

Protein

1. Background Information

1-1. Definitions and Classifications

Proteins are made up of 20 different amino acids linked by peptide bonds. They are important components of organisms, and their types differ depending on the number and types of amino acids, and the sequence of the peptide bonds. Many types of proteins exist, ranging from those with a molecular weight of around 4,000 to viral proteins with molecular weights from several tens of millions to hundreds of millions. Proteins with a small number of amino acids linked by peptide bonds are referred to as peptides. Proteins are composed of 20 different amino acids, which are directly encoded by codons. Of these 20 amino acids, humans can synthesize 11 from other amino acids or intermediate metabolites. The remaining nine amino acids must be consumed from diet, and are referred to as essential amino acids. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

2. To Avoid Inadequacy

2-1. Factors to be Considered in Estimating Requirements

In the previous versions, the DRIs for proteins were calculated on the basis of the amount required to maintain nitrogen balance. To calculate the DRIs for proteins, using the nitrogen balance method, the following must be considered: (1) technical difficulties, (2) metabolic adaptations associated with changes in protein intakes, (3) the protein-sparing effect of energy, (4) lifestyle, and (5) interindividual variability.

2-1-1. Technical Difficulties Associated with the Nitrogen Balance Method

The nitrogen balance method requires all nitrogen intakes, and nitrogen excretion to be accurately quantified. However, as it is difficult to collect data on all foods that were not consumed, such as spilled food and food left on the plate, it is likely that the nitrogen intake may be overestimated. Nitrogen is excreted from the body primarily through urine and feces; however, it can also be eliminated via various bodily secretions such as the skin, sweat, desquamation, hair, and nails. This total excretion is more likely to be underestimated than overestimated. This overestimation of protein intake and underestimation of protein excretion may mistakenly produce a positive nitrogen balance. Therefore, this erroneous calculation may lead to the underestimation of protein or amino acid requirements.

2-1-2. Metabolic Changes Associated with Changes in Protein Intake

When the amount of dietary protein intake is changed, a certain amount of time must normally be given until the change is adapted. This is not only because the human metabolism requires time to adapt to new protein intake, but also because the body's urea pool needs to adjust to the change in protein intake. The urea pool expands or shrinks with increases or

decreases in protein intake with a half-life of approximately 8–12 hours. It takes more than 48 hours for the pool to reach its new size. During this time, urea nitrogen emissions cannot be used as indicators of amino acid oxidation.

In 1985, a joint report by the FAO/WHO/UNU concluded that major adjustments are complete within the first five to seven days, in each sex and age group. On the basis of this conclusion, nitrogen balance studies that allow 1 week or less for adjustments are less likely to produce reliable data; thus, studies that allow a dietary adjustment period of 1–3 weeks must be used.

2-1-3. Protein-Sparing Effect of Energy

Protein utilization efficiency changes with intakes of protein, amino acids, and total nitrogen. Protein metabolism is also affected by intakes of nutrients other than nitrogen compounds. The effect of energy intake on protein metabolism has long been known as the sparing protein effect⁽¹⁾. Energy deficiency reduces protein utilization efficiency, whereas increased energy intake improves nitrogen balance⁽²⁾. The promotion of protein synthesis, and suppression of decomposition due to the increased secretion of insulin contribute to this. In addition, a report on nitrogen balance in adults (361 individuals) revealed a significant positive correlation between energy intake and nitrogen balance⁽³⁾. Moreover, a previous experiment regarding protein requirement found that energy balance tended to be positive, and that protein requirement was underestimated. However, it has recently become possible to successfully measure protein requirement in a state of energy equilibrium.

2-1-4. Lifestyle Behavior

2-1-4-1. Physical Activity and Exercise

In individuals with a large intake, who actively engage in activities, protein requirements can easily be met, and they may not need to worry about the quality of the proteins consumed. However, inactive and elderly individuals are prone to protein or other nutrient deficiencies if they do not pay attention to their diet. Lack of exercise leads to body protein catabolism, while appropriate exercise enhances the use of dietary proteins. Strenuous exercise enhances the breakdown of proteins. Therefore, protein requirement follows a U-shape, depending on the exercise intensity⁽⁴⁾. Some studies in children and adults reported that appropriate exercise promotes growth, and enhances the use of dietary proteins^(5,6).

Transdermal nitrogen loss generally increases due to perspiration during exercise, resulting in the hypercatabolism of amino acids, and decreased synthesis and increased decomposition of proteins by the body. However, once exercise ends, the synthesis of proteins by the body exceeds their decomposition, and the lost nitrogen is often regained. Protein requirements reportedly do not increase when mild or moderate exercise (200–400 kcal/day) is performed^(7,8).

2-1-4-2. Rest and Stress

Daily stress is only addressed in reports on nitrogen balance tests after 48 hours of sleep deprivation, and at the end of the academic year, in university students. The quantitative impact of mild stress on nitrogen balance is not clear. Moreover, daily stress also has an effect on the participants of nitrogen balance tests, and because this effect is already included in the amount required to maintain nitrogen balance, it was decided not to estimate a safety margin for stress.

2-1-4-3. Smoking and Drinking

Smoking causes cellular free-radical damage, while drinking, both directly and indirectly, affects metabolism. However, the quantitative relationship between protein requirement, and smoking or drinking is unclear.

2-1-5. Interindividual Variability

A wide range (10%–40%) of amounts required to maintain nitrogen balance has been reported to date. This range of variability includes intraindividual variability and researcher-related variability, such as experimental conditions and experimental errors, in addition to interindividual variability. According to the results of the data analysis of 235 participants from 19 studies, inter-researcher variability accounted for 40% of the observed variability, while the remaining 60% was due to intra-researcher variability⁽⁹⁾. Furthermore, the outcomes of repeating the measurements in the same participants revealed that two-third of the intra-researcher variability was intraindividual variability, and one-third was true interindividual variability. The coefficient of variation was 12%. However, the coefficient of variation was set at 12.5%, in light of the bias in the variation curve. This was used as the basis for setting an RDA calculation coefficient of 1.25, when calculating the RDA from the EAR.

2-2. Methods Used to Set the Estimated Average Requirement and Recommended Dietary Allowances

2-2-1. Adults

The protein maintenance requirement of high-quality (animal) proteins measured in the nitrogen balance experiment was used as a basis for calculating the reference value for the estimated average requirement (EAR), corrected for the digestive efficiency of mixed proteins in everyday meals. Interindividual variability was then added to this in order to calculate the recommended dietary allowance (RDA). To assess the quality of everyday mixed proteins, amino acid intake was calculated from protein intake and the amino acid compositions of each food group presented in the results of the 2010 and 2011 National Health and Nutrition Survey⁽¹⁰⁾. This amino acid score exceeds 100 even if the 1973 FAO/WHO⁽¹¹⁾ amino acid scoring patterns, the 1985 FAO/WHO/UNU⁽¹²⁾ amino acid scoring patterns, and the 2007 FAO/WHO/UNU⁽¹³⁾ scoring patterns are used as reference. Therefore, no quality correction is necessary.

When the values of the 17 studies that examined the nitrogen balance maintenance dose of high-quality (animal) protein were averaged, the protein maintenance requirement was

determined to be 0.65 g/kg body weight (BW)/day (104 mg nitrogen/kg BW/day)^(14–28). Consequently, this value was decided to be the protein maintenance requirement (Table 1).

A study that measured the digestive efficiency of everyday mixed proteins in women (12 individuals) reported an average efficiency of 92.2%⁽²⁴⁾. In addition, the result for men (6 individuals) was 95.4%⁽²⁹⁾. The digestive efficiency of everyday mixed proteins was, therefore, set at 90%, and the EAR was calculated with the following formula. The RDA was the value for EAR multiplied by an RDA calculation coefficient of 1.25, assuming an interindividual coefficient of variation of 12.5%.

Reference value for EAR calculation (g/kg BW/day) = protein maintenance requirement ÷ digestive efficiency = 0.65 ÷ 0.90 = 0.72

EAR (g/day) = reference value for EAR calculation (g/kg BW/day) × reference BW (kg)

RDA (g/day) = EAR (g/day) × RDA calculation coefficient

2-2-2. Elderly Individuals

During adulthood, physiological functions such as maximum voluntary ventilation, renal blood flow, and vital capacity decline, skeletal muscle mass tends to decrease, and body fat mass tends to increase due to aging. While muscle protein metabolism decreases, visceral protein metabolism remains largely unchanged. Decreases in protein metabolic turnover and physiological functions are thought to influence protein utilization efficiency in the elderly. However, some reports found that there were no differences between the EAR of protein among elderly individuals and that among young adults (aged 18–31 years)⁽⁹⁾. Elderly individuals generally have inactive daily lives, and, thus, have a low dietary intake, and often experience a loss of appetite. These differences in lifestyle are also thought to influence the EAR of protein.

The mean value of the amount of nitrogen balance maintenance observed in healthy elderly individuals, under normal dietary intake conditions, was considered as the reference value in the determination of the EAR.

Of the reports that examined protein reference values for EAR calculation in elderly individuals, a pooled analysis was conducted using 144 data on the nitrogen balance of 60 participants, from five studies, that presented the nitrogen balance results of individuals^(28,30–33). The average value (0.85 g/kg BW/day [136 mg nitrogen/kg BW/day]) obtained from this pooled analysis was set as the reference value for the EAR calculations (Figure 1). However, the digestion-absorption rate of mixed proteins was 90% with this reference value, which is the rate obtained after correcting the other nitrogen loss values, using the actual values and 5 mg/kg BW/day. Moreover, the RDA was calculated by multiplying the EAR and an RDA calculation coefficient of 1.25, assuming that the interindividual coefficient of variation was the same as that of adults (12.5%).

EAR (g/day) = reference value for EAR calculation (g/kg BW/day) × reference BW (kg)

RDA (g/day) = EAR (g/day) × RDA calculation coefficient

A significant amount of elderly individuals living in nursing homes or receiving home

care are undernourished and exhibit a negative nitrogen balance⁽³⁴⁾. Moreover, frailty is frequently observed in elderly individuals with decreased protein intakes⁽³⁵⁾. When the PAL drops, the protein metabolism of the skeletal muscle decreases, which in turn leads to increases in the EAR of protein. Since the EAR of protein also increases when the energy intake is low, a different amount of protein replenishment than that for healthy individuals needs to be considered for such elderly individuals.

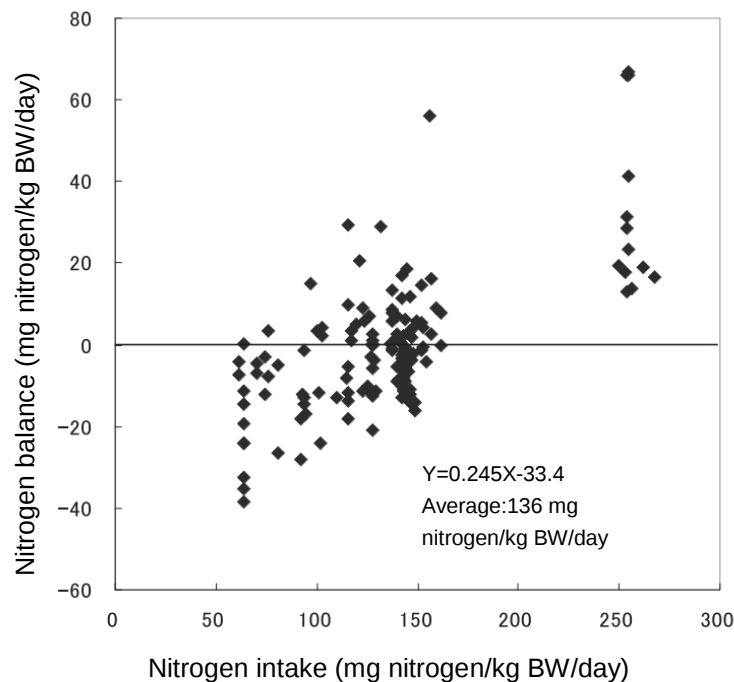


Figure 1 The nitrogen balance of elderly individuals (from 5 studies' results) ^(23,30–33)

2-2-3. Children

The reference value for EAR, in children aged 1–17 years, was calculated using the additive factor method, from the protein maintenance requirement and protein depositions accumulated with growth (Table 2). The utilization efficiency is the protein utilization efficiency during weight maintenance. The EAR was calculated by multiplying the reference value and reference BW. The RDA was calculated by multiplying the EAR and an RDA calculation coefficient of 1.25, assuming the interindividual coefficient of variation was the same as that of adults (12.5%).

Reference value for EAR calculation (g/kg BW/day) = (protein maintenance requirement ÷ utilization efficiency) + (protein deposition ÷ deposition efficiency)

EAR (g/day) = reference value for EAR calculation (g/kg BW/day) × reference BW (kg)

RDA (g/day) = EAR (g/day) × RDA calculation coefficient

In order to obtain the protein maintenance requirement, the mean value (0.67 g/kg BW/day [107 mg nitrogen/kg BW/day]) obtained from the nitrogen balance method results^(36–41) of growing infants (9–62 months), children (8–9 years), and adolescents (12–14 years) was

used (Table 3). However, the nitrogen loss via routes other than urine and feces was set at 6.5 ± 2.3 mg nitrogen/kg BW/day (5–9 mg nitrogen/kg BW/day) on the basis of currently available reports^(36,42–45), and the above-stated maintenance requirement was calculated using this value. Given that no evidence was found stating that the maintenance requirement differs according to the developmental stage (infancy, childhood, and adolescence), this value was used for children of all ages.

Protein deposition was calculated as the amount of accumulated protein, in accordance with growth, using the increase in reference BW in each child age group, and the ratio of body protein to reference BW. The ratio of body protein to a child's BW was calculated on the basis of body composition values from birth to age 10 years⁽⁴⁶⁾, from ages 4 months to 2 years⁽⁴⁷⁾, and from ages 4 to 18 years⁽⁴⁸⁾.

For utilization efficiency, the results of 9–14-month-old infants (with a utilization efficiency of 70% and deposition efficiency of 40% for weight maintenance in 1-year-old infants)⁽³⁶⁾ were used. In addition, the deposition efficiency was considered as 40% throughout childhood, and the utilization efficiency for weight maintenance was considered to be close to the value for adults, in accordance with growth (90%).

Furthermore, values were rounded considering the importance of protein intake in children.

2-2-4. Additional Amount for Pregnant Women

The body protein deposition during pregnancy can be calculated indirectly from an increase in body potassium. The average increase in body potassium in the late-stage of pregnancy is 2.08 mmol/day^(49–52). Using a potassium-to-nitrogen ratio of 2.15 mmol potassium/g nitrogen⁽⁴⁹⁾ and protein conversion factor of 6.25, the body protein deposition was calculated using the following formula.

$$\text{Protein deposition (g/day)} = \text{body potassium increment} \div 2.15 \times 6.25$$

When calculating body protein depositions, changes due to weight gain during pregnancy need to be taken into account. More specifically, the final weight gain was set at 11 kg⁽⁵³⁾, and the weight gains of participants during pregnancy, in various studies, were corrected to find the increase in body potassium, in each study. Accordingly, the body protein deposition was calculated as shown in Table 4.

Based on a report which stated that the protein deposition ratio of the first, second and third trimesters was 0:1:3.9⁽⁵²⁾, the total body protein deposition was found for the second and third trimesters if observations had been made during those trimesters in the report (280 days of pregnancy multiplied by 2/3) and the body protein deposition per day was calculated for each trimester after allocating depositions to the second and third trimesters using the simple ratio stated above.

The depositions calculated by the simple averaging of the values obtained from each study were 0 g/day in the early-stage, 1.94 g/day in the mid-stage, and 8.16 g/day in the late-

stage. The protein deposition efficiency was set at 43%⁽⁴⁹⁾. Using these values, the additional amounts required during pregnancy (EAR) were set at $0 \text{ g/day} \div 0.43 = 0 \text{ g/day}$ (rounded to 0 g/day) in the early-stage, $1.94 \text{ g/day} \div 0.43 = 4.51 \text{ g/day}$ (rounded to 5 g/day) in the mid-stage, and $8.16 \text{ g/day} \div 0.43 = 18.98 \text{ g/day}$ (rounded to 20 g/day) in the late-stage. The additional amount (RDA) was 0 g/day (rounded to 0 g/day) in the early-stage, 5.64 g/day (rounded to 10 g/day) in the mid-stage, and 23.73 g/day (rounded to 25 g/day) in the late-stage. The RDA values were obtained by multiplying the EAR and a RDA calculation coefficient of 1.25, assuming an interindividual coefficient of variation of 12.5%.

2-2-5. Additional Amount for Lactating Women (EAR, RDA)

A substantial portion of the protein accumulated during pregnancy is lost during delivery. However, some of the protein accumulated in the body remains in the mother. Protein is also lost due to weight loss and lactation during the puerperal period. It was, therefore, decided that the amount of protein added is offset against pregnancy-related residual deposits of protein and residual weight gain. Thus, the amount of protein added during the lactation period is only the amount added for lactation.

The average lactation yield per day, given a child is only fed breast milk for the first 6 months until weaning, was set at 0.78 L/day ^(54–60), and the average protein concentration of breast milk during this period was set at 12.6 g/L ^(55,56,61–66). The conversion efficiency from dietary protein to breast milk protein was set at 70% on the basis of Report 1 by the FAO/WHO/UNU in 1985⁽¹²⁾. The additional amount required by lactating women (EAR) was rounded to 15 g/day using these values ($12.6 \text{ g/L} \times 0.78 \text{ L/day} \div 0.70 = 14.04 \text{ g/day}$). The additional amount (RDA) was set at 17.6 g/day (rounded to 20 g/day) by multiplying the EAR and a RDA calculation coefficient of 1.25, assuming an interindividual coefficient of variation of 12.5%.

2-3. Methods Used to Set Adequate Intake

2-3-1. Infants

Since protein requirement cannot be determined using the nitrogen balance method in infants, as is done in adults, it is calculated from the amount of protein contained in the infant formulas and breast milk consumed by healthy infants. This requirement was, therefore, determined based on the concept of adequate intake (AI). Moreover, there is no scientific evidence on the utilization efficiency of protein from infant formulas. The setting of DRIs for protein in artificially fed infants was, therefore, postponed, and a reference value was provided instead.

When infants enter the weaning period, they start to consume protein from sources other than breast milk, and, consequently, a different calculation method of DRIs for protein is used. Therefore, AI was determined for infancy in 3 stages into which the infancy period was divided: 0–5 months, 6–8 months, and 9–11 months.

2-3-1-1. Infants Aged 0–5 Months

No studies have reported on the protein deficiency caused by lactation in infants aged 0–5 months. Therefore, the AI is calculated from the milk intake and the protein concentration of breast milk. In terms of the milk intake among infants, no clear differences were observed between the values in Japan and those in other countries, at 0.63–0.86 L/day^(54–60), so a mean of 0.78 L/day was used. The protein concentration of breast milk is also not considered to differ between races^(55,57,61–66). Thus, the average protein concentration of breast milk during this period was set at 12.6 g/L.

$$\text{AI (g/day)} = 12.6 \text{ (g/L)} \times 0.78 \text{ (L/day)} = 9.83$$

2-3-1-2. Infants Aged 6–8 Months

Once infants enter the weaning period, their nutrient intake greatly changes. The protein intake from baby foods, in infants aged 6–8 months, was estimated at 6.1 g/day, based on studies in Japanese people⁽⁶⁷⁾. Meanwhile, the average milk intake of infants during this period was set at 0.60 L/day^(56,57), and the protein concentration of breast milk was set at 10.6 L/day^(56,61,63). The AI of protein from breast milk, and sources other than breast milk can, therefore, be calculated as follows:

$$\text{AI (g/day)} = \text{protein concentration of breast milk} \times \text{average milk intake} + \text{amount of protein from baby foods other than breast milk} = 10.6 \text{ (g/L)} \times 0.60 \text{ (L/day)} + 6.1 \text{ (g/day)} = 12.5$$

2-3-1-3. Infants Aged 9–11 Months

The protein intake from baby foods, in infants aged 9–11 months, was estimated at 17.9 g/day, based on studies in Japanese people^(67,68). Meanwhile, the average milk volume of infants during this period was set at 0.45 L/day^(56,57), and the protein concentration of breast milk was set at 9.2 L/day^(56,61–63). Therefore, the AI of protein from breast milk, and sources other than breast milk can be calculated as follows.

$$\text{AI (g/day)} = \text{protein concentration of breast milk} \times \text{average milk intake} + \text{amount of protein from baby foods other than breast milk} = 9.2 \text{ (g/L)} \times 0.45 \text{ (L/day)} + 17.9 \text{ (g/day)} = 22.0$$

2-3-1-4. Artificially fed Infants

The DRIs for protein, in artificially-fed infants, was provided as a reference value, taking into account the utilization efficiency of protein from infant formulas. The AI reference value for artificially fed infants was calculated as follows, assuming the utilization efficiency of protein from infant formulas to be 70% of that of breast milk⁽¹²⁾.

$$\text{0–5 months (g/day): } 12.6 \text{ (g/L)} \times 0.78 \text{ (L/day)} \div 0.70 = 14.0$$

$$\text{6–8 months (g/day): } 10.6 \text{ (g/L)} \times 0.60 \text{ (L/day)} \div 0.70 + 6.1 \text{ (g/day)} = 15.2$$

$$\text{9–11 months (g/day): } 9.2 \text{ (g/L)} \times 0.45 \text{ (L/day)} \div 0.70 + 17.9 \text{ (g/day)} = 23.8$$

3. Avoiding Excessive Intake

3-1. Determining the UL (Tolerable Upper Intake Level)

The UL of protein must be determined based on the adverse health events caused by the excessive intake of protein. However, at present, there are an insufficient number of reports providing clear evidence on the determination of the UL of protein. Therefore, a UL for protein was not set.

4. Preventing the Development and Progression of Life-style Related Diseases (LRDs)

The development and progression of LRDs (hypertension, dyslipidemia, diabetes, and chronic kidney disease) are the result of the interaction between environmental factors (lifestyle) and genetic ones. Providing optimal nutrition may be highly significant in preventing the development or progression of these LRDs.

4-2. Methods Used to Set the DG (Tentative Dietary Goal for Preventing LRDs)

The methods used to set the DG are summarized in “Energy Providing Nutrients’ Balance.”

DRIs for Proteins

(EAR, RDA, AI: g/day, DG (median): % energy)

Gender	Males				Females			
Age etc.	EAR	RDA	AI	DG ¹ (Median ²)	EAR	RDA	AI	DG ¹ (Median ²)
0-5 months *	—	—	10	—	—	—	10	—
6-8 months *	—	—	15	—	—	—	15	—
9-11 months *	—	—	25	—	—	—	25	—
1-2 years	15	20	—	13-20(16.5)	15	20	—	13-20(16.5)
3-5 years	20	25	—	13-20(16.5)	20	25	—	13-20(16.5)
6-7 years	25	35	—	13-20(16.5)	25	30	—	13-20(16.5)
8-9 years	35	40	—	13-20(16.5)	30	40	—	13-20(16.5)
10-11 years	40	50	—	13-20(16.5)	40	50	—	13-20(16.5)
12-14 years	50	60	—	13-20(16.5)	45	55	—	13-20(16.5)
15-17 years	50	65	—	13-20(16.5)	45	55	—	13-20(16.5)
18-29 years	50	60	—	13-20(16.5)	40	50	—	13-20(16.5)
30-49 years	50	60	—	13-20(16.5)	40	50	—	13-20(16.5)
50-69 years	50	60	—	13-20(16.5)	40	50	—	13-20(16.5)
70+ years	50	60	—	13-20(16.5)	40	50	—	13-20(16.5)
Pregnant women (additional) Early-stage Mid-stage Late-stage					+0 +5 +20	+0 +10 +25	—	—
Lactating women (additional)					+15	+20	—	—

* AIs for infants are values for breast-fed children.

¹ Ranges are expressed as approximate values.

² Medians indicate the median values for the given range. They do not indicate most desirable values.

References

1. Munro HN (1951) Carbohydrate and Fat as Factors in Protein Utilization and Metabolism. *Physiol Rev* **31**, 449–488.
2. Kishi K, Inoue G, Yoshimura Y, et al. (1983) Quantitative interrelationship between effects of nitrogen and energy intakes on egg protein utilization in young men. *Tokushima J Exp Med* **30**, 17–24.
3. Pellet P & Young V (1991) The effects of different levels of energy intake on protein metabolism and of different levels of protein intake on energy metabolism: A statistical evaluation from the published literature. In *Protein-Energy Interact (International Diet Energy Consult Group)*, pp. 81–136. UNU.
4. Millward D., Bowtell J., Pacy P, et al. (1994) Physical activity, protein metabolism and protein requirements. *Proc Nutr Soc* **53**, 223–240.
5. Young VT, Munro HN, Matthews DE, et al. (1983) Relationship of energy metabolism to protein metabolism. In *New Aspects of Clinical Nutrition*, pp. 43–73. Basel: Karger.
6. Calloway D (1982) Energy-protein relationships. In *Protein Qual humans Assess Vit Estim*, pp. 148–168 [Bodwell C, Adkins J, Hopkins D, editors]. Westport, Connecticut: Avi Publishing Company.
7. Kido Y, Tsukahara T, Rokutan K, et al. (1997) Japanese dietary protein allowance is sufficient for moderate physical exercise in young men. *J Nutr Sci Vitaminol* **43**, 59–71.
8. Kido Y, Tsukahara T, Rokutan K, et al. (1997) Recommended daily exercise for Japanese does not increase the protein requirement in sedentary young men. *J Nutr Sci Vitaminol* **43**, 505–14.
9. Rand WM, Pellett PL & Young VR (2003) Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am J Clin Nutr* **77**, 109–27.
10. 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.
11. FAO/WHO. (1973) *Energy and protein requirements*. WHO Technical Report Series, 522. Geneva: .
12. FAO/WHO/UNU. (1985) *Energy and protein requirements*. WHO Technical Report Series, 724. Geneva.: .
13. FAO/WHO/UNU. (2007) *Protein and amino acid requirements in human nutrition*. WHO Technical Report Series, 935. Geneva: WHO.
14. Bourges H & Lopez Castro BR (1982) Protein requirements of young adult men fed a Mexican rural diet. *Arch Latinoam Nutr* **32**, 630–649.
15. Egaña JI, Uauy R, Cassorla X, et al. (1992) Sweet lupin protein quality in young men. *J Nutr* **122**, 2341–7.

16. Wayler A, Queiroz E, Scrimshaw NS, et al. (1983) Nitrogen balance studies in young men to assess the protein quality of an isolated soy protein in relation to meat proteins. *J Nutr* **113**, 2485–2491.
17. Yáñez E, Uauy R, Ballester D, et al. (1982) Capacity of the Chilean mixed diet to meet the protein and energy requirements of young adult males. *Br J Nutr* **47**, 1–10.
18. Young VR, Taylor YS, Rand WM, et al. (1973) Protein requirements of man: efficiency of egg protein utilization at maintenance and submaintenance levels in young men. *J Nutr* **103**, 1164–74.
19. Young VR, Fajardo L, Murray E, et al. (1975) Protein requirements of man: comparative nitrogen balance response within the submaintenance-to-maintenance range of intakes of wheat and beef proteins. *J Nutr* **105**, 534–42.
20. Young VR, Puig M, Queiroz E, et al. (1984) Evaluation of the protein quality of an isolated soy protein in young men: Relative nitrogen requirements and effect of methionine supplementation. *Am J Clin Nutr* **39**, 16–24.
21. Huang P-C & Po A (1982) Protein requirements of young Chinese male adults on ordinary Chinese mixed diet and egg diet at ordinary levels of energy intake. *J Nutrition* **112**, 897–907.
22. Inoue G, Fujita Y & Niiyama Y (1973) Studies on protein requirements of young men fed egg protein and rice protein with excess and maintenance energy intakes. *J Nutr* **103**, 1673–87.
23. Inoue G, Takahashi T, Kishi K, et al. (1981) The evaluation of soy protein isolate alone and in combination with fish in adult Japanese men. In *Protein-energy requirements of developing countries: evaluation of new data: Report of a working group*, p. 77–87. [Torun B, Young V, Rand W, editors]. Tokyo: United Nations University.
24. Kaneko K & Koike G (1985) Utilization and requirement of egg protein in Japanese women. *J Nutr Sci Vitaminol* **31**, 43–52.
25. Komatsu T, Kishi K, Yamamoto T, et al. (1983) Nitrogen requirement of amino acid mixture with maintenance energy in young men. *J Nutr Sci Vitaminol* **29**, 169–85.
26. Scrimshaw NS, Wayler AH, Murray E, et al. (1983) Nitrogen balance response in young men given one of two isolated soy proteins or milk proteins. *J Nutr* **113**, 2492–7.
27. Tontisirin K, Sirichakawal P & Valyasevi A (1981) Protein requirements of adult Thai males. In *Protein-energy requirements of developing countries: evaluations of new data*, pp. 88–97 [Torun B, Young V, Rand W, editors]. Tokyo: United Nations University.
28. Uauy R, Scrimshaw NS & Young VR (1978) Human protein requirements: Nitrogen balance response to graded levels of egg protein in elderly men and women. *Am J Clin Nutr* **31**, 779–785.
29. Higaki J, Tsukahara M, Kido Y, et al. (1989) Bioavailability of protein in usual-mixed meals among Japanese subjects (in Japanese). *J Japan Soc Nutr Food Sci* **43**, 192.

30. Cheng AH, Gomez A, Bergan JG, et al. (1978) Comparative nitrogen balance study between young and aged adults using three levels of protein intake from a combination wheat-soy-milk mixture. *Am J Clin Nutr* **31**, 12–22.
31. Gersovitz M, Motil K, Munro HN, et al. (1982) Human protein requirements: assessment of the adequacy of the current recommended dietary allowance for dietary protein in elderly men and women. *Am J Clin Nutr* **35**, 6–14.
32. Campbell WW, Crim MC, Dallal GE, et al. (1994) Increased protein requirements in elderly people: new data and retrospective reassessments. *Am J Clin Nutr* **60**, 501–509.
33. Castaneda C, Charnley JM, Evans WJ, et al. (1995) Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. *Am J Clin Nutr* **62**, 30–9.
34. Ebisawa H, Ohzeki T, Ichikawa M, et al. (1992) Protein intake for maintenance of nitrogen balances in the elderly (in Japanese). *Reports Res Comm Essent Amin Acids* **136**, 9–12.
35. Kobayashi S, Asakura K, Suga H, et al. (2013) High protein intake is associated with low prevalence of frailty among old Japanese women: A multicenter cross-sectional study. *Nutr J* **12**, 164.
36. Huang PC, Lin CP & Hsu JY (1980) Protein requirements of normal infants at the age of about 1 year: maintenance nitrogen requirements and obligatory nitrogen losses. *J Nutr* **110**, 1727–1735.
37. Intengan C, Roxas B, Loyola A, et al. (1981) Protein requirements of Filipino Children 20 to 29 months old consuming local diets. In *Protein-energy requirements of developing countries: Evaluation of new data*, pp. 172–181 [Torun B, Young V, Rand W, editors]. Tokyo: United Nations University.
38. Torun B, Cabrera-Santiago M & Viteri F (1981) Protein requirements of pre-school children : Milk and soybean protein isolate. In *Protein-energy requirements of developing countries: Evaluation of new data*, pp. 182–190 [Torun B, Young V, Rand W, editors]. Tokyo: United Nations University.
39. Egana M, Fuenes A & Uauy R (1984) Protein needs of chilean pre-school children fed milk and soy protein isolate diets. In *Protein-energy-requirement studies in developing countries: Results of international*, pp. 249–257 [Rand W, Uauy R, Scrimshaw N, editors]. Tokyo: United Nations University.
40. Intengan C (1984) Protein requirements of Filipino children 20-29 months old consuming local diets. In *Protein-energy-requirement studies in developing countries: Results of international*, pp. 258–264 [Torun B, Young V, Rand W, editors]. Tokyo: United Nations University.
41. Gattás V, Barrera GA, Riumallo JS, et al. (1992) Protein-energy requirements of boys 12-14 y old determined by using the nitrogen-balance response to a mixed-protein diet. *Am J Clin Nutr* **56**, 499–503.

42. Howat PM, Korslund MK, Abernathy RP, et al. (1975) Sweat nitrogen losses by and nitrogen balance of preadolescent girls consuming three levels of dietary protein. *Am J Clin Nutr* **28**, 879–882.
43. Korslund MK, Leung EY, Meiners CR, et al. (1976) The effects of sweat nitrogen losses in evaluating protein utilization by preadolescent children. *Am J Clin Nutr* **29**, 600–603.
44. Viteri F & Martinez C (1981) Integumental nitrogen losses of pre-school children with different levels and sources of dietary protein intake. In *Protein-energy requirements of developing countries: Evaluation of new data*, pp. 164–168 [Torun B, Young V, Rand W, editors]. Tokyo: United Nations University.
45. Torun B & Viteri F (1981) Obligatory nitrogen losses and factorial calculations of protein requirements of pre-school children. In *Protein-energy requirements of developing countries: Evaluation of new data*, pp. 159–163 [Torun B, Young V, Rand W, editors]. Tokyo: United Nations University.
46. Fomon SJ, Haschke F, Ziegler EE, et al. (1982) Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* **35**, 1169–1175.
47. Butte NF, Hopkinson JM, Wong WW, et al. (2000) Body composition during the first 2 years of life: an updated reference. *Pediatr Res* **47**, 578–85.
48. Ellis KJ, Shypailo RJ, Abrams SA, et al. (2000) The reference child and adolescent models of body composition. A contemporary comparison. *Ann N Y Acad Sci* **904**, 374–382.
49. King JC, Howes Galloway D & Margen S (1973) Nitrogen retention, total body 40 K and weight gain in teenage pregnant girls. *J Nutr* **103**, 772–785.
50. Pipe NG, Smith T, Halliday D, et al. (1979) Changes in fat, fat-free mass and body water in human normal pregnancy. *Br J Obs Gynaecol* **86**, 929–940.
51. Forsum E, Sadurskis A & Wager J (1988) Resting metabolic rate and body composition of healthy Swedish women during pregnancy. *Am J Clin Nutr* **47**, 942–947.
52. Butte NF, Ellis KJ, Wong WW, et al. (2003) Composition of gestational weight gain impacts maternal fat retention and infant birth weight. *Am J Obstet Gynecol* **189**, 1423–1432.
53. Takimoto H, Sugiyama T, Fukuoka H, et al. (2006) Maternal weight gain ranges for optimal fetal growth in Japanese women. *Int J Gynecol Obstet* **92**, 272–278.
54. Takai T, Hisahara Y, Aise T, et al. (1968) Observation of ad libitum feeding of breast milk and infant formula (second report) (in Japanese). *J Jpn Pediatr Soc* **72**, 1583–1584.
55. Allen JC, Keller RP, Archer P, et al. (1991) Studies in human lactation - Milk-composition and daily secretion rates of macronutrients in the 1st year of lactation. *Am J Clin Nutr* **54**, 69–80.

56. Nommsen LA, Lovelady CA & Heinig J (1991) Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. *Am J Clin Nutr* **53**, 457–465.
57. Yoneyama K (1998) Growth of breast-fed infants and intake of nutrients from breast-milk (in Japanese). *J Child Heal* **57**, 49–57.
58. Kitamura K, Ochiai F, Shimizu Y, et al. (2002) Sequential change in breast milk composition (in Japanese). *Japanese J Matern Heal* **43**, 493–499.
59. 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.
60. 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.
61. Yamamoto Y, Yonekubo M, Iida K, et al. (1981) Studies on Japanese breast milk composition (first report) — Major nutrient and mineral composition— (in Japanese). *J child Heal* **40**, 468–475.
62. Itoda T, Sakurai T, Ishiyama Y, et al. (1991) The latest survey for the composition of human milk obtained from Japanese mothers. Part I. The contents of gross components and minerals (in Japanese). *Japanese J Pediatr Gastroenterol Nutr* **5**, 145–158.
63. Yoneyama K, Goto I & Nagata H (1995) Changes in the concentrations of nutrient components of human milk during lactation (in Japanese). *Japanese J public Heal* **42**, 472–481.
64. Isomura H (2007) Analysis of breast milk composition: About latest anlysis of breast milk of Japanese mothers (in Japanese). *Obstet Gynecol Pract* **56**, 305–313.
65. Dewey KG & Lönnerdal B (1983) Milk and nutrient intake of breast-fed infants from 1 to 6 months: relation to growth and fatness. *J Pediatr Gastroenterol Nutr* **2**, 497–506.
66. Butte NF, Garza C, Smith EO, et al. (1984) Human milk intake and growth in exclusively breast-fed infants. *J Pediatr* **104**, 187–95.
67. Nakano T, Kato K, Kobayashi N, et al. (2003) Nutrient intake from baby foods infant formula and cow's milk -results from a nation wide infant's dietary survey- (in Japanese). *J Child Heal* **62**, 630–9.
68. Hokama T, Asato Y & Nakazato S (1998) Iron intake from baby foods in Nakagusuku-son in Okinawa, Part II. The result of nutrition survey of later-term of baby food introduction (in Japanese). *J Child Heal* **57**, 45–48.