, serum PTH level after vitamin D administration decreased in those with a serum 25OHD level below 50 nmol/L but not in those with a level above 50 nmol/L (*31*). In a study in Niigata, those with a 25OHD level less than 50 nmol/L had higher serum PTH levels and a higher prevalence of low bone mineral density (*32*). Based on consideration of these results, maintenance of a circulating 25OHD level of at least 50 nmol/L is considered necessary to avoid elevation of serum PTH level and decrease in bone mineral density. In the study conducted in the northern United States, serum PTH level exhibited seasonal variation, reaching a nadir between August and October and a peak between March and May. However, this variation was not observed in those taking 5.5 μg/d or more of vitamin D (*33*), leading to the conclusion that taking at least 5.5 μg/d of vitamin D can prevent elevation of PTH in those living in areas in which they have limited sunshine exposure.

In 7 studies that examined Japanese women (*34–39*) aged 50 to 69 y, the average 25OHD level was found to exceed 50 nmol/L. In contrast, in several studies that examined women aged 18 to 29 y (*32, 34*) and women aged 30 to 49 y (*34*), the average level was found to be below 50 nmol/L. Based on these findings and the findings from US studies, the median vitamin D intake of adults aged 50 to 69 y was determined to be an appropriate basis for determining the adult AI. As the 2005 and 2006 National Health and Nutritional Survey (NHNS) (*40, 41*) found that the median intake of vitamin D in adults aged 50 to 69 y was 5.5 μg/d, the AI was set as 5.5 μg/d. Due to lack of data for those aged 18 to 29 y, 30 to 49 y, and above 70 y, as well as lack of data for males, AI for both males and females in these age groups was also set at 5.5 μg/d.

AI for children

As the findings regarding the relationship between vitamin D intake and plasma 25OHD concentration in children have been inconsistent, they were considered unsuitable as the basis for determining the vitamin D AI for children. Thus, the median vitamin D intake, as reported in the 2005 and 2006 NHNS (*40, 41*), was used as the basis for determining the AI.

AI for infants

In an epidemiological study conducted in Kyoto, 22% of neonates were found to have craniotabes, a mineralization defect of bone, likely due to vitamin D deficiency (*42*). The incidence of craniotabes exhibited seasonal variation, with a peak and nadir between January and May and between July and November, respectively. Circulating 25OHD level was found to be below 25 nmol/L in 37% of all neonates diagnosed with craniotabes at 1 mo after birth. In breast milk-fed neonates, serum concentration of 25OHD was found to be less than 25 nmol/L in 57% of subjects and below 12.5 nmol/L in 17%. In contrast, none of the formula or mixed-fed infants were found to have an inadequate serum 25OHD level. It should be noted that neonates born in a vitamin D-deficient state may not recover to a vitamin D-sufficient state within a short period, and that the serum 25OHD level of breast milk-fed infants was found to decrease further during the winter months (*43*), indicating that the vitamin D delivered from breast milk may have been unsatisfactory. The vitamin D AI for infants was determined to be 2.5 μg/d by multiplying 0.78 L/d (*15, 16*), the average daily milk intake, by 3.05 μg/L (*44*), the vitamin D concentration in breast milk as reported in the *Standard Tables of Food Composition in Japan*, 5th Revised and Enlarged Edition.

However, this AI value is appropriate only for infants with adequate sun exposure, defined as 2 h/wk to the face or 30 min/wk to the face and extremities. Breast-milk-fed infants with little sun exposure are at higher risk of developing rickets. Considering that previous research found that no infants developed rickets after supplementation with 2.5 μg/d of vitamin D for 6 mo and assuming that infants receive an average of 2.38 μg/d of vitamin D from breast milk, it follows that a daily intake of 4.88 μg/d of vitamin D is satisfactory for avoiding rickets. Based on these data, the AI of vitamin D for infants aged 0 to 5 mo with limited sun exposure was determined to be 5 μg/d. Recently, however, a

Table 2. DRIs for vitamin D (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo[1]	—	—	2.5 (5.0)	25	—	—	2.5 (5.0)	25
6–11 mo[1]	—	—	5.0 (5.0)	25	—	—	5.0 (5.0)	25
1–2 y	—	—	2.5	25	—	—	2.5	25
3–5 y	—	—	2.5	30	—	—	2.5	30
6–7 y	—	—	2.5	30	—	—	2.5	30
8–9 y	—	—	3.0	35	—	—	3.0	35
10–11 y	—	—	3.5	35	—	—	3.5	35
12–14 y	—	—	3.5	45	—	—	3.5	45
15–17 y	—	—	4.5	50	—	—	4.5	50
18–29 y	—	—	5.5	50	—	—	5.5	50
30–49 y	—	—	5.5	50	—	—	5.5	50
50–69 y	—	—	5.5	50	—	—	5.5	50
≥70 y	—	—	5.5	50	—	—	5.5	50
Pregnant women (amount to be added)					—	—	+1.5	—
Lactating women (amount to be added)					—	—	+2.5	—

[1] Adequate intakes for an infant who is exposed to appropriate sunlight. The value in parentheses is adequate intakes for those with less sunlight exposure.

study using a novel, highly accurate procedure found the average vitamin D concentration in breast milk to be only 0.6 μg/L (*14*). If this value is employed, the average vitamin D intake of breast-milk-fed infants would be only 0.47 μg/d. Such discrepancies indicate the need for further research into this value (*45*, *46*).

AI for infants aged 6 to 11 mo

The AI of vitamin D for infants aged 6 to 11 mo with adequate sun exposure was determined to be 5 μg/d. This value was also applied to infants aged 6 to 11 mo with limited sun exposure due to lack of evidence for determining the AI.

Additional amount during pregnancy

In a study of pregnant women with limited sun exposure, an inadequate serum 25OHD concentration was observed in those with an average vitamin D intake of less than 5.3 μg/d but not in those an average (*47*) vitamin D intake higher than 7 μg/d (*48*). As these findings indicate that pregnant women require at least 7 μg/d of vitamin D, the additional amount of vitamin D required for pregnant women was determined to be 1.5 μg/d.

Additional amount during lactation

Based on the findings described above, the additional amount of vitamin D required for lactating women was determined to be 2.5 μg/d.

Tolerable upper untake level

Basic considerations

Prolonged intake of excessive quantities of vitamin D can lead to unfavorable outcomes, such as hypercalcemia, renal dysfunction, soft tissue calcification, and growth retardation. As an increased serum 25OHD level itself does not directly cause health problems, the presence of hypercalcemia rather than of a high serum 25OHD level is considered an appropriate indicator for determining the UL.

UL for adults

In an intervention study administering doses of vitamin D for 3 mo, serum calcium concentration was found to exceed the reference value in some subjects receiving 95 μg/d of vitamin D but not in those receiving 60 μg/d of vitamin D (*49*). Thus, the lowest observed adverse effect level (LOAEL) and NOAEL were determined to be 95 μg/d and 60 μg/d, respectively. The latter value was divided by an uncertainty factor of 1.2 yielding a UL for adults of 50 μg/d. Since neither administration of 45 μg/d of vitamin D to elderly subjects for 3 mo (*50*) nor administration of 50 μg/d to pregnant and lactating subjects (*51*) was found to be associated with hypercalcemia, stratification by sex or age group was not performed, and a UL of 50 μg/d was applied to all adult groups.

UL for infants

Based on a study that observed no growth retardation in infants administered an average of 44 μg/d of vitamin D for 6 mo, the NOAEL for infants was determined to be 44 μg/d (*52*), which, assuming an uncertainty factor of 1.8, yielded a UL of 25 μg/d.

UL for children

As data were unavailable for this age group, the UL for children was determined by extrapolating the UL values for adults (50 μg/d) and infants (25 μg/d) based on the reference body weight. Sex differences were not considered.

DRI values for vitamin D are listed in Table 2.

Vitamin E

Background information

Vitamin E is composed of 8 analogues: α-, β-, γ- and

Table 3. DRIs for vitamin E (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	3.0	—	—	—	3.0	—
6–11 mo	—	—	3.5	—	—	—	3.5	—
1–2 y	—	—	3.5	150	—	—	3.5	150
3–5 y	—	—	4.5	200	—	—	4.5	200
6–7 y	—	—	5.0	300	—	—	5.0	300
8–9 y	—	—	6.0	350	—	—	5.5	350
10–11 y	—	—	6.5	450	—	—	6.0	450
12–14 y	—	—	7.0	600	—	—	7.0	600
15–17 y	—	—	8.0	750	—	—	7.0	650
18–29 y	—	—	7.0	800	—	—	6.5	650
30–49 y	—	—	7.0	900	—	—	6.5	700
50–69 y	—	—	7.0	850	—	—	6.5	700
≥70 y	—	—	7.0	750	—	—	6.5	650
Pregnant women (amount to be added)					—	—	+0.0	—
Lactating women (amount to be added)					—	—	+3.0	—

[1] Computation was made on α-tocopherol, not including vitamins E other than α-tocopherol.

δ-forms, of tocopherol and tocotrienol. After intestinal absorption, vitamin E is packaged into chylomicron, transformed into chylomicron remnant by lipoprotein lipase, and transported to the liver. Of the 8 analogues, only α-tocopherol is preferentially bound to α-tocopherol binding protein, whereas the other analogues are metabolized in the liver. Alpha-tocopherol is then formed into very low-density lipoprotein (VLDL), converted into low-density lipoprotein (LDL), and distributed to various tissues (*53*). Due to these metabolic processes, α-tocopherol constitutes the predominant vitamin E analogues present in the blood and various tissues. Based on these facts, only α-tocopherol was considered when determining the current DRI for vitamin E.

Determining DRI

Basis for determining AI

Erythrocytes are susceptible to hemolysis by hydrogen peroxide when the circulating α-tocopherol level is between 6 and 12 μmol/L (*54*), but resistant to it when the serum α-tocopherol level is higher than 14 μmol/L (*55*). Although the data from an intervention study that administered graded doses of vitamin E to vitamin E-deficient subjects are available (*56*), they were not considered appropriate for estimating the EAR and RDA because they were collected many years ago. Several studies that simultaneously studied vitamin E intake and serum α-tocopherol level consistently reported that the average serum α-tocopherol level exceeded 22 μmol/L in all study populations (*40, 41, 57–59*). Average vitamin E intake in these studies ranged from 5.6 to 11.1 mg/d, a range that encompasses the 2005 and 2006 NHNS values (*40, 41*) of an average vitamin E intake of 7.0 mg/d in men and 6.5 mg/d in women. As these findings indicate that the median intake of the Japanese likely yields an adequate vitamin E status, the AI was determined to be the 2005 and 2006 NHNS median values stratified by sex and age group (*40, 41*).

AI for adults

As described above, AI was determined to be the 2005 and 2006 NHNS median values for those aged 18 to 29 y stratified by sex and age group, specifically 7.0 mg/d for men and 6.5 mg/d for women, as these values are expected to yield a blood α-tocopherol level exceeding 12 μmol/L (*40, 41*). As aging has not been reported to be associated with compromised absorption or utilization of vitamin E, the same values were applied to the elderly.

AI for children

The 2005 and 2006 NHNS median values for children stratified by sex and age group were used as the basis for determining the AI for children, as they had been for adults.

AI for infants aged 0 to 5 mo

The AI for infants aged 0 to 5 mo was determined to be 3.0 mg/d by multiplying the average α-tocopherol concentration in breast milk (3.5 to 4.0 mg/L) (*14, 60*) by the average milk intake (0.78 L/d) (*15, 16*).

AI for infants aged 6 to 11 mo

The AI for infants aged 6 to 11 mo old was determined to be 3.5 mg/d by extrapolation from the adult value by the 0.75th power of the BW ratio.

AI during pregnancy

The AI for pregnant women was determined to be the same as that for non-pregnant women because vitamin E deficiency during pregnancy has not been reported.

Additional amount during lactation

Since the average α-tocopherol content provided in breast milk is approximately 3.0 mg/d (*14, 60*), the AI

during lactation was determined to be 3 mg/d.

Tolerable upper intake level

The basis for determining the UL for vitamin E is its possible effect on bleeding tendency. Based on the finding that supplementation with 800 mg/d of α-tocopherol for 28 d did not increase bleeding tendency in healthy males (average body weight, 62.2 kg) (*61*), the NOAEL was determined to be 800 mg/d. Assuming an uncertainty factor of 1.0 and considering that no data regarding LOAEL are available, the sex- and age-group stratified UL was calculated by correcting the 800 mg/d value by BW ratio. Because few data are available regarding the UL for infants aged 0 to 11 mo and because typical feeding with breast milk or baby food does not cause excessive intake, the UL was not determined for this age group.

Additional remarks

Although numerous intervention studies have examined the effect of vitamin E supplementation on the risk of coronary heart diseases, the findings have been inconsistent (*62–65*).

DRI values for vitamin E are listed in Table 3.

Vitamin K

Basic considerations

Naturally occurring vitamin K consists of phylloquinones (PKs; vitamin K_1) and menaquinones (MKs; vitamin K_2). Menaquinones are further subdivided into 11 analogues depending on the number of isoprene units (4–14) in the prenyl side chain. Among the menaquinones, of nutritional importance are menaquinone-4 (MK-4), which is ubiquitously present in animal foods, and menaquinone-7 (MK-7), which is abundantly present in natto, a traditional Japanese food made from soybeans fermented with *Bacillus subtilis*. At present, data are scarce for determining the relative biological activity of these analogues, and no corrections have been made for PK and MK-4 with similar molecular weights. MK-7, which has a much larger molecular weight, can be converted into its MK-4 equivalent using the following formula:

MK-4 equivalent (mg)=MK-7 (mg)×444.7/649.

The sum of the quantity of PK, MK-4, and MK-7 as corrected above was employed in determining the DRI for vitamin K. Although long-chain MKs are produced by intestinal bacteria and MK-4 is also produced by enzymatic conversion from PK, their contribution was not considered sufficiently large to contribute to fulfilling this requirement. Although antibiotic treatment can impair vitamin K status by decreasing the production of MKs by intestinal flora and decreasing vitamin K utilization by inhibiting the enzymatic activity of vitamin K epoxide reductase (*66*), antibiotic treatment itself does not cause vitamin K deficiency if average vitamin K intake is maintained (*67*).

The principal biological action of vitamin K is activation of prothrombin and other serum coagulation factors, thereby enhancing blood coagulation. Other actions include the modulation of bone formation by activation of osteocalcin, a bone matrix protein, and inhibition of arterial calcification by activation of matrix gla protein (MGP), another vitamin-K-dependent matrix protein.

Determining DRI

Evidence for determining AI

Since delayed blood coagulation is the only clinically manifested abnormality attributable to vitamin K deficiency, the intake necessary to maintain normal serum coagulation was considered an appropriate basis for determining the AI for vitamin K. In Japan, however, coagulation abnormalities due to vitamin K deficiency are rarely observed in healthy subjects. An intervention study of young vitamin K-deficient male volunteers weighing 72 kg found that administration of 40 and 32 μg/d of vitamin resulted in a decrease in serum PK level and an elevation in undercarboxylated prothrombin, a serum marker for vitamin K deficiency, respectively, but that administration of 82 μg/d of vitamin K returned these levels to normal values (*68*). Based on these findings, the vitamin K requirement for healthy adults was determined to be approximately 1 μg/[kg·d].

Recent studies have suggested that skeletal vitamin K deficiency is a risk factor for fracture (*69, 70*), indicating that a much higher vitamin K intake is necessary for skeletal action. Although a recent meta-analysis found that vitamin K administration significantly reduced fracture incidence, it employed a high dosage (45 mg/d) of MK-4, which is considered to be pharmacological rather than nutritional (*71*). Based on the findings of previous research, a vitamin K intake of approximately 1.0 μg/[kg·d] was determined to be satisfactory to avoid even mild deficiency, and thus set as the AI for vitamin K.

AI for adults

As described above, a vitamin K intake of 82 μg/d in those weighing 72 kg was found sufficient to avoid deficiency (*68*). Extrapolation of this value by the 0.75th power of the BW ratio was used as the basis for determining the adult AI. Although the elderly may be more susceptible to vitamin K deficiency due to various factors such as impaired intestinal absorption of vitamin K, at present, the data are scarce, and thus the AI for the elderly was the same as that for those aged 50 to 69 y.

AI for children

The AI for children was determined by extrapolating the AI for adults by the 0.75th power of the BW ratio.

AI for infants aged 0 to 5 mo

Neonates are susceptible to vitamin K deficiency for various reasons, such as poor transplacental vitamin K transport (*72*), low vitamin K content in the breast milk (*14, 73*), or low production of vitamin K in the intestinal flora (*74*). As neonatal vitamin K deficiency is known to cause neonatal melena, a form of gastrointestinal bleeding, and intracranial bleeding, vitamin K is orally administered just after birth for their prevention. The AI of 4.0 μg/d for this age group was determined by multiplying the average milk intake (0.78 L/d) by the average vitamin K content of milk (5.17 μg/L) and assuming oral administration of vitamin K just after birth in the clinical setting.

Table 4. DRIs for Vitamin K (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	4	—	—	—	4	—
6–11 mo	—	—	7	—	—	—	7	—
1–2 y	—	—	25	—	—	—	25	—
3–5 y	—	—	30	—	—	—	30	—
6–7 y	—	—	40	—	—	—	40	—
8–9 y	—	—	45	—	—	—	45	—
10–11 y	—	—	55	—	—	—	55	—
12–14 y	—	—	70	—	—	—	65	—
15–17 y	—	—	80	—	—	—	60	—
18–29 y	—	—	75	—	—	—	60	—
30–49 y	—	—	75	—	—	—	65	—
50–69 y	—	—	75	—	—	—	65	—
≥70 y	—	—	75	—	—	—	65	—
Pregnant women (amount to be added)					—	—	+0	—
Lactating women (amount to be added)					—	—	+0	—

AI for infants aged 6 to 11 mo

The AI was determined to be 7 μg/d by considering the amount of vitamin K received from sources other than breast milk.

Additional amount during pregnancy

Increased requirements for vitamin K or alterations in circulating vitamin K levels in pregnant women have not been reported. Because of poor transplacental transport, vitamin K intake in pregnant women is unlikely to affect vitamin K status in the fetuses or neonates. Thus, no additional amount required for pregnant women was determined.

Additional amount during lactation

Since lactating women have not been reported to be at higher risk for vitamin K deficiency, no additional amount required for lactating women was determined.

Tolerable upper intake level

Although menadione, a vitamin K metabolite, can cause toxicity, no toxicity has been reported regarding PKs and MKs. As 45 mg/d of MK-4 is clinically administered to many patients in Japan with osteoporosis with no reports of serious adverse events, the UL for vitamin K was not determined.

Other remarks

Due to the abundant vitamin K content of natto, its intake is contraindicated in patients treated with warfarin. In contrast, patients undergoing long-term antibiotic treatment or experiencing chronic obstruction of the biliary tract or impaired fat absorption are at higher risk of vitamin K deficiency.

DRI values for vitamin K are listed in Table 4.

REFERENCES

1) Moise AR, Noy N, Palczewski K, Blaner WS. 2007. Delivery of retinoid-based therapies to target tissues. *Biochemistry* **46**: 4449–4458.
2) Debier C, Larondelle Y. 2005. Vitamins A and E: metabolism, roles and transfer to offspring. *Br J Nutr* **93**: 153–174.
3) During A, Harrison EH. 2004. Intestinal absorption and metabolism of carotenoids: insights from cell culture. *Arch Biochem Biophys* **430**: 77–88.
4) Food and Nutrition Board, Institute of Medicine. 2002. *In*: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc (Institute of Medicine, ed.), p 82–161. National Academy Press, Washington DC.
5) Sigmundsdottir H, Butcher EC. 2008. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nat Immunol* **9**: 981–987.
6) Sauberlich HE, Hodges RE, Wallace DL, Kolder H, Canham JE, Hood J, Raica N Jr, Lowry LK. 1974. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. *Vitam Horm* **32**: 251–275.
7) Ahmad SM, Haskell MJ, Raqib R, Stephensen CB. 2008. Men with low vitamin A stores respond adequately to primary yellow fever and secondary tetanus toxoid vaccination. *J Nutr* **138**: 2276–2283.
8) Cifelli CJ, Green JB, Green MH. 2007. Use of model-based compartmental analysis to study vitamin A kinetics and metabolism. *Vitam Horm* **75**: 161–195.
9) Furr HC, Green MH, Haskell M, Mokhtar N, Nestel P, Newton S, Ribaya-Mercado JD, Tang G, Tanumihardjo S, Wasantwisut E. 2005. Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans. *Public Health Nutr* **8**: 596–607.
10) Cifelli CJ, Green JB, Wang Z, Yin S, Russell RM, Tang G, Green MH. 2008. Kinetic analysis shows that vitamin A disposal rate in humans is positively correlated with

vitamin A stores. *J Nutr* **138**: 971–977.
11) Raica N Jr, Scott J, Lowry L, Sauberlich HE. 1972. Vitamin A concentration in human tissues collected from five areas in the United States. *Am J Clin Nutr* **25**: 291–296.
12) Joint FAO/WHO Expert Group. 2004. Vitamin A Human Vitamin and Mineral Requirements, 2nd ed, p 17–44. WHO/FAO, Geneva.
13) Canfield LM, Clandinin MT, Davies DP, Fernandez MC, Jackson J, Hawkes J, Goldman WJ, Pramuk K, Reyes H, Sablan B, Sonobe T, Bo X. 2003. Multinational study of major breast milk carotenoids of healthy mothers. *Eur J Nutr* **42**: 133–141.
14) Kamao M, Tsugawa N, Suhara Y, Wada A, Mori T, Murata K, Nishino R, Ukita T, Uenishi K, Tanaka K, Okano T. 2007. Quantification of fat-soluble vitamins in human breast milk by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **859**: 192–200.
15) Suzuki K, Sasaki S, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Jpn J Nutr Diet* **62**: 369–372 (in Japanese).
16) Hirose J, Endo M, Nagao S, Mizushima K, Narita H, Shibata K. 2008. Amount of breast milk sucked by Japanese breast feeding infants. *J Jpn Soc Breastfeeding Res* **2**: 23–28 (in Japanese).
17) Montreewasuwat N, Olson JA. 1979. Serum and liver concentrations of vitamin A in Thai fetuses as a function of gestational age. *Am J Clin Nutr* **32**: 601–606.
18) Strobel M, Tinz J, Biesalski HK. 2007. The importance of beta-carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. *Eur J Nutr* **46** (Suppl 1): I1–20.
19) Penniston KL, Tanumihardjo SA. 2006. The acute and chronic toxic effects of vitamin A. *Am J Clin Nutr* **83**: 191–201.
20) Azais-Braesco V, Pascal G. 2000. Vitamin A in pregnancy: requirements and safety limits. *Am J Clin Nutr* **71** (5 Suppl): 1325S–1333S.
21) Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. 1995. Teratogenicity of high vitamin A intake. *N Engl J Med* **333**: 1369–1373.
22) Minuk GY, Kelly JK, Hwang WS. 1988. Vitamin A hepatotoxicity in multiple family members. *Hepatology* **8**: 272–275.
23) Persson B, Tunell R, Ekengren K. 1965. Chronic vitamin a intoxication during the first half year of life; Description of 5 cases. *Acta Paediatr Scand* **54**: 49–60.
24) Michaelsson K, Lithell H, Vessby B, Melhus H. 2003. Serum retinol levels and the risk of fracture. *N Engl J Med* **348**: 287–294.
25) Männistö S, Smith-Warner SA, Spiegelman D, Albanes D, Anderson K, van den Brandt PA, Cerhan JR, Colditz G, Feskanich D, Freudenheim JL, Giovannucci E, Goldbohm RA, Graham S, Miller AB, Rohan TE, Virtamo J, Willett WC, Hunter DJ. 2004. Dietary carotenoids and risk of lung cancer in a pooled analysis of seven cohort studies. *Cancer Epidemiol Biomarkers Prev* **13**: 40–48.
26) The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. 1994. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* **330**: 1029–1035.
27) Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barrett MJ, Pietinen P, Malila N, Tala E, Liippo K, Salomaa ER, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. 1996. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* **88**: 1560–1570.
28) Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. 1996. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* **334**: 1150–1155.
29) Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. 1996. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* **334**: 1145–1149.
30) Holick MF. 2004. Vitamin D. *In*: Nutrition and Bone Health (Holick MF, Dawson-Hughes B, eds), p 403–440. Humana Press, Totowa, NJ.
31) Malabanan A, Veronikis IE, Holick MF. 1998. Redefining vitamin D insufficiency. *Lancet* **351**: 805–806.
32) Nakamura K, Tsugawa N, Saito T, Ishikawa M, Tsuchiya Y, Hyodo K, Maruyama K, Oshiki R, Kobayashi R, Nashimoto M, Yoshihara A, Ozaki R, Okano T, Yamamoto M. 2008. Vitamin D status, bone mass, and bone metabolism in home-dwelling postmenopausal Japanese women: Yokogoshi Study. *Bone* **42**: 271–277.
33) Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B. 1989. Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. *N Engl J Med* **321**: 1777–1783.
34) Nakamura K, Nashimoto M, Tsuchiya Y, Obata A, Miyanishi K, Yamamoto M. 2001. Vitamin D insufficiency in Japanese female college students: a preliminary report. *Int J Vitam Nutr Res* **71**: 302–305.
35) Nakamura K, Nashimoto M, Matsuyama S, Yamamoto M. 2001. Low serum concentrations of 25-hydroxyvitamin D in young adult Japanese women: a cross sectional study. *Nutrition* **17**: 921–925.
36) Nakamura K, Nashimoto M, Hori Y, Muto K, Yamamoto M. 1999. Serum 25-hydroxyvitamin D levels in active women of middle and advanced age in a rural community in Japan. *Nutrition* **15**: 870–873.
37) Nakamura K, Nashimoto M, Yamamoto M. 2000. Summer/winter differences in the serum 25-hydroxyvitamin D_3 and parathyroid hormone levels of Japanese women. *Int J Biometeorol* **44**: 186–189.
38) Nakamura K, Nashimoto M, Hori Y, Yamamoto M. 2000. Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. *Am J Clin Nutr* **71**: 1161–1165.
39) Nakamura K, Nashimoto M, Yamamoto M. 2001. Are the serum 25-hydroxyvitamin D concentrations in winter associated with forearm bone mineral density in healthy elderly Japanese women? *Int J Vitam Nutr Res* **71**: 25–29.
40) Ministry of Health Labour and Welfare, Japan. 2007. National Health and Nutritional Survey Japan (2005). Tokyo.
41) Ministry of Health Labour and Welfare, Japan. 2008. National Health and Nutritional Survey Japan (2006). Tokyo.

42) Yorifuji J, Yorifuji T, Tachibana K, Nagai S, Kawai M, Momoi T, Nagasaka H, Hatayama H, Nakahata T. 2008. Craniotabes in normal newborns: the earliest sign of subclinical vitamin D deficiency. *J Clin Endocrinol Metab* **93**: 1784–1788.
43) Nakao H. 1988. Nutritional significance of human milk vitamin D in neonatal period. *Kobe J Med Sci* **34**: 121–128.
44) Ministry of Education, Culture, Sports, Science and Technology, Japan. 2005. Standard Tables of Food Composition in Japan, Fifth revised and enlarged ed. 2004. Tokyo.
45) Specker BL, Valanis B, Hertzberg V, Edwards N, Tsang RC. 1985. Sunshine exposure and serum 25-hydroxyvitamin D concentrations in exclusively breast-fed infants. *J Pediatr* **107**: 372–376.
46) Specker BL, Ho ML, Oestreich A, Yin TA, Shui QM, Chen XC, Tsang RC. 1992. Prospective study of vitamin D supplementation and rickets in China. *J Pediatr* **120**: 733–739.
47) MacLennan WJ, Hamilton JC, Darmady JM. 1980. The effects of season and stage of pregnancy on plasma 25-hydroxy-vitamin D concentrations in pregnant women. *Postgrad Med J* **56**: 75–79.
48) Henriksen C, Brunvand L, Stoltenberg C, Trygg K, Haug E, Pedersen JI. 1995. Diet and vitamin D status among pregnant Pakistani women in Oslo. *Eur J Clin Nutr* **49**: 211–218.
49) Narang NK, Gupta RC, Jain MK. 1984. Role of vitamin D in pulmonary tuberculosis. *J Assoc Physicians India* **32**: 185–188.
50) Honkanen R, Alhava E, Parviainen M, Talasniemi S, Monkkonen R. 1990. The necessity and safety of calcium and vitamin D in the elderly. *J Am Geriatr Soc* **38**: 862–866.
51) Ala-Houhala M, Koskinen T, Terho A, Koivula T, Visakorpi J. 1986. Maternal compared with infant vitamin D supplementation. *Arch Dis Child* **61**: 1159–1163.
52) Fomon SJ, Younoszai MK, Thomas LN. 1966. Influence of vitamin D on linear growth of normal full-term infants. *J Nutr* **88**: 345–350.
53) Traber MG, Arai H. 1999. Molecular mechanisms of vitamin E transport. *Annu Rev Nutr* **19**: 343–355.
54) Horwitt MK, Century B, Zeman AA. 1963. Erythrocyte survival time and reticulocyte levels after tocopherol depletion in man. *Am J Clin Nutr* **12**: 99–106.
55) Farrell PM, Bieri JG, Fratantoni JF, Wood RE, di Sant'Agnese PA. 1977. The occurrence and effects of human vitamin E deficiency. A study in patients with cystic fibrosis. *J Clin Invest* **60**: 233–241.
56) Horwitt MK. 1960. Vitamin E and lipid metabolism in man. *Am J Clin Nutr* **8**: 451–461.
57) Sasaki S, Ushio F, Amano K, Morihara M, Todoriki O, Uehara Y, Toyooka E. 2000. Serum biomarker-based validation of a self-administered diet history questionnaire for Japanese subjects. *J Nutr Sci Vitaminol* **46**: 285–296.
58) Hiraoka M. 2001. Nutritional status of vitamin A, E, C, B1, B2, B6, nicotinic acid, B12, folate, and beta-carotene in young women. *J Nutr Sci Vitaminol* **47**: 20–27.
59) Maruyama C, Imamura K, Oshima S, Suzukawa M, Egami S, Tonomoto M, Baba N, Harada M, Ayaori M, Inakuma T, Ishikawa T. 2001. Effects of tomato juice consumption on plasma and lipoprotein carotenoid concentrations and the susceptibility of low density lipoprotein to oxidative modification. *J Nutr Sci Vitaminol* **47**: 213–221.
60) Sakurai T, Furukawa M, Asoh M, Kanno T, Kojima T, Yonekubo A. 2005. Fat-soluble and water-soluble vitamin contents of breast milk from Japanese women. *J Nutr Sci Vitaminol* **51**: 239–247.
61) Morinobu T, Ban R, Yoshikawa S, Murata T, Tamai H. 2002. The safety of high-dose vitamin E supplementation in healthy Japanese male adults. *J Nutr Sci Vitaminol* **48**: 6–9.
62) Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. 2005. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* **142**: 37–46.
63) Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. 2007. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* **297**: 842–857.
64) Asleh R, Blum S, Kalet-Litman S, Alshiek J, Miller-Lotan R, Asaf R, Rock W, Aviram M, Milman U, Shapira C, Abassi Z, Levy AP. 2008. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2–2 genotype. *Diabetes* **57**: 2794–2800.
65) Milman U, Blum S, Shapira C, Aronson D, Miller-Lotan R, Anbinder Y, Alshiek J, Bennett L, Kostenko M, Landau M, Keidar S, Levy Y, Khemlin A, Radan A, Levy AP. 2008. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2–2 genotype: a prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol* **28**: 341–347.
66) Frick PG, Riedler G, Brogli H. 1967. Dose response and minimal daily requirement for vitamin K in man. *J Appl Physiol* **23**: 387–389.
67) Suttie JW. 1995. The importance of menaquinones in human nutrition. *Annu Rev Nutr* **15**: 399–417.
68) Suttie JW, Mummah-Schendel LL, Shah DV, Lyle BJ, Greger JL. 1988. Vitamin K deficiency from dietary vitamin K restriction in humans. *Am J Clin Nutr* **47**: 475–480.
69) Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. 1999. Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr* **69**: 74–79.
70) Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, Wilson PW, Ordovas J, Schaefer EJ, Dawson-Hughes B, Kiel DP. 2000. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr* **71**: 1201–1208.
71) Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, Torgerson DJ. 2006. Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med* **166**: 1256–1261.
72) Shearer MJ, Rahim S, Barkhan P, Stimmler L. 1982. Plasma vitamin K1 in mothers and their newborn babies. *Lancet* **2**: 460–463.
73) Kojima T, Asoh M, Yamawaki N, Kanno T, Hasegawa H, Yonekubo A. 2004. Vitamin K concentrations in the maternal milk of Japanese women. *Acta Paediatr* **93**: 457–463.
74) Greer FR, Mummah-Schendel LL, Marshall S, Suttie JW. 1988. Vitamin K1 (phylloquinone) and vitamin K2 (menaquinone) status in newborns during the first week of life. *Pediatrics* **81**: 137–140.

Case Report

Clinical decision-making for vitamin K-1 and K-2 deficiency and coronary artery calcification with warfarin therapy: are diet, factor Xa inhibitors or both the answer?

Mark L Wahlqvist MD[1,2], Kiyoshi Tanaka MD[3], Bing-Hsiean Tzeng MD[4,5]

[1]*Institute of Population Health Sciences, National Health Research Institutes, Miaoli County, Taiwan, ROC*
[2]*Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia*
[3]*Department of Food and Nutrition, Kyoto Women's University, Kyoto, Japan*
[4]*Department of Cardiology, Tri-Service General Hospital, Taipei, Taiwan, ROC*
[5]*Department of Medicine, National Defence Medical Center, Taipei, Taiwan, ROC*

Coronary artery calcification is a recognised risk factor for ischaemic heart disease and mortality. Evidence is now strong that Mönckeberg's arteriosclerosis, a form of vascular calcification, can be attributable to vitamin K deficiency, but that vitamin K-2, especially the MK-4 form from foods like cheese can be protective. Warfarin blocks the recycling of hepatic and peripheral vitamin K leading to secondary vitamin K deficiency with adverse effects on vasculature, bone, kidneys, brain and other tissues and systems (inflammatory, immune function and neoplasia at least). There is individual susceptibility to vitamin K deficiency and warfarin sensitivity, partly explicable in terms of genetic polymorphisms, epigenetics, diet and pharmacotherapy. The emergence of extensive coronary calcification in a man with atrial fibrillation treated for a decade with warfarin is described by way of illustration and to raise the present clinical management conundrums. Finally, a putative set of recommendations is provided.

Key Words: vitamin K deficiency syndromes, coronary artery calcification, warfarin therapy, cheese and natto, apixaban

INDEX CASE STUDY

At 61 years a Caucasian man developed atrial fibrillation (AF) and went on warfarin as an anticoagulant and verapamil for rate control. Despite 3 attempts at cardioversion the arrhythmia became persistent. There were no apparent risk factors for the AF – no ischaemic or rheumatic heart disease, no thyroid disease, minimal social use of alcohol, no diabetes and no evidence of cardiomyopathy. His diet was varied and of a plant-food orientation, with fish and beans 2-3 times per week, daily whole grains and green leafy vegetables, with low fat dairy and little cheese, and low in salt (<5 g/day). He was variably on an H-2 receptor antagonist or a proton pump inhibitor (PPI) for gastro-oesophageal reflux, which has been considered a risk factor for AF. There were no cardiovascular risk factors apart from a BMI of 26.8 kg/m^2, with blood pressure (BP) generally about 135/85, fasting cholesterol 5.3, HDL cholesterol 1.3, triglyceride 1.8 and glucose 4.8 mmol/L. He was a non-smoker. Non-invasive monitoring of neck and peripheral arteries was unremarkable. A year later, he developed angina in stressful circumstances and on sustained effort, but not on formal exercise ECG and echocardiogram testing. Coronary angiography revealed a single lesion which was stented. Calcification was not in evidence in any radiographic investigation. Post-stent he had triple anti-hemostatic with warfarin, clopidogrel and aspirin for 6 months; he has remained on aspirin. For 8 years after commencement of warfarin, angina-on-effort was occasional, but never during weekly yoga-aerobic and strength training sessions or rarely on recreational hiking. A cardiac thallium scan at this time, because of increased angina frequency revealed a reversible ischaemic region in the infero-lateral myocardium. A second stent was placed at a left coronary-diagonal artery bifurcation and when increased luminal irregularity was noted; triple anti-hemostatic therapy was again implemented, but for 12 months. A post-stent thallium perfusion study showed little benefit from the stent. On warfarin for 10 years and aged 71 years, a cardiac CT showed extensive calcification of the coronary circulation, but quantification was limited by the presence of AF. Imaging also showed peripheral medium distributing arteries (iliofemoral) to be patchily calcified (Figures 1 and 2). Throughout the decade of warfarin therapy, INR (International Normalised Ratio) has been maintained well into the therapeutic range, almost always between 2.0 and 2.5, on doses of about 7 mg daily. There have been 2 episodes of major soft tissue bruising as a result of moderate trauma.

Corresponding Author: Dr Bing-Hsiean Tzeng, Department of Cardiology, Tri-Service General Hospital, No 325, Sec 2, Chenggong Rd, Neihu District, Taipei 114, Taiwan, ROC.
Tel: +886-2-87927160; Fax: +886-2-26345619
Email: 894020086@mail.ndmctsgh.edu.tw
Manuscript received 26 July 2013.
doi: 10.6133/apjcn.2013.22.3.21

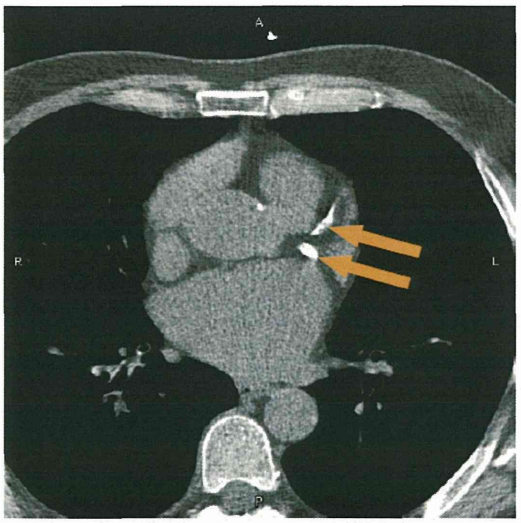

Figure 1. Arrows indicate extensive calcification of the left coronary artery on the cardiac computer tomography (CT).

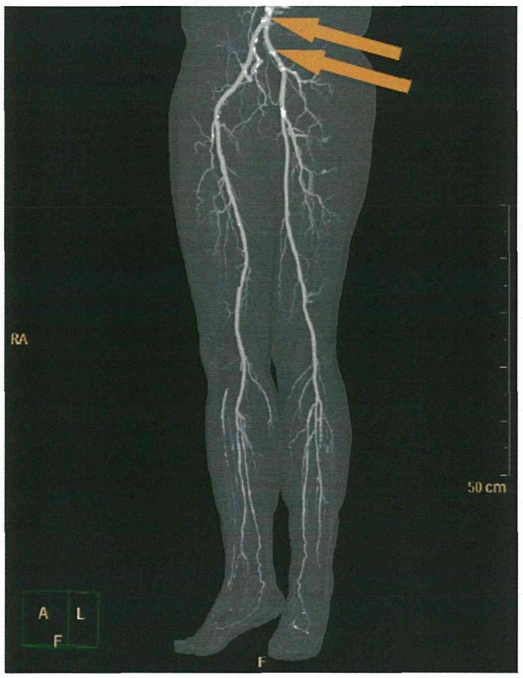

Figure 2. Arrows indicate multiple patchily calcified sites on the distal descending aorta and bilateral ilio-femoral arteries on the limb vascular CT.

Intercurrent illnesses during this decade have included herpes zoster (C2) and temporal arteritis (2 courses of high dose prednisolone with the need for an angiotensin receptor inhibitor, irbesartan for BP control).

The questions now are 1) is the arterial calcification attributable to vitamin K deficiency, both primary (diet) and secondary (warfarin), and is it medial rather than intimal? 2) what, if any, dietary changes might be helpful should vitamin K deficiency be a problem? 3) is the reduced risk of thromboembolic phenomena with warfarin outweighed by the risks of arterial calcification – and the risks of other vitamin K deficiency disorders affecting bone, brain, kidney small vessels and more? 4) is there individual susceptibility to vitamin deficiency which this patient might have exhibited? 5) does this patient's pharmacotherapy represent interactions or synergies which might have exacerbated the risk to vascular biology of warfarin eg aspirin, PPIs, antiarrhythmic agents (verapamil); 6) could an increased intake of vitamin K-2 from foods rich in it reduce the risk of coronary calcification, even in the presence of warfarin therapy? 7) should warfarin use in AF be avoided or used for limited periods only and factor Xa inhibitors like apixaban used instead?

THE ESSENTIALITY OF VITAMINS K-1 AND K-2 IS PLEIOTROPIC WITH STRUCTURAL AND FUNCTIONAL DIFFERENCES

Vitamin K carboxylates, post-translationally, the amino acid glutamate (Glu) in proteins, which then become gamma-carboxy-glutamate (Gla) residues. These residues in turn chelate calcium.[1]

There are 2 naturally-occurring forms of vitamin K, K-1 or phylloquinone (also known as phytomenadione) and K-2 or menaquinone which itself has several forms designated MK-n, where n is the number of side chain isoprenoid residues.[1,2]

Vitamin K-1 is recycled between the reduced hydroquinone and the oxidized epoxide, with epoxide reductase (VKOR) responsible for the formation of the hydroquinone and which can be blocked by warfarin. In this way, warfarin can create an effective vitamin K deficiency in liver and in peripheral tissues. It is the hepatic coagulant proteins II, VII, IX and X which are therapeutically compromised by warfarin in anticoagulation. But so are Gla proteins in other tissues, like osteocalcin in bone and the vascular wall calcification –inhibiting matrix Gla protein MGP and the growth arrest specific gene 6 protein Gas6, among others. In some way, vitamin K deficiency in the CNS, perhaps because of less good transport in apo-E4 lipoproteins, may exacerbate cognitive impairment. Interestingly, whereas vitamin K-1 is transported in VLDL, K-2 is transported in LDL and HDL[3,4] which may make vitamin K-2 of particular relevance for brain. Gas6 plays a role in inflammation, renal function and cell differentiation.[5] There is preferential accumulation of vitamin K-2 in peripheral tissues like the vasculature.[6] But it appears that it is the MK-4 type which can prevent arterial calcification.[7] Thus, the pleiotropic functions of vitamin K are evident and, as yet, not fully recognised.[8]

There is evidence that the clinical manifestations of vitamin K deficiency may be more evident in some tissues, like bone[9,10,11] and the vasculature,[12] than others like liver. Some of this difference may reflect the tissue specificities of the different forms of vitamin K and the local food culture to deliver the forms required.

FOOD AND MICROBIOMIC SOURCES OF VITAMIN K

Vitamin K-1 comes mainly from green leafy vegetables because of its role in photosynthesis. It can be converted to vitamin K-2 of the MK-4 type in animal tissues including the pancreas, testes and vasculature. Other forms of MK-n are produced by bacteria in fermented foods like natto (from soy), cheese and fermented vegetables (eg kimchi and sauerkraut), but are also found in eggs and chicken liver.

Broad spectrum antibiotics can markedly reduce vitamin K status which presumably indicates a major gut microbial source of vitamin K, but the conversion of K-1 to

K-2 may take place in the gut wall.[11] With the present surge in gut microbiomic research, its role in vitamin K nutrition should become clearer.

VITAMIN K DEFICIENCY SYNDROMES

Vitamin K deficiency is widespread among some populations, especially the elderly, even where the food supply would be thought to be adequate as in Japan.[13]

It will be clear from the pleiotropic effects of vitamin K through vitamin K dependent proteins (VKDPs) that its deficiency, whether primary as dietary inadequacy or secondary through the use of the coumarin warfarin, could produce a spectrum of clinical sequelae. But the recognition of vitamin K syndromes is unusual in practice. This probably results from a lack of awareness of food sources of vitamin K-2 as well as the better known K-1, and their differential tissue specificities.

VKDP osteocalcin status is compromised by poor vitamin K intakes as well as warfarin therapy which need to be taken into account in osteoporosis management. For the prevention of vascular calcification, it may be the MK-4 form of K-2 which is most important, with cheese a particularly useful source. Foods like natto and cheese provide other MK-ns as well both MK-4 and MK-7 are favourable for osteocalcin and bone health. This may be indirectly relevant to arterial health. Bone is increasingly recognised as an endocrine organ in its own right with osteocalcin being involved in energy regulation, insulin resistance and cardiovascular risk.[14,15]

Vascular calcification may be an important feature of vitamin K deficiency [5] and of warfarin therapy.[16] Nevertheless, anecdotally, not all patients on warfarin develop arterial calcification, although the incidence and risk are not quantified in any available study. But, presumptively, there would seem to be predisposing factors which might include genetic and epigenetic mechanisms, background diet and use of medications which themselves alter vitamin K status or sensitivity.[1,5] Renal impairment is itself associated with vascular calcification and sufferers will constitute one of the more susceptible groups to vitamin K deficiency.[5] Abdominal obesity and diabetes also increase the likelihood of vascular calcification.[12,17]

For drug interactions, broad spectrum antibiotics (altering the vitamin K producing and transforming gut microbiome), PPIs (which alter divalent cation metabolism, especially of magnesium, and increases risk of fracture), salicylates (which inhibit K-2 less than K-2), steroids (with their osteopenic effects), antihypertensives (which may be associated with vascular calcification although circumstantially) statins (which may protect against vascular calcification and osteoporosis,[12,18] and vitamin D supplements (through hyperphosphatemia). Thus, it is difficult to isolate warfarin effects alone in the pathogenesis of vascular calcification, although the mechanisms are highly plausible.

CORONARY ARTERY CALCIFICATION: IS MEDIAL ARTERIAL (MÖNCKEBERG'S) CALCIFICATION ACCEPTABLE?

In the longitudinal Rotterdam study, increased intake of vitamin K-2, but not K-1, was inversely related to all-cause mortality and aortic calcification.[19] This has been supported by the Beulens cross-sectional study of post-menopausal women.[20]

The pathology is that of medial calcification, known as Mönckeberg's arteriosclerosis, which is not that of atherosclerosis.[12,19,21]

Coronary artery calcification is a significant predictor of risk for ischaemic heart disease and mortality. It would appear that this applies to Mönckeberg's arteriosclerosis as well as to intimal or intimo-medial calcification, although the relative risks of these types of calcification has not been evaluated.

IS INCREASED VITAMIN K-2 INTAKE A WAY TO REDUCE CORONARY CALCIFICATION WITH WARFARIN?

The available experimental and population studies would suggest that higher intakes of vitamin K-2 would reduce the risk of vascular calcification and that they may even allow some reversal.[21] Since foods like cheese might provide a way to this, present ideas about such fatty foods and cardiovascular disease (CVD) might need review, especially since there is no evidence that these foods in their own right present a CVD risk. It is conceivable that they might achieve this in the face of effective warfarin anticoagulation through its hepatic effects, while minimising its adverse vascular effects.

CAN FACTOR XA INHIBITORS (LIKE APIXABAN) REDUCE THE RISKS OF VITAMIN K DEFICIENCY?

If anticoagulation can be confined to factor X, it should allow the other non-coagulant functions of vitamin K to be met by diet and the avoidance of vitamin K deficiency-related vascular calcification, along with other functions as with bone health. At present apixaban looks the most promising agent in this category insofar as reversibility of its action and side effects are concerned, at least insofar as AF is concerned (especially in the presence of ischaemic heart disease). But time will tell whether there are long term, as yet unrecognised complications and whether all-cause and disease-specific mortalities are favourable.

SHOULD WARFARIN BE USED FOR MORE THAN SHORT-TERM THERAPY?

It may be that warfarin could be used for limited time periods with little consequence through vitamin K deficiency, or that its safety might be improved by judicious simultaneous use of vitamin K-2. But, at present, we have little information as to time to effect.

CONCLUSIONS AND RECOMMENDATIONS

1. There is substantial evidence that warfarin-induced vitamin K deficiency can lead to a spectrum of vitamin K deficiency disorders, including vascular calcification.
2. The risk for vitamin K deficiency-related vascular calcification may be minimised by higher intakes of vitamin K-2 which may be sourced from cheese, eggs and fermented foods like natto, although the different MK-n forms of vitamin K-2 may have benefit differentials; cheese with MK-4 has the most evidence at present, challenging the conventional wisdom that cardio-

vascular-protective foods need to be invariably low fat (as has been recognized for fatty fish, nuts and other seed-foods).

3. The clinical identification of individuals susceptible to vascular calcification, by diet, disease or associated pharmacotherapy, may allow better risk-benefit evaluation of warfarin therapy especially in AF.
4. Individuals susceptible to vascular calcification should have a baseline coronary artery calcification assessment and review.
5. Individuals susceptible to vascular calcification with AF are candidates for factor X antagonists instead of warfarin.

REFERENCES

1. Berkner KL. Vitamin K-dependent carboxylation. Vitam Horm. 2008:131-56. doi: 10.1016/s0083-6729(07)00007-6.
2. Guylaine F. Vitamin K. In: Erdman JR, McDonald IA, Zeisel SH, editors. Present Knowledge in Nutrition. Oxford: Wiley Blackwell; 2012. pp. 230-47.
3. Schurgers LJ, Aebert H, Vermeer C, Bultmann B, Janzen J. Oral anticoagulant treatment: friend or foe in cardiovascular disease? Blood. 2004;10:3231-2. doi: 10.1182/blood-2004-04-1277.
4. Schurgers LJ, Vermeer C. Differential lipoprotein transport pathways of K-vitamins in healthy subjects. Biochim Biophys Acta. 2002;1:27-32.
5. Danziger J. Vitamin K-dependent proteins, warfarin, and vascular calcification. Clin J Am Soc Nephrol. 2008;5:1504-10. doi: 10.2215/cjn.00770208.
6. Thijssen HH, Drittij-Reijnders MJ. Vitamin K status in human tissues: tissue-specific accumulation of phylloquinone and menaquinone-4. Br J Nutr. 1996:121-27.
7. Spronk HM, Soute BA, Schurgers LJ, Thijssen HH, De Mey JG, Vermeer C. Tissue-specific utilization of menaquinone-4 results in the prevention of arterial calcification in warfarin-treated rats. J Vasc Res. 2003;6:531-7. doi: 75344.
8. Booth SL. Roles for vitamin K beyond coagulation. Annu Rev Nutr. 2009:89-110. doi: 10.1146/annurev-nutr-080508-141217.
9. Kuwabara A, Fujii M, Kawai N, Tozawa K, Kido S, Tanaka K. Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly. Asia Pac J Clin Nutr. 2011;1:50-5.
10. Nakano T, Tsugawa N, Kuwabara A, Kamao M, Tanaka K, Okano T. High prevalence of hypovitaminosis D and K in patients with hip fracture. Asia Pac J Clin Nutr. 2011;1:56-61
11. Nagae A, Kuwabara A, Tozawa K, Kumode M, Takeuchi Y, Tanaka K. Enteral nutrition and antibiotic use increase the risk for vitamin K deficiency in patients with severe motor and intellectual disabilities. ESPEN J. 2013;1-6. doi: 10.1016/j.clnme.2012.12.002
12. Zhu D, Mackenzie NC, Farquharson C, Macrae VE. Mechanisms and clinical consequences of vascular calcification. Front Endocrinol (Lausanne). 2012:95. doi: 10.3389/fendo.2012.00095.
13. Kuwabara A, Himeno M, Tsugawa N, Kamao M, Fujii M, Kawai N et al. Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly. Asia Pac J Clin Nutr. 2010;1:49-56.
14. Alfadda A, Masood A, Shaik S, Dekhil H, Goran M. Association between osteocalcin, metabolic syndrome, and cardiovascular risk factors: role of total and undercarboxylated osteocalcin in patients with type 2 diabetes. Int J Endocrinol. 2013;2013:197519. doi: 10.1155/2013/197519.
15. Clemens TL, Karsenty G. The osteoblast: an insulin target cell controlling glucose homeostasis. J Bone Miner Res. 2011;4:677-80. doi: 10.1002/jbmr.321.
16. Kaiser C, Agus ZS. Coronary calcium linked to maintenance warfarin. [cited 2013/7/26]; Available from: http://www.medpagetoday.com/Cardiology/Arrhythmias/27768.
17. Lee CD, Jacobs DR, Jr., Schreiner PJ, Iribarren C, Hankinson A. Abdominal obesity and coronary artery calcification in young adults: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am J Clin Nutr. 2007;1:48-54.
18. Danilevicius CF, Lopes JB, Pereira RMR. Bone metabolism and vascular calcification. Braz J Med Biol Res. 2007;40:435-42. doi: 10.1590/S0100-879X2007000400001.
19. Geleijnse JM, Vermeer C, Grobbee DE, Schurgers LJ, Knapen MH, van der Meer IM, Hofman A, Witteman JC. Dietary intake of menaquinone is associated with a reduced risk of coronary heart disease: the Rotterdam Study. J Nutr. 2004;11:3100-5.
20. Beulens JW, Bots ML, Atsma F, Bartelink ML, Prokop M, Geleijnse JM, Witteman JC, Grobbee DE, van der Schouw YT. High dietary menaquinone intake is associated with reduced coronary calcification. Atherosclerosis. 2009;2:489-93. doi: 10.1016/j.atherosclerosis.2008.07.010.
21. Adams J, Pepping J. Vitamin K in the treatment and prevention of osteoporosis and arterial calcification. Am J Health Syst Pharm. 2005;15:1574-81. doi: 10.2146/ajhp040357.

Case Report

Clinical decision-making for vitamin K-1 and K-2 deficiency and coronary artery calcification with warfarin therapy: are diet, factor Xa inhibitors or both the answer?

Mark L Wahlqvist MD[1,2], Kiyoshi Tanaka MD[3], Bing-Hsiean Tzeng MD[4,5]

[1]*Institute of Population Health Sciences, National Health Research Institutes, Miaoli County, Taiwan, ROC*
[2]*Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia*
[3]*Department of Food and Nutrition, Kyoto Women's University, Kyoto, Japan*
[4]*Department of Cardiology, Tri-Service General Hospital, Taipei, Taiwan, ROC*
[5]*Department of Medicine, National Defence Medical Center, Taipei, Taiwan, ROC*

維生素 K 拮抗劑治療而致維生素 K-1 和 K-2 缺乏及冠狀動脈鈣化的臨床決策：飲食、第十凝血因子抑制劑或者兩者是解答嗎?

冠狀動脈鈣化被認為是缺血性心臟病及死亡的危險因子。Mönckeberg 型動脈粥狀硬化是一種血管鈣化，現在有力的證據顯示這可能是維生素 K 缺乏所導致；維生素 K-2，特別是來自於食物，像是乳酪的 MK-4 型式則具保護作用。維生素 K 拮抗劑-warfarin 阻斷肝及周邊的維生素 K 循環，造成次發性維生素 K 缺乏，而引發脈管結構、骨頭、腎臟、腦及其它組織系統的副作用(例如發炎、免疫功能及腫瘤)。個體對維生素 K 缺乏的易感性及 warfarin 敏感性，部分可以基因多型性、表觀遺傳學、飲食及藥物治療所解釋。本文描述一位有心房顫動的男性病患，在過去十年一直以 warfarin 治療，被發現有廣泛性的冠狀動脈鈣化，因而帶出這個目前臨床管理的難題。最後提出一套推斷的建議。

關鍵字：維生素 K 缺乏症狀、冠狀動脈鈣化、維生素 K 拮抗劑治療、乳酪及納豆、apixaban