(2) Water-Soluble Vitamins

Vitamin B_1

1. Background Information

1—1. Definition and Classification

The chemical name of vitamin B_1 is thiamin, and the present DRIs for vitamin B_1 were set as the amount of thiamin hydrochloride; 2-[3-[(4-amino-2-methyl-pyrimidin-5-yl) methyl]-4-methyl]-thiazole-5-yl] ethanol. There are 3 types of thiamin hydrochloride: thiamin monophosphate (TMP), thiamin diphosphate (TDP), and thiamin triphosphate (TTP), and their activity is equimolar to that of vitamin B_1.

1—2. Function

Vitamin B_1 is involved in the metabolism of glucose and branched-chain amino acids. Insufficient vitamin B_1 intake can cause neuritides or brain damage. Beriberi and Wernicke-Korsakoff syndrome are well-known for being caused by vitamin B_1 deficiency.

1—3. Digestion, Absorption and Metabolism

Vitamin B_1 predominantly exists as TDP in living cells in combination with enzyme proteins. TDP is dissociated from proteins through cooking or digestion, and, thereafter, the released TDP undergoes phosphorylation to become thiamin. Thiamin absorption occurs through an active transportation system in the jejunum and ileum. These processes can be affected by the type of food, and other dietary sources. The relative bioavailability of free vitamin B_1 has been reported to be around 60% in the typical Japanese diet[1,2].

2. To Avoid Inadequacy

2—1. Factors to be Considered in Estimating Requirements

There should be a difference in the requirement estimation between the calculation using the dietary intake required for recovery from deficiency, and that using the association between dietary intake and urinary excretion of vitamin B_1.

2—1—1. Estimation of the Dietary Intake Required for Recovery from Deficiency

It was reported that recovery from vitamin B_1 deficiency (caused by the intake of meals with lower than 0.03 mg/day of vitamin B_1) occurred through an intake of 0.7 mg/day of thiamin hydrochloride, in male Japanese student volunteers[3]. Considering the relative bioavailability (60%), an intake of 1.17 mg/day of dietary vitamin B1 is the yield from 0.7 g/day of thiamin hydrochloride. The experimental meals were set at 2,400 kcal/day, and the requirement of dietary vitamin B_1 intake, in the form of thiamin hydrochloride, was considered to be lower than 0.49/1,000 kcal.

2—1—2. Estimation of Requirements from Urinary Thiamin Excretion

Orally administered thiamin is rapidly converted to TDP in the body tissues. Thereafter, excess thiamin is excreted in a free form in the urine. The values obtained through the calculation of excess thiamin may be higher than those required to prevent deficiencies.

The urinary excretion of thiamin sharply increases at an intake of 0.35 mg/1,000 kcal/day of vitamin B_1[(4)]. This value can be considered the body requirement, as the urinary excretion of thiamin increases sharply when the body's requirement is met.

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

These values were determined as amounts per energy intake.

2—2—1. Adults and Children (EAR and RDA)

The DRIs adopted the values obtained from the relation of the inflection points of vitamin B_1 intake and vitamin B_1 excretion. As it is a water-soluble vitamin, excess thiamin is excreted in its free form in the urine. Thus, the EAR of vitamin B_1 was determined as the point at which an increase in urine thiamin excretion is observed.

Since vitamin B_1 plays an important role in energy metabolism, these values were determined as amounts per energy intake. From the data of a meta-analysis of 18 countries[(4)], the EAR was set at 0.35 mg thiamin (0.45 mg thiamin hydrochloride)/1,000 kcal/day. This value was used as a reference for those aged 1 to 69 years, and the EAR was set using estimated energy requirement values. The RDA was set assuming a coefficient of variation of 20%. No report has stated that the calculation of the values for elderly individuals should be specially considered; thus, the EAR and RDA were determined using the reference value of adults and reference body weight (BW), assuming a coefficient of variation of 10%.

2—2—2. Additional Amount for Pregnant Women (EAR, RDA)

The additional amounts were calculated based on the assumption that the requirement of vitamin B_1 increases according to the energy requirement. In other words, the additional EAR and RDA for pregnant women were calculated by multiplying the additional estimated energy requirement values (+50 kcal/ day for early-term, +250 kcal/day for mid-term, and +450 kcal/day for late-term pregnancies, at a level 2 physical activity) and the vitamin B_1 EAR reference values (0.45 mg/1,000 kcal), to yield values of 0.023 mg/day for early-term, 0.11 mg/day for mid-term, and 0.20 mg/day for late-term pregnancies. These reference values were calculated solely assuming an increase in energy expenditure, and that energy expenditure differs between individuals. Since metabolism is enhanced during pregnancy, the value for late-term pregnancy (0.2 mg/day) was adopted as the additional amount required for pregnant women, yielding 0.2 mg/day (rounding 0.24 mg/day), determined as the EAR × 1.2.

2—2—3. Additional Amount for Lactating Women (EAR, RDA)

The additional amount was calculated based on the assumption that the excreted amount in breast milk is supplemented, using a relative availability of 0.6[1,2], as follows: **0.13 mg/L × 0.78 L/day / 0.6 = 0.169 mg/day.**

The EAR was set at 0.2 mg/day by rounding this value.

The additional RDA was determined as the EAR × 1.2, yielding 0.2 mg/day (rounding 0.24 mg/day).

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

2—3—1. Infants (AI)

The average concentration of vitamin B_1 in breast milk is 0.13 mg/L[5–7], and the average milk intake is 0.78 L/day[8,9], representing a daily vitamin B1 intake of about 0.1 mg/day. This value was set as the AI for infants aged 0 to 5 months.

The AI for infants aged 6 to 11 months was calculated using the average of the values from the following 2 expressions:

Expression 1: the AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the EAR for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1+ growth factor)

Thus, the AI was determined as 0.2 mg/day for infants aged 6 to 11 months.

3. To Avoid Excessive Intake

3—1. Dietary Intake

No regular food includes more than 1 mg of vitamin B_1/100 g. In addition, unfavorable outcomes, as a consequence of the excessive intake of regular food, have not been reported.

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

A chronic high dose intake of thiamin (50 mg/kg BW/day) has been reported to cause severe toxicity symptoms[10]. For example, an intake of 10 g of thiamin hydrochloride every day for 2.5 weeks resulted in headaches, irritability, insomnia, pulsus celer, weakness, contact dermatitis, and itchiness. These symptoms disappeared in 2 days, when the intake was discontinued[11]. Nevertheless, there is insufficient evidence for the determination of the UL.

Vitamin B_2

1. Background Information

1—1. Definition and Classification

The chemical name of vitamin B_2 is riboflavin, and the present DRIs were determined as the amount of riboflavin: 7,8-dimethyl-10-[(2R,3R,4S)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4(3H,10H)-dione. The activities of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) are equimolar to that of vitamin B_2.

1—2. Function

Vitamin B_2 functions as coenzymes FMN or FAD, and is involved in several metabolic pathways as well as energy production. Vitamin B_2 deficiency causes growth suppression, as the vitamin works in energy metabolism. Its deficiency also causes canker sores, angular cheilitis, tongue inflammation, and seborrheic dermatitis.

1—3. Digestion, Absorption and Metabolism

Riboflavin predominantly exists as FAD or FMN in combination with enzyme proteins. Riboflavin absorption occurs by an active transportation system in the small-intestinal epithelial cells. These processes can be affected by the type of food and other dietary sources. The relative bioavailability of free vitamin B_2 has been reported to be around 64% in the typical Japanese diet[(1)].

2. To Avoid Inadequacy

2—1. Factors to be Considered in Estimating Requirements

There should be a difference in the requirement estimation between the calculation using the dietary intake required for the recovery from deficiency, and that from the association between dietary intake and urinary excretion of vitamin B_2.

2—1—1. Estimation of the Dietary Intake Required for Recovery from Deficiency

An experimental study examined vitamin B_2 deficiency in 4 Japanese individuals (2 men and 2 women)[(12,13)]. Five to 6 weeks after a vitamin B_2-deficient diet was initiated, the participants complained of sore throat, tongue pain, and pain at the edge of the lips, bleeding from the gums and the oral mucosa, aversion to light, or eye strain[(13)]. The recovery experiment led to acute remission after 0.5 mg/day of vitamin B_2 was administered for 10 days[(12)]. One female participant consuming 1 mg/day of vitamin B_2 reported no complaint. From these results, the vitamin B_2 requirement for the prevention of deficiency can be estimated at about 0.5 mg/day. However, considering the relative bioavailability (64%)[(1)], this value was set as 0.78 mg/day of dietary vitamin B_2.

2—1—2. Estimation of Requirements from Urinary Riboflavin Excretion

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/day was shown to result in a rapid rise in urinary excretion. This value can be considered as the body requirement, as the urinary excretion of thiamin increases sharply when the body requirement is met.

2—2. Method Used to Set the EAR and RDA

These values were determined as amounts per energy intake.

2—2—1. Adults and Children (EAR and RDA)

In the determination of the DRIs for vitamin B_2, the method used was the same as that for vitamin B_1; the values were obtained from inflection point of the relation between vitamin B_2 intake and excretion. Thus, the EAR of vitamin B_2 was determined as the point at which an increase in urine thiamin excretion was observed. A gradual increase in the intake of free riboflavin to 1.1 mg/day was shown to result in a rapid rise in urinary excretion, in healthy men and women when they received 2,200 kcal/day[13,14]. Based on these results, and the involvement of vitamin B_2 in energy metabolism, the EAR was determined as the energy intake/day, i.e., 0.50 mg/1,000 kcal/day for those aged 1-69 years, and the EAR was set using estimated energy requirement values. The RDA was set using a coefficient of variation of 10%.

In terms of elderly individuals, one report stated that the requirement does not differ from that of young adults[15], and another stated that no special consideration is required. Therefore, the EAR and the RDA were determined using the reference value of adults and reference BW, using a coefficient of variation of 10%.

2—2—2. Additional Amount for Pregnant Women (EAR, RDA)

The additional amounts were calculated based on the assumption that the requirement for vitamin B_2 increases according to the estimated energy requirement. In other words, the additional EAR and RDA for pregnant women were calculated by multiplying the additional values of the estimated energy requirement (+50 kcal/ day for early-term, +250 kcal/day for mid-term, and +450 kcal/day for late-term pregnancies at level 2 of physical activity), and the vitamin B_2 EAR reference values (0.50 mg/1,000 kcal), yielding 0.03 mg/day for first-term, 0.13 mg/day for mid-term, and 0.23 mg/day for late-term pregnancies. These reference values were calculated solely assuming an increase in energy expenditure, and that energy expenditure differs between individuals. Since metabolism is enhanced during pregnancy, the value for late-term pregnancy (0.23 mg/day) was adopted as the additional amount required for pregnant women, yielding 0.3 mg/day (rounding 0.27 mg/day) as the additional RDA, determined as the EAR × 1.2.

2—2—3. Additional Amount for Lactating Women (EAR, RDA)

The additional amount was calculated based on the assumption that the excreted amount in breast milk is supplemented, using the relative bioavailability (0.6)[1,2]. The mean concentration of riboflavin in breast milk is 0.40 mg/L, and the average milk volume is 0.78 L/day[5,7–9]. Thus, the additional EAR was 0.5 (rounding 0.52) mg/day. The additional RDA was determined as the EAR × 1.2, yielding 0.6 (rounding 0.62) mg/day.

2—3. Method Used to Set AI

2—3—1. Infants (AI)

The daily vitamin B_2 intake of infants is approximately 0.3 (rounding 0.31) mg/day. This value was set as the AI for infants aged 0 to 5 months.

The AI for infants aged 6 to 11 months was calculated using the average of the values from the following 2 expressions:

Expression 1: the AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the EAR for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1+ growth factor)

Thus, the AI was determined to be 0.4 mg/day for infants aged 6 to 11 months.

3. To Avoid Excessive Intake

3—1. Dietary Intake

No regular food contains more than 1 mg of vitamin B_2/100 g. Additionally, no studies have reported the presence of unfavorable outcomes due to an excessive intake of regular foods.

3—2. Method Used to Set the UL

A chronic high intake of riboflavin has not been reported to cause severe toxicity. For example, a daily intake of 400 mg of riboflavin for 3 months[16], or a single intravenous injection of 11.6 mg of riboflavin[17] caused no deleterious effects. This may be attributed to the rapid excretion of riboflavin in the urine, and also to limited solubility and reduced absorption at higher doses. Thus, there is no evidence for the determination of the UL. Nevertheless, it has been reported that the maximum absorbable amount of riboflavin in a single dose is 27 mg[17]; therefore, a single intake of excess vitamin B_2 is rarely effective.

Niacin

1. Background Information

1—1. Definition and Classification

Niacin activity is predominantly exhibited by nicotinic acid, nicotinamide, and tryptophan. The DRIs for niacin are expressed in niacin equivalents (NEs). The Standard Tables of Food Composition in Japan 2010[18] lists niacin as the sum of nicotinic acid and nicotinamide, and does not include nicotinamide biosynthesized from tryptophan. Therefore, to calculate the NE in a diet, the amount of nicotinamide biosynthesized from dietary tryptophan should be added to the amount of niacin. The tryptophan to nicotinamide conversion ratio 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; therefore, the amount of nicotinamide biosynthesized from tryptophan (mg) is estimated as the amount of protein (g) divided by 6.

1—2. Function

Nicotinic acid and nicotinamide act as coenzymes for enzymes, such as alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, pyruvate dehydrogenase, and 2-oxoglutarate dehydrogenase, in oxidoreduction reactions, after the conversion of pyridine nucleotide. Niacin is involved in many biological reactions including ATP production, antioxidation via vitamin C or E, as well as fatty acid and steroid synthesis. Nicotinamide adenine dinucleotide (NAD^+) is used as a substrate of ADP-ribosylation, and is involved in the repair and synthesis of DNA, as well as cell differentiation. Niacin deficiency causes pellagra, in which dermatitis, diarrhea, and neuropsychiatric abnormality are prominent symptoms.

1—3. Digestion, Absorption, and Metabolism

In living cells, niacin exists mainly as the cofactor nicotinamide adenine dinucleotide phosphate (NAD(P)), which binds weakly to enzyme proteins. During the 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. Nicotinic acid predominantly binds to complex carbohydrates in cereal grains, and, therefore, has a lower digestibility[19]. The relative availability of dietary niacin to free nicotinamide is approximately 60% in a typical Japanese diet[1,2].

2. To Avoid Inadequacy

2—1. Factors to be Considered in Estimating Requirements

The conversion ratio of tryptophan to nicotinamide is set at 1/60, on a weight basis. In other words, 60 mg of tryptophan is equimolar to 1 mg of niacin.

2—2. Method Used to Set the EAR and RDA

Niacin relates to energy metabolism, and, therefore, the EAR for niacin is expressed as mg NE/1,000 kcal.

2—2—1. Adults and Children (EAR and RDA)

The requirement was determined from the minimal amount required for the prevention of pellagra. The conversion ratio of tryptophan to nicotinamide is set at 1/60 on a weight basis, according to human studies[20,21]. Niacin relates to energy metabolism, and, therefore, the EAR for niacin is expressed as amount per energy intake. Another human study showed that a urinary N^1-methylnicotinamide level of 1.0 mg/day reflects pellagra-like clinical niacin deficiency[22]. An analysis of previous studies showed that the niacin intake equivalent to a urinary N^1-methylnicotinamide level of 1.0 mg/day is 4.8 mg NE/1,000 kcal[20–24]. This value was used as the reference for the setting of the EAR for individuals aged 1 to 69 years, and the EAR was determined using estimated energy requirement values. The RDA was determined as the EAR × 1.2. Based on niacin intake and urinary nicotinamide metabolite data, the niacin activity in older individuals is considered to be the same as that in younger individuals[25,26]. Thus, the EAR and RDA were set using the same calculation method as that used in adults.

2—2—2. Additional Amount for Pregnant Women (EAR, RDA)

There is no evidence for the setting of the EAR using a factorial method, and the additional amounts could be set based on the assumption that the requirement for niacin increases according to the estimated energy requirement; however, the amount of nicotinamide biosynthesized from tryptophan increases during pregnancy, and this compensates for the increase in the niacin requirement[27]. Thus, pregnant women do not require additional niacin intake.

2—2—3. Additional Amount for Lactating Women

The conversion rate of tryptophan to nicotinamide returns to a normal level after delivery[27], and, therefore, lactating women require additional niacin intake to compensate for the loss of niacin through breast milk. Using 2.0 mg/L as the concentration of breast milk, 0.78 L/day as the average milk volume, and 60% as the relative availability[1,2], the additional EAR for lactating women was set at 3 mg NE/day (rounding 2.6 mg NE/day). The additional RDA was set at 3 (rounding 3.0) mg NE/day, determined as the EAR × 1.2.

2—3. Method Used to Set the AI

2—3—1. Infants (AI)

The concentration of niacin in the breast milk of Japanese mothers is 2.0 mg/L[5–7]. Considering an average milk intake of 0.78 L/day[6,7], the daily nicotinamide intake is 1.56 mg/day. The AI for infants aged 0 to 5 months was set at 2 mg/day. Nicotinamide is unlikely to be biosynthesized from tryptophan at this stage, and, therefore, the AI is expressed in mg/day[28].

The AI for infants aged 6 to 11 months was calculated using the average of the values from the following 2 expressions:

Expression 1: the AI for infant boys or girls aged 6 to 11 months (extrapolated from the AI of infants) = AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the EAR for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor)

The means of these extrapolated values were determined for each sex. The average of the obtained values for each sex is 3.1 mg NE/day. Thus, the AI for infants aged 6 to 11 months was set as 3 mg NE/day.

3. To Avoid Excessive Intake

3—1. Dietary Intake

A high amount of nicotinamide is present in animal food, at a maximum of approximately 10 mg/100 g. Nicotinic acid exists in plant food at less than 10 mg/100 g. No studies have reported the presence of unfavorable outcomes due to an excessive intake of regular food.

3—2. Method Used to Set the UL

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 obtained from supplements and fortified foods. The large doses of nicotinamide and nicotinic acid used to treat patients with type 1 diabetes and dyslipidemia, respectively, may cause gastrointestinal effects such as dyspepsia, diarrhea, and constipation, and also hepatotoxic symptoms such as dysfunction and fulminant hepatitis. According to previous reports[29–32] the no observed adverse effect levels (NOAELs) for nicotinamide and nicotinic acid were set at 25 mg/kg BW and 6.25 mg/kg BW, respectively. The NOAELs were divided by an uncertainty factor of 5, and the obtained values--5 mg/kg BW and 1.25 mg/kg BW--were set as the ULs for nicotinamide and nicotinic acid, respectively. The ULs were determined using these values, according to age and sex group. A pharmacological dose of nicotinic acid has the transient vasodilatory effect of flushing (reddening of the skin), but does not cause adverse health effects. Thus, it is not appropriate to use flushing as symptom for the setting of the UL for nicotinic acid.

Vitamin B_6

1. Background Information

1—1. Definition and Classification

The chemical substances possessing vitamin B_6 activity are pyridoxine (PN), pyridoxal (PL), and pyridoxamine (PM), and their respective phosphorylated forms. The phosphorylated forms--pyridoxine-5-phosphate (PNP), pyridoxal-5-phosphate (PLP) and pyridoxamine 5-phosphate (PMP)--have an activity that is equimolar to that of vitamin B_6. The current DRIs were determined as the amount of pyridoxine.

1—2. Function

Vitamin B_6 functions as the coenzyme PLP, and is involved in transamination reaction, decarboxylation, and racemization reaction. Vitamin B_6 is important for the maintenance of immune systems, as vitamin B_6 deficiency decreases the rate of the conversion of linoleic acid to arachidonic acid. Deficiency causes pellagra-like symptoms, seborrheic dermatitis, tongue inflammation, angular cheilitis, or hypolymphemia.

1—3. Digestion, Absorption and Metabolism

Vitamin B_6 predominantly exists as PLP or PMP, in combination with enzyme proteins. Once PLP and PMP dissociate, they are absorbed as PL or PM. Living plant cells contain pyridoxine-5'β-glucoside (PNG). PNG is absorbed as PN in humans, and the relative bioavailability of PNG has been estimated to be 50%[33]. The digestion processes associated with this vitamin are affected by the type of food and other dietary sources. The relative bioavailability of free vitamin B_6 has been reported to be 75% in the US[34], and 73% in the typical Japanese diet[1].

2. To Avoid Inadequacy

2—1. Method Used to Set the EAR and RDA

These values were determined as amounts per protein intake.

2—1—1. Adults and Children (EAR and RDA)

Vitamin B_6 is involved in the catabolism of amino acids and formation of bioactive amines, including some neurotransmitters. The plasma PLP concentration has been reported to reflect the body store of vitamin B_6[35]. A low plasma PLP concentration is associated with electroencephalographic changes in young, non-pregnant women[36]. Furthermore, a plasma PLP concentration of 30 nmol/L was required to alleviate vitamin B_6 deficiency-induced disorders[37]. The EAR for vitamin B_6 was based on the amount of vitamin B_6 required for a maintenance of 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 B_6 intake per protein intake[38]. Thus, 0.014 mg pyridoxine/g protein was estimated as the concentration required to maintain a plasma PLP concentration of 30 nmol/L. The EAR reference value was determined (0.014/0.73) using a relative bioavailability of 73%[1]. The EARs were calculated by multiplying this value by the RDAs of protein. The RDA was calculated as the EAR × 1.2. To obtain the daily requirement of vitamin B_6, the EAR of vitamin B_6 was multiplied by the RDA of protein.

In terms of elderly individuals, a previous report stated that plasma PLP levels decrease with increasing age[39]; however, due to a lack of data, the EAR and the RDA were determined using the same method as that used in adults.

2—1—2. Additional Amount for Pregnant Women (EAR, RDA)

The plasma PLP concentration reportedly decreases during pregnancy[40–52].

The additional amount required depends on whether plasma PLP should be maintained at levels that are similar to those in non-pregnant women or those in the first-stage of pregnancy, or taking into consideration if the aforementioned decrease in the PLP concentration occurs as a common physiological response to pregnancy.

The previous DRIs adopted the former method, and set the additional amounts of EAR and RDA for pregnant women using the method used in the US-Canada DRIs[38], and the relative bioavailability in Japan[1]. However, while no study has reported on vitamin B_6 deficiency in this context, the vitamin B_6 intake of pregnant Japanese pregnant women does not exceed the previous EAR of 1.7 mg/day. Therefore, the additional amount of vitamin B_6 required was reconsidered.

Although vitamin B_6 involves the production of tryptophan metabolites, the proportion of some of these metabolites increases, rather than decreases, under conditions of vitamin B_6 insufficiency. Moreover, their effects on pregnant women and fetuses remain unclear.

The decrease in the plasma PLP level during the late term of pregnancy is considered to be caused by the increased requirement of the fetus[49]. This decrease is considered a result of increased placental PL transportation due to the elevated PLP→PL reaction rate that is caused by elevated serum alkaline phosphatase levels in mothers, so as to provide vitamin B_6 to the fetus[42,47,51,52].

During the late stage of pregnancy, to maintain 30 nmol/L of plasma PLP (at a level which is equal to that of women who are not pregnant), an additional 4 to 10 mg/day of pyridoxine is needed[40][47][52][44]. However, these amounts are quite different from the potential intakes for the Japanese population, based on the current intake. Lui et al. recommended maintaining a plasma PLP level of 20 nmol/L to prevent vitamin B_6 deficiency[35]. Abnormal electroencephalograms have been observed at plasma PLP levels lower than 10 nmol/L in non-pregnant women[36]. Another study examined pregnant Japanese women, and reported their mid-term and late-term pregnancy plasma PLP levels (mean±standard deviation) to be 23.3 ± 16.7 nmol/L and 18.3 ± 12.5 nmol/L, respectively[53]. Thus, the additional amount of vitamin

B_6 required would be low, considering the enhanced vitamin B_6 metabolism during pregnancy.

However, the requirement for protein increases according to the body protein storage required during pregnancy, enhancing amino acid metabolism.

From these findings, the additional EAR was determined considering the body protein storage for the placenta and fetus. In other words, the value was calculated based on the EAR reference of pyridoxine for non-pregnant women (0.014 mg/ protein g) and body protein storage during pregnancy, using the relative bioavailability. During pregnancy, the efficiency of various nutrients increases; however, due to a lack of data, the relative bioavailability was set as 73%[(1)]. The additional EAR was calculated as follows:

Early-term pregnancy

(0.014 mg/g protein × 0 g/day = 0 mg/day) / 0.73 = 0 mg/day

Mid-term pregnancy

(0.014 mg/g protein × 1.94 g/day = 0.027 mg/day) / 0.73 = 0.037 mg/day

Late-term pregnancy

(0.014 mg/g protein × 8.16 g/day = 0.114 mg/day) / 0.73 = 0.156 mg/day

The RDAs were determined as these values × 1.2, yielding 0 mg, 0.044 mg and 0.187 mg for early-term, mid-term, and late-term pregnancies, respectively.

These values were calculated solely assuming an increase in the amount of protein required, and that requirement differs between individuals. Since metabolism is enhanced during pregnancy, the value for late-term pregnancy (0.156 mg/day) was adopted as the additional amount required by pregnant women, yielding 0.2 mg/day (rounding 0.156 mg/day). The additional RDA was calculated as the additional EAR × 1.2, yielding 0.2 mg/day (rounding 0.187 mg/day).

2—1—3. Additional Amount for Lactating Women

The additional EAR for pregnant women was calculated based on the mean concentration of vitamin B_6 in breast milk (0.25 mg/L)[(54,55)], the average milk volume (0.78 L/day)[(8,9)], and the relative bioavailability (73%)[(1)], i.e., 0.3 mg/day (rounding 0.267 mg/day). The additional RDA was calculated as the additional EAR × 1.2.

2—2. Method Used to Set AI

2—2—1. Infants (AI)

For infants aged 0 to 5 months, the vitamin B_6 intake is approximately 0.2 mg/day (rounding 0.195 mg/day) based on the mean concentration of vitamin B_6 in breast milk (0.25 mg/L)[(54,55)] and the average milk intake (0.78 L/day)[(8,9)]. This value was set as the AI.

The AI for infants aged 6 to 11 months was calculated using the average of the values from the following 2 expressions:

Expression 1: the AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the EAR for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor)
Thus, the AI was determined to be 0.3 mg/day for infants aged 6 to 11 months.

3. To Avoid Excessive Intake

3—1. Dietary Intake

No regular food contains more than 1 mg of vitamin B_6/100 g. No reports have suggested the development of unfavorable outcomes due to the excessive intake of regular food.

3—2. Method Used to Set the UL

A high intake of pyridoxine over a course of several months was shown to result in sensory neuropathy[56]. This symptom was used as a criterion for the estimation of the UL for pyridoxine. In contrast, the administration of 100-300 mg pyridoxine/day over a period of 4 months did not cause sensory neuropathy in 24 patients with carpal tunnel syndrome[57]. Based on these data, the NOAEL was set at 300 mg/day. Assuming an uncertainty factor of 5, the UL for pyridoxine was set at 60 mg/day-0.86 mg/kg BW. The UL for each age group was obtained by multiplying the UL by the reference BW.

Vitamin B_{12}

1. Background Information

1—1. Definition and Classification

Vitamin B_{12} is a cobamide, and there are various B_{12} compounds with different upper ligands, such as methylcobalamin, sulfitecobalamin, and cyanocobalamin. The DRIs for vitamin B_{12} were set as the amount of cyanocobalamin.

1—2. Function

Vitamin B_{12} is a cofactor for methionine synthetase and L-methylmalonyl-coenzyme A mutase. Vitamin B_{12} deficiency causes megaloblastic anemia, white-matter deficit in the spinal cord and brain, and peripheral neuropathy.

1—3. Digestion, Absorption and Metabolism

Food-bound vitamin B_{12} is dissociated from proteins in the presence of acid and pepsin. The released vitamin B_{12} then binds to the haptocorrins secreted by the salivary glands. Haptocorrins are partially degraded in the duodenum, releasing vitamin $B_{12,}$ which then binds with intrinsic factor. The intrinsic factor-vitamin B_{12} complex enters the enterocyte after binding with the receptors in the ileal mucosa. The dietary absorption was reported to be around 50% in healthy participants[58,59].

2. To Avoid Inadequacy

2—1. Factors to be Considered in Estimating Requirements

The DRIs considered the values required for the treatment of anemia in pernicious anemia patients without intrinsic factors.

2—2. Method Used to Set the EAR and RDA

2—2—1. Adults and Children (EAR and RDA)

It is not possible to determine the EAR of vitamin B_{12} for healthy adults, because of the intrinsic-factor-mediated B_{12} gastrointestinal absorption system and/or the substantial enterohepatic vitamin B_{12} circulation. Thus, the EAR for adults was estimated based on clinical data from vitamin B_{12}-deficient patients with pernicious anemia, that examined the amount of vitamin B_{12} required for the maintenance of an adequate hematological status (mean corpuscular volume < 101 fL) and serum vitamin B_{12} level (100 pmol/L or more). Studies reported that an intramuscular injection with varying concentrations (0.1–10 μg/day) of vitamin B12 showed an increase in the capacity of erythrocyte production at 0.1 μg/day[44], indicating the maximum capacity at 0.5 to 1.0 μg/day[60]. Another study reported that an improvement in the mean corpuscular volume was observed at 1.4 μg/day (range 0.5 to 4.0 μg/day) of vitamin B_2 injection in half of the patients with pernicious anemia[61]. These data suggest an average

intramuscular requirement of 1.5 mg/day for the maintenance of an adequate hematological status.

Vitamin B_{12}-deficient patients with pernicious anemia cannot reabsorb vitamin B_{12} (0.5 μg/day) from the bile, due to the lack of an intrinsic-factor-mediated vitamin B_{12} absorption system. Thus, under normal physiological conditions, an average intake of 1.0 μg/d is required to compensate for the estimated extra losses of biliary vitamin B_{12} (0.5 μg/day) from the average intramuscular requirement (1.5 μg/day). Adjusting for this value with a 50% absorption rate of dietary vitamin B_{12}, the EAR was set at 2.0 μg/day for adults. The RDA was calculated as 2.4 mg/day, by multiplying the EAR and 1.2.

Although serum vitamin B_{12} levels are known to be higher in women than men[62–64], data on this are insufficient. Therefore, the same values were adopted for both sexes.

The EAR for children was calculated from the EAR for adults aged 18 to 29 years, using the following equation for body surface area, at each age:

(Reference BW at each age/reference BW of adults aged 18 to 29 years) 0.75 × (1 + growth factor).

The EARs and DRIs for those aged over 50 years were set at values that were identical to those set for adults aged 18 to 49 years, due to a lack of detailed information on the decrease in vitamin B_{12} absorption in elderly individuals[65,66].

2—2—2. Additional Amount for Pregnant Women (EAR, RDA)

The human fetus is estimated to accumulate 0.1 to 0.2 μg/day of vitamin B_{12}[67,68]. Using the median (0.15 μg/day) of the fetal deposition, and the 50% absorption rate of dietary vitamin B_{12} in healthy adults, the additional EAR for pregnant women was set at 0.3 μg/day. The additional RDA was estimated as 0.4 μg/day (rounding 0.36 μg/d) by multiplying the additional EAR and 1.2.

2—2—3. Additional Amount for Lactating Women

Using the average vitamin B_{12} concentration and secretion of breast milk, and the 50% absorption rate of dietary vitamin B_{12} in healthy adults (0.45 μg/L × 0.78 L/day/0.5), the additional EAR for lactating women was set at 0.7 μg/day (rounding 0.702 mg/day). The additional RDA was calculated as 0.8 μg/day (rounding 0.84 μg/d) by multiplying the additional EAR and 1.2.

2—3. Method Used to Set AI

2—3—1. Infants (AI)

For infants aged 0 to 5 months, the mean concentration of vitamin B_{12} in breast milk is 0.45 μg/L[6,7,69]. The average milk volume is 0.78 L/d[8,9], representing a daily vitamin B_{12} intake of about 0.4 μg/day (rounding 0.35 μg/day). This value was set as the AI.

The AI for infants aged 6 to 11 months was calculated using the average of the values

from the following 2 expressions:

Expression 1: the AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the EAR for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor)

Thus, the AI was determined to be 0.5 μg/day for infants aged 6 to 11 months.

3. To Avoid Excessive Intake

3—1. Dietary Intake

Vitamin B_{12} absorption is regulated by the intrinsic factor secreted by the stomach in the intestinal absorption system. No reports till date have suggested the presence of unfavorable outcomes due to the excessive intake of regular foods.

3—2. Method Used to Set the UL

Vitamin B_{12} cannot be absorbed when its intake is excessive, and the intrinsic-factor-regulating absorption system is saturated[61,70]. The oral administration of substantial amounts (500 μg) of vitamin B_{12} was shown to result in only about 1% absorption in the intestine[61]. No harmful effect was observed even when a mega dose (2.5 mg) of vitamin B_{12} was administrated parenterally[71]. Thus, the UL was not determined for vitamin B_{12}.

Folate

1. Background Information

1—1. Definition and Classification

The basic skeleton of folate is pteroylmonoglutamate, which comprises p-aminobenzoic acid with pterin rings and glutamate. Folate naturally occurs in combination with 1 or more molecules of glutamate (γ-binding).

In its narrowest sense, folate is referred to as pteroylmonoglutamate. In a broader sense, it includes coenzyme species in their reduced form, as well as single-carbon compounds and their polyglutamate forms. The present DRIs used the broader definition, as equivalents of pteroylmonoglutamate, in accordance with The Standard Tables of Food Composition 2010.

1—2. Function

Folate functions as a coenzyme in single-carbon transfers, in the metabolism of nucleic and amino acids. Folate deficiency causes megaloblastic anemia. Folate deficiency in mothers can lead to fetal neural tube defects (NTDs) and anencephalia.

1—3. Digestion, Absorption and Metabolism

Most naturally occurring folates (food folates) are pteroylpolyglutamates, the activities of which are more easily lost during cooking than those of pteroylmonoglutamates--the form used in vitamin supplements. Pteroylpolyglutamates are hydrolyzed to monoglutamate forms in the gut before absorption across the intestinal mucosa. The digestion processes can be affected by the type of food and other dietary sources. The relative bioavailability of food folate is reported to be 25-81% that of pteroylmonoglutamate[72–74]. The relative bioavailability of free-pteroylmonoglutamate is reported to be 50% in the typical Japanese diet[2].

2. To Avoid Inadequacy

2—1. Factors to be Considered in Estimating Requirements

The relative bioavailability of dietary folate depends on its food sources, and is influenced by other dietary intakes. Naturally occurring folates include various reduced forms, which are a combination of polyglutamate chains and 1 carbon fragment. Polyglutamate is hydrolyzed by conjugase in the jejunum, and is converted to monoglutamate. This is then actively absorbed by specific transporters, and is present in the mucosal cell in its monoglutamate form. Conjugase is an enzyme that comprises zinc as a prosthetic group. It is well-known that orange juice and banana contain the conjugase activity inhibitor[75].

2—2. Method Used to Set the EAR and RDA

2—2—1. Adults and Children (EAR and RDA)

The concentrations of red blood cell folate (300 nmol/L) and plasma total homocysteine (14 μmol/L) were applied as biomarkers to reflect the middle- to long-term folate nutritional status[76–79]. The EAR for adults aged 18 to 29 years old was estimated as 200 μg/day. The RDA was calculated as 240 μg/day, by multiplying the EAR and 1.2. The lower value was adopted if the values for the men and women, in each group, were different.

The EAR for children was calculated from the EAR for adults (200 μg/day), using the following equation for body surface area, in each age group:
(Reference BW at each age/reference BW of those aged 18 to 29 years) 0.75 × (1 + growth factor).

For adults aged over 50 years old, folate bioavailability was estimated to be equivalent to that of younger adults[79]; therefore, the same value as that of adults aged 18 to 29 years old was adopted.

2—2—2. Additional Amount for Pregnant Women (EAR, RDA)

Women with macrocytic anemia during pregnancy recover naturally after delivery[78], indicating a considerable increase in the demand for folate during pregnancy. The addition of 100 μg/day of pteroylmonoglutamate to a diet adequate in food folate has been reported to result in adequate levels of red cell folate[80,81]. Thus, this value was set as the additional EAR (200 μg/day; 100/bioavailability rate 0.5[2,72]). The additional RDA was calculated by multiplying the additional EAR and 1.2, yielding 240 μg/day.

2—2—3. Additional Amount for Lactating Women

The additional EAR for pregnant women was calculated based on the mean concentration of folate in breast milk (54 μg/L[5–7]), the average milk volume (0.78 L/day)[8,9], and the relative bioavailability (50%)[2,72], yielding 80 μg/day (rounding 84 μg/day). The additional RDA was calculated by multiplying the additional EAR and 1.2, yielding 100 μg/day.

2—3. Method Used to Set AI

2—3—1. Infants (AI)

For infants aged 0 to 5 months, the mean concentration of vitamin folate in breast milk is 54 μg/L[5–7]. The average milk intake is 0.78 L/day[8,9], representing a daily folate intake of 40 μg/day (rounding 42 μg/day). This value was set as the AI.

The AI for infants aged 6 to 11 months was calculated using the average of the values from the following 2 expressions:

Expression 1: AI for infants aged 6 to 11 months (extrapolated from the AI for infants) = AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the EAR for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor)
Thus, the AI was determined to be 60 μg/day for infants aged 6 to 11 months.

3. To Avoid Excessive Intake

3—1. Dietary Intake

No regular food contains more than 300 μg of folate/100 g except for liver. No study till date has reported the presence of unfavorable outcomes due to an excessive intake of regular food.

3—2. Method Used to Set the Tolerable Upper Intake level

In the United States (US), adverse health effects such as the masking of pernicious anemia and neurological damage resulting from elevated serum folate levels, caused by the intake of folic acid-supplemented foods, have been reported[82]. This masking of pernicious anemia[83–86] is the biggest factor involved in the setting of the UL for folate intake. When individuals with insufficient vitamin B_{12} levels consume large amounts of pteroylmonoglutamate, the development of pernicious anemia is masked; in addition, it also leads to the progression of more severe disease as well as posterior spinal degeneration[83–86].

These adverse effects may be induced by the dihydropteroylmonoglutamate derived from pteroylmonoglutamate, which inhibits the activities of thymidylate synthase[87], phosphoribosylaminoimidazolecarboxamide transformylase[88], and 5,10-methylenetetrahydrogenase[89]. Thus, consuming excessive amounts of pteroylmonoglutamate may inhibit the single-carbon transfer pathways of folate metabolism.

Pteroylmonoglutamate intake is now common, as folate supplementation is recommended before or in the early term of pregnancy, for the prevention of NTDs. However, while supplementation may prevent NTDs, unfavorable outcomes (neurological damage) have been reported. Therefore, the UL for pteroylmonoglutamate should be determined.

The UL of folate intake was determined according to the US-Canada DRIs[90]. Women of reproductive age who were administered 0.36-5 mg/day of pteroylmonoglutamate from the preconception period till the gestational age of 3 months had no serious side effects[90]. Based on this finding, the adverse effect level was estimated to be 5 mg/day, equivalent to 88 mg/kg BW/day using the reference BW of women aged 19 to 30 years[91]. The UL reference was estimated as 18 μg/kg BW/day, by dividing the value by an uncertainty factor of 5. The UL was determined using the reference value and reference BW in each age group. Related studies on this topic have been limited to those on women; therefore, the UL for men was the same as that for women.

3—3. Additional Concerns regarding Women of Reproductive Age

Fetal NTDs are disorders pertaining to the closure of the neural tube (which occurs approximately 28 days after conception), and are clinically diagnosed as anencephaly, spina bifida, and myelomeningocele. Abundant evidence suggests that the preconceptual intake of pteroylmonoglutamate decreases the risk of fetal NTDs[92–102]. Genetic polymorphisms of the enzymes related to folate metabolism (e.g., methylene tetrahydrofolate reductase) may be associated with NTD risk[92–102].

Other congenital disorders that can be avoided through the administration of pteroylmonoglutamate are cleft lip/palate[103,104] and congenital heart disease[104]. Thus, maintaining an adequate maternal folate status is essential for the prevention of NTDs. To estimate the minimum effective dose for the risk reduction of NTDs, the lowest reported preconception dose (0.36 mg/day; at 0.36 to 5 mg/day for over 3 months[92–102]) was applied. This value was rounded to 0.4 mg/day, i.e., a dietary folate equivalent of 800 mg/day.

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

4—1. The Association with LRDs

4—1—1. Prevention of Disease Development

4—1—1—1. The Association between Plasma Homocysteine Levels and Cardiovascular or Cerebrovascular Diseases

Higher folate intakes have been reported to be associated with a decreased risk of stroke or heart disease[105,106]. Several randomized controlled trials have investigated the preventive effect of folic acid, but the results are inconsistent[107,108]. Inconsistencies in the intervention and observation, and the results of each of those studies must be further studied. The amount of vitamin consumed exceeded the possible dietary intake in the intervention studies; in addition, other types of vitamin B or various polyphenols may have influenced the results of the observational studies.

4—1—1—2. Association between Folate Intake and Cancer

Previous epidemiological studies have shown that the intake of pteroylmonoglutamate during pregnancy protects against the development of NTDs; however, the risk of cancer is considered to increase with intake. A meta-analysis of approximately 50,000 individuals showed that the risk neither increased nor decreased with long-term pteroylmonoglutamate supplementation[109].

4—1—2. Prevention of Disease Progression

No data were available in this regard.

4—2. Tentative Dietary Goal for Preventing LRDs

The DG was not determined due to a lack of data.

Pantothenic acid

1. Background Information

1—1. Definition and Classification

Pantothenic acid exists mainly as coenzyme A (CoA) derivatives, acetyl CoA, acyl CoA, acyl-carrier protein (ACP) and 4-phosphopantetheine, the activities of which are equimolar to that of pantothenic acid. The present DRIs were determined as the amount of pantothenic acid.

1—2. Function

Pantothenic acid functions as a component of CoA and phosphopantetheine, which are involved in carbohydrate and fatty acid metabolism. Pantothenic acid is widely distributed in foods, and cases of deficiency are rare.

1—3. Digestion, Absorption and Metabolism

CoA in the diet is hydrolyzed in the intestinal lumen to dephospho-CoA and pantetheine, and these are hydrolyzed to pantothenic acid in its absorbable form. The digestion of this vitamin is affected by the type of food, and other dietary sources. The relative bioavailability of pantothenic acid is reported as 70% in the typical Japanese diet[1,2].

2. To Avoid Inadequacy

2—1. Factors to be Considered in Estimating Requirements

Pantothenic acid is involved in fatty acid metabolism.

2—2. Method Used to Set AI

2—2—1. Adults and Children (AI)

There is no evidence for the setting of the EAR for pantothenic acid, as there are no reports on this vitamin's deficiency in humans. Thus, we estimated the AIs based on the Japanese intake. According to the National Health and Nutrition Survey (NHNS) 2010 and 2011[110], the median dietary pantothenic acid intake among adults and adolescents is 3-7 mg/day. In another dietary assessment study, the mean pantothenic acid intake was reported to be 4.6 mg/day in young Japanese women[111]. A study on Japanese individuals aged 32-76 years reported that the mean intakes were 7 mg/day and 6 mg/day in the men and women, respectively[112]. There is no evidence that these intake levels lead to pantothenic acid deficiency. Thus, the AIs were adopted from the median dietary pantothenic acid intake determined in the NHNS 2010 and 2011, corresponding to participants' sex and age. The AIs for elderly individuals were set as the same median value, as there are no data indicating the need for special consideration in terms of pantothenic acid nutrition in this population.

2—2—2. Infants (AI)

For infants aged 0 to 5 months, the mean concentration of pantothenic acid in breast milk is 5.0 mg/L[5,7]. The average milk volume is 0.78 L/day[8,9], representing a daily pantothenic acid intake of 4.0 mg/day (rounding 3.9 mg/day). This value was set as the AI.

The AI for infants aged 6 to 11 months was calculated using the average of the values from the following 2 expressions:

Expression 1: the AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the AI for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor)

Thus, the AI was determined to be 3 mg/day for infants aged 6 to 11 months.

2—2—3. Pregnant Women (AI)

There is no evidence for the determination of the additional pantothenic acid amount for pregnant women by the factorial method. Moreover, there is no indication that the pantothenic acid requirement rises with increases in the energy requirement during pregnancy. Thus, the pantothenic acid intake for pregnant women was estimated using the median of the dietary pantothenic acid intake determined in the NHNS 2010 and 2011[113]. The AI for pregnant women was set at 5 mg/day.

2—2—4. Lactating Women (AI)

For pantothenic acid, the estimated AIs are in excess of the pantothenic acid requirement. Thus, the pantothenic acid intakes for lactating women are estimated using the median dietary pantothenic acid intake determined in the NHNS 2010 and 2011[113]. The AI for lactating women was set at 5 mg/day.

3. To Avoid Excessive Intake

3—1. Dietary Intake

No regular food contains more than 5 mg of pantothenic acid/100 g except for liver. No study till date has reported unfavorable outcomes due to the excessive intake of regular food.

3—2. Method Used to Set the UL

A pharmacological dose of pantothenic acid, administered over 3 months, in combination with nicotinamide, ascorbic acid, and pyridoxine, was reported to cause adverse effects such as nausea, poor appetite, and abdominal pain in children[114]. However, there are no reports stating that a pharmacological dose of pantothenic acid causes adverse health effects. Thus, the UL for pantothenic acid was not set at present.

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

4—1. The Association with LRDs

No data were available in this context. The DG was not determined due to a lack of data.

Biotin

1. Background Information

1—1. Definition and Classification

Biotin is a compound formally known as 5-[(3aS, 4S, 6aR)-2-oxysohexysahydro-1H-cheno[3, 4d-]imidazole-4-yl] pentatonic acid, and only its d-isomer shows physiological activity. The present DRIs were determined as the amount of biotin.

1—2. Function

Biotin functions as a coenzyme in bicarbonate-dependent carboxylation reactions. Biotin deficiency can cause immune deficiency disorders such as rheumatism, Sjogren's syndrome and Crohn’s disease. Insufficient biotin intake can also cause various symptoms such as dermatitis, atrophic gingivitis, lack of appetite, nausea, and facial pallor.

1—3. Digestion, Absorption and Metabolism

Biotin predominantly exists as protein-bound forms in food. Released biotin is absorbed mainly from the jejunum. The digestion of this vitamin can be affected by the type of food, and other dietary sources. The relative bioavailability of free biotin has been reported to be 80% in the typical Japanese diet[(2)].

2. To Avoid Inadequacy

2—1. Method Used to Set AI

2—1—1. Adults and Children (AI)

There are currently no data on which the EAR for adults can based. The average daily biotin intake among Americans is 35.5 μg/day[(115)]. The average daily biotin intakes among Japanese individuals are 45.1 μg/day[(116)] and 60.7 μg/day[(117)]. According to the Standard Tables of Food Composition in Japan 2010[(18)] that listed biotin for the first time, the biotin intakes are approximately 30 μg/day[(118)] and 50 μg/day[(119)]. However, in many standard tables, the biotin component values of several foods are still not listed. Thus, the AIs were set based on the average dietary biotin intakes for adults from the previous total dietary assessment methods, i.e., 50 μg/day for adults aged 18 to 69 years.

The AI for children was calculated from the AI for adults (50 μg/day), using the following equation:

The AI for adults aged 18 to 29 years × (reference BW for children/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor).

Few studies have investigated the biotin requirements of elderly individuals. There are no data indicating that the biotin requirements of healthy individuals, aged over 70 years, differ from those of young adults. Thus, the AI for those aged over 70 years is the same as that for adults aged 18 to 29 years.

There were insufficient data to allow for the differences in requirements to be discerned between men and women, across all age groups. The lower value was adopted if the values of the men and women varied, in each age group.

2—1—2. Infants (AI)

For infants aged 0 to 5 months, the mean concentration of biotin in breast milk is 5 μg/L[6,7,120,121]. The average milk intake is 0.78 L/day[8,9], representing a daily biotin intake of 4.0 μg /day (rounding 3.9 μg /day). This value was set as the AI.

The AI for infants aged 6 to 11 months was calculated using the average of the values from following 2 expressions:

Expression 1: The AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75

Expression 2: the AI for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor)

Thus, the AI was determined to be 10 μg/day for infants aged 6 to 11 months.

2—1—3. Pregnant Women (AI)

Pregnant women have reduced serum biotin concentrations, as well as reduced biotin excretion in the urine. In contrast, the urinary excretion of organic acids such as 3-hydroxyisovaleric acid increases during late pregnancy[122]. These findings indicate that pregnancy increases biotin requirements. However, there are no data on the additional amount required by pregnant women. Thus, the AI for pregnant women was set at the AI of non-pregnant women.

2—1—4. Lactating Women (AI)

The amount of biotin required during lactation should be calculated from the differences in the biotin requirements of lactating and non-lactating women of a similar age. However, no such data are available. Thus, the AI for lactating women was set at the AI of non-lactating women.

3. To Avoid Excessive Intake

3—1. Dietary Intake

No regular food contains more than several dozen μg of folate/100 g except for liver. No study till date has reported unfavorable outcomes due to an excessive intake of regular food.

3—2. Method Used to Set the UL

There was insufficient evidence for the determination of the UL for healthy individuals. Excessive biotin intake of 200 mg/day is not associated with adverse effects, even in patients with biotin-responsive inborn errors of metabolism[114].

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

4—1. The Association with LRDs

No relevant data were available. The DG was not determined due to a lack of data.

Vitamin C

1. Background Information

1—1. Definition and Classification

The present DRIs were determined as the amount of ascorbic acid. Vitamin C (ascorbic acid) is a compound of (R)-3, 4-Dihydroxy-5-[(S)-1, 2-Dihydroxyethyl] furan-2(5H)-one, commonly known as L-ascorbic acid or ascorbic acid. Vitamin C freely exists as L-ascorbic acid in its reduced form, or L-dehydroascorbic acid in its oxidized form.

1—2. Function

Vitamin C is essential for the biosynthesis of collagen, skin, and cells. Vitamin C deficiency cause scurvy. Vitamin C also has antioxidant functions.

1—3. Digestion, Absorption and Metabolism

Vitamin C is transported to the blood after intestinal absorption. The digestion processes related to this vitamin can be affected by the type of food, and other dietary sources. The relative bioavailability of vitamin C is 90% up to an intake of 200 mg/day, and less than 50% at more than 1 g/day, pointing to differences between dietary intake and intake in the form of supplements[123]. The body's vitamin C level is maintained through various mechanisms, and the plasma concentration is saturated at an intake of about 400 mg/day[124,125].

2. To Avoid Inadequacy

2—1. Factors to be Considered in Estimating Requirements

The demand is higher for smokers and passive smokers than non-smokers[121,126–128]. Compared to others in the same age group, the vitamin C intakes of these individuals must be higher amounts than the RDA.

2—2. Method Used to Set the EAR and RDA

2—2—1. Adults and Children (EAR and RDA)

Severe vitamin C deficiency results in scurvy, which may be preventable by an ascorbic acid intake of 6-12 mg/day[129,130]. Optimal antioxidant activity in the plasma, and the prevention of cardiovascular disease are achieved at a plasma ascorbic acid concentration of 50 μmol/L[131].

A meta-analysis of 36 studies (participants' age: 15 to 96 years) that examined the association between vitamin C intake and plasma concentration reported that an vitamin C intake of 83.4 mg/day was necessary for the plasma vitamin C level to be maintained at 50 μmol/L[125,132]. From these findings, the EAR for adults aged 18 to 29 years was determined to be 83.4 mg/day; this method was preferred for the setting of a value for the prevention of scurvy.

The RDA was calculated as the EAR × 1.2. The differences in the requirements between sexes were not considered[125].

The EAR and RDA for children was calculated from the EAR and RDA for adults aged 18 to 29 years, using the following equation:

EAR (RDA) for adults aged 18 to 29 years × (reference BW for children/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor).

The lower value was adopted if the values differed between the men and women, in each age group.

The meta-analysis stated above conducted a separate analysis using studies examining individuals aged 15-65 years, and those examining adults aged 60-96 years. The intake required for the achievement of the same plasma vitamin C level was higher in the latter analysis[132]. Therefore, elderly individuals may need to consume a higher amount of vitamin C; however, it was difficult to set a value specifically for this age group. Thus, the EAR and RDA values were adopted from those applicable to adults aged 18 to 69 years.

In a vitamin C depletion–repletion study conducted in men and women, the excretion of unmetabolized ascorbic acid into the urine was not detectable at an intake of 50 to 60 mg/day, but was detectable at an intake of 100 mg/day, under conditions in which the leukocyte vitamin C, as an indicator of body store, was saturated[124,125]. This finding supports the setting of an RDA value of 100 mg/day.

2—2—2. Additional Amount for Pregnant Women (EAR, RDA)

The additional amounts were calculated based on the intake of vitamin C required to prevent infant scurvy. Thus, the additional EAR was set at 10 mg/day[133]. The additional RDA was set by assuming a coefficient of 1.2, yielding 10 mg/day (rounding 12 mg/day).

2—2—3. Additional Amount for Lactating Women (EAR, RDA)

The additional EAR for lactating women was calculated based on the mean concentration of vitamin C in breast milk (50 mg/L[6,7,69]), the average milk volume (0.78 L/day)[8,9], and the relative bioavailability (100%)[1], yielding 40 mg/day (rounding 39 mg/day). The additional RDA was calculated as the EAR × 1.2, yielding 50 mg/day (rounding 46.8 mg/day).

2—3. Method Used to Set AI

2—3—1. Infants (AI)

The mean concentration of vitamin C in breast milk is 50 mg/L. The average milk intake is 0.78 L/day[8,9], representing a daily vitamin C intake of about 40 mg/day (rounding 39 mg/day). This value was set as the AI.

The AI for infants aged 6 to 11 months was calculated using the average of the values from the following 2 expressions:

Expression 1: the AI for infants aged 0 to 5 months × (reference BW for infants aged 6 to 11 months/reference BW for infants aged 0 to 5 months) 0.75
Expression 2: the EAR for adults aged 18 to 29 years × (reference BW for infants aged 6 to 11 months/reference BW for adults aged 18 to 29 years) 0.75 × (1 + growth factor)
Thus, the AI was determined to be 40 mg/day for infants aged 6 to 11 months.

3. To Avoid Excessive Intake

3—1. Dietary Intake

Few regular foods contain more than 100 mg of vitamin C/100 g; however, no studies till date have reported unfavorable outcomes due to the excessive intake of regular food.

3—2. Method Used to Set the Tolerable Upper Intake level

Generally, the intake of vitamin C intake is regarded as safe for healthy individuals, as the excess intake merely results in a lower absorption rate from the intestine, and enhanced excretion in the urine following absorption[124,125,134]. Thus, no UL for vitamin C was set at present.

However, for patients with renal dysfunction, the intake of several grams of vitamin C may increase the risk of kidney stones[135,136]. Acute gastrointestinal intolerance was observed following excess intake; for example, an intake of 3 to 4 g/day induced diarrhea[137]. An intake higher than 1 g/day from supplements is not advised[124,125,138].

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

4—1. The Association with LRDs

4—1—1. Prevention of Disease Development

Several reports have stated that there is no benefit in consuming more than 1 g/day of vitamin C[124–126,136]. The positive effects of vitamin C supplementation have not been clearly studied[132].

4—1—2. Prevention of Disease Progression

No relevant data were available. The DG was not determined due to a lack of data.

5. Future Dietary Reference Intakes for Japanese

It is important to reconsider if the use of EAR and RDA, or DG is more appropriate for vitamin C in the DRIs. Additionally, the outcomes used in the setting of the DRIs should also be reviewed in the future.

DRIs for Vitamin B_1 (mg/day) [1]

Gender	Males			Females		
Age etc.	EAR	RDA	AI	EAR	RDA	AI
0-5 months	—	—	0.1	—	—	0.1
6-11 months	—	—	0.2	—	—	0.2
1-2 years	0.4	0.5	—	0.4	0.5	—
3-5 years	0.6	0.7	—	0.6	0.7	—
6-7 years	0.7	0.8	—	0.7	0.8	—
8-9 years	0.8	1.0	—	0.8	0.9	—
10-11 years	1.0	1.2	—	0.9	1.1	—
12-14 years	1.2	1.4	—	1.1	1.3	—
15-17 years	1.3	1.5	—	1.0	1.2	—
18-29 years	1.2	1.4	—	0.9	1.1	—
30-49 years	1.2	1.4	—	0.9	1.1	—
50-69 years	1.1	1.3	—	0.9	1.0	—
70+ years	1.0	1.2	—	0.8	0.9	—
Pregnant women (additional)				+0.2	+0.2	—
Lactating women (additional)				+0.2	+0.2	—

[1] Calculated using estimated energy requirement for PAL II.

Notice: EARs are calculated from the intake where urinary excretion of vitamin B_1 starts to increase (i.e. internal saturation intake), not from the minimum intake required to prevent beriberi (one of the major vitamin B_1 deficiency diseases).

DRIs for Vitamin B_2 (mg/day) [1]

Gender	Males			Females		
Age etc.	EAR	RDA	AI	EAR	RDA	AI
0-5 months	—	—	0.3	—	—	0.3
6-11 months	—	—	0.4	—	—	0.4
1-2 years	0.5	0.6	—	0.5	0.5	—
3-5 years	0.7	0.8	—	0.6	0.8	—
6-7 years	0.8	0.9	—	0.7	0.9	—
8-9 years	0.9	1.1	—	0.9	1.0	—
10-11 years	1.1	1.4	—	1.1	1.3	—
12-14 years	1.3	1.6	—	1.2	1.4	—
15-17 years	1.4	1.7	—	1.2	1.4	—
18-29 years	1.3	1.6	—	1.0	1.2	—
30-49 years	1.3	1.6	—	1.0	1.2	—
50-69 years	1.2	1.5	—	1.0	1.1	—
70+ years	1.1	1.3	—	0.9	1.1	—
Pregnant women (additional)				+0.2	+0.3	—
Lactating women (additional)				+0.5	+0.6	—

[1] Calculated using estimated energy requirement for PAL II.

Notice: EARs are calculated from the intake where urinary excretion of vitamin B_2 starts to increase (i.e. internal saturation intake), not from the minimum intake required to prevent dermatitis such as cheilitis, perleche and glossitis (some of the major vitamin B_2 deficiency diseases).

DRIs for Niacin (mg NE/day) [1]

Gender	Males				Females			
Age etc.	EAR	RDA	AI	UL [2]	EAR	RDA	AI	UL [2]
0-5 months [3]	—	—	2	—	—	—	2	—
6-11 months	—	—	3	—	—	—	3	—
1-2 years	5	5	—	60（15）	4	5	—	60（15）
3-5 years	6	7	—	80（20）	6	7	—	80（20）
6-7 years	7	9	—	100（30）	7	8	—	100（25）
8-9 years	9	11	—	150（35）	8	10	—	150（35）
10-11 years	11	13	—	200（45）	10	12	—	200（45）
12-14 years	12	15	—	250（60）	12	14	—	250（60）
15-17 years	14	16	—	300（75）	11	13	—	250（65）
18-29 years	13	15	—	300（80）	9	11	—	250（65）
30-49 years	13	15	—	350（85）	10	12	—	250（65）
50-69 years	12	14	—	350（80）	9	11	—	250（65）
70+ years	11	13	—	300（75）	8	10	—	250（60）
Pregnant women (additional)					—	—	—	—
Lactating women (additional)					+3	+3	—	—

NE = niacin equivalent = niacin + 1/60 tryptophan.

[1] Calculated using estimated energy requirement for PAL II.

[2] Quantity as nicotinamide (mg). Values in parentheses are quantities as nicotinic acid (mg). Calculated using the reference weight.

[3] The unit is mg/day.

DRIs for Vitamin B_6 (mg/day) [1]

Gender	Males				Females			
Age etc.	EAR	RDA	AI	UL [2]	EAR	RDA	AI	UL [2]
0-5 months	—	—	0.2	—	—	—	0.2	—
6-11 months	—	—	0.3	—	—	—	0.3	—
1-2 years	0.4	0.5	—	10	0.4	0.5	—	10
3-5 years	0.5	0.6	—	15	0.5	0.6	—	15
6-7 years	0.7	0.8	—	20	0.6	0.7	—	20
8-9 years	0.8	0.9	—	25	0.8	0.9	—	25
10-11 years	1.0	1.2	—	30	1.0	1.2	—	30
12-14 years	1.2	1.4	—	40	1.1	1.3	—	40
15-17 years	1.2	1.5	—	50	1.1	1.3	—	45
18-29 years	1.2	1.4	—	55	1.0	1.2	—	45
30-49 years	1.2	1.4	—	60	1.0	1.2	—	45
50-69 years	1.2	1.4	—	55	1.0	1.2	—	45
70+ years	1.2	1.4	—	50	1.0	1.2	—	40
Pregnant women (additional)					+0.2	+0.2	—	—
Lactating women (additional)					+0.3	+0.3	—	—

[1] Calculated using RDAs in DRIs for proteins (excludes additional values for pregnant or lactating women).

[2] Quantity as pyridoxine, not as dietary vitamin B_6.

DRIs for Vitamin B_{12} (μg/day)

Gender	Males			Females		
Age etc.	EAR	RDA	AI	EAR	RDA	AI
0-5 months	—	—	0.4	—	—	0.4
6-11 months	—	—	0.5	—	—	0.5
1-2 years	0.7	0.9	—	0.7	0.9	—
3-5 years	0.8	1.0	—	0.8	1.0	—
6-7 years	1.0	1.3	—	1.0	1.3	—
8-9 years	1.2	1.5	—	1.2	1.5	—
10-11 years	1.5	1.8	—	1.5	1.8	—
12-14 years	1.9	2.3	—	1.9	2.3	—
15-17 years	2.1	2.5	—	2.1	2.5	—
18-29 years	2.0	2.4	—	2.0	2.4	—
30-49 years	2.0	2.4	—	2.0	2.4	—
50-69 years	2.0	2.4	—	2.0	2.4	—
70+ years	2.0	2.4	—	2.0	2.4	—
Pregnant women (additional)				+0.3	+0.4	—
Lactating women (additional)				+0.7	+0.8	—

DRIs for Folic Acid (μg/day) [1]

Gender	Males				Females			
Age etc.	EAR	RDA	AI	UL [2]	EAR	RDA	AI	UL [2]
0-5 months	—	—	40	—	—	—	40	—
6-11 months	—	—	60	—	—	—	60	—
1-2 years	70	90	—	200	70	90	—	200
3-5 years	80	100	—	300	80	100	—	300
6-7 years	100	130	—	400	100	130	—	400
8-9 years	120	150	—	500	120	150	—	500
10-11 years	150	180	—	700	150	180	—	700
12-14 years	190	230	—	900	190	230	—	900
15-17 years	220	250	—	900	220	250	—	900
18-29 years	200	240	—	900	200	240	—	900
30-49 years	200	240	—	1,000	200	240	—	1,000
50-69 years	200	240	—	1,000	200	240	—	1,000
70+ years	200	240	—	900	200	240	—	900
Pregnant women (additional)					+200	+240	—	—
Lactating women (additional)					80	+100	—	—

[1] In order to reduce the risk of neural tube closure, an additional intake of 400 μg/day of pteroylmonoglutamic acid is recommended for women who are planning to become pregnant or may be pregnant.

[2] Quantity as pteroylmonoglutamic acid contained in dietary supplement and vitamin-enriched food.

DRIs for Pantothenic Acid (mg/day)

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

DRIs for Biotin (μg/day)

Gender	Males	Females
Age etc.	AI	AI
0-5 months	4	4
6-11 months	10	10
1-2 years	20	20
3-5 years	20	20
6-7 years	25	25
8-9 years	30	30
10-11 years	35	35
12-14 years	50	50
15-17 years	50	50
18-29 years	50	50
30-49 years	50	50
50-69 years	50	50
70+ years	50	50
Pregnant women		50
Lactating women		50

DRIs for Vitamin C (mg/day)

Gender	Males			Females		
Age etc.	EAR	RDA	AI	EAR	RDA	AI
0-5 months	—	—	40	—	—	40
6-11 months	—	—	40	—	—	40
1-2 years	30	35	—	30	35	—
3-5 years	35	40	—	35	40	—
6-7 years	45	55	—	45	55	—
8-9 years	50	60	—	50	60	—
10-11 years	60	75	—	60	75	—
12-14 years	80	95	—	80	95	—
15-17 years	85	100	—	85	100	—
18-29 years	85	100	—	85	100	—
30-49 years	85	100	—	85	100	—
50-69 years	85	100	—	85	100	—
70+ years	85	100	—	85	100	—
Pregnant women (additional)				+10	+10	—
Lactating women (additional)				+40	+45	—

Notice: EARs are calculated from cardiovascular disease prevention effects and antioxidative effects, not from intake sufficient enough to avoid scurvy.

References

1. Fukawatari T & Shibata K (2008) Relative availability of B-group vitamins in a test diet to free vitamins (in Japanese). *J Home Econ Japan* **59**, 403–410.
2. Fukuwatari T & Shibata K (2009) Relative availability of water-soluble vitamins in a white bread diet to free vitamins (in Japanese). *J Home Econ Japan* **60**, 57–63.
3. Nishio M, Fujiwara M, Kitamura S, et al. (1948) Symptoms with experimental B1 deficiency and B1 requirements (in Japanese). *Vitam* **1**, 256–257.
4. FAO/WHO, a joint FAO/WHO Expert Group (1967) *World Health Organization Technical Report Series No.362. FAO Nutrition Meetings Report Series No.41. Requirements of Vitamin A, thiamine, riboflavine and niacin. Report of a Joint FAO/WHO Expert Group, Rome, Italy, 6-17 September 1965.* Geneva: WHO.
5. Itoda T, Sugawara M, Yakabe T, et al. (1996) The latest survey for the composition of human milk obtained from Japanese mothers. Part X. Content of water-soluble vitamins (in Japanese). *Japanese J Pediatr Gastroenterol Nutr* **10**, 11–20.
6. 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.
7. Shibata K, Endo M, Yamauchi M, et al. (2009) Distribution of the water-soluble vitamin content of Japanese breast milk (in Japanese). *J Japanese Soc Food Nutr* **62**, 179–184.
8. 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.
9. 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.
10. Iber FL, Blass JP, Brin M, et al. (1982) Thiamin in the elderly--relation to alcoholism and to neurological degenerative disease. *Am J Clin Nutr* **36**, 1067–82.
11. Mills CA. (1941) Thiamine overdosage and toxicity. *J Am Med Assoc* **116**, 2101.
12. Nakagawa I (1952) Effects of dietary depletion of Vitamin B2 (in Japanese). *Vitam* **5**, 1–5.
13. Horwitt MK, Harvey CC, Â W, et al. (1950) Correlation of urinary excretion of riboflavin with dietary intake and symptoms of ariflavinosis. *J Nutr* **41**, 247–264.
14. Davis M V, Oldham HG & Roberts LJ (1946) Riboflavin excretions of young women on diets containing varying levels of the B vitamins. *J Nutr* **32**, 143–61.
15. Boisvert WA, Mendoza I, Castañeda C, et al. (1993) Riboflavin requirement of healthy elderly humans and its relationship to macronutrient composition of the diet. *J Nutr* **123**, 915–25.
16. Schoenen J, Lenaerts M & Bastings E (1994) High-dose riboflavin as a prophylactic treatment of migraine: results of an open pilot study. *Cephalalgia* **14**, 328–9.
17. Zempleni J, Galloway JR & McCormick DB (1996) Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* **63**, 54–66.

18. 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.
19. Carter EG & Carpenter KJ (1982) The bioavailability for humans of bound niacin from wheat bran. *Am J Clin Nutr* **36**, 855–861.
20. Horwitt MK, Harper AE & Henderson LM (1981) Niacin-tryptophan relationships for evaluating niacin equivalents. *Am J Clin Nutr*.
21. Fukuwatari T, Ohta M, Kimtjra N, et al. (2004) Conversion ratio of tryptophan to niacin in Japanese women fed a purified diet conforming to the Japanese Dietary Reference Intakes. *J Nutr Sci Vitaminol* **50**, 385–91.
22. Goldsmith GA, Sarett HP, Register UD, et al. (1952) Studies of niacin requirement in man. I. Experimental pellagra in subjects on corn diets low in niacin and tryptophan. *J Clin Invest* **31**, 533–42.
23. Goldsmith GA, Rosenthal HL, Gibbens J, et al. (1955) Studies of niacin requirement in man. II. Requirement on wheat and corn diets low in tryptophan. *J Nutr* **56**, 371–386.
24. Horwitt M, Harvey C, Rothwell W, et al. (1956) Tryptophan-niacin relationships in man: Studies with diets deficient in riboflavin and niacin, together with observations on the excretion of nitrogen and niacin metabolites. *J Nutr* **60**, 1–43.
25. Shibata K, Sanada H, Yuyama S, et al. (1994) Evaluation of niacin nutrition in persons of advanced age supposed by the urinary excretion of niacin metabolites (in Japanese). *Vitam* **68**, 365–372.
26. Wada H, Fukuwatari T, Sasaki R, et al. (2006) Blood NAD and NADP levels in the elderly (in Japanese). *Vitam* **80**, 125–127.
27. Fukuwatari T, Murakami M, Ohta M, et al. (2004) Changes in the urinary excretion of the metabolites of the tryptophan-niacin pathway during pregnancy in Japanese women and rats. *J Nutr Sci Vitaminol* **50**, 392–8.
28. Shibata K. (1990) Effects of ethanol feeding and growth on the tryptophan-niacin metabolism in rats. *Agric Biol Chem* **54**, 2953–2959.
29. Rader JI, Calvert RJ & Hathcock JN (1992) Hepatic toxicity of unmodified and time-release preparations of niacin. *Am J Med* **92**, 77–81.
30. Winter SL & Boyer JL (1973) Hepatic toxicity from large doses of vitamin B3 (nicotinamide). *N Engl J Med* **289**, 1180–2.
31. McKenney JM, Proctor JD, Harris S, et al. (1994) A comparison of the efficacy and toxic effects of sustained- vs immediate-release niacin in hypercholesterolemic patients. *JAMA* **271**, 672–677.
32. Pozzilli P, Visaili N, Signore A, et al. (1995) Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). *Diabetologia* **38**, 848–852.
33. Gregory JF (1997) Bioavailability of vitamin B-6. *Eur J Clin Nutr* **51 Suppl 1**, S43-8.
34. Tarr JB, Tamura T & Stokstad ELR (1981) Availability of vitamin B6 and

pantothenate in an average American diet in man. *Am J Clin Nutr* **34**, 1328–37.

35. Lui A, Lumeng L, Aronoff GR, et al. (1985) Relationship between body store of vitamin B6 and plasma pyridoxal-P clearance: metabolic balance studies in humans. *J Lab Clin Med* **106**, 491–7.
36. Kreisch MJ, Sauberlich HE & Newbrun E (1991) Electroencephalographic changes and periodontal status during short-term vitamin B-6 depletion of young, nonpregnant women. *Am J Clin Nutr* **53**, 1266–1274.
37. Leklem JE (1990) Vitamin B-6: a status report. *J Nutr* **120 Suppl**, 1503–7.
38. Food and Nutrition Board Institute of Medicine (1998) Vitamin B6. In *Dietary reference intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic Acid, biotin, and choline*, pp. 150–195. Washington DC.: National Academies Press.
39. Bates CJ, Pentieva KD, Prentice A, et al. (1999) Plasma pyridoxal phosphate and pyridoxic acid and their relationship to plasma homocysteine in a representative sample of British men and women aged 65 years and over. *Br J Nutr* **81**, 191–201.
40. Cleary RE, Lumeng L & Li TK (1975) Maternal and fetal plasma levels of pyridoxal phosphate at term: adequacy of vitamin B6 supplementation during pregnancy. *Am J Obstet Gynecol* **121**, 25–8.
41. Brophy MH & Siiteri PK (1975) Pyridoxal phosphate and hypertensive disorders of pregnancy. *Am J Obs Gynecol* **121**, 1075–1079.
42. Chang SJ (1999) Adequacy of maternal pyridoxine supplementation during pregnancy in relation to the vitamin B6 status and growth of neonates at birth. *J Nutr Sci Vitaminol* **45**, 449–58.
43. Hansen CM, Shultz TD, Kwak HK, et al. (2001) Assessment of vitamin B-6 status in young women consuming a controlled diet containing four levels of vitamin B-6 provides an estimated average requirement and recommended dietary allowance. *J Nutr* **131**, 1777–86.
44. Hamfelt A & Tuvemo T (1972) Pyridoxal phosphate and folic acid concentration in blood and erythrocyte aspartate aminotransferase activity during pregnancy. *Clin Chim Acta* **41**, 287–98.
45. Fcirth-Walker D, Leibman D & Smolen A (1989) Changes in pyridoxal phosphate and pyridoxamine phosphate in blood, liver and brain in the pregnant mouse. *J Nutr* **119**, 750–6.
46. Shane B & Contractor SF (1975) Assessment of vitamin B 6 status. Studies on pregnant women and oral contraceptive users. *Am J Clin Nutr* **28**, 739–47.
47. Schuster K, Bailey LB & Mahan CS (1984) Effect of maternal pyridoxine X HCl supplementation on the vitamin B-6 status of mother and infant and on pregnancy outcome. *J Nutr* **114**, 977–88.
48. Satowa S, Misawa M, Kamiyama I, et al. (1978) Studies on serum vitamin B6 and PLP

status in pregnant women. (in Japanese). *Vitam* **63**, 361–368.
49. Reinken L & Dapunt O (1978) Vitamin B6 nutriture during pregnancy. *Int J Vitam Nutr Res* **48**, 341–347.
50. Trumbo PR & Wang JW (1993) Vitamin B-6 status indices are lower in pregnant than in nonpregnant women but urinary excretion of 4-pyridoxic acid does not differ. *J Nutr* **123**, 2137–41.
51. Barnard HC, de Kock JJ, Vermaak WJ, et al. (1987) A new perspective in the assessment of vitamin B-6 nutritional status during pregnancy in humans. *J Nutr* **117**, 1303–6.
52. Lumeng L, Cleary RE, Wagner R, et al. (1976) Adequacy of vitamin B6 supplementation during pregnancy: a prospective study. *Am J Clin Nutr* **29**, 1376–83.
53. Shibata K, Tachiki A, Mukaeda K, et al. (2013) Changes in plasma pyridoxal 5'-phosphate concentration during pregnancy stages in Japanese women. *J Nutr Sci Vitaminol* **59**, 343–6.
54. Isa Y, Kaitou A, Hayakawa T, et al. (2004) The vitamin B6 content in breast milk of Japanese women. (in Japanese). *Vitam* **78**, 437–440.
55. Shibata K, Sugimoto E, Hirose J, et al. (2009) Differences in measured amounts of vitamin B6 in breast milk accrding to determination method. (in Japanese). *J Japanese Soc Food Nutr* **62**, 131–135.
56. Schaumburg H, Kaplan J, Windebank A, et al. (1983) Sensory neuropathy from pyridoxine abuse. A new megavitamin syndrome. *N Engl J Med* **309**, 445–8.
57. Del Tredici A, Bernstein A & Chinn K (1985) Carpal tunnel syndrome and vitamin B6 therapy. In *Vitamin B6: Its role in health and desease Current topics in nutrition and disease*, pp. 459–462 [Reynolds R, Leklem J, editors]. New York.: Alan R. Liss.
58. Food and Nutrition Board, Institute of Medicine (1998) The B vitamins and choline: overview and methods. In *Dietary Reference Intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic Acid, biotin, and choline*, pp. 27–40. Washington, D.C.: National Academies Press.
59. Watanabe F (2007) Vitamin B 12 sources and bioavailability. *Exp Biol Med* **232**, 1266–1274.
60. Sullivan LW & Herbert V (1965) Studies on the minimum daily requirement for vitamin B12. Hematopietic responses to 0.1 micorogm. of cyanocobalamin of coenzyme B12, and comparison of their relative potency. *N Engl J Med* **272**, 340–6.
61. Berlin H, Berlin R & Brante G (1968) Oral treatment of pernicious anemia with high doses of vitamin B12 without intrinsic factor. *Acta Med Scand* **184**, 247–258.
62. Fernandes-Costa F, Van Tonder S & Metz J (1985) A sex difference in serum cobalamin and transcobalamin levels. *Am J Clin Nutr* **41**, 784–786.
63. Shibata K, Fukuwatari T, Ohta M, et al. (2005) Values of water-soluble vitamins in blood and urine of Japanese young men and women consuming a semi-purified diet

based on the Japanese Dietary Reference Intakes. *J Nutr Sci Vitaminol* **51**, 319–28.

64. Fukui T, Hirose J, Fukuwatari T, et al. (2009) Sex difference of blood levels of water-soluble vitamins of Japanese college students talking self-selected food (in Japanese). *Japanese J Nutr Diet* **67**, 284–290.
65. Krasinski SD, Russell RM, Samloff IM, et al. (1986) Fundic atrophic gastritis in an elderly population. Effect on hemoglobin and several serum nutritional indicators. *J Am Geriatr Soc* **34**, 800–6.
66. Scarlett JD, Read H & O'Dea K (1992) Protein‐bound cobalamin absorption declines in the elderly. *Am J Hematol* **39**, 79–83.
67. Loría A, Vaz-Pinto A, Arroyo P, et al. (1977) Nutritional anemia. VI. Fetal hepatic storage of metabolites in the second half of pregnancy. *J Pediatr* **91**, 569–573.
68. Vaz Pinto A, Torras V, Sandoval JF, et al. (1975) Folic acid and vitamin B12 determination in fetal liver. *Am J Clin Nutr* **28**, 1085–6.
69. Watanabe T, Taniguti A, Kayako S, et al. (2005) The concentrations of water-soluble vitamins in milk of Japanese women (in Japanese). *Vitam* **79**, 573–581.
70. Scott JM. (1997) Bioavailability of vitamin B12. *Eur J Clin Nutr* **51**, S49-53.
71. Mangiarotti G, Canavese C, Salomone M, et al. (1986) Hypervitaminosis B12 in maintenance hemodialysis patients receiving massive supplementation of vitamin B12. *Int J Artif Organs* **9**, 417–20.
72. Tamura T & Stokstad EL (1973) The availability of food folate in man. *Br J Haematol* **25**, 513–32.
73. Konings EJM, Troost FJ, Castenmiller JJM, et al. (2002) Intestinal absorption of different types of folate in healthy subjects with an ileostomy. *Br J Nutr* **88**, 235.
74. Sauberlich HE, Kretsch MJ, Skala JH, et al. (1987) Folate requirement and metabolism in nonpregnant women. *Am J Clin Nutr* **46**, 1016–1028.
75. Bhandari SD & Gregory JF (1990) Inhibition by selected food components of human and porcine intestinal pteroylpolyglutamate hydrolase activity. *Am J Clin Nutr* **51**, 87–94.
76. Milne DB, Johnson LK, Mahalko JR, et al. (1983) Folate status of adult males living in a metabolic unit: possible relationships with iron nutriture. *Am J Clin Nutr* **37**, 768–73.
77. O'Keefe C a, Bailey LB, Thomas EA, et al. (1995) Controlled dietary folate affects folate status in nonpregnant women. *J Nutr* **125**, 2717–2725.
78. McPartlin J, Weir DG, Halligan A, et al. (1993) Accelerated folate breakdown in pregnancy. *Lancet* **341**, 148–149.
79. Wolfe JM, Bailey LB, Herrlinger-Garcia K, et al. (2003) Folate catabolite excretion is responsive to changes in dietary folate intake in elderly women. *Am J Clin Nutr* **77**, 919–923.
80. Chanarin I, Rothman D, Ward A, et al. (1968) Folate status and requirement in pregnancy. *Br Med J* **2**, 390–394.

81. Daly S, Mills JL, Molloy AM, et al. (1997) Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet* **350**, 1666–9.
82. Smith AD (2007) Folic acid fortification: the good, the bad, and the puzzle of vitamin B-12. *Am J Clin Nutr* **85**, 3–5.
83. Butterworth CE & Tamura T (1989) Folic acid safety and toxicity: A brief review. *Am J Clin Nutr* **50**, 353–358.
84. Dickinson CJ (1995) Does folic acid harm people with vitamin B12 deficiency? *QJM* **88**, 357–64.
85. Campbell NR (1996) How safe are folic acid supplements? *Arch Intern Med* **156**, 1638–44.
86. Smith AD, Kim Y-I & Refsum H (2008) Is folic acid good for everyone? *Am J Clin Nutr* **87**, 517–33.
87. Dolnick BJ & Cheng Y (1978) Human thymidylate synthetase. II. Derivatives of pteroylmono- and -polyglutamates as substrates and inhibitors. *J Biol Chem* **253**, 3563–3567.
88. Allegra CJ, Drake JC, Jolivet J, et al. (1985) Inhibition of phosphoribosylaminoimidazolecarboxamide transformylase by methotrexate and dihydrofolic acid polyglutamates. *Proc Natl Acad Sci U S A* **82**, 4881–4885.
89. Matthews RG & Baugh CM (1980) Interactions of pig liver methylenetetrahydrofolate reductase with methylenetetrahydropteroylpolyglutamate substrates and with dihydropteroylpolyglutamate inhibitors. *Biochemistry* **19**, 2040–5.
90. Food and Nutrition Board Institute of Medicine (1998) Folate. In *Dietary reference intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic Acid, biotin, and choline*, pp. 196–305. Washington, D.C.: National Academies Press.
91. Institute of Medicine of The National Academies (2006) *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. [Otten JJ, Hellwig JP, Meyers LD, editors]. Washington, D.C.: National Academies Press.
92. Tamura T & Picciano MF (2006) Folate and human reproduction. *Am J Clin Nutr* **83**, 993–1016.
93. Berry RJ, Li Z, Erickson JD, et al. (1999) Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* **341**, 1485–90.
94. Daly LE, Kirke PN, Molloy A, et al. (1995) Folate levels and neural tube defects. Implications for prevention. *JAMA* **274**, 1698–702.
95. Mulinare J, Cordero JF, Erickson JD, et al. (1988) Periconceptional use of multivitamins and the occurrence of neural tube defects. *JAMA* **260**, 3141–5.
96. Milunsky A, Jick H, Jick SS, et al. (1989) Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA* **262**, 2847–52.
97. Laurence KM, James N, Miller MH, et al. (1981) Double-blind randomised controlled

trial of folate treatment before conception to prevent recurrence of neural-tube defects. *Br Med J* **282**, 1509–1511.

98. Smithells RW, Nevin NC, Seller MJ, et al. (1983) Further experience of vitamin supplementation for prevention of neural tube defect recurrences. *Lancet* **1**, 1027–31.
99. Vergel RG, Sanchez LR, Heredero BL, et al. (1990) Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. *Prenat Diagn* **10**, 149–152.
100. A.E. Czeizel ID (1992) Prevention of the first occurrence of neural tube defected by periconceptional vitamin supplementation. *N Engl J Med* **327**, 1832–1834.
101. Lumley J, Watson L, Watson M, et al. (2011) WITHDRAWN: Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. *Cochrane database Syst Rev*, CD001056.
102. Goldberg BB, Alvarado S, Chavez C, et al. (2006) Prevalence of periconceptional folic acid use and perceived barriers to the postgestation continuance of supplemental folic acid: Survey results from a teratogen information service. *Birth Defects Res Part A - Clin Mol Teratol* **76**, 193–199.
103. Martínez De Villarreal LE, Delgado-Enciso I, Valdéz-Leal R, et al. (2001) Folate levels and N(5), N(10)-methylenetetrahydrofolate reductase genotype (MTHFR) in mothers of offspring with neural tube defects: a case-control study. *Arch Med Res* **32**, 277–282.
104. Copp AJ, Stanier P & Greene NDE (2013) Neural tube defects: Recent advances, unsolved questions, and controversies. *Lancet Neurol* **12**, 799–810.
105. Voutilainen S, Rissanen TH, Virtanen J, et al. (2001) Low dietary folate intake is associated with an excess incidence of acute coronary events: The Kuopio Ischemic Heart Disease Risk Factor Study. *Circulation* **103**, 2674–2680.
106. Weng LC, Yeh WT, Bai CH, et al. (2008) Is ischemic stroke risk related to folate status or other nutrients correlated with folate intake? *Stroke* **39**, 3152–3158.
107. Ishihara J, Iso H, Inoue M, et al. (2008) Intake of folate, vitamin B6 and vitamin B12 and the risk of CHD: The Japan Public Health Center-based Prospective Study cohort I. *J Am Coll Nutr* **27**, 127–136.
108. Toole JF, Malinow MR, Chambless LE, et al. (2004) Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* **291**, 565–75.
109. Vollset SE, Clarke R, Lewington S, et al. (2013) Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: Meta-analyses of data on 50 000 individuals. *Lancet* **381**, 1029–1036.
110. 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_eiyou_chousa_tokubetsushuukei_h22

.pdf.

111. Kimura N, Fukuwatari T, Sasaki R, et al. (2003) Vitamin intake in Japanese women college students. *J Nutr Sci Vitaminol* **49**, 149–155.
112. Kobayashi S, Honda S, Murakami K, et al. (2012) Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. *J Epidemiol* **22**, 151–159.
113. 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_eiyou_chousa_tokubetsushuukei_ninpu_h19.pdf.
114. Haslam RH, Dalby JT & Rademaker AW (1984) Effects of megavitamin therapy on children with attention deficit disorders. *Pediatrics* **74**, 103–111.
115. Iyengar G V., Woif WR, Tanner JT, et al. (2000) Content of minor and trace elements, and organic nutrients in representative mixed total diet composites from the USA. *Sci Total Environ* **256**, 215–226.
116. Saitoh Y & Fusao U (2004) Estimate of the daily dietary intake of biotin, vitamin B6 and niacin from the 1999 Tokyo Total Diet Study (in Japanese). *Japanese J Nutr Diet* **62**, 165–169.
117. Watanabe T & Taniguchi A (2006) Study on the estimate of dietary intake of biotin by total diet study (in Japanese). *J Japanese Soc Clin Nutr* **27**, 304–312.
118. Shibata K, Tsuji T & Fukuwatari T (2013) Intake and urinary amounts of biotin in Japanese elementary school children, college students, and elderly persons. *Nutr Metab Insights* **6**, 43–50.
119. Imaeda N, Kuriki K, Fujiwara N, et al. (2013) Usual dietary intakes of selected trace elements (Zn, Cu, Mn, I, Se, Cr, and Mo) and biotin revealed by a survey of four-season 7-consecutive day weighed dietary records in middle-aged Japanese dietitians. *J Nutr Sci Vitaminol* **59**, 281–8.
120. Hirano M, Honma K, Daimatsu T, et al. (1992) Longitudinal variations of biotin content in human milk. *Int J Vitam Nutr Res* **62**, 281–282.
121. Watanabe T, Taniguchi A, Fukui T, et al. (2004) The contents of biotin, pantothenic acid and niacin in mature milk of Japanese women (in Japanese). *Vitam* **78**, 399–407.
122. Mock DM, Gerald Quirk J & Mock NI (2002) Marginal biotin deficiency during normal pregnancy. *Am J Clin Nutr* **75**, 295–299.
123. Tsujimura M, Higasa S, Aono K, et al. (2006) 'Vitamin C activity of L-dehydroascorbic acid in human' -Time-dependent Vitamin C urinary excretion after the oral lord- (in Japanese). *Vitam* **80**, 281–285.
124. Levine M, Conry-Cantilena C, Wang Y, et al. (1996) Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci* **93**, 3704–3709.

125. Levine M, Wang Y, Padayatty SJ, et al. (2001) A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci U S A* **98**, 9842–6.
126. Kallner AB, Hartmann D & Hornig DH (1981) On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. *Am J Clin Nutr* **34**, 1347–1355.
127. Tribble DL, Giuliano LJ & Fortmann SP (1993) Reduced plasma ascorbic acid concentrations in nonsmokers regularly exposed to environmental tobacco smoke. *Am J Clin Nutr* **58**, 886–90.
128. Preston AM, Rodriguez C, Rivera CE, et al. (2003) Influence of environmental tobacco smoke on vitamin C status in children. *Am J Clin Nutr* **77**, 167–72.
129. Grandon J, Lund C & Dill D (1940) Experimental human scurvy. *New Engl J Med* **223**, 353–369.
130. Hodges RE, Hood J, Canham JE, et al. (1971) Clinical manifestations of ascorbic acid aericuency in man. *Am J Clin Nutr* **24**, 432–43.
131. Gey KF (1998) Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *BioFactors* **7**, 113–74.
132. Brubacher D, Moser U & Jordan P (2000) Vitamin C concentrations in plasma as a function of intake: A meta-analysis. *Int J Vitam Nutr Res* **70**, 226–237.
133. Food and Nutrition Board Institute of Medicine (2000) Vitamin C. In *Dietary reference intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*, pp. 95–185. Washington, D.C.: National Academies Press.
134. Blanchard J, Tozer TN & Rowland M (1997) Pharmacokinetic perspectiveson megadoses of ascorbic. *Am J Clin Nutr* **66**, 1165–71.
135. Traxer O, Huet B, Poindexter J, et al. (2003) Effect of ascorbic acid consumption on urinary stone risk factors. *J Urol* **170**, 397–401.
136. Massey LK, Liebman M & Kynast-Gales SA (2005) Ascorbate increases human oxaluria and kidney stone risk. *J Nutr* **135**, 1673–7.
137. Cameron E & Campbell A (1974) The orthomolecular treatment of cancer II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. *Chem Biol Interact* **9**, 285–315.
138. Melethil S, Mason WD & Chang CJ (1986). (1986) Dose dependent absorption and excretion of vitamin C in humans. *Int J Pharm* **31**, 83–89.