

Vitamins

(1) Fat-soluble Vitamins

Vitamin A

1. Background Information

1-1. Definition and Classification

The vitamin A compounds with potent vitamin A activity *in vivo* after oral intake include retinol, retinal, carotenoids, and 50 types of provitamin A carotenoids, such as β -carotene, α -carotene, and β -cryptoxanthin. In the current DRIs, we used retinol activity equivalents (RAE) as the vitamin A unit, instead of retinol equivalents (REs) that were used in the previous DRIs.

1–2. Function

Retinol and retinal are necessary for the protection of retinal cells, and photostimulated reaction in the photoreceptor cells. Vitamin A deficiency can cause blindness via corneal xerosis in infants or children, and night blindness due to impaired adaptation to darkness in adults.

1–3. Digestion, absorption, and metabolism

Retinyl ester and provitamin A carotenoids are the main forms of vitamin A contained in animal and plant foods, respectively. Retinyl ester hydrolase catalyzes the hydrolysis of retinyl ester to retinol in the intestinal brush border, and this retinol is then absorbed at a rate of 70% to 90%^(1,2). Most β -carotenes produce 2 mol of vitamin A (retinal) in the small intestine, while other provitamin A carotenoids produce 1 mol of retinal. The absorption rate of β -carotene is about one-seventh of a supplement that contains purified β -carotene and oils. In the present DRIs, the absorption rate of β -carotene is one-sixth its total value, which is in accordance with the rate described in the US-Canada DRIs⁽³⁾.

Assuming that the conversion rate of β -carotene to retinol is 50%, the bioavailability of β -carotene as vitamin A is $1/12(1/6 \times 1/2)$, such that 12 μg of food-derived β -carotene would correspond to 1 μg in RAE. Thus, the following formula can be used to convert the values of food-derived vitamin A-related compounds to RAEs:

Retinol activity equivalent (μgRAE)

$$= \text{retinol } (\mu\text{g}) + \beta\text{-carotene } (\mu\text{g}) \times 1/12 + \alpha\text{-carotene } (\mu\text{g}) \times 1/24 + \beta\text{-cryptoxanthin } (\mu\text{g}) \times 1/24 + \text{other provitamin A carotenoids } (\mu\text{g}) \times 1/24$$

Caution is to be exercised in calculating the corresponding value for oil-solubilized β -carotene, as its bioavailability as a form of vitamin A is half of its total value; 2 μg of fat-solubilized β -carotene would correspond to 1 μg of retinol.

2. To Avoid Inadequacy

2–1. Factors to be Considered in Estimating Requirements

Classical vitamin A deficiency leads to corneal xerosis in infants, and possibly blindness and night blindness in adults. Other signs of deficiency include growth retardation, skeletal and neurological developmental defects, disturbed growth and differentiation of epithelial cells, dryness, thickening, and keratinization of the skin, immunodeficiency, and susceptibility to infection⁽⁴⁾. Due to the abundant storage of vitamin A in the liver, inadequate intake does not lead to decreased plasma retinol concentrations, unless the hepatic vitamin A storage is lower than 20 µg⁽⁵⁾. 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.

2–2. Method Used to Set the Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA)

2–2–1. Calculation of EAR and RDA

Plasma retinol concentrations are maintained at normal levels unless the hepatic vitamin A storage is below 20 µg, even if adults take only meals without vitamin A for up to 4 months. Therefore, as long as the hepatic vitamin A storage is maintained at the minimum level (20 µg/g), relatively minor deficiencies such as immunodeficiency and night blindness will not result^(5,6). Thus, the vitamin A intake required to maintain minimal hepatic vitamin A storage can be used to estimate the EAR for vitamin A⁽⁷⁾. Compartment analyses, assuming the existence of 3 compartments—serum, liver, and other tissues—have shown that the daily disposal rate of vitamin A is approximately 2%, regardless of the vitamin A storage in the body^(8–10). 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:

$$\text{DDA } (\mu\text{g/day}) = \text{BS } (\mu\text{g}) \times \text{DDR } (2/100)^{(8)}$$

BS/BW can be calculated as follows:

$$\text{BS/BW } (\mu\text{g}/[\text{kg BW/day}]) = \text{HS } (20 \mu\text{g/g}) \times \text{liver weight/ BW } (21 \text{ g/kg BW})^{(8,11)} \times 10/9^{(8,12)}$$

DDA/BW can be calculated as follows:

$$\text{DDA/BW} = \text{BS } (20 \mu\text{g/g} \times 21 \text{ g/kg} \times 10/9) \times \text{DDR } (2/100) = 9.3 \mu\text{g}/[\text{kg BW/day}]$$

Thus, the amount of vitamin A intake required to compensate for its daily elimination, thereby ensuring that the hepatic storage of vitamin A is maintained and vitamin A deficiency is avoided, is estimated to be 9.3 µgRAE/kg BW/day. This value was set as the reference value for the calculation of the DRIs.

2–2–2. Adult (EAR, RDA)

The EARs of vitamin A for those aged 18 years and above, as calculated by the multiplication of the reference value (9.3 µgRAE/kg BW/day) and the reference BW, are 550

to 650 µgRAE/day for men and 450 to 500 µgRAE/day for women.

Assuming a coefficient of variation of 20%⁽³⁾, the multiplication of these EAR values by 1.4 yields an RDA of 800 to 900 µgRAE/day ($\hat{=}$ 550 to 600 \times 1.4) for men and 650 to 700 µgRAE/day ($\hat{=}$ 450 to 500 \times 1.4) for women.

2–2–3. Children (EAR, RDA)

There are currently no data that can be used for the estimation of the EAR for children. The extrapolation of the reference value (9.3 µgRAE/kg BW/day) to children aged 1-5 years, based on the BW ratio, may yield a value of 150 to 200 µgRAE/day, at which plasma retinol levels are maintained below 20 µg/100 mL, thus rendering children susceptible to corneal xerosis⁽¹³⁾. Therefore, the RDA for those children must be above 200 µgRAE/day, for the prevention of unfavorable outcomes; the EAR for children was determined through the extrapolation of the EAR for adults aged 18 to 29 years by the 0.75th power of the BW ratio, which represents the ratio of the body surface area, considering the growth factors⁽³⁾. For the determination of the RDA for children aged less than 6 years, the DDA was calculated assuming the liver weight/BW ratio to be 42 g/kg BW^(8,11):

DDA/BW (µgRAE/kg BW/day) = BS (20 µg/g \times 42 g/kg \times 10/9) \times DDR (2/100) = 18.7 µg/kg BW.

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

EAR = 18.7 µg/kg BW/day \times reference BW \times (1 + growth factor)

Assuming the coefficient of variation in the vitamin A requirement to be 20%⁽⁶⁾, the RDA was determined as the EAR \times 1.4.

2–2–4. Additional Amount for Pregnant and Lactating Women (EAR, RDA)

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 a gestational age of 37 to 40 weeks, the amount of vitamin A deposited in the fetal liver was found to be 1,800 µg; therefore, the total amount of vitamin A transported to the fetus during pregnancy was estimated at 3,600 µg^(14,15). Assuming that the vitamin A absorption rate of the mother is 70%, this amount is accumulated during the last 3 months of pregnancy⁽¹⁵⁾; therefore, the additional amounts required during the early and mid-stages were not determined. The EAR for the additional vitamin A required during the late stage was determined to be 60 µgRAE/day, by rounding 56.3 µgRAE, which, assuming a coefficient of variation of 20%⁽³⁾, yields an RDA of 80 µgRAE/day (by rounding the 78.9 µgRAE/day observed during the late stage).

Based on the average concentration of vitamin A in breast milk (320 µgRAE/day), the EAR for the additional vitamin A required during lactation was estimated at 300 mg RAE/day, which, assuming a coefficient of variation of 20%⁽³⁾, yielded an RDA of 450 mg RAE/day by rounding 449 µgRAE/day.

2–3. Method Used to Set the Adequate Intake (AI)

2–3–1. Infants (AI)

The retinol level secreted in breast milk has been reported to be $352 \pm 18 \mu\text{g/L}$ (mean \pm standard deviation [SD]) at 98 ± 7 days after delivery, in Japanese individuals⁽¹⁶⁾. Another study on more than 600 Japanese mothers with healthy babies reported that the vitamin A concentration in breast milk is $525 \pm 314 \mu\text{gRE/L}$ (mean \pm SD)⁽¹⁷⁾. A more recent study presented precise and detailed data on the concentrations of vitamin A and β -carotene in the breast milk of Japanese mothers, using liquid chromatography-tandem mass spectrometry analysis⁽¹⁸⁾. This study reported that the levels are $1,026 \pm 398 \mu\text{gRE/L}$ 0 to 10 days after delivery, $418 \pm 138 \mu\text{gRE/L}$ at 11 to 30 days, $384 \pm 145 \mu\text{gRE/L}$ at 31 to 90 days, $359 \pm 219 \mu\text{gRE/L}$ at 91 to 180 days, and $267 \pm 117 \mu\text{gRE/L}$ at 181 to 270 days (the calculation of the RE in Reference No. 17 is similar to that of the RAE used in the current DRIs; therefore, it is used as the RAE). The concentration of β -carotene is high in colostrum (0.35 to $0.70 \mu\text{mol/L}$ at 0 to 10 days after delivery), and this decreases to $0.062 \mu\text{mol/L}$ at 3 months after delivery^(16,18). Based on the average vitamin A concentration (411 mg RAE/L)⁽¹⁸⁾, and average milk intake (0.78 L/day)^(19,20), the vitamin A intake in breast milk-fed infants aged 0 to 5 months was estimated at 320 mg RAE/day . Thus, the AI for this age group was determined to be 300 mg/day . Based on the extrapolation from the AI for infants aged 0 to 5 months, the AI for infants aged 6 to 11 months was determined to be 400 mg RAE/day by the 0.75^{th} power of the BW ratio. The level of provitamin A carotenoids was not taken into account, as its availability is unknown.

3. To Avoid Excessive Intake

3–1. Dietary Intake

The use of a supplement, or having a high intake of liver has been reported to lead to health problems associated with excessive intake⁽³⁾.

3–2. Method Used to Set the Tolerable Upper Intake level (UL)

An elevated plasma level of retinoic acid is considered responsible for most clinical signs⁽²¹⁾ and symptoms of vitamin A intoxication, such as headaches. In cases of acute vitamin A intoxication, the cerebrospinal pressure is notably elevated, while increased intracranial pressure, hair loss or muscle pain is observed in cases of chronic intoxication.

Based on a study that focused on the hepatotoxicity caused by excessive vitamin A deposition⁽²²⁾, the no observed adverse effect level (NOAEL) in adults was estimated at $13,500 \text{ mg RAE/day}$, which yielded a UL of $2,700 \text{ mg RAE/day}$, assuming an uncertainty factor of 5. Based on the clinical observation of increased intra-cranial pressure in infants caused by excessive vitamin A intake⁽²³⁾, the NOAEL in infants was estimated at $6,000 \text{ mg RAE/day}$, which yielded a UL of 600 mg RAE/day , assuming an uncertainty factor of 10.

The UL for children aged 1 to 17 years was determined by the extrapolation of the UL for those aged 18 to 29 years based on the BW ratio. For safety reasons, the values for men

were applied to women because of the difference in the reference BW. The extrapolation to infants aged 1 to 2 years old yielded a UL of 500 mg RE/day, which is lower than that for infants aged 6 to 11 months (600 mg RAE/day). Thus, the UL for infants aged 1 to 2 years old was revised to 600 mg RAE/day.

Although a recent study found that ingesting approximately 1,500 mg RAE/day of retinol for 30 years doubled the fracture risk in elderly individuals⁽²⁴⁾, data from other studies⁽²⁵⁾ contradict this finding. Thus, the determination of a separate UL for vitamin A intake in elderly individuals was not considered in the development of the present DRIs. Moreover, as the excessive intake of β -carotene has not been reported to be associated with the unfavorable consequences of vitamin A intoxication described above^(21,24,26), the level of provitamin A carotenoids was also not included in the estimation of the UL.

4. Other Remarks

4–1. Carotenoid Intake and DRIs for Japanese

Due to the strict regulations pertaining to 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 the digestion of these carotenoids, including anti-oxidant activity and immune potentiation.

Although the results of cohort studies suggest that a higher intake of carotenoids is associated with a lower incidence of lung cancer⁽²⁷⁾, supplementary intervention with β -carotene has been reported to be ineffective or even harmful in the prevention of cancer, especially lung cancer^(28–31). Regarding the benefits of specific carotenoids, the prevention of prostate cancer by lycopene^(32,33), improvement in age-related macular degeneration by lutein and zeaxanthin^(34,35), and maintenance of the retinal pigment by lutein and zeaxanthin have also been reported. The anti-oxidation of carotenoids can aid in the photoprotection of the skin⁽³⁶⁾. Thus, further research into the efficacy and safety of carotenoids is required. In developing the present DRIs, carotenoids were not considered separately because their deficiency has not been reported.

Vitamin D

1. Background Information

1–1. Definition and Classification

Vitamin D₂ and vitamin D₃ are naturally occurring compounds with potent vitamin D activity. The human body obtains vitamin D from 2 sources--ultraviolet irradiation, which converts the pro-vitamin D₃ (7-dehydrocholesterol) in the skin to pre-vitamin D₃, which in turn is converted to vitamin D₃ by thermal isomerization; and the dietary intake of vitamin D₂ and vitamin D₃ from sources such as mushrooms and fish, which are good sources of vitamin D₂ and vitamin D₃, respectively. The current DRIs do not discriminate between vitamin D₂ and D₃ intakes because the compounds have similar characteristics and molecular weights, as well as biological activities that are almost equal. Therefore, the indices for the DRIs of vitamin D are based on the summation of the values of these 2 compounds.

1–2. Function

Vitamin D is first metabolized to 25-hydroxy vitamin D (25OHD) before being metabolized to 1 α ,25-dihydroxy vitamin D (1 α ,25(OH)₂D)--its active form. In turn, 1 α ,25(OH)₂D combines with the vitamin D receptor in the core of the target cells and induces the gene expression of vitamin D-dependent proteins. The major actions of vitamin D include the enhancement of the absorption of calcium and phosphate in the intestine and kidneys, and stimulation of bone formation and growth. Vitamin D deficiency impairs calcium absorption from the intestine and kidney, and thus decreases calcium availability, resulting in rickets in children and osteoporosis in adults. Excess vitamin D intake causes hypercalcemia, kidney damage, or calcification disorders in the soft tissues.

1–3. Digestion, Absorption and Metabolism

Although circulating 25OHD levels are dependent on the summation of the vitamin D obtained through the action of ultraviolet radiation and diet⁽³⁷⁾, 1 α ,25(OH)₂D is a hormone that regulates calcium metabolism and maintains its blood level in a stable range. Thus, the circulating 25OHD level is the best index of vitamin D status. As vitamin D deficiency and the resultant hypocalcemia cause elevated levels of serum parathyroid hormone (PTH), the serum concentration of PTH can also be a good index of vitamin D deficiency⁽³⁸⁾.

2. To Avoid Inadequacy

2–1. Factors to be Considered in Estimating Requirements

Vitamin D deficiency may result in rickets in children, and osteoporosis in adults. In adults, especially elderly adults, even so-called “vitamin D insufficiency,” which is milder than vitamin D deficiency, can result in decreased bone mineral density.

The current US-Canada DRIs determined the EARs and RDAs for calcium and vitamin

D, while their previous DRIs determined the AIs for both⁽³⁹⁾. As vitamin D can be induced in the skin through ultraviolet irradiation, there is a lack of scientific evidence on the dose-response relationship between vitamin D intake and the maintenance of bone health. The US-Canada DRIs determined the 25OHD level to be a good index of vital vitamin D, produced through both diet and ultraviolet irradiation. A 25OHD level lower than 30 nmol/L increases the risk of rickets in children and osteoporosis in adults, decreases the absorption of calcium in children and adults, decreases the bone mineral density in children and adults, and increases the prevalence of bone fractures in elderly individuals. For the prevention of bone fractures, based on the maximal effects obtained at 50 nmol/L, the amount of 50% for the requirement was determined to be 40 nmol/L and the amount of 97.5% for the requirement was determined to be 50 nmol/L. These values were determined under circumstances of minimal exposure to sunlight. However, in Japan, few studies have reported data on both serum 25OHD levels and the intake of vitamin D in the same set of individuals, making it difficult to determine an EAR and RDA based on the method stated above.

A cohort study conducted in Nagano, which followed-up 1,470 post-menopausal women (63.7 ± 10.7) for a mean of 7.2 years, showed that 49.6% of the participants had serum 25OHD levels lower than 50 nmol/L⁽⁴⁰⁾. The relative risk for long-bone fractures was 2.20 (95% confidence interval [CI] 1.37-3.53) in those who had serum 25OHD levels lower than 62.5 nmol/L compared to the reference (62.5 nmol/L or higher), showing that vitamin D deficiency could increase the risk of osteoporosis-related bone fractures.

Although no intervention studies have been conducted for the prevention of bone fractures in Japan, many such studies have been conducted in other countries. Some of those studies reported that, while a vitamin D intake of 10 µg/day is insufficient, taking more than 17.5 µg/day may inhibit femur fractures^(41,42). A recent meta-analysis reported that the effects of vitamin D supplementation on bone mineral density were limited to femur bone, and the effect was insignificant⁽⁴³⁾. Thus, the results are inconsistent.

2-2. Method Used to Set the AI

2-2-1. Adults (AI)

In the US-Canada DRIs, the EAR (10 µg/day for adults aged over 19 years) and RDA (15 µg/day for adults aged over 19 years and 20 µg/day for adults aged over 70 years) were determined as stated above. However, these values were determined without consideration of the production due to ultraviolet irradiation, and therefore, cannot be applied to the AI in the present DRIs.

One study focused on the duration of sun exposure required to produce 5.5 µg of vitamin D₃, with the arms and face uncovered, in 3 areas of Japan (Sapporo, Tsukuba and Naha). This study reported that, while vitamin D₃ may be produced even in winter in Naha, this is not the case in Sapporo in December, except around noon⁽⁴⁴⁾. Based on the results for Sapporo in December, the production of 5.5 µg took 94.7 minutes at noon, without limitation for sunny

days; this suggests that approximately 7.5 µg of vitamin D₃ can be produced through 2 hours of sun exposure around noon. Thus, the required amount was calculated by subtracting the amount produced by sun exposure (7.5 µg) from the daily requirement (15 µg), yielding 7.5 µg. However, there is no evidence on the daily sun exposure among healthy Japanese adults, and a study reported that 25OHD levels may be affected by walking outdoors in addition to vitamin D intake⁽⁴⁵⁾. Many factors should be taken into account. Therefore, the AI values of the previous DRIs could not be updated based on the scientific evidence, and the AI for adults was set at 5.5 µg/day. Due to a lack of data, the AI values for both men and women were the same.

Osteoporosis increases the risk of bone fractures at various sites, and vitamin D deficiency increases the risk of non-vertebral body fractures, especially femur bone fractures, and these bone fractures predominantly occur in elderly individuals⁽⁴⁶⁾. In Japan, a high prevalence of fractures was observed in vitamin D-deficient elderly individuals^(40,47). Moreover, an intervention study conducted on elderly individuals in a nursing home, who had limited access to sun exposure, showed that 5 µg/day of supplementation was insufficient⁽⁴⁸⁾, and in only 40% of the individuals, 50 nmol/L of serum 25OHD was maintained, with 20 µg/day of supplementation⁽⁴⁹⁾. Based on these results, the Japanese 2011 guidelines for the prevention and treatment of osteoporosis 2011 (Japan Osteoporosis Society) recommends an intake of 10 to 20 µg/day (Grade B)⁽⁴⁶⁾. However, as most of the aforementioned studies targeted elderly individuals in nursing homes⁽⁴⁷⁻⁴⁹⁾, their results cannot be generalized to the elderly population as a whole. Therefore, the AI was set as 5.5 µg/day for those aged over 70 years, as well as for those aged 18 to 69 years.

A vitamin D intake that is higher than the AI set above may be favorable for those living in areas with shorter daylight hours in winter, those whose performance of outdoor activities is severely limited, or elderly individuals. It is difficult to set these recommendation values at this time.

2-2-2. Children (AI)

One study evaluated the intake of vitamin D and plasma 25OHD levels in 1,380 Japanese children aged 12 to 18 years (672 boys and 718 girls)⁽⁵⁰⁾. The study reported that the mean intake of vitamin D was approximately 10 µg/day, and median plasma 25OHD level was 50 nmol/L, regardless of age or sex. As findings on the relationship between vitamin D intake and plasma 25OHD concentration in children are limited, they were considered unsuitable for the setting of the vitamin D AI for children. Thus, the AI for children was determined through the extrapolation of the AI for adults by the 0.75th power of the BW ratio and the growth factors.

2-2-3. Infants (AI)

In infants, the development of rickets due to vitamin D deficiency is commonly observed both in Japan and other countries⁽⁵¹⁻⁵³⁾, suggesting that limited sun exposure or breast feeding are risk factors. In Japan, although the prevalence rate of rickets is unclear, in an

epidemiological study conducted in Kyoto, 22% of neonates were found to have craniotabes, a mineralization defect of the bone likely caused by vitamin D deficiency⁽⁵⁴⁾. The incidence of craniotabes exhibited seasonal variation, with a peak and nadir between January and May, and between July and November, respectively. The circulating 25OHD levels were found to be below 25 nmol/L in 37% of all neonates diagnosed with craniotabes, at 1 month after birth.

In breast milk-fed newborn infants, the serum concentration of 25OHD was found to be lower than 25 nmol/L in 57% of the population, and below 12.5 nmol/L in 17%. In contrast, none of the formula-fed infants or those with a combination feed 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 decreases further during the winter months⁽⁵⁵⁾, indicating that the vitamin D delivered from breast milk may be unsatisfactory. Similar cases were reported in Iowa, US. The rates of breast milk-fed infants without vitamin D supplementation who showed plasma 25OHD levels lower than 27.5 nmol/L were 70%, 57%, 33% and 23% at 112, 168, 224 and 280 days after birth, respectively⁽⁵⁶⁾.

The concentration of vitamin D in breast milk, including the active metabolite, is 3.0 µg/L in Japanese individuals⁽¹⁷⁾. Although a recent study using a novel, highly accurate procedure found the average vitamin D concentration in breast milk to be only 0.6 mg/L⁽¹⁸⁾, no consequent data followed. In contrast, a concentration of 0.3 µg/100 g was reported in the Standard Tables of Food Composition in Japan, 2010, through the use of a traditional method⁽⁵⁷⁾. The concentrations of vitamin D and its metabolite, which has vitamin D activity, depend on the vitamin D nutrition status of lactating mothers, lactating stage, or season. Therefore, an AI was set for the prevention of rickets.

Breast milk-fed infants with a low degree of sun exposure are at a higher risk of developing rickets⁽⁵⁸⁾. Considering that previous research found that infants did not develop rickets after supplementation with 2.5, 5 or 10 µg/day of vitamin D for 6 months, and assuming that infants receive an average of 2.38 µg/day of vitamin D from breast milk, the daily intakes would be 4.88, 7.38 or 12.38 µg/day of vitamin D; therefore, an intake of 4.88 µg/day is satisfactory for the prevention of rickets.

Based on these data, the AI of vitamin D for infants aged 0 to 5 months was determined to be 5 µg/day. The 2003 guideline of the American Pediatric Society set the requirement intake at 5 µg/day for the prevention of rickets⁽⁵⁹⁾; in the updated guideline (2008), the value was set at 10 µg/day⁽⁶⁰⁾. However, considering that this value requires vitamin D supplementation, and the fact that the actual compliance to this guideline is low⁽⁶¹⁾, the present DRIs did not adopt this value.

The average plasma 25OHD level of 150 18-month-old infants was reported to be higher than 25 nmol/L, and their vitamin D intakes were 8.6 and 3.9 µg/day at 6 months and 12 months, respectively⁽⁶²⁾. In Norway, infants fed 10 µg/day of vitamin D in winter (the amount of milk intake is unknown) had plasma 25OHD levels at nearly the median of the level

measured in late summer and the level of infants who were fed infant formula. Based on the average milk intake of Japanese infants who were fed infant formula (0.8 L/day)⁽⁶³⁾, this amount would be equivalent to 8 µg/day. However, achieving this value requires vitamin D supplementation. Moreover, under circumstances of appropriate sun exposure, lower intakes are not considered a severe risk of deficiency.

The AI of vitamin D for 6-11-month-old infants with adequate sun exposure was determined to be 5 µg/day. This value was also applied to infants aged 6 to 11 months with limited sun exposure, due to a lack of evidence on AI determination.

2–2–4. Pregnant and Lactating Women (AI)

During pregnancy, the requirement of calcium and capacity to produce 1 α ,25(OH)₂D increases; this decreases after delivery. 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 0.75 to 5.3 µg/day⁽⁶⁴⁾, but not in those with an average vitamin D intake higher than 7 µg/day⁽⁶⁵⁾. As these findings indicate that pregnant women require at least 7 µg/day of vitamin D, the AI of vitamin D required for pregnant women was determined to be 7 µg/day.

For lactating women, as the AI for infants was set based on the prevention of rickets and since rickets and hypocalcemia resulting from vitamin D deficiency have been reported among breast-milk-fed infants⁽⁵³⁾, the additional amount of vitamin D required for lactating women was determined to be 2.5 µg/day, by multiplying the concentration of vitamin D (including that of the active metabolite) in breast milk (3.0 µg/L)⁽¹⁷⁾ and the average milk intake (0.78 L/day)^(19,20). The AI for lactating women was set as 8.0 µg/day by adding the additional value to the AI for adults aged over 18 years.

3. To Avoid Excessive Intake

3–1. Dietary Intake

The production of vitamin D in the skin due to ultraviolet irradiation is controlled, and excess vitamin D is not produced. Thus, an excessive intake of vitamin D is unlikely. Vitamin D is activated in the liver and kidneys in which activation is strictly controlled. Once hypercalcemia occurs, further activation is regulated.

3–2. Method Used to Set the UL

A 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 plasma 25OHD level by itself does not directly cause health problems, the presence of hypercalcemia rather than a high serum 25OHD level, is considered an appropriate indicator for the determination of the UL. For infants, excessive vitamin D intake can cause growth retardation, and this is set as an unfavorable outcome in some studies.

3–2–1. Adults (UL)

In an intervention study that included 10, 20, 30, 60, and 95 µg/day of vitamin D supplementation for 3 months, the serum calcium concentration was found to exceed the reference value in some participants receiving 95 µg/day of vitamin D, but not in those receiving 60 µg/day of vitamin D⁽⁶⁶⁾. However, this study had a small sample size, limited to patients with granulomatous diseases that cause hypercalcemia. As no other study has reported the presence of hypercalcemia at vitamin D intake levels lower than 250 µg/day, the NOAEL was determined to be 250 µg/day. This value was divided by an uncertainty factor of 1.2 based on the US-Canada DRIs⁽³⁹⁾, yielding a UL of 100 µg/day for adults. Since some case reports pointed to the presence of hypercalcemia at a vitamin D intake level of 1,250 µg/day^(67,68), using this value as the lowest observed adverse effect level (LOAEL) and an uncertainty factor of 10, a similar value was calculated. Age and sex-related differences were not considered. There is currently no evidence for the setting of other ULs for elderly individuals; therefore, the UL for them was set as the same value as that for adults⁽³⁹⁾.

An intervention study on pregnant women reported no unfavorable outcomes including hypercalcemia at intake levels lower than 100 µg/day⁽⁶⁹⁾. Since there is evidence on pregnant or lactating women having a higher risk of hypercalcemia, the UL for these individuals was set at 100 µg/day^(39,70).

3–2–2. Infants (UL)

Based on a study that observed no growth retardation in infants administered an average of 44 (34.5–54.3) µg/day of vitamin D for 6 months⁽⁷¹⁾, the NOAEL for infants was determined to be 44 µg/day, which, assuming an uncertainty factor of 1.8, yielded a UL of 24.4 µg/day in the US-Canada DRIs⁽³⁹⁾. Accordingly, the UL for infants was set at 25 µg/day.

3–2–3. Children (UL)

As relevant data are unavailable for this age group, the UL for children was determined by extrapolating the UL values for adults aged 18 to 29 years (100 µg/day), and infants (25 µg/day) based on the reference BW. The calculation was conducted by age and sex, and the lowest values in each age group were adopted.

4. For the Prevention of the Development and Progression of Lifestyle-Related Diseases

Vitamin D deficiency is a risk factor for bone fracture, and is indicated to be associated with various lifestyle-related diseases. Since these data remain insufficient, the present DRIs did not consider these associations.

5. Future Dietary Reference Intakes for Japanese Individuals

Reliable data on the sun exposure hours of Japanese individuals, and the association between daily vitamin D intake and plasma 25OHD levels are required.

Vitamin E

1. Background Information

1–1. Definition and Classification

Vitamin E is composed of 8 analogues: α -, β -, γ - and δ -forms, of tocopherol and tocotrienol. Only α -tocopherol presents in the human blood and various tissues. Therefore, α -tocopherol was considered in the current DRIs.

1–2. Function

Vitamin E is located in the phospholipid bilayer of the cell membrane, and prevents the propagation of the lipid peroxidation of unsaturated fatty acid or other components of the biological membrane. Animal studies have shown that vitamin E deficiency causes not only infertility, but also cerebromalacia, hepatic necrosis, kidney disorder, hemolytic anemia, and muscular dystrophy. Excess vitamin E can increase an individual's bleeding tendency. An intake of regular food prevents vitamin E deficiency or excess.

1–3. Digestion, Absorption and Metabolism

Digested vitamin E analogues are absorbed via the intestine to the lymphatic system after forming micelles due to bile acid. The absorption rate of this vitamin has been estimated to be 51 to 86 %⁽⁷²⁾; however, one study reported a rate of only 21 to 29%⁽⁷³⁾. The actual absorption rate of vitamin E in humans is unknown. After intestinal absorption, vitamin E is packaged into chylomicron, transformed into chylomicron remnants by lipoprotein lipase, and transported to the liver. Of the 8 analogues, only α -tocopherol is preferentially bound to α -tocopherol-binding proteins, while the other analogues are metabolized in the liver. α -tocopherol is then transformed into very low-density lipoprotein (VLDL), and distributed again in the blood flow⁽⁷⁴⁾.

2. To Avoid Inadequacy

2–1. Factors to be Considered in Estimating Requirements

Erythrocytes are susceptible to hemolysis by hydrogen peroxide when the circulating α -tocopherol level is between 6 and 12 mmol/L⁽⁷⁵⁾, when the plasma α -tocopherol level is 16.2 μ mol/L (697 μ g/dL). However, they are resistant to hemolysis when the serum α -tocopherol level is higher than 14 mmol/L⁽⁷⁶⁾.

An intervention study that administered graded doses (0 to 320 mg/day) of vitamin E to vitamin E-deficient participants reported that 12 μ mol/L of circulating α -tocopherol corresponds to 12 mg/day of vitamin E intake⁽⁷⁷⁾. These values were not considered appropriate for the estimation of EAR and RDA values, as they were collected many years ago.

2–2. Method Used to Set the AI

2–2–1. Calculation Method Used for the AI

Several studies that analyzed vitamin E intake and circulating α -tocopherol levels consistently reported that the average serum α -tocopherol level exceeded 22 $\mu\text{mol/L}$ in all study populations^(78–80). The average vitamin E intake in those studies ranged from 5.6 to 11.1 mg/day, a range that encompasses the values of the 2010 and 2011 National Health and Nutrition Survey (NHNS)⁽⁸¹⁾ of median vitamin E intakes (6.2–6.8 mg/day in men and 5.5–6.6 mg/day in women), in each sex and age group. As these findings indicate that the median intake of Japanese individuals likely yields an adequate vitamin E status, the AI values were determined to be the 2010 and 2011 NHNS median values, stratified by sex and age group⁽⁸¹⁾.

2–2–2. Adults (AI)

As described above, the AI was determined to be 6.5 mg/day for men and 6.0 mg/day for women, using the weighted average of the median values of the 2010 and 2011 NHNS⁽⁸¹⁾, stratified by sex and age group, as these values are expected to yield a blood α -tocopherol level exceeding 12 $\mu\text{mol/L}$. As aging has not been reported to be associated with the compromised absorption or utilization of vitamin E, the same values were applied to elderly individuals.

Table 1. Reports of the association between blood α -tocopherol level and dietary intake among healthy Japanese subjects

Reference No.	Gender	n	Age (years)	Serum α -tocopherol level ($\mu\text{mol/L}$) ¹	Dietary intake (mg/day) ¹	NHNS ²	
						Age (years)	Intake (mg/day)
78	male	42	31-58	25.4 \pm 5.6	11.1 \pm 4.9	30-49	6.2
	female	44	24-67	31.8 \pm 10.5	9.5 \pm 3.9	30-49	5.7
79	female	150	21-22	32.0 \pm 10.5	7.0 \pm 2.4 ³	18-29	5.5
80	female	10	21.6 \pm 0.8	22.2 \pm 2.2	7.1 \pm 2.0 ⁴		
		11	21.2 \pm 0.8	26.3 \pm 4.2	6.2 \pm 2.4 ⁴		
		10	21.0 \pm 0.7	28.5 \pm 3.6	5.6 \pm 2.0 ⁴		

¹ Mean \pm standard deviation

² Dietary intake value among those in relevant age categories reported in NHNS2010, 2011

³ α -tocopherol equivalent

⁴ α -tocopherol values calculated from α -tocopherol intake (mg/kg BW/day) and mean BW (kg).

2–2–3. Children (AI)

As data were unavailable for this age group, the median values of the 2010 and 2011 NHNS for children, stratified by sex and age group, were used as the bases for the determination of the AI for children, like in the case of adults. For children aged under 12 years, as there is little difference in body height and weight between boys and girls, the average value of the boys and girls was set as the AI.

2–2–4. Infants (AI)

The concentration of vitamin E in breast milk gradually decreases, from 6.8 to 23 mg/L in colostrum, and 1.8 to 9 mg/L in mature milk⁽⁸²⁾. This is not associated with full-term birth or preterm birth, and does not show diurnal variations⁽⁸³⁾. The AI for infants aged 0 to 5 months was determined to be 3.0 mg/day by multiplying the average α -tocopherol concentration in the breast milk of Japanese individuals (3.5 to 4.0 mg/L)^(17,18) and the average milk intake (0.78 L/day)^(19,20).

The AI for infants aged 6 to 11 months old was determined to be 4.0 mg/day through the extrapolation of the adult value by the 0.75th power of the BW ratio, yielding 3.85 mg/day for boys and 3.80 mg/day for girls.

2–2–5. Pregnant or Lactating Women (AI)

During pregnancy, the elevated blood lipid level increases the blood α -tocopherol level⁽⁸⁴⁾. The AI for pregnant women was determined to be 6.5 mg/day using the median value of the 2007-2011 NHNS (6.25 mg/day)⁽⁸⁵⁾ for pregnant women, as there are no reports on vitamin E deficiency during pregnancy. For lactating women, the AI was determined to be 7.0 mg/day using the median value of the 2007-2011 NHNS (6.55 mg/day)⁽⁸⁵⁾ for lactating women.

3. To Avoid Excessive Intake

3–1. Dietary Intake

The intake of regular foods does not lead to vitamin E deficiency or excess.

3–2. Method Used to Set the UL

The basis for determining the UL for vitamin E is its possible effect on an individual's bleeding tendency. Based on the finding that supplementation with 800 mg/day of α -tocopherol for 28 days did not increase the bleeding tendency in healthy men (average BW, 62.2 kg)⁽⁸⁶⁾, the NOAEL was determined to be 800 mg/day. 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/day value by the BW ratio. As few data are available regarding the UL for infants, and because typical feeding with breast milk or baby food does not cause excessive intake, the UL was not determined for infants.

4. For the Prevention of the Development and Progression of LRDs

Although numerous intervention studies have examined the effect of vitamin E supplementation on the risk of coronary heart diseases, the findings are inconsistent^(87–90). A recent study showed an association between excessive vitamin E intake and osteoporosis⁽⁹¹⁾; however, we did not use these data, as the study was conducted on animals, and there are no clinical data supporting this.

Vitamin K

1. Background Information

1–1. Definition and Classification

Naturally occurring vitamin K consists of phyloquinones (PKs; vitamin K₁) and menaquinones (MKs; vitamin K₂). Phyloquinones have a phytyl group in the side chain. 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 on the determination of the relative biological activity of these analogues are scarce, and no corrections have been made for PKs and MK-4, which have similar molecular weights. MK-7, which has a much larger molecular weight, can be converted into its MK-4 equivalent using the following formula:

$$\text{MK-4 equivalent (mg)} = \text{MK-7 (mg)} \times 444.7/649.0$$

The sum of the quantity of PK, MK-4, and MK-7 as corrected above was employed in determining the DRIs for vitamin K.

1–2. Function

The principal biological action of vitamin K is the activation of prothrombin and other serum coagulation factors, for the enhancement of blood coagulation. Other actions include the modulation of bone formation by the activation of osteocalcin, a bone matrix protein, and inhibition of arterial calcification by the activation of matrix gla protein (MGP), another vitamin-K-dependent matrix protein. Vitamin K deficiency delays blood coagulation. The average dietary pattern of Japanese individuals does not lead to vitamin deficiency.

1–3. Digestion, Absorption and Metabolism

Although long-chain MKs are produced by intestinal bacteria⁽⁹²⁾, and MK-4 is also produced by enzymatic conversion from PK⁽⁹³⁾, it is not known to what extent these amounts fulfill requirements. As an intake of 0.8 to 1.0 µg/day kg BW of PK from the regular diet can cause potential vitamin K deficiency⁽⁹⁴⁾, the production of MKs by intestinal bacteria in various tissues is not considered to be large enough to contribute to the fulfillment of this requirement.

2. To Avoid Inadequacy

2–1. Factors to be Considered in Estimating Requirements

Delayed blood coagulation is the only clinically manifested abnormality attributable to vitamin K deficiency. In Japan, coagulation abnormalities due to vitamin K deficiency are rarely observed in healthy individuals. The requirement of vitamin K is considered to be almost fulfilled, except in the case of patients after surgery or those receiving warfarin. The amount of

vitamin K intake required for the activation of blood coagulation is unknown. An intervention study of male volunteers (28.3±3.2 years old) provided vitamin K-deficient meals for 40 days⁽⁹⁴⁾; however, the sample size was small, and so the data cannot be used for the determination of the DRIs.

A recent cohort study examined the association between femur bone fractures and vitamin K intake, and reported that individuals taking 100 µg/day or more of vitamin K had a lower risk of fractures than those with a lower intake^(95,96). Under-γ-carboxylated osteocalcin (ucOC) has been considered as an indicator of suboptimal vitamin K status, and is reported as an independent risk factor for bone fractures. A much greater amount of vitamin K (more than 500 µg/day) is required to decrease ucOC levels than to activate the coagulation factor in the liver^(97,98). The vitamin K intake required for the prevention of bone fractures may be increased, compared to when plasma uncarboxylated prothrombin is used as an index. A meta-analysis reported that vitamin K supplementation decreased the number of bone fracture events, as a result of high doses of MKs (45 mg/day) being administered⁽⁹⁹⁾.

Based on these findings, the vitamin K requirement for the prevention of bone fractures is considered to be greater than the amount required for the maintenance of normal blood coagulation function. Here, AIs were set due to a lack of scientific evidence.

2–2. Method Used to Set the AI

2–2–1. Adults (AI)

As described above, the vitamin K intake of healthy Japanese individuals is considered to be almost sufficient. The average values of vitamin K intake from the 2010 and 2011 NHNS data were 185 µg/day and 280 µg/day, respectively⁽⁸¹⁾. The intake of natto has a significant impact on vitamin K intake in Japanese individuals. The vitamin K intakes have been reported to be 336.2±138.2 µg/day and 154.1±87.8 µg/day for those who consumed natto and those did not, respectively⁽¹⁰⁰⁾. As remarkable unfavorable outcomes have not been reported among those who do not consume natto, based on the value above, 150 µg/day was set as the AI.

Elderly individuals may be more susceptible to vitamin K deficiency due to various factors such as impaired intestinal absorption of vitamin K due to the decreased secretion of bile salts and pancreatic juice, or decreased dietary fat intake. Additionally, the presence of chronic diseases or use of antibiotics decreases the MK production in the intestine, and vitamin K activity due to the inhibition of the activity of vitamin K epoxide reductase. Therefore, the AIs for elderly individuals are considered to be elevated, with a study reporting that these individuals require a higher level of vitamin K⁽¹⁰¹⁾; however, at present, relevant data are scarce, and thus, the AI for elderly individuals--aged above 69 years--was the same as that for those aged 50 to 69 years.

2–2–2. Children (AI)

The AI for children was determined by extrapolating the AI for adults by the 0.75th

power of the BW ratio.

2–2–3. Infants (AI)

The average concentration of vitamin K in the breast milk of Japanese mothers has been reported to be 5.17 µg/L⁽¹⁰²⁾. Recent studies showed that the concentrations were 3.771 ng/mL for PK, and 1.795 ng/mL for MK-7, using newly-developed measurement methods⁽¹⁸⁾. Newborn infants are susceptible to vitamin K deficiency for various reasons, such as poor transplacental vitamin K transport⁽¹⁰³⁾, low vitamin K content in the breast milk^(18,102), or low production of vitamin K in the intestinal flora⁽¹⁰²⁾. 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⁽¹⁰⁴⁾. An AI of 4.0 mg/day for infants aged 0-5 months was determined by multiplying the average milk intake (0.78 L/day)^(19,20) and the average vitamin K content of milk (5.17 µg/L), and assuming the presence of the oral administration of vitamin K just after birth in clinical settings. For infants aged 6 to 11 months, the AI was determined to be 7 µg/day, considering the amount of vitamin K received from sources other than breast milk.

2–2–4. Pregnant or Lactating Women (AI)

There is no evidence stating that the vitamin K requirement is increased or that the circulating vitamin K levels are altered in pregnant women. Due to poor transplacental transport, the vitamin K intake in pregnant women is unlikely to affect the vitamin K status of fetuses or neonates. The requirements are technically the same for women who are pregnant and those who are not, and therefore, fulfilling the AI for women who are not pregnant should be sufficient for the prevention of vitamin K deficiency. Thus, the AI for pregnant women was determined to be 150 µg/day.

Since lactating women are not at a higher risk for vitamin K deficiency, the AI for them was determined to be 150 µg/day.

3. To Avoid Excessive Intake

Although menadione--a vitamin K metabolite--can cause toxicity, no toxicity has been reported with regards to 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⁽⁴⁶⁾, the UL for vitamin K was not determined.

4. For the Prevention of the Development and Progression of LRDs

Vitamin K deficiency has been reported to increase the risk of bone fractures. However, due to a lack of scientific evidence on its preventive effect against bone fracture in intervention studies, DGs were not determined.

II Energy and Nutrients
 Vitamins (1) Fat-soluble Vitamins

DRIs for Vitamin A (µg RAE/day) ¹

Gender	Males				Females			
Age etc.	EAR ²	RDA ²	AI ³	UL ³	EAR ²	RDA ²	AI ³	UL ³
0-5 months	—	—	300	600	—	—	300	600
6-11 months	—	—	400	600	—	—	400	600
1-2 years	300	400	—	600	250	350	—	600
3-5 years	350	500	—	700	300	400	—	700
6-7 years	300	450	—	900	300	400	—	900
8-9 years	350	500	—	1,200	350	500	—	1,200
10-11 years	450	600	—	1,500	400	600	—	1,500
12-14 years	550	800	—	2,100	500	700	—	2,100
15-17 years	650	900	—	2,600	500	650	—	2,600
18-29 years	600	850	—	2,700	450	650	—	2,700
30-49 years	650	900	—	2,700	500	700	—	2,700
50-69 years	600	850	—	2,700	500	700	—	2,700
70+ years	550	800	—	2,700	450	650	—	2,700
Pregnant women (additional)	/							
Early-stage					+0	+0	—	—
Mid-stage					+0	+0	—	—
Late-stage					+60	+80	—	—
Lactating women (additional)	/				+300	+450	—	—

¹ Retinol activity equivalent (µgRAE)
 = 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 Includes provitamin A carotenoids.

³ Excludes provitamin A carotenoids.

II Energy and Nutrients
 Vitamins (1) Fat-soluble Vitamins

DRIs for Vitamin D ($\mu\text{g}/\text{day}$)

Gender	Males		Females	
Age etc.	AI	UL	AI	UL
0-5 months	5.0	25	5.0	25
6-11 months	5.0	25	5.0	25
1-2 years	2.0	20	2.0	20
3-5 years	2.5	30	2.5	30
6-7 years	3.0	40	3.0	40
8-9 years	3.5	40	3.5	40
10-11 years	4.5	60	4.5	60
12-14 years	5.5	80	5.5	80
15-17 years	6.0	90	6.0	90
18-29 years	5.5	100	5.5	100
30-49 years	5.5	100	5.5	100
50-69 years	5.5	100	5.5	100
70+ years	5.5	100	5.5	100
Pregnant women			7.0	—
Lactating women			8.0	—

II Energy and Nutrients
 Vitamins (1) Fat-soluble Vitamins

DRIs for Vitamin E (mg/day) ¹

Gender	Males		Females	
Age etc.	AI	UL	AI	UL
0-5 months	3.0	—	3.0	—
6-11 months	4.0	—	4.0	—
1-2 years	3.5	150	3.5	150
3-5 years	4.5	200	4.5	200
6-7 years	5.0	300	5.0	300
8-9 years	5.5	350	5.5	350
10-11 years	5.5	450	5.5	450
12-14 years	7.5	650	6.0	600
15-17 years	7.5	750	6.0	650
18-29 years	6.5	800	6.0	650
30-49 years	6.5	900	6.0	700
50-69 years	6.5	850	6.0	700
70+ years	6.5	750	6.0	650
Pregnant women			6.5	—
Lactating women			7.0	—

¹ Calculated for α -tocopherol. These do not include vitamin E other than α -tocopherol.

II Energy and Nutrients
 Vitamins (1) Fat-soluble Vitamins

DRIs for Vitamin K (µg/day)

Gender	Males	Females
Age etc.	AI	AI
0-5 months	4	4
6-11 months	7	7
1-2 years	60	60
3-5 years	70	70
6-7 years	85	85
8-9 years	100	100
10-11 years	120	120
12-14 years	150	150
15-17 years	160	160
18-29 years	150	150
30-49 years	150	150
50-69 years	150	150
70+ years	150	150
Pregnant women	/	150
Lactating women		150

References

1. Moise AR, Noy N, Palczewski K, et al. (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.
3. Food and Nutrition Board, Institute of Medicine (2002) *Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc*. 2nd ed. Washington, D.C.: National Academies Press.
4. Sigmundsdottir H & Butcher EC (2008) Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nat Immunol* **9**, 981–987.
5. Sauberlich HE, Hodges RE, Wallace DL, et al. (1974) Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. *Vitam Horm* **32**, 251–75.
6. Ahmad SM, Haskell MJ, Raqib R, et al. (2008) Men with low vitamin A stores respond adequately to primary yellow fever and secondary tetanus toxoid vaccination. *J Nutr* **138**, 2276–83.
7. Olson JA (1987) Recommended dietary intakes (RDI) of vitamin A in humans. *Am J Clin Nutr* **45**, 704–16.
8. Cifelli CJ, Green JB, Wang Z, et al. (2008) Kinetic analysis shows that vitamin A disposal rate in humans is positively correlated with vitamin A stores. *J Nutr* **138**, 971–7.
9. Cifelli CJ, Green JB & Green MH (2007) Use of model-based compartmental analysis to study vitamin A kinetics and metabolism. *Vitam Horm* **75**, 161–95.
10. Furr HC, Green MH, Haskell M, et al. (2005) Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans. *Public Health Nutr* **8**, 896–607.
11. Shimada K (2002) *Naikagakusyo*. 6th ed. Tokyo: Nakayama Shoten Co., Ltd.
12. Raica N, Scott J, Lowry L, et al. (1972) Vitamin A concentration in human tissues collected from five areas in the United States. *Am J Clin Nutr* **25**, 291–296.
13. Joint FAO/WHO Expert Group (2004) Vitamin A. In *Human vitamin and mineral requirements*, 2nd ed., pp. 17–44. WHO/FAO.
14. 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**, 601–6.
15. Strobel M, Tinz J & Biesalski HK (2007) The importance of β -carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. *Eur J Nutr* **46**, 1–20.
16. Canfield LM, Clandinin MT, Davies DP, et al. (2003) Multinational study of major breast milk carotenoids of healthy mothers. *Eur J Nutr* **42**, 133–41.

17. Sakurai T, Furukawa M, Asoh M, et al. (2005) Fat-soluble and water-soluble vitamin contents of breast milk from Japanese women. *J Nutr Sci Vitaminol* **51**, 239–47.
18. Kamao M, Tsugawa N, Suhara Y, et al. (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.
19. Suzuki K, Sasaki S, Shizawa K, et al. (2004) Milk intake by breast-fed infants before weaning. (in Japanese). *Japanese J Nutr* **62**, 369–372.
20. Hirose J, Endo M, Shibata K, et al. (2008) Amount of breast milk sucked by Japanese breast feeding infants. (in Japanese). *J Japanese Soc Breastfeed Res* **2**, 23–28.
21. Penniston KL & Tanumihardjo SA (2006) The acute and chronic toxic effects of vitamin A. *Am J Clin Nutr* **83**, 191–201.
22. Minuk GY, Kelly JK & Hwang W-S (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, et al. (2003) Serum retinol levels and the risk of fracture. *N Engl J Med* **348**, 287–294.
25. Ribaya-Mercado JD & Blumberg JB (2007) Vitamin A: Is it a risk factor for osteoporosis and bone fracture? *Nutr Rev* **65**, 425–438.
26. Azais-Braesco V & Pascal G (2000) Vitamin A in pregnancy: requirements and safety limits. *Am J Clin Nutr* **71**, 1325S–33S.
27. Mannisto S (2004) Dietary carotenoids and risk of lung cancer in a pooled analysis of seven cohort studies. *Cancer Epidemiol Biomarkers Prev* **13**, 40–48.
28. 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**, 1029–35.
29. Albanes D, Heinonen OP, Taylor PR, et al. (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.
30. Ilbert G, Menn SO, Ary G, et al. (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* **334**, 1150–1155.
31. Hennekens CH, Buring JE, Manson JE, et al. (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**, 1145–9.
32. Kavanaugh CJ, Trumbo PR & Ellwood KC (2007) The U.S. Food and Drug Administration’s evidence-based review for qualified health claims: tomatoes, lycopene, and cancer. *J Natl Cancer Inst* **99**, 1074–85.
33. Van Patten CL, de Boer JG & Tomlinson Guns ES (2008) Diet and dietary supplement

- intervention trials for the prevention of prostate cancer recurrence: a review of the randomized controlled trial evidence. *J Urol* **180**, 2314-21; discussion 2721-2.
34. W-T Chong E, Wong TY, of ophthalmology P, et al. (2007) Dietary antioxidants and primary prevention of age related macular degeneration: systematic review and meta-analysis. *BMJ* **335**, 755.
 35. Leung IY-F (2008) Macular pigment: New clinical methods of detection and the role of carotenoids in age-related macular degeneration. *Optom - J Am Optom Assoc* **79**, 266-272.
 36. Stahl W & Sies H (2007) Carotenoids and flavonoids contribute to nutritional protection against skin damage from sunlight. *Mol Biotechnol* **37**, 26-30.
 37. Brustad M, Alsaker E, Engelsen O, et al. (2004) Vitamin D status of middle-aged women at 65-71 degrees N in relation to dietary intake and exposure to ultraviolet radiation. *Public Health Nutr* **7**, 327-35.
 38. Holick MF. (2004) Vitamin D. In *Nutr Bone Heal*, pp. 403-440 [Holick M, Dawson-Hughes B, editors]. Totowa: NJ: Humana Press.
 39. Food and Nutrition Board Institute of Medicine. (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, D.C.: National Academies Press.
 40. Tanaka S, Kuroda T, Yamazaki Y, et al. (2014) Serum 25-hydroxyvitamin D below 25 ng/mL is a risk factor for long bone fracture comparable to bone mineral density in Japanese postmenopausal women. *J Bone Miner Metab* **32**, 514-23.
 41. Bischoff-Ferrari HA, Willett WC, Orav EJ, et al. (2012) A pooled analysis of vitamin D dose requirements for fracture prevention. *N Engl J Med* **367**, 40-9.
 42. Bischoff-Ferrari HA, Willett WC, Wong JB, et al. (2009) Prevention of nonvertebral fractures with oral vitamin D and dose dependence: A meta-analysis of randomized controlled trials. *Arch Intern Med* **169**, 551-561.
 43. Reid IR, Bolland MJ & Grey A (2014) Effects of vitamin D supplements on bone mineral density: A systematic review and meta-analysis. *Lancet* **383**, 146-155.
 44. Miyauchi M, Hirai C & Nakajima H (2013) The solar exposure time required for vitamin D₃ synthesis in the human body estimated by numerical simulation and observation in Japan. *J Nutr Sci Vitaminol* **59**, 257-63.
 45. Ohta H, Kuroda T, Onoe Y, et al. (2009) The impact of lifestyle factors on serum 25-hydroxyvitamin D levels: A cross-sectional study in Japanese women aged 19-25 years. *J Bone Miner Metab* **27**, 682-688.
 46. Orimo H, Nakamura T, Hosoi T, et al. (2012) Japanese 2011 guidelines for prevention and treatment of osteoporosis--executive summary. *Arch Osteoporos* **7**, 3-20.
 47. Kuwabara A, Himeno M, Tsugawa N, et al. (2010) Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly. *Asia Pac J Clin Nutr* **19**, 49-56.
 48. Himeno M, Tsugawa N, Kuwabara A, et al. (2009) Effect of vitamin D

- supplementation in the institutionalized elderly. *J Bone Miner Metab* **27**, 733–737.
49. Kuwabara A, Tsugawa N, Tanaka K, et al. (2009) Improvement of vitamin D status in Japanese institutionalized elderly by supplementation with 800 IU of vitamin D(3). *J Nutr Sci Vitaminol* **55**, 453–8.
 50. Shibata K (2007) *Study for development of Japanese Dietary Reference Intakes. in Reports for the Japanese Ministry of Health Labour and Welfare (2007) (in Japanese)*. .
 51. Ward LM, Gaboury I, Ladhani M, et al. (2007) Vitamin D-deficiency rickets among children in Canada. *CMAJ* **177**, 161–166.
 52. Ozono K (2012) Today's nutritional deficiencies-Vitamin D deficiency. (in Japanese). *Vitam* **86**, 28–31.
 53. Matsuo K, Mukai T, Suzuki S, et al. (2009) Prevalence and risk factors of vitamin D deficiency rickets in Hokkaido, Japan. *Pediatr Int* **51**, 559–562.
 54. Yorifuji J, Yorifuji T, Tachibana K, et al. (2008) Craniotabes in normal newborns: The earliest sign of subclinical vitamin D deficiency. *J Clin Endocrinol Metab* **93**, 1784–1788.
 55. Nakao H. (1988) Nutritional significance of human milk vitamin D in neonatal period. *Kobe J Med Sci* **34**, 121–128.
 56. Ziegler EE, Hollis BW, Nelson SE, et al. (2006) Vitamin D deficiency in breastfed infants in Iowa. *Pediatrics* **118**, 603–610.
 57. The Council for Science and Technology, Ministry of Education, Culture, Sports and Technology (2010) *Standard tables of food composition in Japan - 2010*. Tokyo: Official Gazette Co-operation.
 58. Specker BL, Ha ML, Oestreich A, et al. (1992) Prospective study of vitamin D supplementation and rickets in China. *J Pediatr* **120**, 733–739.
 59. Gartner LM, Greer FR & Section on Breastfeeding and Committee on Nutrition. American Academy of Pediatrics (2003) Prevention of rickets and vitamin D deficiency: new guidelines for vitamin D intake. *Pediatrics* **111**, 908–10.
 60. Wagner CL, Greer FR & Section on Breastfeeding and Committee on Nutrition. American Academy of Pediatrics (2008) Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics* **122**, 1142–1152.
 61. Perrine CG, Sharma AJ, Jefferds MED, et al. (2010) Adherence to Vitamin D Recommendations Among US Infants. *Pediatrics* **125**, 627–632.
 62. Leung SSF, Lui S & Swaminathan R (1989) Vitamin D Status of Hong Kong Chinese Infants. *Acta Paediatr* **78**, 303–306.
 63. Kanno T, Jinno S & Kaneko T (2013) Survey of the anthropometric growth, nutritional intake, fecal properties, and morbidity of infants, in relation with feeding methods (XI). *J child Heal* **72**, 253–60.
 64. 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**, 75–9.
65. Henriksen C, Brunvand L, Stoltenberg C, et al. (1995) Diet and vitamin D status among pregnant Pakistani women in Oslo. *Eur J Clin Nutr* **49**, 211–218.
 66. Narang NK, Gupta RC & Jain MK (1984) Role of vitamin D in pulmonary tuberculosis. *J Assoc Physicians India* **32**, 185–8.
 67. Schwartzman MS & Franck WA (1987) Vitamin d toxicity complicating the treatment of senile, postmenopausal, and glucocorticoid-induced osteoporosis. Four case reports and a critical commentary on the use of vitamin D in these disorders. *Am J Med* **82**, 224–230.
 68. Davies M & Adams PH (1978) The continuing risk of vitamin-D intoxication. *Lancet* **2**, 621–3.
 69. Hollis BW, Johnson D, Hulsey TC, et al. (2011) Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res* **26**, 2341–57.
 70. EFSA Panel on Dietetic Products Nutrition and Allergies (NDA) (2012) Scientific opinion on the tolerable upper intake level of vitamin D. *EFSA J* **10**, 2813.
 71. Fomon SJ, Kabir M, And Y, et al. (1966) Influence of vitamin D on linear growth of normal full-term infants. *J Nutr* **88**, 345–350.
 72. Kelleher J & Losowsky MS (1970) The absorption of alpha-tocopherol in man. *Br J Nutr* **24**, 1033–1047.
 73. Blomstrand R & Forsgren L (1968) Labelled tocopherols in man. Intestinal absorption and thoracic-duct lymph transport of dl-alpha-tocopheryl-3,4-14C2 acetate dl-alpha-tocopheramine-3,4-14C2 dl-alpha-tocopherol-(5-methyl-3H) and N-(methyl-3H)-dl-gamma-tocopheramine. *Int Z Vitaminforsch* **38**, 328–44.
 74. Traber MG & Arai H (1999) Molecular mechanisms of vitamin E transport. *Annu Rev Nutr* **19**, 343–55.
 75. 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.
 76. Farrell PM, Bieri JG, Fratantoni JF, et al. (1977) The occurrence and effects of human vitamin E deficiency. A study in patients with cystic fibrosis. *J Clin Invest* **60**, 233–241.
 77. Horwitt MK (1960) Vitamin E and lipid metabolism in man. *Am J Clin Nutr* **8**, 451–61.
 78. Sasaki S, Ushio F, Amano K, et al. (2000) Serum biomarker-based validation of a self-administered diet history questionnaire for Japanese subjects. *J Nutr Sci Vitaminol* **46**, 285–96.
 79. 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**, 20–7.
 80. Maruyama C, Imamura K, Oshima S, et al. (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.
81. Ministry of Health, Labour and Welfare, National Health and Nutrition Survey in Japan, Results of 2010-2011.
http://www.mhlw.go.jp/bunya/kenkou/dl/kenkou_eiyoubousa_tokubetsushuukei_h22.pdf.
 82. Jansson L, Akesson B & Holmberg L (1981) Vitamin E and fatty acid composition of human milk. *Am J Clin Nutr* **34**, 8–13.
 83. Lammi-Keefe CJ, Jensen RG, Clark RM, et al. (1985) Alpha tocopherol, total lipid and linoleic acid contents of human milk at 2, 6, 12 and 16 weeks. In *Composition and Physiological Properties of Human Milk.*, pp. 241–245 [Scaub J, editor]. New York.: Elsevier Science.
 84. Herrera E, Ortega H, Alvino G, et al. (2004) Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. *Eur J Clin Nutr* **58**, 1231–1238.
 85. Ministry of Health Labour and Welfare National Health and Nutrition Survey in Japan, Results of 2007-2011.
http://www.mhlw.go.jp/bunya/kenkou/dl/kenkou_eiyoubousa_tokubetsushuukei_ninpu_h19.pdf.
 86. Morinobu T, Ban R, Yoshikawa S, et al. (2002) The safety of high-dose vitamin E supplementation in healthy Japanese male adults. *J Nutr Sci Vitaminol* **48**, 6–9.
 87. Miller Iii ER, Pastor-Barriuso R, Dalal D, et al. (2005) Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* **142**, 37–46.
 88. Bjelakovic G, Nikolova D, Gluud LL, et al. (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: Systematic review and meta-analysis. *J Am Med Assoc* **297**, 842–857.
 89. Asleh R, Blum S, Kalet-Litman S, et al. (2008) Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2-2 genotype. *Diabetes* **57**, 2794–2800.
 90. Milman U, Blum S, Shapira C, et al. (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.
 91. Fujita K, Iwasaki M, Ochi H, et al. (2012) Vitamin E decreases bone mass by stimulating osteoclast fusion. *Nat Med* **18**, 589–594.
 92. Shearer MJ (1995) Vitamin K. *Lancet*, 229–234.
 93. Okano T, Shimomura Y, Yamane M, et al. (2008) Conversion of phyloquinone (vitamin K1) into menaquinone-4 (vitamin K2) in mice: Two possible routes for menaquinone-4 accumulation in cerebra of mice. *J Biol Chem* **283**, 11270–11279.

94. Suttie J (1988) Vitamin K deficiency from dietary vitamin K restriction in humans. *Am J Clin Nutr* **47**, 475–480.
95. Feskanich D, Weber P, Willett WC, et al. (1999) Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr* **69**, 74–9.
96. Booth SL, Tucker KL, Chen H, et al. (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**, 1201–8.
97. Binkley NC, Krueger DC, Kawahara TN, et al. (2002) A high phylloquinone intake is required to achieve maximal osteocalcin γ -carboxylation. *Am J Clin Nutr* **76**, 1055–1060.
98. Bügel S, Sørensen AD, Hels O, et al. (2007) Effect of phylloquinone supplementation on biochemical markers of vitamin K status and bone turnover in postmenopausal women. *Br J Nutr* **97**, 373–380.
99. Cockayne S, Adamson J, Lanham-New S, et al. (2006) Vitamin K and the prevention of fractures: Systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med* **166**, 1256–1261.
100. Kamao M, Suhara Y, Tsugawa N, et al. (2007) Vitamin K content of foods and dietary vitamin K intake in Japanese young women. *J Nutr Sci Vitaminol* **53**, 464–70.
101. Tsugawa N, Shiraki M, Suhara Y, et al. (2006) Vitamin K status of healthy Japanese women: age-related vitamin K requirement for gamma-carboxylation of osteocalcin. *Am J Clin Nutr* **83**, 380–386.
102. Kojima T, Asoh M, Yamawaki N, et al. (2004) Vitamin K concentrations in the maternal milk of Japanese women. *Acta Paediatr* **93**, 457–463.
103. Shearer MJ, Rahim S, Barkhan P, et al. (1982) Plasma vitamin K1 in mothers and their newborn babies. *Lancet* **2**, 460–3.
104. Puckett RM & Offringa M (2000) Prophylactic vitamin K for vitamin K deficiency bleeding in neonates. *Cochrane database Syst Rev*, CD002776.