Table 4. Energy for tissue increase associated with growth (energy deposition).

Sex	Males				Females			
			Tissue increase				Tissue increase	
Age	A. Reference body weight (kg)	B. Body weight increase (kg/y)	C. Energy density (kcal/g)	D. Energy deposition (kcal/d)	A. Reference body weight (kg)	B. Body weight increase (kg/y)	C. Energy density (kcal/g)	D. Energy deposition (kcal/d)
0–5 mo	6.4	9.5	4.4	120	5.9	8.7	5.0	120
6–8 mo	8.5	3.4	1.5	15	7.8	3.4	1.8	15
9–11 mo	9.1	2.4	2.7	15	8.5	2.5	2.3	15
1–2 y	11.7	2.1	3.5	20	11.0	2.1	2.4	15
3–5 y	16.2	2.1	1.5	10	16.2	2.2	2.0	10
6–7 y	22.0	2.5	2.1	15	22.0	2.5	2.8	20
8–9 y	27.5	3.4	2.5	25	27.2	3.1	3.2	25
10–11 y	35.5	4.5	3.0	35	34.5	4.1	2.6	30
12–14 y	48.0	4.2	1.5	20	46.0	3.1	3.0	25
15–17 y	58.4	2.0	1.9	10	50.6	0.8	4.7	10

Body weight increase (B) was calculated using the reference body weight (A) and the proportional distribution method, as shown in the following example:

Weight increase (kg/y) in females from 9 to 11 mo (X)

=[(reference weight between 9 and 11 mo (=reference weight at 10.5 mo)
−(reference weight between 6 and 8 mo (=reference weight of 7.5 mo))]/[0.875 (y)−0.625 (y)]+[(reference weight between 1 and 2 y)−(reference weight between 9 and 11 mo)]/[2 (y)−0.875 (y)].

Body weight increase=X/2
=[(8.5−7.8)/0.25+(11.0−8.5)/1.125]/2
≒2.5.

The energy density for tissue increase (C) was computed based on the DRIs for the United States and Canada (*1*).

The energy deposition for tissue increase (D) was calculated by multiplying weight increase (B) and by the energy density of tissue increase (C), as in the following example:

Energy (kcal/d) for tissue increase for females aged 9 and 11 mo
=[(2.5 kg/y)×1,000/365)]×2.3 (kcal/g)
=16
≒15.

=difference between pre-pregnancy total energy expenditure and pregnancy total energy expenditure (kcal/d)+energy deposition (kcal/d).

When the final values are rounded into 50-kcal units, an additional 50 kcal/d is required during the early stage, 250 kcal/d during the mid-stage and 450 kcal/d during the late stage.

10. Additional values for lactating women

The EER of lactating women is calculated as follows:

EER (kcal/d)
=EER before pregnancy (kcal/d)+additional energy required by lactating women (kcal/d).

Although BMR is considered to be elevated immediately after delivery, primarily due to the 2 processes of maintenance of increased body weight compared to pre-pregnancy weight and breast milk production, an obvious increase in BMR is not observed. Of 4 longitudinal studies using the DLW method, 1 reported that energy expenditure by physical activity decreased significantly (*78*) whereas the other 3 reported a 10% decrease in absolute quantity but no significant difference was observed (*79*, *81*, *84*). These findings indicate that total energy expenditure during lactation is the same as that during pregnancy (*77*, *79*, *81*, *84*). Regarding change in total energy expenditure, there is no need to calculate an additional value for lactating women. Meanwhile, lactating women must intake additional energy for breast milk production since it is not included in total energy expenditure.

Assuming that the amount of breast milk secreted is equal to the amount suckled by the infant (0.78 L/d) (*85*, *86*) and that breast milk provides 663 kcal/L (*87*), the following equation can be used to determine the total energy provided by breast milk:

Total energy provided by breast milk (kcal/d)
=0.78 L/d×663 kcal/L
≒517 kcal/d.

Recognizing that the energy requirement decreases due to energy obtained from weight loss (decomposition of tissue) and assuming that the energy corresponding to the body weight reduction is 6,500 kcal/kg and the amount of body weight loss is 0.8 kg/mo (*76–80*), the energy to be subtracted in the equation shown above can be calculated as follows:

Table 5. PAL of adults aged 15 to 69 y during daily activities for typical durations.[1]

PAL[2]	Low level (I)	Moderate level (II)	High level (III)
	1.50 (1.40–1.60)	1.75 (1.60–1.90)	2.00 (1.90–2.20)
Description of activity[3]	Subjects largely remain sedentary and perform activities that require low expenditure.	Subjects largely remain sedentary but perform any of the following: moving within the workplace, working while standing, serving customers, commuting, shopping, housekeeping, and participating in light sport activities.	Subjects engage in work that requires moving or standing or habitually engage in active athletic activities.
Types of each activity (h/d)			
Sleeping (0.9)[4]	7–8	7–8	7
Remaining sedentary or remaining still while standing (1.5: 1.0–1.9)[4]	12–13	11–12	10
Engaging in slow walking or light intensity activities, such as housekeeping (2.5: 2.0–2.9)[4]	3–4	4	4–5
Performing moderate-intensity activities that can be sustained for an extended period, including normal walking (4.5: 3.0–5.9)[4]	0–1	1	1–2
Performing vigorous activities that require frequent rest (7.0: ≥6.0)[4]	0	0	0–1

PAL, physical activity level.
[1] The values presented are the standard values for each activity based on the PALs obtained using the DLW method and BMR, and the hours from 3 d of activity records for adult subjects living in Tokyo and its suburbs.
[2] Representative values. The range is shown in parentheses.
[3] Prepared using Black et al. (*17*) as a reference and giving due consideration to the significant effects of occupation on PAL.
[4] Data in parentheses are MET values (representative value: lower threshold–upper threshold).

6,500 kcal/kg body weight
×0.8 kg/mo÷30 d
≒173 kcal/d.

Therefore, the additional energy required by lactating women who have experienced a normal pregnancy and delivery is calculated as follows:

Additional energy required by lactating women (kcal/d) = breast milk energy (kcal/d) − energy of weight loss (kcal/d).

Thus, the additional energy required for breast-feeding is 517−173=344 kcal/d, which, when rounded by 50-kcal units, is 350 kcal/d.

Application

Concept of reference basal metabolic rate

Reference basal metabolic rate (reference BMR) is designed such that the estimated value corresponds to a measured value for a reference physique. Therefore, for individuals with a body physique largely different from the reference physique, the prediction error tends to be large. Among the Japanese, for example, the BMR tends to be overestimated when the reference BMR is applied to obese individuals (*88*) and underestimated when applied to lean individuals. An EER obtained by multiplying an overestimated or underestimated BMR and PAL would have a high possibility of being above the

Table 6. Dietary Reference Intakes for energy: estimated energy requirement (kcal/d).[1]

Sex	Males			Females		
PAL	I	II	III	I	II	III
0–5 mo	—	550	—	—	500	—
6–8 mo	—	650	—	—	600	—
9–11 mo	—	700	—	—	650	—
1–2 y	—	1,000	—	—	900	—
3–5 y	—	1,300	—	—	1,250	—
6–7 y	1,350	1,550	1,700	1,250	1,450	1,650
8–9 y	1,600	1,800	2,050	1,500	1,700	1,900
10–11 y	1,950	2,250	2,500	1,750	2,000	2,250
12–14 y	2,200	2,500	2,750	2,000	2,250	2,550
15–17 y	2,450	2,750	3,100	2,000	2,250	2,500
18–29 y	2,250	2,650	3,000	1,700	1,950	2,250
30–49 y	2,300	2,650	3,050	1,750	2,000	2,300
50–69 y	2,100	2,450	2,800	1,650	1,950	2,200
≥70 y[2]	1,850	2,200	2,500	1,450	1,700	2,000
Pregnant women (amount to be added)						
Early stage				+50	+50	+50
Mid-stage				+250	+250	+250
Late stage				+450	+450	+450
Lactating women (amount to be added)				+350	+350	+350

[1] The estimated energy requirement (EER) for adults is calculated as follows:
EER (kcal/d)=BMR (kcal/d)×PAL.
The PALs were 1.50 (Level I), 1.75 (Level II), and 2.00 (Level III) for adults aged 18 to 69 y and 1.45 (Level I), 1.70 (Level II), and 1.95 (Level III) for adults aged over 70 y, respectively.
[2] Calculation of PAL was largely based on research findings regarding relatively healthy, independently living elderly subjects aged 70 to 75 y.

true requirement for an obese individual and below that for a lean individual. Thus, designing an energy intake plan based on such an EER would increase the probability of further obesity or leanness in such individuals.

Relationship between reference BMR and fat-free mass

BMR has been found to be more strongly associated with fat-free mass (FFM) than body weight (*5*, *8*, *11*, *89*). In the future, the combined use of adequate body composition assessment and corresponding predictive equations will likely yield more accurate estimation of BMR.

Measurement errors in the EER

In the DRIs for the United States and Canada (*1*, *2*), the standard error of estimate of total energy expenditure is approximately 300 kcal/d for males. Assuming this variability is divided into biological and experimental variances, such as measurement error in using the DLW method, and that both variances are equal, biological variability can be estimated at approximately±200 kcal/d as a standard deviation. Thus, when EER is calculated as 2,500 kcal/d, the probability of the true energy requirement being between 2,300 and 2,700 kcal/d is approximately 68% and of being between 2,100 and 2,900 kcal/d approximately 95%. In other words, if the EER were 2,500 kcal/d, 1 out of 3 individuals' true energy requirement would be below 2,300 kcal/d or above 2,700 kcal/d.

Physical activity level

Metabolic equivalent (MET), a multiple of the resting metabolic rate in the sitting position, was used as physical activity intensity to estimate PAL rather than activity factor (Af), a multiple of BMR (*90*). This was done to avoid confusion in using MET and Af representing physical activity intensity. As fasting BMR in the sitting position is approximately 10% higher than the resting metabolic rate in the supine position (*1*, *90*), MET is calculated as follows:

MET value×1.1≒Af.

The PAL of adults aged 15 to 69 y during the performance of daily activities for typical durations is shown in Table 5.

Effect of excessive post-exercise oxygen consumption on total energy expenditure

In the DRIs for the United States and Canada, excessive post-exercise oxygen consumption (EPOC), which is assumed to be 15% of certain activities, was added to calculate the EER in addition to energy expenditure during physical activity. However, EPOC was not added to the DRIs-J because it is considered to be very small in daily life (*91*). Therefore, only energy expenditure during certain activity was considered energy expended during physical activity in the DRI-Js. The EER values for

each sex and age group are shown in Table 6.

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Dietary Reference Intakes for Japanese 2010: Protein

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Summary Proteins form the most important structural component of cells that constitute the various types of tissue, such as muscle, skin, and bone. Proteins also function as enzymes and hormones to regulate various metabolic processes in the body. The estimated average requirement (EAR) of protein for both men and women who habitually consume mixed protein was evaluated as 0.72 g/kg body weight/d by nitrogen balance studies as the value to maintain nitrogen equilibrium with high quality protein, revised with digestibility of mixed protein in habitual food intake. The recommended intake of protein for infants is normally based on the adequate intake (AI) standard, which reflects the observed mean protein intake of infants fed principally with breast milk for up to 6 mo of age. The EAR of children aged 1–17 y was estimated by the factorial method, which adds the amount required for protein storage because of growth and protein requirement for maintenance. The EAR of protein in the elderly was calculated by meta-analysis, employing 144 data sets obtained from 5 published reports, with 60 subjects, and was found to be 0.85 g of habitual mixed protein/kg body weight/d. The tolerable upper intake level (UL) of protein must be established based on the health risk caused by excessive protein intake. However, no clear evidence to establish this value is available at present, and therefore, the UL of protein cannot be determined.

Key Words protein, nitrogen balance studies

1. *Background Information*

1-1. Function and metabolism

The most important structural components of cells that constitute the various types of tissue, such as muscle, skin, and bone, are proteins. Proteins also function as enzymes and hormones to regulate various metabolic processes in the body. Some proteins, such as hemoglobin, albumin, transferrin, and apolipoprotein, contribute to material transport within the body, whereas some others, such as γ-globulins, function as antibodies in non-specific defense reactions of the body, known as biophylaxis. Amino acids, which are the fundamental units of protein structure, are not only the constituents of the proteins, but they also function as precursors of neurotransmitters, vitamins, and other bioactive materials. Furthermore, proteins are utilized as an energy source when oxidized.

Organisms take in oxygen, water, and nutrients from outside the body and maintain a dynamic equilibrium by excreting carbon dioxide, metabolic products, and water out of the body. Similarly, body proteins maintain a steady state by continuous synthesis and breakdown, although the metabolic turnover rate differs depending on the nature of the protein. Body proteins finally degrade into amino acids, some of which are form urea and are excreted. Therefore, protein has to be supplied from food even in adults. For growing children, increased quantity of dietary protein is required for construction and accumulation of newly synthesized tissues.

1-2. Energy intake

Protein bioavailability is affected by the amount of ingested protein, amino acids, and total nitrogen. Protein metabolism is also influenced by non-nitrogenous dietary compounds in addition to such nitrogenous compounds. Energy intake is known to affect protein metabolism by the "protein-sparing action of energy" (*1*). Energy deficiency decreases protein utilization, which is reflected in a decreasing nitrogen balance. On the other hand, protein utilization, i.e. nitrogen balance, is improved when energy intake increases (*2*). Based on the mechanisms of the effect of energy on protein utilization, energy intake increases might accelerate the reduction of protein synthesis and breakdown through an increase in insulin secretion. A study on 361 adult subjects showed a significant positive correlation between energy intake and nitrogen (*3*). Presently, protein requirements are measured in a state of energy equilibrium, in consideration of the fact that protein

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requirements used to be underestimated because the nitrogen balance study employed for calculating protein requirements was conducted in a state of positive energy balance.

At present, the protein requirement is estimated on the assumption that the intake of energy and other nutrients is sufficient. Therefore, sufficient attention should be paid to the fact that protein deficiency can occur under conditions where there is a deficiency in the intake of energy and/or other nutrients, even if the required amount of protein is ingested. Moreover, it should be recognized that protein deficiency might exist among older individuals, or those with low physical activity, or low body weight, even if the protein intake is sufficient to meet the protein requirement.

1-3. Lifestyle

1-3-1. Physical activity/exercise. Persons with a high physical activity and enough food consumption can satisfy the protein requirement with ease. However, sedentary and elderly persons can easily develop deficiencies of either protein or other nutrients. The protein requirement responds to the intensity of exercise, forming a U curve (*4*), because insufficient exercise causes a catabolic state of body protein, and appropriate exercise augments the utilization of dietary protein, while vigorous exercise promotes a catabolic state of protein in the body. Appropriate exercise promotes growth as well as augments dietary protein utilization in children (*5*, *6*).

Following exercise, we observed augmentation of subcutaneous nitrogen losses because of sweating, enhancement of amino acid degradation, reduction of protein synthesis, and enhancement of protein degradation in the body. However, after exercise, the body begins to promote protein synthesis and recover from degradation. Mild and moderate levels of exercise (200–400 kcal/d) do not increase the protein requirement (*7*, *8*). Based on the protein requirement at the various levels of physical activity and exercise shown in the "Exercise Guideline for Health in Japan-2006," the protein requirement might not increase if the energy supply is sufficient.

1-3-2. Rest/Stress. The effect of mild daily life stress on the nitrogen balance has not been fully clarified. Only few reports have shown data on the relationship between stress and nitrogen balance, for example, a study in university students on the effects of sleep deprivation for 48 h and term-end examinations. Since the subjects that participated in that nitrogen balance study suffered from such stress, no compensation was conducted.

1-3-3. Smoking/drinking. Smoking affects cells, creating lesions with free radicals. Drinking affects metabolism, both directly and indirectly. However, the quantitative relationship between smoking and drinking and the protein requirement remains to be clarified.

1-4. Estimation of variability

There is a large range of variation, about 10–40%, in the reported nitrogen balance data (*9*). This variation arises from both intra-individual and inter-individual experimental variances and experimental error. According to the results of analyzing data from 235 subjects across 19 studies, 40% of the observed variances can be attributed to the variance between studies and the remaining 60% are due to variations within the studies (*9*). According to the results of analysis of variance on that data, it was shown that two-thirds of the variances were within individuals, with the remaining one-third representing true between-individual variances. Although the calculated coefficient of variation was 12%, 12.5% was employed here considering the skewed distribution of the data. Accordingly, the conversion factor of 1.25 was employed to calculate the recommended dietary allowance (RDA) from the estimated average requirement (EAR).

2. Determining DRIs

2-1. EAR/RDA/adequate intake (AI)

2-1-1. Adult (EAR/RDA). The protein EAR was evaluated by nitrogen balance studies as the value required for maintaining the nitrogen equilibrium with high quality protein, and we revised it to account for the digestibility of mixed protein in habitual food intake. The quality of the mixed protein was evaluated by employing the data obtained from the national nutrition survey. The data on protein intake was categorized into separate food groups and amino acid intake was calculated using the amino acid composition tables for each food group to evaluate their amino acid score. The amino acid score for mixed protein of habitual intake was over 100, even after employing several available evaluation criteria, such as the FAO/WHO provisional amino acid pattern published in 1973 (*10*), the FAO/WHO/UNU amino acid scoring pattern published in 1985 (*11*), and the WHO/FAO/UNU amino acid pattern published in 2007 (*12*). Therefore, it was assumed that further considerations on mixed protein quality were not necessary.

An average protein intake of 0.65 g/kg body weight/d (104 mgN/kg/d) was found to maintain nitrogen equilibrium in 17 studies on high quality protein (*13–27*). Therefore, this value was adopted as the protein intake required for maintaining nitrogen equilibrium.

The average digestibility of habitually ingested mixed proteins was evaluated as 92.2% in a study conducted on 12 female (*18*) and as 95.4% in a study on 6 males (*28*). Accordingly, the digestibility of mixed protein in daily food was set at 90%.

The EAR (g/kg body weight/d) was considered as being equal to the minimum protein intake required in order to allow nitrogen equilibrium (g/kg body weight/d)÷digestibility=0.65/0.90=0.72.

The EAR (g/d) was considered as being equal to the EAR (g/kg body weight/d)×reference body weight (kg).

The RDA (g/d) was considered as being equal to the EAR (g/d)×calculation coefficient.

2-1-2. Elderly (EAR/RDA). A decline of physiological functions, such as the maximal breathing capacity, renal blood flow, and vital capacity, as well as the decrease in skeletal muscles and the relative increase in adipose, is associated with aging. Although protein metabolism is lowered in skeletal muscles along with aging, it does

Table 1. EAR and RDA of protein determined using the factorial method for children.

Males

Age (y)	Reference body weight (A) (kg)	Body weight gain (B) (kg/y)	Body protein (C) (%)	Protein storage requirement (D) (g/kg/d)	Efficiency of protein utilization for growth (E) (%)	Protein maintenance requirement (F) (g/kg/d)	Efficiency of protein utilization for maintenance (G) (%)	EAR (g/d)	RDA (g/d)
1–2	11.7	2.1	13.2	0.065	40	0.67	70	13.1	16.4
3–5	16.2	2.1	14.7	0.052	40	0.67	70	17.6	22.0
6–7	22.0	2.5	15.5	0.048	40	0.67	70	23.7	29.6
8–9	27.5	3.4	14.5	0.049	40	0.67	70	29.7	37.1
10–11	35.5	4.5	13.9	0.048	40	0.67	75	36.0	45.0
12–14	48.0	4.2	13.9	0.033	40	0.67	80	44.2	55.3
15–17	58.4	2.0	15.0	0.014	40	0.67	85	48.1	60.1

Females

Age (y)	Reference body weight (A) (kg)	Body weight gain (B) (kg/y)	Body protein (C) (%)	Protein storage requirement (D) (g/kg/d)	Efficiency of protein utilization for growth (E) (%)	Protein maintenance requirement (F) (g/kg/d)	Efficiency of protein utilization for maintenance (G) (%)	EAR (g/d)	RDA (g/d)
1–2	11.0	2.1	13.0	0.068	40	0.67	70	12.4	15.5
3–5	16.2	2.2	14.1	0.052	40	0.67	70	17.6	22.0
6–7	22.0	2.5	14.1	0.044	40	0.67	70	23.5	29.4
8–9	27.2	3.1	13.7	0.043	40	0.67	70	28.9	36.1
10–11	34.5	4.1	14.6	0.048	40	0.67	75	34.9	43.6
12–14	46.0	3.1	14.8	0.027	40	0.67	80	41.7	52.1
15–17	50.6	0.8	11.9	0.005	40	0.67	85	40.5	50.6

Protein storage requirement (D)=B×1,000÷365×C÷100÷A.
EAR (g/d)=(D÷E×100+F÷G×100)×A, RDA (g/d)=EAR×1.25.
EAR, estimated average requirement; RDA, recommended dietary allowance.

not change in the visceral organs. Although decreases in protein turnover and physiological function in the elderly may have an influence on protein utilization, it has been reported that there is no difference observed in the EAR between young adults and the elderly (*9*). Generally, physical inactivity combined with decreased appetite causes a reduction in food intake in the elderly. These types of lifestyle-related characteristics may have an influence on the EAR of protein.

The EAR for the elderly is normally evaluated as the average value required in maintaining the nitrogen equilibrium under ordinary diet conditions in apparently healthy elderly people.

In this study, the estimated average protein requirement in the elderly was calculated by employing a meta-analysis on 144 data sets published in 5 reports (*22*, *29–32*), with 60 subjects, and we obtained a value of 0.85 g/kg body weight/d (136 mgN/kg body weight/d). In order to calculate this value, the digestibility of the mixed protein in habitual meals was estimated as 90%. With regard to miscellaneous nitrogen losses, the measured values of each study were adopted. In cases where no data was available, we employed a value of 5 mgN/kg body weight/d.

The incidence of malnutrition with a negative nitrogen balance is not rare among institutionalized elderly persons or those who are provided home health care (*33*). Since both lower physical activity and lower energy intake increase the EAR of protein, care should be taken to ensure that persons in such situations receive sufficient protein.

2-1-3. Children (EAR/RDA). The EAR for children of 1–17 y old was estimated by the factorial method, which adds the amount of protein required for storage due to growth to the protein maintenance requirement (Table 1). The efficiency of protein utilization, shown in Table 1 (G), was adopted in the calculations for the protein maintenance requirement.

The EAR (g/kg body weight/d) was considered as being equal to the protein maintenance requirement ÷efficiency of protein utilization for maintenance+the protein storage requirement÷efficiency of protein utilization for growth.

The EAR (g/d) was considered as being equal to the EAR (g/kg body weight/d)×the reference body weight (kg).

Table 2. Protein storage during pregnancy.

Reference	Number of individuals studied	Increase in whole body potassium (mmol/d)	Protein storage (g/d)[1]	Body weight gain (kg)
63	10	3.41	9.91	12.9
65	27	1.71	4.97	10.4
66	22	2.02	5.87	13.6
67	34	1.18	3.43	12.8
Mean	—	2.08	6.05	12.4

[1] Protein storage (g/d)=Potasium accumulated (mmol/d)÷2.15×6.25.

RDA (g/d) was considered as being equal to the EAR (g/d)×the calculation coefficient.

A value of 0.67 g/kg/d (107 mgN/kg body weight/d) was adopted for the protein maintenance requirement. This was the mean value obtained by multiple nitrogen balance studies on growing subjects, including children and adolescents (*34–40*). Regarding miscellaneous nitrogen losses other than that in feces and urine, the value of 6.5±2.3 mgN/kg body weight/d (range, 5–9 mgN/kg body weight/d) obtained in current reports (*34, 41–44*), was adopted. The same value adopted for the protein maintenance requirement was used in all age groups composed of growing subjects, since there was no evidence to suggest any differences among these age groups.

The protein storage associated with growth was calculated from the amount of increase in reference body weight and the ratio of body protein in each age group. The ratio of body protein to body weight was based on the body compositions obtained from 3 groups with subjects in the following age ranges: birth–10 y (*45*), 4 mo–2 y (*46*), and 4 y–18 y (*47*).

Regarding the efficiency of protein utilization required for maintenance and for growth, the values of 70% and 40%, respectively, were adopted for 1-y-old infants. A value of 40% was adopted for the efficiency of protein utilization required for maintenance in infants, and it is considered that this value will increase with growth toward the value for adults (90%).

Considering the importance of protein nutrition, it is necessary to gather as much data on the subject as possible.

2-1-4. Infants (AI). Since it is not possible to estimate the protein requirement for infants by the nitrogen balance method as is done for adults, this value is normally calculated using protein intake from breast milk or modified milk in normal healthy infants. Therefore, this value is based on the concept of AI.

As weaning infants develop, they begin to consume protein from foods other than breast milk. Therefore, the AI for infants was calculated by dividing their life stages into 3 groups, ranging 0–5 mo, 6–8 mo, and 9–11 mo.

No reports have been published showing protein deficiency in breastfeeding babies aged 0–5 mo. Therefore, the ingested amount of breast milk and protein concentration of breast milk were used for related calculations. Since the intake of breast milk was reported as being about 0.63–0.86 L/d (*48–54*), with no clear difference between the values for Japan and other countries, we employed a value of 0.78 L/d (*53, 54*). It was assumed that there was no difference in the protein concentration of breast milk among different races (*49, 51, 55–61*), and the protein concentration of breast milk in this stage was considered as 12.6 g/L. Therefore, the AI was calculated as follows:

AI (g/d)=12.6 (g/L)×0.78 (L/d)=9.83

During the weaning period, the nutrient intake situation for infants is greatly altered. The protein intake from weaning food, except for breast milk, in infants of 6–8 mo was estimated to be 6.1 g/d, based on a study report in Japanese infants (*56*). On the other hand, the average consumption of breast milk at this stage was about 0.6 L/d (*51, 57*), which corresponds to 10.6 g/L of protein from breast milk (*45, 50, 52*). Therefore, the AI of protein was calculated as follows:

AI of protein (g/d) was taken as being equal to the protein concentration in breast milk×the average consumption of breast milk + the protein intake from weaning food=10.6 (g/L)×0.60 (L/d)+6.1 (g/d)=12.5.

Protein intake from weaning food, except for breast milk, in infants aged 9–11 mo was estimated to be 17.9 g/d based on studies conducted in Japanese infants (*61, 62*). On the other hand, the average consumption of breast milk at this stage was about 0.45 L/d (*51, 57*), which corresponds to 9.2 g/L of protein from breast milk (*50, 55–57*). Therefore, the AI of protein was calculated as follows.

AI of protein (g/d) was taken as being equal to the protein concentration in breast milk×the average consumption of breast milk+the protein intake from weaning food=9.2 (g/L)×0.45 (L/d)+17.9 (g/d)=22.0.

The values for the AI of protein for infants with an intake of modified milk (g/d) in the 3 age groups were taken as reference value as follows, and the protein utilization value of modified milk was considered to be 70% (*11*).

0–5 mo: 12.6 (g/L)×0.78 (L/d)×100/70=14.0

6–8 mo: 10.6 (g/L)×0.60 (L/d)×100/70+6.1 (g/d) =15.2

9–11 mo: 9.2 (g/L)×0.45 (L/d)×100/70+17.9 (g/d) =23.8

Table 3. DRIs for protein (g/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	10	—	—	—	10	—
6–8 mo	—	—	15	—	—	—	15	—
9–11 mo	—	—	25	—	—	—	25	—
1–2 y	15	20	—	—	15	20	—	—
3–5 y	20	25	—	—	20	25	—	—
6–7 y	25	30	—	—	25	30	—	—
8–9 y	30	40	—	—	30	40	—	—
10–11 y	40	45	—	—	35	45	—	—
12–14 y	45	60	—	—	45	55	—	—
15–17 y	50	60	—	—	45	55	—	—
18–29 y	50	60	—	—	40	50	—	—
30–49 y	50	60	—	—	40	50	—	—
50–69 y	50	60	—	—	40	50	—	—
≥70 y	50	60	—	—	40	50	—	—
Pregnant women (amount to be added)								
Early-stage					+0	+0	—	—
Mid-stage					+5	+5	—	—
Late-stage					+20	+25	—	—
Lactating women (amount to be added)					+15	+20	—	—

EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.

2-1-5. Pregnancy: Additional requirement (EAR/RDA). It is possible to estimate protein accretion indirectly from the increase in whole body potassium. In addition to the increase in whole body potassium, using a potassium/nitrogen ratio of 2.15 mmol of potassium/g of nitrogen (*63*), and the factor of 6.25 g of protein/g of nitrogen, we were able to calculate protein storage as follows.

Protein storage (g/d)=potassium accumulated (mmol/d)÷2.15×6.25

In order to apply the formula shown above, it is necessary to estimate the body weight gain accompanying pregnancy, since protein storage changes according to body weight gain. A value of 11 kg was considered as the total body weight gain during pregnancy (*64*), and the protein storage for each stage of pregnancy was estimated as shown in Table 2, using available reports on body potassium storage during each stage of pregnancy (*63*, *65–67*).

The daily body protein storage in each stage of pregnancy was calculated according to a report that revealed that the ratio of amount of protein storage was 0, 1, and 3.9 for the early, mid, and late-stage, respectively (*67*). The data from the other reports studied for the mid and late-stage were also used for the calculation of daily protein storage, by calculating the same ratio for the corresponding stage.

The average values obtained from the calculations were 0 g/d for the early-stage, 1.94 g/d for the mid-stage, and 8.16 g/d for the late-stage. These values were divided by the efficiency of protein utilization for a growth ratio of 43% (*63*), and then rounded off. As a result, the additional requirement for each stage of pregnancy (EAR) was 0 g/d for the early-stage, 5 g/d for the mid-stage, and 20 g/d for the late-stage.

2-1-6. Lactating women: Additional requirement (EAR/RDA). Although a significant amount of the protein accumulated during pregnancy is lost with delivery, a portion of the accumulated protein remains in the mother's body. On the other hand, body weight decreases during the puerperal period, and protein secreted through lactation. Therefore, it was considered that the accumulated protein and body weight gain due to pregnancy were counterbalanced with these losses during the puerperal and lactation periods. Therefore, the additional requirement during the lactation period was calculated only for the secretion of milk.

A value of 0.78 L/d was adopted for the average intake of breast milk for the 6-mo breastfeeding period before the onset of weaning (*53*, *54*), and 12.6 g/L was adopted for the protein concentration of breast milk in this period (*49*, *51*, *55–61*). The efficiency for the conversion of dietary protein to breast milk protein was assumed to be 70%, based on the FAO/WHO/UNU report published in 1985 (*11*). The additional requirement for lactating women (EAR) was calculated as 12.6 g/L×0.78 L/d÷0.70=14.04 g/d, and adopted as 15 g/d according to the rounding off process employed. The additional requirement for lactating women (RDA)

was calculated as 17.6 g/d by multiplying by 1.25, the calculation coefficient, and we obtained a final value of 20 g/d according to the rounding off process employed.

2-2. Tolerable upper intake level (UL)

The UL of protein must be established based on the health risks due to excessive protein intake. However, there is no clear evidence available to establish this value at present. Therefore, we were not able to establish a TU value for protein.

However, unfavorable metabolic alterations, such as a reduction in insulin sensitivity, increases in the renal excretion of acid/oxalate and calcium, increases in the glomerular filtration rate, increases in bone resorption, and a decrease in the plasma glutamine concentration in healthy adults under 40-y-old fed 1.9–2.2 g/kg of protein (*68*), have been reported. In addition, a report showed hyperuremia with an elevated blood urea nitrogen value of over 10.7 mmol/L in subjects older than 65 y who were fed protein at a ratio of more than 2 g/kg body weight/d (*69*). These results suggest that not more than 2 g/kg body weight/d of protein should be consumed by adults, regardless of their age.

The DRIs for protein are summarized in Table 3.

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Dietary Reference Intakes for Japanese 2010: Fat

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Summary In the Dietary Reference Intakes (DRIs) for fat, adequate intake (AI) and tentative dietary goal for preventing lifestyle-related disease (DGs) were used. AIs were set for *n*-6 and *n*-3 polyunsaturated fatty acids, which are essential fatty acids because they are not produced by the human body and their deficiency leads to dermatitis. DGs have been set for total fat, saturated fat, *n*-6 fatty acids, *n*-3 fatty acids, and cholesterol, whose consumption levels affect risk of lifestyle-related disease, including obesity, diabetes mellitus, cardiovascular disease, and stroke. As AI for *n*-6 and *n*-3 polyunsaturated fatty acids, the 50th percentile of *n*-6 and *n*-3 fatty acid intake was set. In the Japanese population, 98% of dietary *n*-6 fatty acids come from linoleic acid; therefore the amount of *n*-6 fatty acid intake is considered to be that of linoleic acid. Both α-linolenic (60% of total *n*-3 fatty acids) acid and fish oils are considered essential fatty acids because it has been difficult to conclude that only α-linolenic acid is essential for humans. The prevention of diabetes mellitus and stroke was emphasized. For example, an increase in saturated fatty acids intake leads to increased incidences in obesity, diabetes, and myocardial infarction, whereas a decrease of saturated fatty acids intake is associated with increased incidence in brain hemorrhage. Therefore, DG of saturated fatty acids in those more than 18 y of age was set between 4.5 and 7% energy.

Key Words total fat, saturated fat, monounsaturated fat, *n*-6 fatty acids, *n*-3 fatty acids, cholesterol, trans fatty acids

Background Information

In the Dietary Reference Intakes for Japanese (DRIs-J) 2010 for fat, the adequate intakes (AIs) and tentative dietary goal for preventing lifestyle-related disease (DGs) for fat were determined. Specifically, AIs were set for *n*-6 and *n*-3 polyunsaturated fatty acids, which are essential fatty acids because they are not produced by the human body and their deficiency leads to disease. DGs have been set for total fat, saturated fat, *n*-6 fatty acids, *n*-3 fatty acids, and cholesterol, whose consumption levels affect risk of lifestyle-related disease, including obesity, diabetes mellitus, cardiovascular disease, and stroke.

Total fatty acids, saturated fat, and *n*-6 fatty acids are major fuels that supply energy to humans. Therefore, they are expressed as percentage of energy (%en) from total energy intake. Essential fatty acids, including metabolites of α-linolenic acid are expressed as absolute values (g/d) but not relative values (en% of total energy) due to their essentiality.

To estimate the average amount of fatty acid intake in the Japanese which was used for DRIs, it was calculated using the original data that had been collected by the 2005 and 2006 NHNS. The 50th percentiles of the major fatty acids and cholesterol are presented in the original Japanese DRIs. For the determination of DGs in the DRIs-J 2010, systematic reviews were conducted by using appropriate key words in PubMed. From these publications, 437 related to DRIs were selected for careful reading and, along with those that had been used for the DRIs-J 2005, were used for a review of the DRIs-J 2010.

In this paper, the original version of the Japanese DRIs has been summarized and only selected sections discussed for the sake of brevity.

Determining DRIs

1. Total fat

1-1. DG (lower boundary). A low fat/high carbohydrate diet leads to increased postprandial glucose and fasting triacylglycerol (TG) concentrations and decreased fasting high-density lipoprotein (HDL)-cholesterol concentration (*1*). Although there is no definite evidence that average daily fat intake in a low fat/high carbohydrate diet increases risk of obesity and diabetes mellitus, unfavorable metabolite profiles in low fat/high carbohydrate diets indicate that a lower boundary of adequate total fat intake exists.

As described in the following sections, the AI of *n*-6

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Table 1. Dietary Reference Intakes for total fat [Ratio of total fat to total energy (percentage of fat energy): % energy].

Sex	Males		Females	
Age	AI	DG (range)	AI	DG (range)
0–5 mo	50	—	50	—
6–11 mo	40	—	40	—
1–2 y	—	20≤, <30	—	20≤, <30
3–5 y	—	20≤, <30	—	20≤, <30
6–7 y	—	20≤, <30	—	20≤, <30
8–9 y	—	20≤, <30	—	20≤, <30
10–11 y	—	20≤, <30	—	20≤, <30
12–14 y	—	20≤, <30	—	20≤, <30
15–17 y	—	20≤, <30	—	20≤, <30
18–29 y	—	20≤, <30	—	20≤, <30
30–49 y	—	20≤, <25	—	20≤, <25
50–69 y	—	20≤, <25	—	20≤, <25
≥70 y	—	20≤, <25	—	20≤, <25
Pregnant women			—	—
Lactating women			—	—

AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases.

Table 2. Dietary Reference Intakes for saturated fatty acids (% energy).

Sex	Males	Females
Age	AI (range)	AI (range)
0–5 mo	—	—
6–11 mo	—	—
1–2 y	—	—
3–5 y	—	—
6–7 y	—	—
8–9 y	—	—
10–11 y	—	—
12–14 y	—	—
15–17 y	—	—
18–29 y	4.5≤, <7.0	4.5≤, <7.0
30–49 y	4.5≤, <7.0	4.5≤, <7.0
50–69 y	4.5≤, <7.0	4.5≤, <7.0
≥70 y	4.5≤, <7.0	4.5≤, <7.0
Pregnant women		—
Lactating women		—

AI, adequate intake.

fatty acids was set at approximately 5 en%, the AI (or DG) of *n*-3 fatty acids at approximately 1 en%, and the lower DG (lower boundary) of saturated fat at approximately 5 en%. The 50th percentile value for monounsaturated fat was found to be approximately 6 en% and the total fatty acid level was 17 en% (=5+1+5+6). Considering the glycerol portion of TG (approximately 10% of total fat), approximately 20 en% was set as the lower boundary for total fat (Table 1).

1-2. DG (upper boundary). The prevention of obesity, which leads to diabetes and other diseases, is a major concern for public health. There might be an optimal dietary fat to carbohydrate ratio for prevention and treatment of obesity. In a meta-analysis of general populations under free-living conditions, a reduction in the percentage of energy as fat was found to be positively and independently associated with weight loss (*2*). Another meta-analysis of intervention studies provided support for this conclusion (*3*). However, obese subjects with hyperinsulinemia (or insulin resistance) lost more weight on a moderately low-carbohydrate (or low-glycemic load) diet consisting of 40 en% carbohydrates and 30 to 35 en% fat than on a low-fat diet consisting of 55 to 60 en% carbohydrate and 20% fat, whereas those without hyperinsulinemia lost more weight on the low-fat diet than the moderately low-carbohydrate diet (*4–6*). The optimal dietary fat to carbohydrate ratio may differ in populations depending on the prevalence of obesity.

Considering the lower prevalence of obesity in the Japanese population, the upper boundary of total fat was set as the 50th percentile of fat en% of Japanese nationwide survey, which is 30 en% for individuals aged 1 to 29 y and 25 en% for individuals aged 30 y and over (Table 1).

2. Saturated fat

2-1. DG (lower boundary). In 3 Japanese cohort studies, subjects who ate less saturated fat showed an increased risk of hemorrhagic stroke (*7–9*). First, in the Ni-Hon-San Study, which followed males aged 45 to 69 y (n=1,366) in Hiroshima and Nagasaki for 4 y (1972 to 1976), subjects who ate less than 5 g/d of saturated fat showed an increased incidence of intracranial hemorrhage (*9*). Second, in the Honolulu Heart Program, a 10-y cohort study of male Hawaiians of Japanese descent that examined the relationship between dietary fat and cholesterol and mortality, subjects who ate less than 10 g/d of saturated fat showed a 2-fold increase in the incidence of stroke (bleeding and infarction were not identified separately) than subjects who ate more than 10 g/d of saturated fat (*8*). Third, in a 14-y prospective study (1983 to 1997) of 4,775 Japanese aged 40 to 69 y who participated in a single 24-h dietary recall survey, a low intake of saturated fat (approximately <10 g/d) was found to be associated with increased risk of intraparenchymal hemorrhage after adjusting for known cardiovascular risk factors (*7*). No study found an association between saturated fat intake and risk of brain infarction (*10*).

To determine the lower DG boundary for saturated fat, the results of 2 studies were examined. In a cohort study in Hawaii, subjects who ate less than 10 g/d (=3.9 en%) of saturated fat showed an increase in total mortality and mortality due to cancer, coronary heart disease, and stroke relative to subjects who ate more than 10 g/d of saturated fat (*8*). In a cohort study of Japanese subjects, the multivariate relative risk was found to be 3.37 for the lowest quartile (5.0 g/d), 2.60 for the second

lowest quartile (8.5 g/d), and 2.21 for the third lowest quartile (11.9 g/d=5.3 en%) compared to the highest quartile (18.3 g/d) (*7*). As these findings indicate that individuals who eat less than 4.6 en% (=(3.9+5.3)/2) saturated fat may have an increased risk of death and lifestyle-related diseases, the rounded value of 4.5 en% was set as the lower boundary of the DG for saturated fat for adults aged 18 y and over (Table 2). Because the amount of animal protein was not adjusted for further examination in these 2 studies, it is possible that the increase in hemorrhagic stroke observed had been due to a shortage of animal protein rather than a shortage of saturated fat. Therefore, to prevent hemorrhagic stroke, consumption of saturated fat from dairy products and animal meat is recommended.

2-2. DG (upper boundary). An increased intake of saturated fat has been hypothesized to elevate low-density lipoprotein (LDL)-cholesterol concentration and, ultimately, promote the development of atherosclerosis. However, cohort studies in the United States have not supported this hypothesis. In the Nurses' Health Study, the significantly positive association that had been found between saturated fat intake and mortality due to coronary heart disease (CHD) disappeared after adjusting for confounding factors (*11*). In a cohort of US males, the positive association that had been found between intake of saturated fat and incidence of myocardial infarction disappeared after adjusting for dietary fiber intake (*12*). However, age may affect these associations. Two studies found a positive association between intake of saturated fat and incidence of CHD for adults aged 60 y and over but not for adults aged under 60 y (*13*, *14*). In contrast, several intervention studies demonstrated that reduction of saturated fat intake led to reduced incidence of ischemic heart disease, degree of atherosclerosis, and LDL-cholesterol concentration (*15–17*). In a meta-analysis to examine the effects of dietary changes on blood lipid profile, intake of less than 10 en% (National Cholesterol Education Program Step I diet) or less than 7 en% (National Cholesterol Education Program Step II diet) of saturated fat resulted in significant reductions in blood LDL-cholesterol concentrations over a period of 1 mo to 2 y (*3*).

Several cross-sectional studies showed a positive association between intake of saturated fat and prevalence of obesity (*18*). Observational studies have reported a positive association between saturated fat intake and the prevalence of diabetes, but these positive associations disappeared after adjusting for body mass index (BMI) (*19–21*). However, cross-sectional studies have reported a positive association between saturated fat intake and prevalence of insulin resistance (a cause of Type 2 diabetes) even after adjusting for BMI (*22–24*). Furthermore, intervention studies have observed a positive association between dietary saturated fat intake and insulin resistance (*25*, *26*). These results indicate that increased intake of saturated fat may increase body weight and insulin resistance (independent of obesity) and eventually lead to the development of diabetes mellitus.

In summary, saturated fat intake has been associated with increased incidence of myocardial infarction, obesity, and diabetes mellitus in a dose-dependent manner. Thus, although it is not clear that increased intake of saturated fat is a cause of these diseases due to a lack of large scale intervention study, research suggests that a diet high in saturated fat may promote these diseases. A meta-analysis of intervention studies in the United States and Europe indicates that a diet of 10 en% or less saturated fat decreases LDL-cholesterol concentration by 12% while a diet of 7 en% or less saturated fat decreases in LDL-cholesterol concentration by 16% (*3*). These data indicate that lower intake of saturated fat leads to lower incidence of myocardial infarction, obesity, and diabetes mellitus.

In the Japanese population, the 50th percentile value of dietary saturated fat, which is approximately 7 en%, was set as the upper boundary of the saturated fat DG for adults (Table 2). In younger individuals, the associations between saturated fat and lifestyle-related diseases are unclear, but it has been reported that subjects whose total blood cholesterol concentrations were high at age 22 y experienced high incidence of cardiovascular disease 27 to 42 y later (*27*). Therefore, 7 en% was also set as the upper boundary for saturated fat intake for subjects aged 18 to 19 y.

3. Monounsaturated fat

3-1. DG (lower and upper boundaries). In intervention studies conducted over relatively short periods, metabolic markers (LDL-cholesterol or insulin resistance) in subjects fed a high-monounsaturated fat diet were found to be better than those fed a high-saturated fat diet or a high-carbohydrate diet. However, in diabetic subjects, a high-monounsaturated fat diet (25 en%) resulted in a greater increase in body weight than a high-carbohydrate diet (*28*). The results of long-term cohort studies are mixed, with some finding a negative association (*29*), others no association (*11*), and yet others a positive association (*13*, *14*, *30*, *31*) between monounsaturated fat intake and incidence of CHD.

Increasing dietary monounsaturated fat may lead to obesity and atherosclerosis when total energy intake is not restricted. However, when total fat intake is below 25 to 30 en% and the lower boundary of saturated fat, *n*-6, and *n*-3 fatty acids is maintained, intake of monounsaturated fat will be below 15 to 20 en% and overconsumption of monounsaturated fat will be avoided. Therefore, lower and upper boundaries of monounsaturated fat were not set.

4. n-6 fatty acids

4-1. AI. As the human body is unable to synthesize *n*-6 fatty acids, they are classified as essential fatty acids, thus requiring that an AI be set for these lipids. However, there are no data available to elucidate the appropriate AI value in healthy Japanese. In the Japanese population, 98% of dietary *n*-6 fatty acids come from linoleic acid. Patients deficient in *n*-6 fatty acids develop dermatitis, which can be improved by supplementation of 7.4 to 8.0 g/d or 2 en% of linoleic acid. Considering that most Japanese do not suffer from diseases due to *n*-6 fatty acid deficiency, the 50th percentile

Table 3. Dietary Reference Intakes for *n*-6 fatty acids.

Sex	Males		Females	
Age	AI (g/d)	DG (% energy)	AI (g/d)	DG (% energy)
0–5 mo	4	—	4	—
6–11 mo	5	—	5	—
1–2 y	5	—	5	—
3–5 y	7	—	6	—
6–7 y	8	—	7	—
8–9 y	9	—	8	—
10–11 y	10	—	9	—
12–14 y	11	—	10	—
15–17 y	13	—	11	—
18–29 y	11	<10	9	<10
30–49 y	10	<10	9	<10
50–69 y	10	<10	8	<10
≥70 y	8	<10	7	<10
Pregnant women (amount to be added)			+1	—
Lactating women (amount to be added)			+0	—

AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases.

Table 4. Dietary Reference Intakes for *n*-3 fatty acids (g/d).

Sex	Males		Females	
Age	Al	DG	Al	DG
0–5 mo	0.9	—	0.9	—
6–11 mo	0.9	—	0.9	—
1–2 y	0.9	—	0.9	—
3–5 y	1.2	—	1.2	—
6–7 y	1.6	—	1.3	—
8–9 y	1.7	—	1.5	—
10–11 y	1.8	—	1.7	—
12–14 y	2.1	—	2.1	—
15–17 y	2.5	—	2.1	—
18–29 y	—	2.1≤	—	1.8≤
30–49 y	—	2.2≤	—	1.8≤
50–69 y	—	2.4≤	—	2.1≤
≥70 y	—	2.2≤	—	1.8≤
Pregnant women			1.9	—
Lactating women			1.7	—

AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases.
Note: In the DG, it is advised to have more than 1 g/d of EPA+DHA.

of *n*-6 fatty acid intake was set as the AI for *n*-6 fatty acids (Table 3).

4-2. DG (lower boundary). As there is no strong evidence that low intake of *n*-6 fatty acids increases risk of disease, a DG (lower boundary) was not set.

4-3. DG (upper boundary). Despite some concern that excessive intake of *n*-6 fatty acids may lead to increased incidence of cancer (*32*), recent meta-analyses do not support this concern (*33*, *34*). Because delta-6 desaturase competitively acts on both linoleic acid and α-linolenic acid, increased intake of linoleic acid may decrease production of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the metabolites of α-linolenic acid. However, adequate intake of EPA and DHA could counteract this unfavorable effect.

The effects of high intake of *n*-6 fatty acids (more than 10 en%) on mortality and mobility have not been studied in detail. Because linoleic acid produces inflammatory fat, such as prostaglandin and leukotriene (*35*), high intake of *n*-6 fatty acids could be a risk to health. Indeed, a recent Japanese cross-sectional study of school children found that the odds ratio of the prevalence of wheezing for the highest quintile of intake (14.5 g/d) was 1.2 (95% CI, 1.06 to 1.37) relative to the lowest quintile (5.7 g/d) (*36*).

Although there is no definite evidence that high intake of *n*-6 fatty acids is a risk factor, an upper boundary was set at 10 en% for adults in recognition of the possible association between high intake and chronic inflammation (Table 3).

5. n-3 fatty acids

5-1. Background information. Dietary *n*-3 fatty acids are primarily found in 2 sources: vegetable oil, which contains α-linolenic acid, and fish oil, which contains EPA, DHA, and docosahexaenoic acid (DPA). A portion of α-linolenic acid is metabolized to EPA and DHA in humans and 59% of total *n*-3 fatty acid in diet is in the form of α-linolenic acid, as well as that DHA intake is 1.8-fold larger than EPA intake and that DPA intake is only 30% of EPA intake. Moreover, according to a Japanese nationwide survey, there are marked differences between the 50th percentile median and mean values of EPA, DPA, and DHA intake, with the former approximately half the latter (data not shown). Therefore, it is uncertain whether the 50th percentile values of fish oil intake are a good index of the average amount of fish oil intake by a population.

Because the beneficial physiological effects of *n*-3 fatty acids might be due to the direct effects of *n*-3 fatty acids rather than their metabolic competition with *n*-6 fatty acids, the ratio of *n*-3/*n*-6 fatty acids was not used to set the DRIs for *n*-3 fatty acids. Epidemiologic observations support this notion. In the Nurses' Health Study, the inverse association that had been found between α-linolenic acid and risk of coronary artery disease (CAD) was not affected by linoleic acid intake (*37*). In the Health Professional Study, the inverse association that had been found between α-linolenic acid or EPA and DHA intake and risk of coronary artery disease was not confounded by linoleic acid intake (*38*).

5-2. AI. Since *n*-3 fatty acids are essential fatty acids, an AI for *n*-3 fatty acid intake should be set. Because administering both α-linolenic acid and fish oil to patients deficient in *n*-3 fatty acids has been found

to result in improvement of dermatitis and increase in body weight (*39*), it has been difficult to conclude that only α-linolenic acid is essential for humans. Therefore, all *n*-3 fatty acids, including both α-linolenic acid and fish oils, are considered essential fatty acids. Although there are no data with which to elucidate the appropriate AI value for healthy Japanese, the 50th percentile of *n*-3 fatty acid intake was set as the AI in consideration of the fact that most Japanese do not suffer from diseases due to *n*-3 fatty acid deficiency (Table 4).

5-3. DG (lower boundary) of α-linolenic acid. Intervention studies in France and India identified 1.8 g/d as the intake of α-linolenic acid that reduces the mortality of patients with CHD (*40, 41*). The Iowa Women's Health Study, a prospective cohort study of postmenopausal women, found an inverse association between intake of α-linolenic acid and total mortality (*42*). Several cohort studies have shown an inverse association between intake of α-linolenic acid and incidence of CHD in the United States (*12, 37, 43*). Recognizing that these favorable effects may apply to the Japanese population, intake of α-linolenic acid for adults aged 18 y and over is advised to be equal to or higher than the current 50th percentile values of the Japanese population (in men, 50th percentile values of α-linolenic acid are 1.49 (in 18–29 y old), 1.42 (30–49 y old), 1.32 (50–69 y old) and 1.06 g/d (70 y old and over), respectively, and in women, 1.24 (in 18–29 y old), 1.19 (30–49 y old), 1.14 (50–69 y old) and 0.96 g/d (70 y old and over), respectively).

5-4. DG (upper boundary) of α-linolenic acid. A long-term intervention study in Japanese elderly subjects showed that an increase of 3.0 g/d of α-linolenic acid (total intake of α-linolenic acid of 4.8 g/d) had no adverse effects on lipid profiles or major metabolites in blood (*44*). Although the DG (upper boundary) of α-linolenic acid was not set, large habitual intake of α-linolenic acid in males should be avoided due to concern that it may increase the incidence of prostate cancer (*45*).

5-5. DG (lower boundary) of EPA and DHA. Many studies have found a positive association between intake of *n*-3 fatty acids and reduced risk of CAD (*46*). A recent review that examined the association between the intake of EPA and DHA and mortality due to CAD identified a threshold of EPA and DHA intake—0.5 g/d—above which no further reduction in CAD mortality resulted (*47*). Likewise, clinical studies have identified a threshold of 0.75 g/d for reducing blood pressure and risk of arrhythmia (*47*). However, no threshold regarding intake and nonfatal coronary events has been identified in Japanese subjects. In a Japanese cohort study (the JPHC Study), the multivariable hazard ratio of nonfatal coronary events of the highest quintile (EPA and DHA intake of 2.1 g/d) was found to be 67% lower than that of the lowest quintile (EPA and DHA intake of 0.3 g/d) (*48*), while the hazard ratio of the middle quintile (EPA and DHA intake of 0.9 g/d) was found to decrease significantly (39%). In the Japan Eicosapentaenoic Acid Lipid Intervention Study (the JELIS), in which 18,645 patients with a total cholesterol of 250 mg/dL or greater were randomly assigned to receive 1.8 g/d EPA with statins or statins only, a 19% relative reduction in major coronary events was observed in the EPA with statins group over a 5-y follow-up period (*49*). However, this reduction was only observed regarding unstable angina, not coronary death.

The findings of other studies indicate that EPA and DHA intake may reduce the incidence of heart failure. In a Japanese cohort study (the JACC Study), the hazard ratio for the highest quintile (EPA, DHA, and DPA intake of 2.11 to 5.06 g/d) was found to be 0.58 (95% CI, 0.36 to 0.93) relative to the lowest quintile (EPA, DHA, and DPA intake of 0.05 to 1.18 g/d) (*50*). In an intervention study in Italy, supplementation of 1 g/d of EPA and DHA significantly reduced risk of death and rate of hospital re-admission for heart failure patients (*51*), while several US studies have found an inverse association between fish intake and the incidence of brain infarction (*52–54*). The JELIS found that supplementation of 1.8 g/d of EPA decreased the relative risk of stroke recurrence by 20% (*55*). Other studies have found an inverse association between EPA and DHA intake and incidence of age-related macular degeneration (*56–58*), as well as that high EPA+DHA intake has favorable effects on allergic rhinitis (*59*), peak bone mineral density (*60*), and aged-induced cognitive decline (*61, 62*).

These findings indicate that high EPA and DHA intake could reduce the incidence of CAD, stroke, and age-related macular degeneration. One study found that Japanese subjects whose average intake of EPA and DHA was 0.9 g/d showed a significant reduction in hazard ratio (0.61; 95% CI, 0.38 to 0.98) for nonfatal cardiac events compared subjects whose intake was 0.3 g/d (*48*). Rounding this value (0.9 g/d), the DG for the lower boundary of EPA and DHA was set at 1 g/d, which is equivalent to approximately 90 g/d of fish (Table 4).

5-6. DG (upper boundary) of EPA and DHA. The possible adverse effects of EPA and DHA intake on bleeding time, LDL-cholesterol concentration, blood glucose level, immune functions, lipid peroxide level, and plasminogen activator inhibitor-1 (PAI-1) have been reviewed systematically (*46*). Intake at typical daily levels has not been found to result in increased occurrence of clinically significant adverse effects (*46*). In the JELIS, administration of 1.8 g/d EPA did not increase hemorrhagic stroke, stomach cancer, lung cancer, colon cancer, breast cancer, or LDL-cholesterol concentration (*49*). Therefore, a DG (upper boundary) of EPA and DHA was not set.

In setting the DRIs, the safety of incidental intake of heavy metals, such as mercury, cadmium, lead, and tin, and of chemical environmental pollutants, such as dioxins and polychlorinated biphenyls (PCBs), which are generally present in fish, was not considered because other regulations apply to these compounds. In addition, the amount of toxic compounds varies between fish species and the areas where fish are caught. Guidelines for the safety of toxic compounds in food have been issued by the Japanese Government and should also be referred to.

Table 5. Dietary Reference Intakes for cholesterol (mg/d).

Sex	Males	Females
Age	DG	DG
0–5 mo	—	—
6–11 mo	—	—
1–2 y	—	—
3–5 y	—	—
6–7 y	—	—
8–9 y	—	—
10–11 y	—	—
12–14 y	—	—
15–17 y	—	—
18–29 y	<750	<600
30–49 y	<750	<600
50–69 y	<750	<600
≥70 y	<750	<600
Pregnant women		—
Lactating women		—

DG, tentative dietary goal for preventing lifestyle-related diseases.

5-7. DG (lower and upper boundary) of n-3 fatty acids. Questions such as "If sufficient amounts of EPA and DHA are consumed, is it unnecessary to consume α-linolenic acid?" and "When very low amounts of EPA and DHA are consumed, should intake of α-linolenic acid be increased ?" are difficult to answer because of insufficient data regarding the optimal ratio of α-linolenic acid to EPA and DHA intake. Therefore, the DG (lower boundary) of total *n*-3 fatty acid intake (including α-linolenic acid, EPA, and DHA) for adults aged 18 y and over was set at the 50th percentile value of the dietary intake of the Japanese population. However, as both the JPHC study and the JELIS observed beneficial effects of fish oil intake on CAD (albeit without considering basal intake of α-linolenic acid), more than 1 g/d intake of EPA and DHA is advised, regardless of intake of α-linolenic acid. A DG for the upper boundary of total *n*-3 fatty acids was not set because the values for α-linolenic acid and fish oils were not set (Table 4).

6. Dietary cholesterol

6-1. DG (lower boundary). Either increased or decreased blood cholesterol concentration has been associated with elevated mortality from stroke in a U-shaped-curve manner (*63*). The increased mortality from ischemic stroke observed in subjects with high blood cholesterol concentrations was due in part to increased LDL-cholesterol concentration, which promotes atherosclerosis. Observation of elevated mortality from intracerebral hemorrhage in patients with lower blood cholesterol concentrations does not confirm that low blood cholesterol concentration is a cause of hemorrhagic stroke (*64, 65*). Japanese cohort studies have found no association between dietary cholesterol intake and incidence of stroke, including hemorrhagic stroke (*7, 8, 10, 66*). Interestingly, one study that had identified an inverse association between dietary cholesterol intake and incidence of stroke found that this association disappeared after adjusting for intake of animal protein and fat (*66*). As a meta-analysis found that treatment to reduce blood cholesterol concentration did not increase incidence of stroke (*67*), a DG (lower boundary) for cholesterol was not set.

6-2. DG (upper boundary). In cohort studies in the United States, no association was found between intake of cholesterol (or egg consumption) and incidence of CAD (*12, 68–70*). However, in the Honolulu Heart Program Study, Japanese whose intake of cholesterol was more than 325 mg/1,000 kcal (747 mg/d expressed on a daily basis), showed a significant increase in mortality from CHD (*8*). In one of the NIPPON DATA 80 studies, a series of cohort studies conducted in Japan, no association was found between egg consumption and death due to ischemic heart disease in subjects who had undergone dietary assessment in 1980 and been followed up to 1994 (*71*). In a study in which subjects underwent dietary assessment between 1990 and 1994 and were followed up to 2001, those who ate fewer eggs were found to have increased incidence of CHD (*72*). However, this finding could be attributed to reverse causation; that is, the subjects with high blood cholesterol tended to reduce egg consumption due to exposure to a public campaign advising them to do so to lower their blood cholesterol. Therefore, it is difficult to interpret the results of recent studies that examined the association between cholesterol intake and cardiovascular disease. In the NIPPON DATA 80 study, women who ate more than 2 eggs per day were found to have a 2-fold higher risk of mortality from cancer compared with women who seldom ate eggs (*71*). Recent studies have supported this finding, having found a positive association between intake of cholesterol and incidence of ovarian and endometrial cancer (*73, 74*) as well as lung, pancreatic, and colon/rectal cancer (*75*). Thus, a high intake of cholesterol is not recommended for the public at large. Using the data from the Honolulu Heart Program Study (*8*), the DG for the upper boundary of cholesterol intake was set at 750 mg/d for men and 600 mg/d for women, with these different values reflecting adjustment by differences in daily energy intake (Table 5).

7. Trans fatty acids

7-1. Background information. Trans fatty acids are mostly derived from 3 sources: 1) partially hydrogenated foods, such as margarine; 2) geometrical isomers of linoleic and α-linolenic acid resulting from the deodorization process; and 3) naturally occurring trans fatty acids from beef, lamb, and dairy fat resulting from biohydrogenation in ruminants. In humans, high intake of partially hydrogenated vegetable oils has been associated with increased incidence of CHD, obesity, allergies, lower birth weight, and fetal loss (*76*). As high intake of trans fatty acids derived from ruminants has not been associated with CHD, obesity, or diabetes, it is considered less harmful than high intake of other forms of trans fatty acids (*77–80*).

7-2. DG (upper boundary). High intake of trans