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.

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Dietary Reference Intakes for Japanese 2010: Water-Soluble Vitamins

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Summary A potential approach for determining the estimated average requirement (EAR) is based on the observation that a water-soluble vitamin or its catabolite(s) can be detected in urine. In this approach, the urinary excretion of a water-soluble vitamin or its catabolite(s) increase when the intake exceeds the requirement. This approach is applied to vitamin B_1, vitamin B_2 and niacin. A second approach is to determine the blood concentration. In this case, the requirement is indicated by a value rather than a threshold level. The second approach is applied to vitamin B_6, vitamin B_{12}, folate, and vitamin C. The recommended dietary allowance (RDA) was calculated by multiplying the EAR by 1.2. For pantothenic acid and biotin, there were insufficient data for determining the EAR. Thus, adequate intakes were set based on food surveillance data.

Key Words water-soluble vitamins, DRI, urine, blood, requirement

Vitamin B_1

Background information

The chemical name of vitamin B_1 is thiamin, and the active form is thiamin diphosphate (TDP). Severe thiamin deficiency results in a nerve and heart disease, termed beriberi. Less severe deficiency results in nonspecific symptoms such as malaise, loss of weight, irritability, and confusion.

In foods, thiamin exists mainly as a TDP-protein complex. Thus, the absorption of thiamin in the digestive tract involves 2 stages: (1) the release of TDP from the complex by the action of proteases and (2) the release of thiamin from TDP by the action of phosphatases and pyrophosphatases. There are 2 mechanisms of absorption. At low luminal concentrations (<2 μmol/L), the process is carrier-mediated; at higher concentrations (e.g., a 2.5 mg dose for humans) passive diffusion also occurs.

Most of the thiamin in serum is bound to protein, mainly albumin. Thiamin is taken up by blood cells and body tissues via active transport. Intracellular thiamin occurs predominantly (80%) as TDP, most of which is bound to proteins. The relative availability of dietary vitamin B_1 to free thiamin in a typical Japanese diet is around 60% (*1*, *2*).

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Determining DRIs

Evidence for determining the estimated average requirement (EAR)

Orally administered thiamin is rapidly converted to TDP in the body tissues. Thereafter, excess thiamin is excreted as free form in the urine. Urinary excretion of thiamin has been shown sharply to increase at a concentration >0.35 mg thiamin/1,000 kcal/d (*3*). Based on this evidence, the EAR of thiamin ($C_{12}H_{17}N_4OS$, molecular weight 265.3) was determined. It should be noted that the Standard Tables of Food Composition in Japan give the content of vitamin B_1 as the value of thiamin hydrochloride ($C_{12}H_{17}ClN_4OS{\cdot}HCl$, molecular weight 337.3). Thus, the EAR of vitamin B_1 becomes 0.45 mg thiamin hydrochloride/1,000 kcal/d. The recommended dietary allowance (RDA) is set by assuming a coefficient of variation of 10%. Thus the RDA becomes 0.54 mg thiamin hydrochloride/1,000 kcal/d.

Table 1. DRIs for vitamin B_1 (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.1	—	—	—	0.1	—
6–11 mo	—	—	0.3	—	—	—	0.3	—
1–2 y	0.5	0.5	—	—	0.4	0.5	—	—
3–5 y	0.6	0.7	—	—	0.6	0.7	—	—
6–7 y	0.7	0.8	—	—	0.7	0.8	—	—
8–9 y	0.8	1.0	—	—	0.8	1.0	—	—
10–11 y	1.0	1.2	—	—	0.9	1.1	—	—
12–14 y	1.1	1.4	—	—	1.0	1.2	—	—
15–17 y	1.2	1.5	—	—	1.0	1.2	—	—
18–29 y	1.2	1.4	—	—	0.9	1.1	—	—
30–49 y	1.2	1.4	—	—	0.9	1.1	—	—
50–69 y	1.1	1.3	—	—	0.9	1.1	—	—
≥70 y	1.0	1.2	—	—	0.8	0.9	—	—
Pregnant women (amount to be added)								
Early-stage					+0.0	+0.0	—	—
Mid-stage					+0.1	+0.1	—	—
Late-stage					+0.2	+0.2	—	—
Lactating women (amount to be added)					+0.2	+0.2	—	—

DRIs, Dietary Reference Intakes; EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.
[1] Calculated by using PAL II of the EER.

For example, the RDAs for 18- to 29-y-old males and females are 1.4 mg/d and 1.1 mg/d, respectively, assuming a physical activity level (PAL) II, i.e., within the estimated energy requirement (EER).

Life stages

0–5 mo. The mean concentration of thiamin hydrochloride in breast milk is 0.13 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily vitamin B_1 intake of about 0.1 mg/d. This value was set as the adequate intake (AI).

6–11 mo. The AI for infants aged 6–11 mo is calculated using the average of the values from the following 2 expressions: Expression 1, AI for infant boy or girl aged 6–11 mo (extrapolated AI from infants)=AI for infants (0–5 mo)×(average reference infant boy or girl body weight of 6–11 mo/average reference infant boy or girl body weight of 0–5 mo)$^{0.75}$; Expression 2, AI for infant boy or girl aged 6–11 mo (extrapolated AI from adults)=RDA×(average reference infant boy or girl body weight of 6–11 mo/average reference male or female weight of 18–29 y old)$^{0.75}$×(1+growth factor). Thus, the AI of infants aged 6–11 mo is 0.3 mg/d.

Pregnant women. The additional amounts are calculated based on the assumption that the requirement for vitamin B_1 increases according to energy expenditure. In other words, the additional EAR and RDA for pregnant women are calculated by multiplying the EAR or RDA by the additional energy expenditure resulting from pregnancy.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. But, the availability of dietary vitamin B_1 is low compared with the free form of vitamin B_1. The relative availability of dietary vitamin B_1 to free thiamin in a typical Japanese diet is around 60% (*1, 2*). Thus, the EAR is divided by 0.6. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

Chronic intake of thiamin (50 mg/kg body weight/d) has been reported to cause severe toxicity symptoms (*9*). For example, intake of 10 g of thiamin hydrochloride for 2.5 wk daily resulted in headaches, irritability, insomnia, pulsus celer, weakness, contact dermatitis, and itchiness. These symptoms disappeared in 2 d when the intake was discontinued (*10*). Nevertheless, there is insufficient evidence for determining the tolerable upper intake level (UL).

The Dietary Reference Intakes (DRIs) for vitamin B_1 are summarized in Table 1.

Vitamin B_2

Background information

The chemical name of vitamin B_2 is riboflavin, and the active forms are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Riboflavin deficiency results in angular cheilitis, glossitis (magenta tongue), seborrheic dermatitis, and other disorders.

Table 2. DRIs for vitamin B_2 (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.3	—	—	—	0.3	—
6–11 mo	—	—	0.4	—	—	—	0.4	—
1–2 y	0.5	0.6	—	—	0.5	0.5	—	—
3–5 y	0.7	0.8	—	—	0.6	0.8	—	—
6–7 y	0.8	0.9	—	—	0.7	0.9	—	—
8–9 y	0.9	1.1	—	—	0.9	1.0	—	—
10–11 y	1.1	1.4	—	—	1.0	1.2	—	—
12–14 y	1.3	1.5	—	—	1.1	1.4	—	—
15–17 y	1.4	1.7	—	—	1.1	1.4	—	—
18–29 y	1.3	1.6	—	—	1.0	1.2	—	—
30–49 y	1.3	1.6	—	—	1.0	1.2	—	—
50–69 y	1.2	1.5	—	—	1.0	1.2	—	—
≥70 y	1.1	1.3	—	—	0.9	1.0	—	—
Pregnant women (amount to be added)								
Early-stage					+0.0	+0.0	—	—
Mid-stage					+0.1	+0.2	—	—
Late-stage					+0.2	+0.3	—	—
Lactating women (amount to be added)					+0.3	+0.4	—	—

[1] Calculated by using PAL II of the EER.

In foods, riboflavin exists mainly as a complex of FMN or FAD, non-covalently bound to related enzyme proteins. During digestion, FAD and FMN are firstly liberated in acidic conditions, and are then hydrolyzed by pyrophosphatase and phosphatase. Finally, riboflavin is released and absorbed from the small intestine (*11*). The absorbed riboflavin is incorporated into the body tissues, and used for FAD synthesis. In the rat liver, for example, about 90% of riboflavin exists as FAD, about 10% as FMN, and the remaining 1% as riboflavin.

In the blood, riboflavin exists mainly in the form of FAD, with ~10% FMN and ~4% riboflavin. A large portion of riboflavin is associated with immunoglobulins, but some is bound to albumin (*12*). The absorbed riboflavin is incorporated into the body tissues, and converted mainly to FAD via FMN.

Excess riboflavin is rapidly excreted in the urine, primarily as free riboflavin.

Determining DRIs

Evidence for determining the EAR

Usually only a small amount of riboflavin is excreted in the urine; the level of excretion varies according to the intake of vitamin B_2. If the body requirement is met, urinary excretion shows a rapid increase. A gradual increase in the intake of free riboflavin to ≥1.1 mg/d was shown to result in a rapid rise in urinary excretion by healthy males and females (*13*, *14*). Based on these results, and the involvement of vitamin B_2 in energy metabolism, EAR was determined as the energy intake/d, i.e., 0.50 mg riboflavin/1,000 kcal/d. For example, the EARs for 18- to 29-y-old males and females are 1.3 mg/d and 1.0 mg/d, respectively, assuming a PAL II, i.e., within the EER.

Life stages

0–5 mo. For infants of 0–5 mo, breast milk is the sole source of vitamin B_2. The mean concentration of riboflavin in breast milk is 0.40 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily vitamin B_2 intake of about 0.3 mg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 0.4 mg/d.

Pregnant women. The additional amounts are calculated based on the assumption that the requirement for vitamin B_2 increases according to energy expenditure. In other words, the additional EAR and RDA for pregnant women are calculated by multiplying the EAR or RDA by the additional energy expenditure resulting from pregnancy.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. The mean concentration of riboflavin in breast milk is 0.40 mg/L (4–6) and the average secretion of breast milk is 0.78 L/d (*7*, *8*). Thus, the additional EAR becomes 0.3 mg/d. The additional RDA is calculated by multiplying the additional EAR by

1.2.

Tolerable upper intake level

Chronic use of riboflavin has not been reported to cause severe toxicity. For example, a daily intake of 400 mg of riboflavin for 3 mo (*15*), supplemental oral intake of up to 60 mg riboflavin, or single intravenous injection of 11.6 mg riboflavin (*16*) caused no deleterious effects. This may be attributed to rapid excretion of riboflavin in the urine, and also to limited solubility and reduced absorption at higher doses. Stripp demonstrated limited absorption of 50–500 mg of riboflavin, and consequently no adverse effects (*17*). Zempleni et al. reported that the maximum absorbable amount of riboflavin in a single dose was 27 mg (*16*). Moreover, there are no data indicating that riboflavin administration during pregnancy is potentially dangerous. Thus, there is no evidence for determining the UL.

The DRIs for vitamin B_2 are summarized in Table 2.

Niacin

Background information

The main compounds showing niacin activity are nicotinic acid, nicotinamide, and tryptophan. The DRIs for niacin are expressed in niacin equivalent (NE).

The Standard Tables of Food Composition in Japan, (*18*) list niacin as the sum of nicotinic acid and nicotinamide, and do not include nicotinamide biosynthesized from tryptophan. Therefore, to calculate NE in a diet, the amount of nicotinamide biosynthesized from dietary tryptophan should be added to the amount of niacin. The conversion ratio for tryptophan to nicotinamide is set at 1/60 on a weight basis. The NE is calculated using the following formula:

Niacin equivalent (mg NE)

=niacin intake (mg)+(1/60) tryptophan intake (mg)

Most protein contains approximately 1% of tryptophan, and therefore the amount of nicotinamide biosynthesized from tryptophan (mg) is estimated as the amount of protein (g) divided by 6.

In living cells, niacin exists mainly as the cofactor NAD(P), which binds weakly to enzyme proteins. During cooking and processing of animal and plant foods, NAD(P) is hydrolyzed to nicotinamide and nicotinic acid, respectively. Any remaining NAD(P) is hydrolyzed to nicotinamide in the gastrointestinal tract. Nicotinamide and nicotinic acid are absorbed in the small intestine. Most nicotinic acid binds to complex carbohydrates in cereal grains, and is therefore less digestible (*19*). The relative availability of dietary niacin to free nicotinamide is approximately 60% in a typical Japanese diet (*1*, *2*).

Determining DRIs

Evidence for determining the EAR

The conversion ratio of tryptophan to nicotinamide is set at 1/60 on a weight basis (*20*, *21*). Niacin relates to energy metabolism, and therefore the EAR for niacin is expressed as mg NE/1,000 kcal. Human studies show that NE intake correlates well with urinary nicotinamide metabolite N^1-methylnicotinamide, and that a urinary N^1-methylnicotinamide of 1.0 mg/d reflects clinical niacin deficiency (*20*, *22–25*). Analysis of previous studies shows that the niacin intake equivalent to a urinary N^1-methylnicotinamide of 1.0 mg/d is 4.8 mg NE/1,000 kcal. This value was set as the EAR for subjects aged 1–69 y. The RDA is determined as 5.8 mg NE/1,000 kcal, calculated by multiplying the EAR by 1.2. Based on niacin intake and urinary nicotinamide metabolite data, niacin activity in older subjects is considered to be the same as that in younger subjects. Thus, the EAR and RDA were set at 4.8 mg NE/1,000 kcal and 5.8 mg NE/1,000 kcal, respectively, for adults >70 y old. To express the EAR and RDA in mg NE/d, each value is multiplied by the estimated energy requirement corresponding to a subject's sex, age, and physical activity.

Life stages

0–5 mo. The mean nicotinamide concentration in breast milk is 2.0 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily nicotinamide intake of ~1.6 mg/d. The AI for infants aged 0–5 mo was set at 2 mg/d. Nicotinamide is unlikely to be biosynthesized from tryptophan at this stage, and therefore the AI is expressed in mg/d.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. The average of the obtained values for each sex is 3.1 mg NE/d. Thus, the AI for infants aged 6–11 mo becomes 3 mg NE/d.

Pregnant women. The additional amounts are set based on the assumption that the requirement for niacin increases according to energy expenditure. There is no evidence for setting the EAR by factorial method. Thus, the EAR and RDA for niacin are expressed as mg NE/1,000 kcal. However, the amount of nicotinamide biosynthesized from tryptophan increases during pregnancy, and this compensates for the increase in niacin requirement (*16*). Thus, pregnant women do not require additional niacin intake.

Lactating women. The conversion rate of tryptophan to nicotinamide returns to a normal level after delivery (*26*), and therefore lactating women require additional niacin intake to compensate for the loss of niacin to breast milk. Daily niacin secretion to milk of 1.6 mg/d is adjusted by the relative availability of dietary niacin to free nicotinamide 60% (*1*, *2*). Thus, the additional EAR for lactating women was set at 3 mg NE/d (rounded up from 2.6 mg NE/d). The additional RDA was set at 3 mg NE/d, calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

Nicotinic acid and nicotinamide are often used in niacin supplements and fortified foods. The UL for niacin therefore takes into account the nicotinic acid and nicotinamide taken from supplements and fortified foods. The large doses of nicotinamide and nicotinic acid used to treat patients with type I diabetes and hypercholesterolemia, respectively, may cause gastrointestinal effects such as dyspepsia, diarrhea, and constipation, and also

Table 3. DRIs for niacin (mgNE/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL[2]	EAR	RDA	AI	UL[2]
0–5 mo[3]	—	—	2	—	—	—	2	—
6–11 mo	—	—	3	—	—	—	3	—
1–2 y	5	6	—	60 (15)	4	5	—	60 (15)
3–5 y	6	7	—	80 (20)	6	7	—	80 (20)
6–7 y	7	9	—	100 (30)	7	8	—	100 (30)
8–9 y	9	10	—	150 (35)	8	10	—	150 (35)
10–11 y	11	13	—	200 (45)	10	12	—	150 (45)
12–14 y	12	14	—	250 (60)	11	13	—	250 (60)
15–17 y	13	16	—	300 (70)	11	13	—	250 (65)
18–29 y	13	15	—	300 (80)	9	11	—	250 (65)
30–49 y	13	15	—	350 (85)	10	12	—	250 (65)
50–69 y	12	14	—	350 (80)	9	11	—	250 (65)
≥70 y	11	13	—	300 (75)	8	10	—	250 (60)
Pregnant women (amount to be added)					+0	+0	—	—
Lactating women (amount to be added)					+3	+3	—	—

[1] NE=niacin equivalents (mgNE)=niacin intake (mg)+1/60 of tryptophan intake (mg). Calculated by using PAL II of the EER.

[2] The ULs were the amounts of nicotinamide (mg) and mg of nicotinic acid in parentheses. Values were calculated using reference body weight.

[3] Values were expressed as mg/d.

hepatotoxic symptoms such as dysfunction and fulminant hepatitis. According to previous reports (*26–30*), the no observed adverse effect levels (NOAELs) for nicotinamide and nicotinic acid were set at 25 mg/kg body weight and 6.25 mg/kg body weight, respectively. The NOAELs were divided by an uncertainty factor of 5, and the obtained values of 5 mg/kg body weight and 1.25 mg/kg body weight were set as the ULs for nicotinamide and nicotinic acid, respectively. A pharmacological dose of nicotinic acid has the transient vasodilatory effect of flushing (reddening of the skin), but no adverse health effects. Thus, it is not appropriate to use flushing for setting a UL for nicotinic acid.

The DRIs for niacin are summarized in Table 3.

Vitamin B_6

Background information

The chemical substances possessing vitamin B_6 activity are pyridoxine, pyridoxal, and pyridoxamine and their respective phosphorylated forms. The functional form is pyridoxal 5′-phosphate (PLP). Vitamin B_6 deficiency results in seborrheic dermatitis, epileptiform convulsions, and microcytic anemia. In foods, vitamin B_6 exists mainly as a complex of PLP or pyridoxamine 5′-phosphate (PMP), associated with protein. During digestion, PLP and PMP are released and hydrolyzed by phosphatase, after which pyridoxal and pyridoxamine are released and absorbed. Plants possess pyridoxine 5′β-glucoside (PNG), which, if ingested, is partially hydrolyzed to pyridoxine and absorbed. The bioavailability of vitamin B_6 in humans is estimated to be 50% (*31*). The bioavailability in typical American foods is estimated to be 75% (*32*), while that in a typical rice-based Japanese diet is 73% (*1*).

In serum, PLP and pyridoxal are the dominant B_6 vitamers. PLP is bound to protein, predominantly albumin. Erythrocytes possess pyridoxal kinase and pyridoxamine 5′-phosphate/pyridoxine 5′-phosphate oxidase, and therefore PLP can be synthesized from pyridoxal and PMP. Pyridoxal is incorporated into the body tissues and converted to PLP.

Pyridoxal is metabolized in the liver to 4-pyridoxic acid, and excreted in the urine.

Determining DRIs

Evidence for determining the EAR

Vitamin B_6 is involved in the catabolism of amino acids and formation of bioactive amines, including some neurotransmitters such as γ-aminobutyric acid. The plasma PLP concentration has been reported to reflect the body store of vitamin B_6 (*33*). A low plasma PLP concentration was shown to be associated with electroencephalographic changes in young, non-pregnant women (*34*). Furthermore, a plasma PLP concentration of 30 nmol/L was required to alleviate vitamin B_6 deficiency-induced disorders (*35*). The EAR for vitamin B_6 is based on the amount of vitamin B_6 that can maintain a plasma PLP level of 30 nmol/L. The vitamin B_6 requirement increases as the protein intake increases, and the plasma PLP concentration correlates well with vitamin

Table 4. DRIs for vitamin B_6 (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL[2]	EAR	RDA	AI	UL[2]
0–5 mo	—	—	0.2	—	—	—	0.2	—
6–11 mo	—	—	0.3	—	—	—	0.3	—
1–2 y	0.4	0.5	—	10	0.4	0.5	—	10
3–5 y	0.5	0.6	—	15	0.5	0.6	—	15
6–7 y	0.7	0.8	—	20	0.6	0.7	—	20
8–9 y	0.8	0.9	—	25	0.8	0.9	—	25
10–11 y	0.9	1.0	—	30	0.9	1.0	—	30
12–14 y	1.0	1.3	—	40	1.0	1.3	—	40
15–17 y	1.1	1.4	—	50	1.0	1.3	—	45
18–29 y	1.1	1.4	—	55	1.0	1.1	—	45
30–49 y	1.1	1.4	—	60	1.0	1.1	—	45
50–69 y	1.1	1.4	—	55	1.0	1.1	—	45
≥70 y	1.1	1.4	—	50	1.0	1.1	—	40
Pregnant women (amount to be added)					+0.7	+0.8	—	—
Lactating women (amount to be added)					+0.3	+0.3	—	—

[1] Calculated by using recommended dietary allowance of protein (except for additional amount for pregnant and lactating women).
[2] Quantity as pyridoxine, not indicating values in dietary vitamin B_6.

B_6 intake per protein intake (*36*). Thus, 0.014 mg pyridoxine/g protein was estimated as the concentration required to maintain a plasma PLP concentration of 30 nmol/L. Based on the bioavailability of vitamin B_6 in a typical rice-based Japanese diet (*1*), the EAR becomes 0.019 mg pyridoxine/g protein. The RDA is calculated by multiplying the EAR by 1.2, to give 0.023 mg pyridoxine/g protein. To obtain the daily requirement of vitamin B_6, the EAR of vitamin B_6 is multiplied to a RDA of protein. For example, the EAR for 18- to 29-y-old males and females are 1.1 mg pyridoxine/d and 1.0 mg pyridoxine/d, assuming that RDAs of protein is 60 g/d and 50 g/d, respectively.

Life stages

0–5 mo. For infants of 0–5 mo, breast milk is the sole source of vitamin B_6. The mean concentration of pyridoxine in breast milk is 0.25 mg/L (*4–6, 37*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily vitamin B_6 intake of about 0.2 mg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 0.3 mg/d.

Pregnant women. The plasma PLP concentration has been reported to decrease during pregnancy (*38*). However, during the last stage, it must be maintained at 30 nmol/L. Thus, the additional amount is set at 0.5 mg/d (*36*). The additional EAR during pregnancy is set at 0.7 mg/d including a bioavailability of 73%. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. The additional EAR for pregnant women is calculated based on the mean concentration of vitamin B_6 in breast milk (0.25 mg/L) (*8*), the average secretion (0.78 L/d) of breast milk (*7, 8*), and a bioavailability of 73%, i.e., 0.3 mg/d. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

A continuously high intake of pyridoxine for several months was shown to result in sensory neuropathy (*39*). This symptom was used as a criterion for estimating the UL for pyridoxine. By contrast, administration of 100–300 mg pyridoxine/d over a period of 4 mo did not cause sensory neuropathy in 24 patients with carpal tunnel syndrome (*40*). Based on these data, the NOAEL was set at 300 mg/d. Assuming an uncertainty factor of 5, the UL for pyridoxine was set at 60 mg/d, namely 0.8 mg/kg body weight. The UL for each age group was obtained by multiplying the UL by the respective weight.

The DRIs for vitamin B_6 are summarized in Table 4.

Vitamin B_{12}

Background information

Vitamin B_{12} (B_{12}) belongs to the corrinoids, which are compounds having in common a corrin nucleus. There are various B_{12} compounds with different upper ligands; in particular, methylcobalamin and 5′-deoxya-

Table 5. DRIs for vitamin B_{12} (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.4	—	—	—	0.4	—
6–11 mo	—	—	0.6	—	—	—	0.6	—
1–2 y	0.8	0.9	—	—	0.8	0.9	—	—
3–5 y	0.9	1.1	—	—	0.9	1.1	—	—
6–7 y	1.1	1.4	—	—	1.1	1.4	—	—
8–9 y	1.3	1.6	—	—	1.3	1.6	—	—
10–11 y	1.6	1.9	—	—	1.6	1.9	—	—
12–14 y	2.0	2.4	—	—	2.0	2.4	—	—
15–17 y	2.0	2.4	—	—	2.0	2.4	—	—
18–29 y	2.0	2.4	—	—	2.0	2.4	—	—
30–49 y	2.0	2.4	—	—	2.0	2.4	—	—
50–69 y	2.0	2.4	—	—	2.0	2.4	—	—
≥70 y	2.0	2.4	—	—	2.0	2.4	—	—
Pregnant women (amount to be added)					+0.3	+0.4	—	—
Lactating women (amount to be added)					+0.7	+0.8	—	—

denosylcobalamin function as B_{12} coenzymes. The DRIs for B_{12} were set as cyanocobalamin (molecular weight 1,355.4).

Humans possess a complex process for gastrointestinal absorption of dietary B_{12} (*41*). B_{12} released from food protein is first bound to haptocorrin (salivary B_{12}-binding protein) in the stomach. After proteolysis of the haptocorrin–B_{12} complex by pancreatic proteases in the duodenum, the released B_{12} binds to intrinsic factor (IF, gastric B_{12}-binding protein) in the proximal ileum. The IF–B_{12} complex can enter mucosal cells in the distal ileum by receptor-mediated endocytosis.

The bioavailability of dietary B_{12} is highly dependent on this IF-mediated absorption system. Under physiological conditions, 50% of dietary B_{12} is assumed to be absorbed by healthy adults (*42*). The IF-mediated B_{12} absorption system becomes saturated at a dietary concentration of about 2 μg of B_{12} (*43*). Ingestion of a large quantity of B_{12} from certain foods results in a significant decrease in the absorption rate of B_{12}.

Substantial amounts of B_{12} are excreted in bile (average excretion of 2.5 μg/d) (*44*). Approximately 50% of biliary B_{12} is re-absorbed by the intestine, with the remainder excreted in the feces.

Determining DRIs

Evidence for determining the EAR

It is not possible to determine the EAR of B_{12} for healthy adults, because of the saturable IF-mediated B_{12} gastrointestinal absorption system and/or substantial amounts of enterohepatic B_{12} circulation. Thus, the EAR for adults was estimated based on clinical data (the amount of B_{12} required for maintenance of adequate hematological status and serum B_{12} level) from B_{12}-deficient patients with pernicious anemia, following intramuscular injection with varying concentrations (0.1–10 μg/d) of B_{12} (*45*, *46*). The data suggest an average intramuscular requirement of 1.5 μg/d for maintenance of adequate hematological status. B_{12}-deficient patients with pernicious anemia cannot reabsorb B_{12} (0.5 μg/d) from the bile, because of the lack of an IF-mediated B_{12} absorption system (*42*). Thus, under normal physiological conditions, an average intake of 1.0 μg/d is required to compensate for the estimated extra losses of biliary B_{12} (0.5 μg/d) from the average intramuscular requirement (1.5 μg/d). We adjusted this value with a 50% absorption rate of dietary B_{12}, to obtain an EAR (2.0 μg/d) for healthy adults. The RDA was calculated as 2.4 μg/d, by multiplying the EAR by 1.2.

The EAR for children was calculated from the EAR for adults (2.0 μg/d), using the following equation for body surface area at each age: [(reference weight at each age/reference weight of 18- to 29-y-olds)$^{0.75}$×(1+growth factor)].

The EARs and DRIs for >50-y-olds were set at identical values to those for 18- to 49-y-olds, because of the lack of detailed information concerning the decrease in B_{12} absorption in elderly persons.

Life stages

0–5 mo. The mean concentration of B_{12} in breast milk is 0.45 μg/L (*5*, *6*, *47*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily B_{12} intake of 0.35 μg/d. The AI was rounded up to 0.4 μg/d.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 0.6 μg/d (rounded down from 0.61 μg/d).

Table 6. DRIs for folate (μg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL[2]	EAR	RDA	AI	UL[2]
0–5 mo	—	—	40	—	—	—	40	—
6–11 mo	—	—	65	—	—	—	65	—
1–2 y	80	100	—	300	80	100	—	300
3–5 y	90	110	—	400	90	110	—	400
6–7 y	110	140	—	600	110	140	—	600
8–9 y	130	160	—	700	130	160	—	700
10–11 y	160	190	—	900	160	190	—	900
12–14 y	200	240	—	1,200	200	240	—	1,200
15–17 y	200	240	—	1,300	200	240	—	1,300
18–29 y	200	240	—	1,300	200	240	—	1,300
30–49 y	200	240	—	1,400	200	240	—	1,400
50–69 y	200	240	—	1,400	200	240	—	1,400
≥70 y	200	240	—	1,300	200	240	—	1,300
Pregnant women (amount to be added)					+200	+240	—	—
Lactating women (amount to be added)					+80	+100	—	—

[1] Women planning pregnancy or possibly pregnant are advised to take 400 μg/d of supplemental pteroyl monoglutamate to reduce risks for fetal NTDs.
[2] ULs were estimated as pteroyl monoglutamates.

Pregnant women. Based on the liver B_{12} content of infants, the human fetus is estimated to accumulate 0.1–0.2 μg/d of B_{12} (*48*, *49*). Using the median (0.15 μg/d) of the fetal deposition and the 50% absorption rate for dietary B_{12} in healthy adults, the additional EAR for pregnant women becomes 0.3 μg/d. The additional RDA is calculated as 0.4 μg/d (rounded up from 0.36 μg/d) by multiplying the additional EAR by 1.2.

Lactating women. Using the average values for breast milk B_{12} concentration and secretion, and the 50% absorption rate for dietary B_{12} in healthy adults (0.45 μg/L×0.78 L/d÷0.5), the additional EAR for lactating women becomes 0.7 μg/d (rounded up from 0.702 μg/d). The additional RDA is calculated as 0.8 μg/d (rounded down from 0.84 μg/d) by multiplying the additional EAR by 1.2.

Tolerable upper intake level

Oral administration of substantial amounts (>500 μg) of B_{12} was shown to result in only about 1% absorption in the intestine (*50*). Even when a mega dose (2.5 mg) of B_{12} was administrated parenterally, no harmful effect of the excess intake was observed (*51*). Thus, in the present study, we did not determine the UL for B_{12}.

The DRIs for vitamin B_{12} are summarized in Table 5.

Folate

Background information

In its narrowest sense, folate is referred to as pteroylmonoglutamate. In broader terms, it includes coenzyme species in their reduced form, and also single-carbon compounds and their polyglutamate forms. The Standard Tables of Food Composition (*18*) list food folates, and also their DRIs, in their broader terms, as equivalents of pteroylmonoglutamate.

Cellular folate is mostly bound to enzyme proteins in their single-carbon polyglutamate coenzyme form. In comparison with monoglutamates, these polyglutamates readily lose their activities during heat processing (*52*). Most of the folate coenzymes are released through cooking and digestion by gastric acid. Following digestion by intestinal enzymes, they are converted to 5-methyltetrahydrofolate, and absorbed through the surface cells of the small intestine.

The relative bioavailability of food folate varies considerably (25–81%) (*53–55*). In a bioavailability study of wheat bread, the bioavailability was estimated to be 50% (*2*, *54*).

Determining DRIs

Evidence for determining the EAR

Red blood cell folate (≥300 nmol/L) and plasma total homocysteine (<14 μmol/L) concentrations were applied as biomarkers to reflect middle- to long-term folate nutritional status (*54*, *56–59*). The EAR for adults (18–49 y) was estimated as 200 μg/d. The RDA was calculated as 240 μg/d, by multiplying the EAR by 1.2. The EAR for children was calculated from the EAR for adults (200 μg/d), using the following equation for body surface area at each age: [(reference weight at each age/reference weight of 18- to 29-y-olds)$^{0.75}$×(1+growth factor)]. The values were rounded to the nearest 10 μg. For adults aged ≥50 y, folate bioavailability was estimated to be equivalent to that of younger adults (*60*),

and therefore the same values were applied.

Life stages

0–5 mo. The mean concentration of folate in breast milk is 54 μg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily folate intake of folate of about 40 μg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 65 μg/d.

Pregnant women. Macrocytic anemia in pregnancy recovers naturally after delivery (*61*), indicating a considerable increase in demand for folate during pregnancy. The addition of 100 μg/d of pteroylmonoglutamate to a diet adequate in food folate has been reported to result in adequate levels of red cell folate (*62, 63*). Thus, this value was set as the additional EAR (200 μg/d=100/bioavailability rate 0.5). The additional RDA was calculated by multiplying the additional EAR by 1.2.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. Thus, the additional EAR is calculated using the following formula: (breast milk consumption×breast milk content)÷folate bioavailability, which becomes (0.78 L×54 μg/L)÷0.5. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

In the United States, there have been reports of adverse health effects resulting from elevated serum folate, caused by intake of folic acid-supplemented foods (*64*). These adverse effects may be induced by dihydropteroylmonoglutamate derived from pteroylmonoglutamate, which inhibits the activities of thymidylate synthase, phosphoribosylaminoimidazolecarboxamide transformylase, and 5,10-methylenetetrahydrogenase (*65–67*). Thus, excess pteroylmonoglutamate may inhibit the single-carbon transfer pathways of folate metabolism.

In order to develop the upper limit of folate intake, we considered the US and Canadian DRIs. It has been reported that women of reproductive age who were given 0.36–5 mg/d of folic acid during preconception to 3-mo gestation suffered no serious side-effects (*68–74*). Based on this finding, the adverse effect level was estimated to be 5 mg/d, equivalent to 80 μg/kg body weight/d. The UL was estimated as 27 μg/kg body weight/d, by dividing by an uncertainty factor of 3.

Additional concerns regarding women of reproductive age

Fetal neural tube defects (NTDs) are disorders of the closure of the neural tube (which occurs approximately 28 d after conception), and are clinically diagnosed as anencephaly, spina bifida, and myelomeningocele. Abundant evidence suggests that preconceptual intake of pteroylmonoglutamate decreases fetal NTD risk (*68–74*). Genetic polymorphisms of enzymes related to folate metabolism (e.g., methylene tetrahydrofolate reductase) may be associated with NTD risk (*75–80*). Other congenital disorders that can be avoided by administering folic acid are cleft lip/palate (*81, 82*) and congenital heart disease (*83*). Thus, adequate maternal folate status is essential for the prevention of NTDs. In order to estimate the minimum effective dose for risk reduction of NTDs, the lowest reported preconception dose (0.36 mg/d) was applied. This value was rounded up to 0.4 mg/d (400 μg/d), i.e., a dietary folate equivalent of 800 μg/d.

Association between cardiovascular disease and folate

Higher folate intake is associated with decreased risk of strokes or heart disease. Several randomized controlled trials have investigated the preventive effect of folic acid, but with inconsistent results (*84–88*). Thus, we did not determine any specific values for modifying DRI values.

The DRIs for folate are summarized in Table 6.

Pantothenic acid

Background information

Pantothenic acid exists mainly as the coenzyme A (CoA) derivatives, acetyl CoA and acyl CoA. Additionally, some pantothenic acid, such as phosphopantetheine, binds to enzyme proteins in living cells. Most CoA and phosphopantetheine derivatives separate from proteins during cooking and processing of food, and also under the acidic conditions of the stomach. Free CoA and phosphopantetheine derivatives are digested to release pantothenic acid, which is absorbed in the intestine. The relative availability of dietary pantothenic acid to free pantothenic acid is approximately 70% in a typical Japanese diet (*1, 2*).

Determining DRIs

Evidence for determining the AI

There is no evidence for setting an EAR for pantothenic acid, because deficiency of this vitamin has not been reported to occur in humans. Thus, we estimated the AIs based on food surveillance data. According to the National Health and Nutrition Survey 2005 and 2006, (*89, 90*), the median dietary pantothenic acid intake for adults and adolescents is 3–7 mg/d. In another dietary assessment study, the mean pantothenic acid intake of young Japanese females was reported to be 4.6 mg/d (*91*). There is no evidence that such intake levels cause pantothenic acid deficiency. Thus, the AIs were set at the median dietary pantothenic acid intake determined in the National Health and Nutrition Survey Japan 2005 and 2006, corresponding to a subject's sex and age. The AIs for elderly subjects were set at the same median value, because there are no data indicating specific consideration for pantothenic acid nutrition in the elderly.

Life stages

0–5 mo. The mean pantothenic acid concentration in breast milk is 5.0 mg/L (*6, 47*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily pantothenic acid intake of 3.9 mg/d. The AI was rounded up to 4 mg/d.

6–11 mo. To set the AI for infants aged 6–11 mo,

Table 7. DRIs for pantothenic acid (mg/d).

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

the extrapolated values are calculated from the AI for infants aged 0–5 mo, using the weight ratio method. The average of the obtained values for each sex is 5.0 mg/d. Thus, the AI for infants aged 6–11 mo was set at 5 mg/d.

Pregnant women. There is no evidence for determining the amount of additional pantothenic acid for pregnant women by factorial method. Moreover, there is no indication that the pantothenic acid requirement increases with the increase in energy requirement during pregnancy. Thus, the pantothenic acid intake for pregnant women is estimated using the median of dietary pantothenic acid intake determined in the National Health and Nutrition Survey Japan 2005 and 2006 (*89*, *90*). The additional AI for pregnant women was set at 1 mg/d.

Lactating women. The additional water-soluble vitamin intake for lactating women is determined based on the assumption that the excreted amount in breast milk is supplemented, with adjustment according to relative bioavailability. However, for pantothenic acid, the estimated AIs are in excess of the pantothenic acid requirement. Thus, the pantothenic acid intakes for lactating and non-lactating women are estimated using the median dietary pantothenic acid intake determined in the National Health and Nutrition Survey Japan 2005 and 2006 (*89*, *90*). The additional AI for lactating women was set at 1 mg/d.

Tolerable upper intake level

A pharmacological dose of pantothenic acid, administered over a 3-mo period in combination with nicotinamide, ascorbic acid, and pyridoxine, was reported to cause adverse effects such as nausea, poor appetite, and abdominal pain in children (*92*). However, there are no reports that a pharmacological dose of pantothenic acid causes any adverse health effects. Thus, in the present study, no UL for pantothenic acid was set.

The DRIs for pantothenic acid are summarized in Table 7.

Biotin

Background information

Biotin is involved in gluconeogenesis, amino acid catabolism, and fatty acid synthesis. Biotin deficiency is known as "egg white injury," and is characterized by symptoms such as dermatitis, alopecia, and nervous irritability in humans and experimental animals. Biotin is also essential for reproduction. Maternal biotin deficiency during gestation results in congenital malformations such as cleft palate, micromelia, and micrognathia in mammalian fetuses.

Determining DRIs

Evidence for determining the AI

Biotin in foods exists not only in a free form, but also in a protein-bound form. Biotin generally binds to the lysine in proteins, and is converted to the free form during cooking and processing. In the digestive tract, intestinal hydrolysis of protein-bound biotin yields biotinyl oligopeptide and biocytin, which are cleaved to free biotin by biotinidase prior to absorption. Free biotin is mainly absorbed from the small intestine. There are no reports concerning the bioavailability of biotin in foods. However, the proportions of free biotin and protein-bound biotin are likely to vary substantially, even within food groups. The bioavailability of biotin in a typical Japanese meal is reported to be about 80% (*1*).

There are no data on which to base an EAR for adults. It has been reported that the average daily biotin intake for Americans is 35.5 μg. A number of studies have

Table 8. DRIs for biotin (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	4	—	—	—	4	—
6–11 mo	—	—	10	—	—	—	10	—
1–2 y	—	—	20	—	—	—	20	—
3–5 y	—	—	25	—	—	—	25	—
6–7 y	—	—	30	—	—	—	30	—
8–9 y	—	—	35	—	—	—	35	—
10–11 y	—	—	40	—	—	—	40	—
12–14 y	—	—	50	—	—	—	50	—
15–17 y	—	—	50	—	—	—	50	—
18–29 y	—	—	50	—	—	—	50	—
30–49 y	—	—	50	—	—	—	50	—
50–69 y	—	—	50	—	—	—	50	—
≥70 y	—	—	50	—	—	—	50	—
Pregnant women (amount to be added)					—	—	+2	—
Lactating women (amount to be added)					—	—	+5	—

determined the average daily biotin intake for Japanese as 45.1 μg, 60.7 μg, and 70.1 μg (*93–97*). Thus, the AI were set based on the average dietary biotin intake for adult males and females, i.e., 50 μg/d.

The AI for children is calculated from the AI for adults (50 μg/d), using the following equation: AI for 18- to 29-y-olds×(reference body weight for children/reference body weight for 18- to 29-y-olds)$^{0.75}$×(1+growth factor).

Few studies have investigated biotin requirements in the elderly. There are no data indicating that the biotin requirements of healthy subjects aged ≥70 y differ from those of young adults. Thus, the AI for subjects aged ≥70 y is the same as that for adults aged 18–29 y.

There were insufficient data to enable differences in requirements to be discerned between males and females of all age groups.

Life stages

0–5 mo. The mean biotin content of breast milk is 5 μg/L (*5, 6, 47, 98*). The average intake of milk is 0.78 L/d) (*7, 8*), representing a daily biotin intake of ~3.9 μg/d. The AI was rounded up to 4 μg/d.

6–11 mo. The AI for infants aged 6–11 mo is calculated from the average of values extrapolated from the AI for infants aged 0–5 mo and the AI for adults aged 18–29 y. This gives a value of 10.4 μg/d (14.9 μg/d for males and 16.6 μg/d for females). The AI was rounded down to 10 μg/d.

Pregnant women. Pregnant women have been demonstrated to exhibit reduced biotin concentration in the serum, and also reduced biotin excretion in the urine. By contrast, urinary excretion of organic acids such as 3-hydroxyisovaleric acid increases during late pregnancy (*99*). These findings indicate that pregnancy increases biotin requirements. However, there are no data on the additional amount required by pregnant women. Thus, the additional AI for pregnant women is calculated using the following formula: AI of biotin for infants aged 0–5 mo×average additional amount of energy for pregnant women/average additional amount of energy for male and female infants aged 0–5 mo. The additional AI for pregnant women was set at 2 μg/d.

Lactating women. The additional amount of biotin required during lactation should be calculated from the difference in biotin requirements for lactating and nonlactating women of a similar age. However, no such data are available. Thus, the increased requirement during lactation is based on the estimated biotin concentration in breast milk and the average milk secretion (0.78 L/d), adjusted by the bioavailability (*1*) (5 μg/L×0.78 L/d/0.8=4.875 μg/d). The additional AI for lactating women was set at 5 μg/d.

Tolerable upper intake level

There was insufficient evidence for determining the UL for healthy individuals. No adverse effects are associated with excess biotin intake, even in patients with biotin-responsive inborn errors of metabolism (*100*).

The DRIs for biotin are summarized in Table 8.

Vitamin C

Background information

Vitamin C refers to ascorbic acid and its oxidized form, dehydroascorbic acid, which exerts a biological effect through immediate reduction into ascorbic acid in the body (*101*). Severe vitamin C deficiency results in scurvy, which may be preventable by an ascorbic acid intake of 6–12 mg/d (*102*). Intake of a higher dose of vitamin C exerts an antioxidant effect, thereby helping

Table 9. DRIs for vitamin C (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	40	—	—	—	40	—
6–11 mo	—	—	40	—	—	—	40	—
1–2 y	35	40	—	—	35	40	—	—
3–5 y	40	45	—	—	40	45	—	—
6–7 y	45	55	—	—	45	55	—	—
8–9 y	55	65	—	—	55	65	—	—
10–11 y	65	80	—	—	65	80	—	—
12–14 y	85	100	—	—	85	100	—	—
15–17 y	85	100	—	—	85	100	—	—
18–29 y	85	100	—	—	85	100	—	—
30–49 y	85	100	—	—	85	100	—	—
50–69 y	85	100	—	—	85	100	—	—
≥70 y	85	100	—	—	85	100	—	—
Pregnant women (amount to be added)					+10	+10	—	—
Lactating women (amount to be added)					+40	+50	—	—

to prevent cardiovascular disease (*103*).

Ascorbic acid is readily absorbed by the intestine at a dose of <200 mg/d. Absorption is reduced at higher doses, and is <50% at a dose of >1 g/d (*104*). Vitamin C is reused within the body and excreted from the kidneys as unmetabolized ascorbic acid; the plasma is saturated at a dose of approximately 400 mg/d (*105*, *106*).

Determining DRIs

Evidence for determining the EAR

Optimal antioxidant activity in plasma, and prevention of cardiovascular disease, is achieved at a plasma ascorbic acid concentration of 50 μmol/L (*103*). This can be maintained by an ascorbic acid intake of approximately 85 mg/d (*107*), which is recognized as the EAR. The RDA is calculated by multiplying the EAR by 1.2, to give 100 mg/d. In a vitamin C depletion–repletion study, excretion of unmetabolized ascorbic acid into the urine was not detectable at an intake of 50–60 mg/d, but was detectable at an intake of 100 mg/d, where leukocyte vitamin C as an indicative of body store was saturated (*105*, *106*). This finding supports an RDA value of 100 mg/d. Levine et al. (*106*) did not consider differences in requirement according to sex.

Life stages

0–5 mo. The mean concentration of vitamin C in breast milk is 50 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily vitamin C intake of about 40 mg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 40 mg/d.

Pregnant women. The additional amounts are calculated based on the intake of vitamin C required to prevent infant scurvy. Thus, the additional EAR becomes 10 mg/d. The additional RDA is set by assuming a coefficient of variation of 10%.

Lactating women. The additional amounts are calculated based on the assumption that the excreted amount in breast milk is supplemented. The additional RDA is set by assuming a coefficient of variation of 10%.

Elderly. Vitamin C requirement appears to be higher in elderly subjects (aged 60–96 y old) than in younger subjects (aged 15–65 y old) (*107*). However, it is difficult to determine the required intake for the elderly subjects, because of insufficient data.

Tolerable upper intake level

Vitamin C is safe for healthy subjects, because excess intake results in a lower absorption rate from the intestine, and enhanced excretion in the urine following absorption (*105*, *106*, *108*). However, for patients with renal dysfunction, intake of several grams of vitamin C may increase the risk of kidney stones (*109*, *110*). Acute gastrointestinal intolerance was observed following excess intake; for example, intake of 3–4 g/d induced diarrhea (*111*). There are insufficient data with which to determine the UL. Absorption of vitamin C is saturated at high doses. By contrast, intake of ≥1 g/d from supplements is not advised (*102*, *105*, *106*).

Special consideration for smokers

There is evidence that smokers require more vita-

min C than do nonsmokers (*107*, *112*). This is also the case for passive smokers (*113*, *114*). Thus, smokers would require more vitamin C than nonsmokers, while they should recognize that smoking cessation is a basic countermeasure.

The DRIs for vitamin C are summarized in Table 9.

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