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 others such as \( \gamma \)-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...
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)\times digestibility=0.65/0.90=0.72.

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

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

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Reference body weight (A) (kg)</th>
<th>Body weight gain (B) (kg/y)</th>
<th>Body protein (C) (%)</th>
<th>Protein storage requirement (D) (g/kg/d)</th>
<th>Efficiency of protein utilization for growth (E) (%)</th>
<th>Protein maintenance requirement (F) (g/kg/d)</th>
<th>Efficiency of protein utilization for maintenance (G) (%)</th>
<th>EAR (g/d) (g/kg/d)</th>
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<td>40</td>
<td>0.67</td>
<td>85</td>
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</tbody>
</table>

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.
Japanese DRIs for Protein

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 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:

\[
AI (g/d) = 12.6 \times 0.78 (L/d) = 9.83
\]

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:

\[
AI (g/d) = 10.6 \times 0.60 (L/d) + 6.1 (g/d) = 12.5
\]

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:

\[
AI (g/d) = 9.2 \times 0.45 (L/d) + 17.9 (g/d) = 22.0
\]

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 2. Protein storage during pregnancy.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of individuals studied</th>
<th>Increase in whole body potassium (mmol/d)</th>
<th>Protein storage (g/d)</th>
<th>Body weight gain (kg)</th>
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<td>3.41</td>
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<td>67</td>
<td>34</td>
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<td>3.43</td>
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</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.08</td>
<td>6.05</td>
<td>12.4</td>
</tr>
</tbody>
</table>

1 Protein storage (g/d)=Potassium accumulated (mmol/d)×2.15×6.25.
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.

\[
\text{Protein storage (g/d)} = \frac{\text{potassium accumulated (mmol/d)}}{2.15} \times 6.25
\]

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

\[
\text{EAR} = \frac{0.78 \times 12.6}{0.70} = 14.04 \text{ g/d}
\]

and adopted as 15 g/d according to the rounding off process employed. The additional requirement for lactating women (RDA)

<table>
<thead>
<tr>
<th>Age</th>
<th>EAR</th>
<th>RDA</th>
<th>AI</th>
<th>UL</th>
<th>EAR</th>
<th>RDA</th>
<th>AI</th>
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</thead>
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**Table 3. DRIs for protein (g/d).**

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

REFERENCES


26) Young VR, Fajardo L, Murray E, Rand WM, Scrimshaw NS. 1975. Protein requirements of man: comparative nitrogen balance response within the submaintenance-to-maintenance range of intakes of wheat and beef pro-


