

of intake using the AI should consider that intake equal to or above the AI poses nearly 0% risk of inadequacy. Even if intake is below AI, risk of inadequacy cannot, by its nature, be quantitatively judged. As the UL is used for preventing excessive intake, an intake above the UL is evaluated as excessive. DGs are used for primary prevention of lifestyle-related diseases. However, as lifestyle-related diseases have many causes, dietary improvement by adherence to DGs should not be overly emphasized.

1-3. Development and use of dietary improvement plans (Table 3). Planning for dietary improvement consists of evaluation of nutrient intake by dietary assessment and implementation of dietary changes based on the results. However, because conducting these procedures is often difficult, several compromises may be taken into consideration according to the situation. For assessment of insufficient or excessive intake of energy, BMI or body weight change should be used, planning should be focused on maintaining a normal range of BMI, and measurement should be performed at least twice within several months (at least twice a year) and reviewed using changes in body weight as indices. For assessment of nutrient intake, the RDA should be used. If intake is close to or above the RDA, planning should aim to maintain this intake, and if intake is below the RDA, it should aim to approach the RDA. The AI should be used for assessment of nutrients for which the AI has been established. If intake is close to or above the AI, it should be maintained, and if below the AI, it should be increased to approach the AI. As intake above the UL should strictly be avoided, a plan for the reduction of the intake of any nutrient whose intake is above the UL should be promptly developed and implemented. If intake is out of a range of a DG, the goal of planning should be to come within the range.

While conducting such planning, comprehensive consideration of other nutrition- and non-nutrition-related factors associated with lifestyle-related diseases, as well as the sustainability of a particular plan over many years, as prevention of lifestyle-related diseases is a life-long endeavor, is recommended.

2. *Dietary improvement of groups*

2-1. Basic concepts. The basic concepts in applying the DRIs for dietary improvement of a group are shown in Table 4. These concepts are based on DRIs of the United States and Canada (1, 2, 33) and the application patterns of the DRIs in Japan. The following 3 procedures are important in these concepts: assessment of dietary intake, development of a plan for dietary improvement based on the results of the assessment, and implementation of the plan for dietary improvement.

2-2. Dietary assessment (Table 4). For assessment of insufficient or excessive intake of energy, the BMI should be used. Energy is calculated from the distribution of the percentage of individuals within and outside the range of normal BMI, defined by the Japan Society for the Study of Obesity as BMI between 18.5 and 25.0 (32). For determination of nutrient intake, the distribution of nutrient intake as obtained from dietary assessment is used. Such assessment should be performed

with full understanding of measurement errors, especially those due to under- and over-reporting and day-to-day variation.

For nutrients for which the EAR has been established, the percentage of individuals for whom intake is below the EAR should be calculated. Theoretically, the probability method should be used to obtain the correct percentage. However, as it is rarely applicable because it can be used only under strict conditions (1), the cut-point method is usually used instead (13). In cases in which the distribution curve of requirement is very different from the normal distribution, the value calculated using the cut-point method differs from the true value, as does the value for iron (1). Moreover, when mean intake and its distribution differ from the EAR, the value obtained using the cut-point method may differ from the true percentage. When, in using the AI, the percentage of individuals whose intake is below the AI is calculated, it does not theoretically match the true percentage of those with inadequate intake. However, because no other indices exist, the AI must be used for practical reasons. In using the UL, the percentage of those at risk of excessive intake should be calculated from the intake distribution and the UL. In using a DG, the percentage of those whose intake is out of range of the DG should be calculated from the intake distribution and the DG.

2-3. Development and use of plans for dietary improvement (Table 4). For assessment of insufficient or excessive intake of energy, the BMI or change in body weight is used as an index. Planning should focus on increasing the percentage of individuals with a BMI within the normal range, measurement should be performed at least twice within a period of several months (at least twice a year), and change in body weight should be used for making and revising plans.

For assessment of sufficiency of nutrient intake, the EAR or AI is used. When the EAR is used, planning should aim to decrease the percentage of individuals with an intake below the EAR. When the AI is used, planning should aim to increase the mean intake of the group to approach the AI. For prevention of excessive nutrient intake, the UL is used. Planning should aim to reduce individual intake below the UL, as intake above the UL should strictly be avoided. For evaluation of nutrients related to lifestyle-related diseases, the DG is used. Planning should aim to increase the percentage of individuals whose intake is within or close to the DG while considering other nutrition- and non-nutrition-related factors related to lifestyle-related diseases and the sustainability of a particular plan over a long period.

3. *Management of food services*

3-1. Basic concept. The term *management of food service* refers here to planning for the provision of a continuous food supply with appropriate quality control based on evaluation of intake of a specific group of individuals. Maintenance and improvement of health, healthy growth of children, and primary prevention of lifestyle-related diseases are the key goals of management of food service. Therefore, it is necessary to plan for the serving of foods based on the DRIs.

3-2. Characteristics of target groups. Management of food services for a target group requires determination of the distribution of sex, age, body height and weight, and PAL and the percentage of individuals with a BMI outside the normal range of 18.5 to 25.0 (34). Using reference data, such as those contained in student health records, rather than conducting an independent assessment is recommended. When such reference data are not available, those obtained from similar groups can be used. It is desirable to repeat assessment of individual characteristics periodically for revision of the food service plan.

3-3. Dietary assessment. Not only are the meals provided by food services but all meals subject to assessment. It is preferable to use data regarding total intake to determine the extent of nutrient contribution by food services. If such data are difficult to obtain, data obtained by assessment of a single meal or a sample of individuals may be used. To prevent insufficient intake of nutrients, the percentage of individuals with an intake below the EAR is estimated from the measured intake distribution. When the AI is used, the percentage of individuals with intake below the AI is estimated. To prevent excessive intake, the percentage of individuals with an intake above the UL is estimated from the measured intake distribution. For primary prevention of lifestyle-related diseases, the percentage of individuals with an intake outside of a range of a DG is calculated from measured intake distribution.

3-4. Dietary planning. Dietary planning should be conducted using the DRIs, be based on individual characteristics and intakes, and consider whether every meal or a single daily meal is served. Determination of energy provided per serving should be based on sex, age group, and PAL distribution and on standard indices, such as the BMI. Changes in the BMI and body weight should also be used when useful.

Not all individuals in a group must meet the EAR or AI, which may increase the percentage of individuals with excessive intake. Menus should be planned to avoid the risk of approaching the UL. For primary prevention of lifestyle-related diseases, menus should be planned such that nearly no individual's intake falls outside of a range of a DG where possible. It is also important to consider the existence and degree of other nutrition- and non-nutrition-related factors in lifestyle-related diseases; the sustainability of a menu plan over a long period, as prevention of lifestyle-related diseases is a life-long endeavor; and the fact that a DRI is not a standard of nutrient provision but rather of nutrient intake, which requires flexibility in its use.

3-5. Supplementary note regarding dietary planning. As required energy and nutrient intakes differ among groups when individuals are classified into more than one group according to sex, age group, and PAL, preparation of a specific menu for each group is desirable. If doing so is difficult, the method described here may be used as a practical alternative. The EER is calculated based on sex, age group, and PAL. When there is more than one EER for a number of groups, they are grouped

together such that one EER may be used as a representative value for these groups, such as when the difference in energy requirement among several groups is within a range of 200 kcal/d. When doing so, the energy intake of each individual should preferably be within $\pm 10\%$ of the EER.

In order of increasing priority, dietary planning of should be conducted as follows: planning for (1) energy; (2) protein, with attention to prevention of deficiency; (3) fat; (4) vitamins A, B₁, B₂, and C; calcium; and iron; (5) saturated fatty acid, dietary fiber, sodium (salt), and potassium; and (6) other nutrients considered important for a particular group.

Closing Comments

The DRIs-J 2010 is not merely a scientific report describing the intake of energy and nutrients necessary for prevention of deficiency/insufficiency and excess but also a source of practical guidelines in planning for dietary improvement in general and in food services by dietitians and other health professionals. Reliable and comprehensive data regarding energy and nutrient intakes obtained by evaluation of representative samples of the Japanese population have been indispensable in both determining DRI values and establishing methods for their application. Nevertheless, compared to research into determination of the intake of energy and nutrients in the DRIs, research into application of the DRIs has been extremely scarce in Japan, limiting the availability of data and raising questions concerning its quality (35). Due to lack of evidence, current application of the DRIs is conceptual rather than scientific and practical. Highly scientific research into application of the DRIs is thus urgently needed.

REFERENCES

- 1) Food and Nutrition Board, Institute of Medicine. 2001. Dietary Reference Intakes: Applications in Dietary Assessment (Dietary Reference Intakes). National Academies Press, Washington DC.
- 2) Barr SI. 2006. Applications of Dietary Reference Intakes in dietary assessment and planning. *Appl Physiol Nutr Metab* **31**: 66–73.
- 3) Beaton GH. 2006. When is an individual versus a member of a group? An issue in the application of the dietary reference intakes. *Nutr Rev* **64**: 211–225.
- 4) Ozeki O, Ebisawa L, Ichikawa M, Nagasawa N, Sato F, Fujita Y. 2000. Physical activities and energy expenditures of institutionalized Japanese elderly women. *J Nutr Sci Vitaminol* **46**: 188–192.
- 5) Gaillard C, Alix E, Sallé A, Berrut G, Ritz P. 2007. Energy requirements in frail elderly people: a review of the literature. *Clin Nutr* **26**: 16–24.
- 6) Bertoli S, Battezzati A, Merati G, Margonato V, Maggioni M, Testolin G, Veicsteinas A. 2006. Nutritional status and dietary patterns in disabled people. *Nutr Metab Cardiovasc Dis* **16**: 100–112.
- 7) Katsura E. 1954. Clinical presentation of experiment of vitamin B₁ deficiency in human. *Vitamin* **7**: 708–713 (in Japanese).
- 8) Intersalt Cooperative Research Group. 1988. Intersalt: an international study of electrolyte excretion and blood

- pressure. Results for 24 hour urinary sodium and potassium excretion. *BMJ* **297**: 319–328.
- 9) Tokudome Y, Imaeda N, Nagaya T, Ikeda M, Fujiwara N, Sato J, Kuriki K, Kikuchi S, Maki S, Tokudome S. 2002. Daily, weekly, seasonal, within- and between-individual variation in nutrient intake according to four season consecutive 7 day weighed diet records in Japanese female dietitians. *J Epidemiol* **12**: 85–92.
 - 10) Nelson M, Black AE, Morris JA, Cole TJ. 1989. Between- and within-subject variation in nutrient intake from infancy to old age: estimating the number of days required to rank dietary intakes with desired precision. *Am J Clin Nutr* **50**: 155–167.
 - 11) Ogawa K, Tsubono Y, Nishino Y, Watanabe Y, Ohkubo T, Watanabe T, Nakatsuka H, Takahashi N, Kawamura M, Tsuji I, Hisamichi S. 1999. Inter- and intra-individual variation of food and nutrient consumption in a rural Japanese population. *Eur J Clin Nutr* **52**: 781–785.
 - 12) Egami I, Wakai K, Kaitoh K, Kawamura T, Tamakoshi A, Lin Y, Nakayama T, Sugimoto K, Ohno Y. 1999. Intra- and inter-individual variations in diets of the middle-aged and the elderly. *Nippon Kosho Eisei Zasshi* **46**: 828–837 (in Japanese with English summary).
 - 13) Harris JA, Benedict FG. 1919. A Biometric Study of Basal Metabolism in Man. Publication No. 279. Carneric Institute of Washington, Washington DC.
 - 14) FAO/WHO/UNU. 1985. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Technical Report Series, No. 724. WHO, Geneva.
 - 15) Ganpule AA, Tanaka S, Ishikawa-Takata K, Tabata I. 2007. Interindividual variability in sleeping metabolic rate in Japanese subjects. *Eur J Clin Nutr* **61**: 1256–1261.
 - 16) Case KO, Brahler CJ, Heiss C. 1997. Resting energy expenditures in Asian women measured by indirect calorimetry are lower than expenditures calculated from prediction equations. *J Am Diet Assoc* **97**: 1288–1292.
 - 17) Yamamura C, Kashiwazaki H. 2002. Factors affecting the post-absorptive resting metabolic rate of Japanese subjects: reanalysis based on published data. *Eiyogaku Zasshi (Jpn J Nutr Diet)* **60**: 75–83 (in Japanese).
 - 18) Brooks GA, Butte NF, Rand WM, Platt JP, Caballero B. 2004. Chronicle of the Institute of Medicine physical activity recommendation: how a physical activity recommendation came to be among dietary recommendations. *Am J Clin Nutr* **79** (Suppl): 921S–930S.
 - 19) Japanese Society of Hypertension. 2006. Japanese Society of Hypertension guidelines for the management of hypertension (JSH 2004). *Hypertens Res* **29** (Suppl): S1–105.
 - 20) Okubo H, Sasaki S, Hirota N, Notsu A, Todoriki H, Miura A, Fukui M, Date C. 2006. The influence of age and body mass index on relative accuracy of energy intake among Japanese adults. *Public Health Nutr* **9**: 651–657.
 - 21) Livesey G, Elia M. 1988. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *Am J Clin Nutr* **47**: 608–628.
 - 22) Forbes GB. 1987. Influence of nutrition. In: Human Body Composition. Growth, Aging, Nutrition, and Activity, p 209–247. Springer-Verlag, New York.
 - 23) Zhang J, Temme EH, Sasaki S, Kesteloot H. 2000. Under- and overreporting of energy intake using urinary cations as biomarkers: relation to body mass index. *Am J Epidemiol* **152**: 453–462.
 - 24) Murakami K, Sasaki S, Takahashi Y, Uenishi K, Yamasaki M, Hayabuchi H, Goda T, Oka J, Baba K, Ohki K, Kohri T, Watanabe R, Sugiyama Y. 2008. Misreporting of dietary energy, protein, potassium and sodium in relation to body mass index in young Japanese women. *Eur J Clin Nutr* **62**: 111–118.
 - 25) Ishiwaki A, Yokoyama T, Fujii H, Saito K, Nozue M, Yoshita K, Yoshiike N. 2007. A statistical approach for estimating the distribution of usual dietary intake to assess nutritionally at-risk populations based on the new Japanese Dietary Reference Intakes (DRIs). *J Nutr Sci Vitaminol* **53**: 337–344.
 - 26) Sasaki S, Takahashi T, Itoi Y, Iwase Y, Kobayashi M, Ishihara J, Akabane M, Tsugane S; JPHC. 2003. Food and nutrient intakes assessed with dietary records for the validation study of a self-administered food frequency questionnaire in JPHC Study Cohort I. *J Epidemiol* **13** (Suppl 1): S23–50.
 - 27) Ministry of Education, Culture, Sports, Science and Technology, Japan. 2005. Report of the Subdivision on Resources. The Council for Science and Technology: Standard Tables of Food Composition in Japan, 5th revised and enlarged ed. Tokyo (in Japanese).
 - 28) Miller WC, Koceja DM, Hamilton EJ. 1997. A meta-analysis of the past 25 years of weight loss research using diet, exercise of diet plus exercise intervention. *Int J Obes* **21**: 941–947.
 - 29) Food and Nutrition Board, Institute of Medicine. 2001. Iron. In: Dietary Reference Intakes: For Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (Institute of Medicine, ed), p 290–393. National Academies Press, Washington DC.
 - 30) Asakura K, Sasaki S, Murakami K, Takahashi Y, Uenishi K, Yamakawa M, Nishiwaki Y, Kikuchi Y, Takebayashi T; Japan Dietetic Students' Study for Nutrition and Biomarkers Group. 2009. Iron intake does not significantly correlate with iron deficiency among young Japanese women: a cross-sectional study. *Public Health Nutr* **12**: 1373–1383.
 - 31) Barr SI, Murphy SP, Agurs-Collins TD, Poos MI. 2003. Planning diets for individuals using the dietary reference intakes. *Nutr Rev* **61**: 352–360.
 - 32) Japan Society for the Study of Obesity. 2006. Guidelines for the Treatment of Obesity 2006. *Journal of Japan Society for the Study of Obesity* **12**: 1–91 (in Japanese).
 - 33) Murphy SP, Barr SI. 2005. Challenges in using the dietary reference intakes to plan diets for groups. *Nutr Rev* **63**: 267–271.
 - 34) Buzzard M. 1998. 24-hour dietary recall and food record methods. In: Nutritional Epidemiology (Willett W, ed), 2nd ed, p 50–73. Oxford University Press, New York.
 - 35) Sasaki S. 2011. The value of the National Health and Nutrition Survey in Japan. *Lancet* **378** (9798): 1205–1206.

Dietary Reference Intakes for Japanese 2010: Energy

Izumi TABATA¹, Naoyuki EBINE², Yukiko KAWASHIMA³, Kazuko ISHIKAWA-TAKATA⁴,
Shigeo TANAKA^{5,*}, Mitsuru HIGUCHI⁶ and Yutaka YOSHITAKE⁷

¹Faculty of Sport and Health Science, Ritsumeikan University, 1-1-1 Noji-higashi, Kusatsu,
Shiga 525-8577, Japan

²Department of Health and Sports Science, Doshisha University, 1-3 Tatara-Miyakodani, Kyotanabe,
Kyoto 610-0394, Japan

³Dietary Department, St. Marianna University School of Medicine Hospital, 2-16-1 Sugao,
Miyamae-ku, Kawasaki, Kanagawa 216-8511, Japan

⁴Department of Nutritional Education, National Institute of Health and Nutrition, 1-23-1 Toyama,
Shinjuku-ku, Tokyo 162-8636, Japan

⁵Department of Nutritional Science, National Institute of Health and Nutrition, 1-23-1 Toyama,
Shinjuku-ku, Tokyo 162-8636, Japan

⁶Faculty of Sport Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa,
Saitama 359-1192, Japan

⁷Department of Physical Education, National Institute of Fitness and Sports in Kanoya,
Shiramizu-1, Kanoya, Kagoshima 891-2393, Japan

(Received October 26, 2012)

Summary For energy of Dietary Reference Intakes for Japanese (DRIs-J), the concept of Estimated Energy Requirement (EER) is applied. The EER has been established as an index for individuals and groups. The definition of EER for individuals is “habitual energy intake in a day which is predicted to have the highest probability that energy balance (energy intake–energy expenditure, in adults) becomes zero in an individual of a given age, gender, height, body weight, and level of physical activity in good health.” In contrast, the definition of EER for a group is “habitual energy intake in a day which is predicted to have the highest probability that energy balance (energy intake–energy expenditure, in adults) becomes zero in a group.” The EER is calculated as follows: $EER \text{ (kcal/d)} = \text{basal metabolic rate (BMR) (kcal/d)} \times \text{physical activity level (PAL)}$. Representative values for BMR per kg body weight are determined based on a number of reports for Japanese. This is called the reference value of BMR (reference BMR). Total energy expenditure measured by the doubly labeled water (DLW) method is utilized to determine PAL for each sex and age group. For adults, physical activity levels are determined based on data for Japanese adults. For children, energy deposition is added to the total energy expenditure. For pregnant and lactating women, additional values compared to EER before pregnancy for each stage of pregnancy and during lactation are calculated. Excess post-exercise oxygen consumption is not added to calculate EER in addition to energy expenditure during physical activity.

Key Words estimated energy expenditure (EER), total energy expenditure, basal metabolic rate (BMR), physical activity level (PAL), doubly labeled water method

Background Information

Daily energy expenditure (total energy expenditure) consists of basal metabolic rate (BMR), physical activity energy expenditure, and thermic effect of food (diet-induced thermogenesis). In children and infants, the need for additional energy for growth also requires determination of not only the energy necessary for meeting daily needs but also the energy necessary for increased tissue for growth (energy deposition) and the energy necessary for tissue formation. Of the two forms of energy required for growth, only energy for tissue formation is currently included in determination of total energy expenditure for children and infants. Therefore, to determine energy requirement, energy deposition

needs to be added to total energy expenditure. Determining the energy requirement for pregnant women requires determination of the energy expenditure of the fetus and the energy necessary for the growth of fetal tissues. Determining the energy requirement for lactating women requires determination of the energy required to produce breast milk and consideration of weight loss corresponding to breast milk production. Therefore, increased or decreased energy requirements corresponding to an increase or decrease in tissue growth must be considered in addition to total energy expenditure, as reflected in the formula used to calculate energy requirements:

Energy requirement

= total energy expenditure + energy for the increased or decreased tissue.

For adults undergoing no body weight change, no

*To whom correspondence should be addressed.

E-mail: tanakas@nih.go.jp

Table 1. Basal metabolic rate of the Japanese population.

Sex	Males			Females		
	Reference BMR (kcal/kg weight/d)	Reference weight (kg)	BMR (kcal/d)	Reference BMR (kcal/kg weight/d)	Reference weight (kg)	BMR (kcal/d)
Age						
1–2 y	61.0	11.7	710	59.7	11.0	660
3–5 y	54.8	16.2	890	52.2	16.2	850
6–7 y	44.3	22.0	980	41.9	22.0	920
8–9 y	40.8	27.5	1,120	38.3	27.2	1,040
10–11 y	37.4	35.5	1,330	34.8	34.5	1,200
12–14 y	31.0	48.0	1,490	29.6	46.0	1,360
15–17 y	27.0	58.4	1,580	25.3	50.6	1,280
18–29 y	24.0	63.0	1,510	22.1	50.6	1,120
30–49 y	22.3	68.5	1,530	21.7	53.0	1,150
50–69 y	21.5	65.0	1,400	20.7	53.6	1,110
≥70 y	21.5	59.7	1,280	20.7	49.0	1,010

BMR, basal metabolic rate.

additional energy is required above that for meeting daily needs. Therefore, when energy intake exceeds energy requirements, the unutilized energy substrate is accumulated mainly in adipose tissue as triglycerides. An increase in adipose tissue may increase body weight and body fat in the short term and lead to obesity, a risk factor for many lifestyle-related diseases and increased total mortality, in the long term. In contrast, an energy intake less than that of energy expenditure may cause a decrease in the amount of accumulated fat in adipose tissues and in the amount of body protein, such as that contained in muscle tissue; a decrease in bodily functioning and quality of life; and an increase in morbidity due to infectious disease and certain cancers as well as in total mortality. Therefore, the optimal energy intake of adults—their true energy requirement—is that equal to the amount of energy expended when they are at an appropriate body weight.

Determining DRI

Estimated energy requirement

1. Definition of estimated energy requirement

In the determination of the Dietary Reference Intakes for Japanese (DRIs-J) for energy, the concept of estimated energy requirement (EER) was applied in the same way as it had been in determining the DRIs for the United States and Canada (1, 2). The EER is established for individuals and groups; the EER for individuals is defined as “habitual energy intake in a day which is predicted to have the highest probability that energy balance (energy intake—energy expenditure, in adults) becomes zero in an individual of a given age, sex, height, body weight, and level of physical activity in good health.”

When the energy intake of an individual is the same as the EER, the probability of inadequate intake—that the individual's energy intake is below his/her true energy requirement—is 50% and the probability of excessive intake is 50%. For many nutrients, the probability of adequate energy intake decreases as energy intake decreases, and the probability of adequate energy intake increases as intake increases while remaining

sufficiently below the UL. However, the probability of inadequate energy balance increases equally whether intake is below or above the EER. That is, the probability of weight gain increases when an individual's energy intake is above the EER and the probability of weight loss increases when the individual's energy intake is below the EER. For this reason, the DRI concepts used for determination of other nutrients cannot be applied to determination of energy requirements.

In contrast to that for individuals, the EER for a group is defined as “habitual energy intake in a day which is predicted to have the highest probability that energy balance (energy intake—energy expenditure, in adults) becomes zero in a group.” When the energy intake of a defined group is the same as the EER, the probability that the energy intake is below a group member's true energy requirement is 50% and probability that the energy intake is above the requirement is 50%. The components with great impact on total energy expenditure are BMR and energy expenditure for physical activities. Therefore, determination of an accurate EER requires determination of the defined individuals' or groups' BMR and the amount of physical activity.

2. Basal metabolic rate

As shown in Table 1, BMR in kcal/d is calculated as follows:

$$\text{BMR (kcal/d)} = \text{Reference BMR (kcal/kg body weight/d)} \times \text{reference body weight (kg)}$$

BMR is measured early in the morning while resting in the supine position in a comfortable indoor environment at a comfortable room temperature. The reference BMR is based on the reference BMR reported in the 2005 DRIs-J as well as the BMR values that have been reported by several studies conducted since 1980 (3–15).

3. Physical activity level

Physical activity level (PAL) is an index of level of physical activity that considers diet-induced thermogenesis, also. PAL is calculated as total energy expenditure divided by BMR (16–18), as shown in the following

Table 2. BMI and PAL at each physical activity level (mean±SD).

PAL (range)	n	Sex ratio (% male)	Age (y)	BMI (kg/m ²)	PAL
Level I (<1.6)	38	55	40±11	23.9±2.5	1.50±0.08
Level II (≥1.6, ≤1.9)	65	52	39±11	22.8±3.1	1.74±0.08
Level III (>1.9)	36	39	40±9	21.3±2.6	2.03±0.13
Total	139	50	39±10	22.7±2.9	1.75±0.22

n, number of subjects; BMI, body mass index; PAL, physical activity level.

formula:

$PAL = \text{total energy expenditure (kcal/d)} / \text{BMR (kcal/d)}$.

The doubly labeled water (DLW) method, the most accurate method for measuring total energy expenditure that was employed in determining the DRIs of the United States and Canada, was utilized to determine the PAL for each sex and age group. Considering the range of inter-individual variability in energy expenditure based on individual characteristics and evidence, a number of PALs were established to calculate a more accurate EER.

4. Calculation of EER

Using PALs obtained from daily total energy expenditure of Japanese measured using the DLW method (19), the EER is calculated as follows:

$EER \text{ (kcal/d)} = \text{BMR (kcal/d)} \times \text{PAL}$.

For children, pregnant women, and lactating women, energy deposition is added to the EER to account for increased tissue due to growth, the products of conception and accretion of maternal tissues, and the energy costs corresponding to postpartum lactation and weight change, respectively.

5. Adults

In a study aimed at determining the PAL of Japanese adults ($n=139$, aged 20 to 59 y) (19), the subjects were divided into 3 groups using the 25th and 75th percentile values (1.60 and 1.90, respectively; Table 2). Based on the results of the stratification, the groups were labeled according to activity level as Level I (low activity level, representative value=1.50), Level II (moderate activity level, representative value=1.75), and Level III (high activity level, representative value=2.00). According to this classification, the ratio of individuals allocated to each level could be roughly expressed as 1 : 2 : 1. As shown in Table 2, the mean±standard deviation (SD) for the PAL of all subjects was 1.75 ± 0.22 . The representative value (or mean) for Level I generally corresponds to the value (mean−1×SD) for the entire group and the representative value (or mean) for Level III to the value of (mean+1×SD).

According to the results of studies of total energy expenditure and PAL of the Japanese using the DLW method (19–33), the use of these 3 levels appears appropriate.

6. The elderly

Among the many studies that have attempted to determine the PAL of healthy, independently living elderly subjects (33–42), the mean value was 1.69, leading the reference PAL for elderly subjects to be set as 1.70. How-

ever, the subjects' mean age in most of these reports (11 out of 13) ranged from 70 to 75 y, and many examined only relatively healthy independently living elderly subjects. These facts, as well as the fact that few studies have examined the average PAL of subjects in their 90 s, makes it difficult to identify reference PALs for the elderly over 70 y. One report (43) found that the PAL of subjects in their 90 s tends to be low.

7. Children

Children in the growth stage require energy not only for physical activity but also for tissue formation and increased tissue (energy deposition). As the energy used for tissue formation is included in the calculation of total energy expenditure, the EER (kcal/d) was calculated as follows:

$EER \text{ (kcal/d)} = \text{BMR (kcal/d)} \times \text{PAL} + \text{energy deposition (kcal/d)}$.

As PALs differ by age group, a systematic review was conducted of reports of children's PALs using the DLW method. Values of PAL were determined based on reports with measured BMR data (44–66). For children younger than 5 for whom such data were unavailable, PAL values were also based on estimated BMR (31, 67–74). The mean PAL was found to be 1.36, 1.47, 1.57, 1.59, 1.63, 1.66, and 1.76 for ages 1 to 2 y, 3 to 5 y, 6 to 7 y, 8 to 9 y, 10 to 11 y, 12 to 14 y, and 15 to 17 y, respectively, showing a tendency to increase with age (Fig. 1). The Grouping of PALs at each age group is shown in Table 3. The similar tendency was observed in a systematic review (75).

Although individual variability was observed for ages 1 to 2 y and ages 3 to 5 y, the PALs for these groups were not categorized into levels due to the lack of data for categorizing PAL for individuals or groups. In contrast, the PALs for those aged 6 and over were categorized into 3 levels to consider individual variability. The means of the standard deviation of selected references weighted by the number of subjects based on age group differed in the range 0.17 to 0.25, with a mean value of 0.21. Therefore, the PAL in each age group of children was increased or decreased by 0.20 from the corresponding group's "moderate" value. As there were no data regarding PAL for these age groups in Japan, Level I (low) was established for school-age children for the first time, with consideration of the wide variations in PAL reported in previous studies conducted in foreign countries. In the future, the status and determinants of the PALs of Japanese school-age children need to be studied.

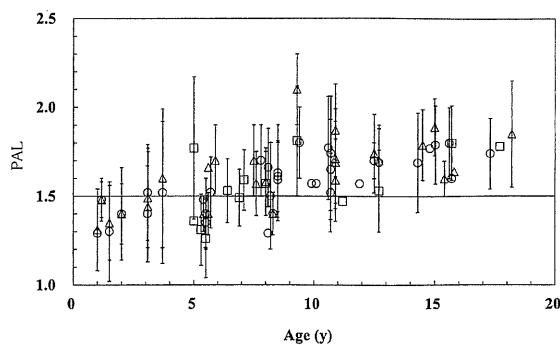


Fig. 1. PAL of children. The data presented for all age groups were taken only from studies that measured basal metabolic rate except for those for children aged 3 to 5 y, for whom data were also taken from studies that estimated basal metabolic rate, and children aged 1 to 2 y, for whom data were also taken from studies that measured sleeping metabolic rate and estimated basal metabolic rate, due to the lack of studies for these age groups. Δ , boys; \circ , girls; \square , boys and girls; mean \pm SD. PAL: physical activity level.

Energy for increased tissue was determined as the product of increased weight per day calculated from the reference weight and the energy density of increased tissue (1) (refer to Table 4 for details).

8. Infants

For infants, as for older children, energy is required for not only physical activity but also tissue formation and energy deposition. As the energy required for tissue formation is included in total energy expenditure, the EER was calculated as follows:

$$\begin{aligned} \text{EER (kcal/d)} \\ = \text{total energy expenditure (kcal/d)} + \text{energy deposition (kcal/d)}. \end{aligned}$$

For determining the total energy expenditure of infants, the Food and Agricultural Organization (FAO), World Health Organization (WHO), and United Nations University (UNU) have reported that total energy expenditure of breast-fed infants can be modeled by the following regression equation, which uses body weight as an independent variable and considering the relationships among sex, age (months), body weight, body height, and total energy that were identified in previous studies (76, 77):

$$\begin{aligned} \text{Total energy expenditure (kcal/d)} \\ = 92.8 \times \text{reference weight (kg)} - 152.0. \end{aligned}$$

As no study has determined Japanese infants' total energy expenditure using the DLW method, total energy expenditure was determined by substituting the reference weights of the Japanese into the regression equation. As with children, energy deposition is calculated as the product of increased weight per day as calculated using the reference weight and energy density of increased tissue for infants (67) (Table 4).

The EER is determined for infants at 3 different ages: 0 to 5 mo, 6 to 8 mo, and 9 to 11 mo. For infants aged 0 to 5 mo who undergo large weight changes, atten-

Table 3. PAL by physical activity level of each age group of both males and females.

PAL	Level I (low)	Level II (moderate)	Level III (high)
1–2 y	—	1.35	—
3–5 y	—	1.45	—
6–7 y	1.35	1.55	1.75
8–9 y	1.40	1.60	1.80
10–11 y	1.45	1.65	1.85
12–14 y	1.45	1.65	1.85
15–17 y	1.55	1.75	1.95
18–29 y	1.50	1.75	2.00
30–49 y	1.50	1.75	2.00
50–69 y	1.50	1.75	2.00
≥ 70 y	1.45	1.70	1.95

PAL: physical activity level.

tion must be placed on the large difference in the EER between the first and second half of this period. As formula-fed infants typically have greater total energy expenditure than breast-fed infants (76), the FAO, WHO, and UNU have reported that the EER of formula-fed infants should be determined using the following regression equation (76, 77).

$$\begin{aligned} \text{Total energy expenditure (kcal/d)} \\ = 82.6 \times \text{body weight (kg)} - 29.0. \end{aligned}$$

9. Additional values for pregnant women

The EER of pregnant women is calculated as follows: EER (kcal/d)

$$= \text{EER before pregnancy (kcal/d)} + \text{additional energy required by pregnant women (kcal/d)}.$$

Considering that the female reproductive period encompasses several age groups, it is necessary to determine the additional amounts of energy needed to maintain good health during pregnancy and for normal delivery for each stage of pregnancy. Longitudinal studies using the DLW method found that although PAL decreases during the early and late stage of pregnancy, increased rates of total energy expenditure during the early, mid, and late stage of pregnancy correspond to increased rates of weight gain, as does an increase in BMR during the late stage of pregnancy (76–82). Therefore, differences between pre-pregnancy EER and total energy expenditure during each stage (76, 77) adjusted by an average total weight gain of 11 kg during pregnancy (83) are as follows: +19 kcal/d during the early stage, +77 kcal/d during the mid-stage, and +285 kcal/d during the late stage. Total energy deposition is calculated as the sum of energy deposition of protein and fat that yields a final weight gain of 11 kg, based on protein deposition and body fat deposition on a per-stage basis (76, 77). Thus, energy deposition is 44 kcal/d during the early stage, 167 kcal/d during the mid-stage, and 170 kcal/d during the late stage.

As a result, total additional energy for each stage is calculated as follows:

$$\text{Additional energy for pregnant women (kcal/d)}$$

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).

$$6,500 \text{ kcal/kg body weight} \\ \times 0.8 \text{ kg/mo} \div 30 \text{ d} \\ = 173 \text{ kcal/d.}$$

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

$$\text{Additional energy required by lactating women (kcal/d)} \\ = \text{breast milk energy (kcal/d)} - \text{energy of weight loss (kcal/d).}$$

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		
	I	II	III	I	II	III
PAL						
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:

$$\text{EER (kcal/d)} = \text{BMR (kcal/d)} \times \text{PAL}$$

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:

$$\text{MET value} \times 1.1 = \text{Af}$$

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.

REFERENCES

- 1) Food and Nutrition Board, Institute of Medicine. 2005. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acid, Cholesterol, Protein, and Amino Acids, p 107–264. National Academies Press, Washington DC.
- 2) Brooks GA, Butte NF, Rand WM, Flatt JP, Caballero B. 2004. Chronicle of the Institute of Medicine physical activity recommendation: how a physical activity recommendation came to be among dietary recommendations. *Am J Clin Nutr* **79**(Suppl): S921–930.
- 3) Yanai R, Masuda T, Kitagawa S, Nagao N, Nagao M, Matsueda S. 2006. Relationship between overreporting or underreporting among young males and females and physical factors, psychosocial factors, and lifestyle habits. *Journal of Kawasaki Medical Welfare Society* **16**: 109–119.
- 4) Shimada M, Nishimuta M, Kodama N, Yoshitake Y. 2006. Existence in subject of low plasma triiodothyronine correlated with post-absorptive resting metabolism measurement of T3 is essential for determining standard basal metabolic rate. *Jpn J Phys Fitness Sports Med* **55**: 295–306 (in Japanese).
- 5) Taguchi M, Higuchi M, Oka J, Yoshiga C, Ishida Y, Matsushita M. 2001. Basal metabolic rate in Japanese female endurance athletes. *Jpn J Nutr Diet* **59**: 127–134 (in Japanese).
- 6) Usui C, Takahashi E, Gando Y, Sanada K, Oka J, Miyachi M, Tabata I, Higuchi M. 2007. Relationship between blood adipocytokines and resting energy expenditure in young and elderly women. *J Nutr Sci Vitaminol* **53**: 529–535.
- 7) Yamamura C, Tanaka S, Futami J, Oka J, Ishikawa Takata K, Kashiwazaki H. 2003. Activity diary method for predicting energy expenditure as evaluated by a whole-body indirect human calorimeter. *J Nutr Sci Vitaminol* **49**: 262–269.
- 8) Ganpule AA, Tanaka S, Ishikawa-Takata K, Tabata I. 2007. Interindividual variability in sleeping metabolic rate in Japanese subjects. *Eur J Clin Nutr* **61**: 1256–1261.
- 9) Hirose M. 1989. Study of basal metabolism of modern middle age Japanese. *Ehime Medical* **8**: 192–210 (in Japanese).
- 10) Hioki C, Arai M. 2007. Bofutsushosan use for obesity with IGT: search for scientific basis and development of effective therapy with Kampo medicine. *J Trad Med* **24**: 115–127.
- 11) Usui C, Oka J, Yamakawa J, Sasaki Y, Higuchi M. 2003. Basal metabolic rate and its determinants in postmenopausal women. *Jpn J Phys Fitness Sport Med* **52**: 189–198.
- 12) Yokozeki T. 1993. Basal metabolic rate and physical activity in the elderly. *J Jpn Soc Nutr Food Sci* **46**: 451–458 (in Japanese).
- 13) Yokozeki T. 1993. Basal metabolic rate and energy requirement of bed-ridden elderly women. *J Jpn Soc Nutr Food Sci* **46**: 459–466 (in Japanese).
- 14) Tahara Y. 1983. Seasonal variation of heat production by body composition in basal metabolic condition and cold exposure. *J Jpn Soc Nutr Food Sci* **36**: 255–263 (in Japanese).
- 15) Maeda T, Fukushima T, Ishibashi K, Higuchi S. 2007. Involvement of basal metabolic rate in determination of type of cold tolerance. *J Physiol Anthropol* **26**: 415–418.
- 16) Shetty PS, Henry CJ, Black AE, Prentice AM. 1996. Energy requirements of adults: an update on basal metabolic rates (BMRs) and physical activity levels (PALs). *Eur J Clin Nutr* **50**: S11–23.
- 17) Black AE, Coward WA, Cole TJ, Prentice AM. 1996. Human energy expenditure in affluent societies: an analysis of 574 doubly-labelled water measurements. *Eur J Clin Nutr* **50**: 72–92.
- 18) Schutz Y, Weinsier RL, Hunter GR. 2001. Assessment of free-living physical activity in humans: an overview of currently available and proposed new measures. *Obes Res* **9**: 368–379.
- 19) Ishikawa-Takata K, Tabata I, Sasaki S, Rafamantanantsoa HH, Okazaki H, Okubo H, Tanaka S, Yamamoto S, Shirota T, Uchida K, Murata M. 2008. Physical activity level in healthy free-living Japanese estimated by doubly-labelled water method and International Physical Activity Questionnaire. *Eur J Clin Nutr* **62**: 885–891.
- 20) Ebine N, Feng JY, Homma M, Saitoh S, Jones PJ. 2000. Total energy expenditure of elite synchronized swimmers measured by the doubly labeled water method. *Eur J Appl Physiol* **83**: 1–6.
- 21) Ebine N, Rafamantanantsoa HH, Nayuki Y, Yamanaka K, Tashima K, Ono T, Saitoh S, Jones PJ. 2002. Measurement of total energy expenditure by the doubly labelled water method in professional soccer players. *J Sports Sci* **20**: 391–397.
- 22) Ebine N, Shimada M, Tanaka H, Nishimuta M, Yoshitake Y, Saitoh S, Jones PJ. 2002. Comparative study of total energy expenditure in Japanese men using doubly labeled water method against activity record, heart rate monitoring, and accelerometer methods. *Jpn J Phys Fitness Sport Med* **51**: 151–163 (in Japanese).
- 23) Rafamantanantsoa HH, Ebine N, Yoshioka M, Higuchi H, Yoshitake Y, Tanaka H, Saitoh S, Jones PJ. 2002. Validation of three alternative methods to measure total energy expenditure against the doubly labeled water method for older Japanese men. *J Nutr Sci Vitaminol* **48**: 517–523.
- 24) Touno M, Rafamantanantsoa HH, Ebine N, Peng HY, Yoshitake Y, Tanaka H, Saitoh S. 2003. Measurement of total energy expenditure of firefighters on normal work shift. *Jpn J Phys Fitness Sport Med* **52**: 265–274 (in Japanese).
- 25) Rafamantanantsoa HH, Ebine N, Yoshioka M, Yoshitake Y, Tanaka H, Saitoh S. 2003. The effectiveness of three-day dietary records with advanced photo system camera for measuring energy intake in Japanese men as determined by doubly labeled water technique. *J Clin Biochem Nutr* **33**: 33–38.
- 26) Rafamantanantsoa HH, Ebine N, Yoshioka M, Yoshitake Y, Tanaka H, Saitoh S, Jones PJ. 2003. The role of exercise physical activity in varying the total energy expenditure in healthy Japanese men 30 to 69 years of age. *J Nutr Sci Vitaminol* **49**: 120–124.
- 27) Peng HY, Yoshitake Y, Saitoh S. 2004. Validity of methods to measure total energy expenditure of middle-aged women: a validation study against doubly labeled water method. *J Jpn Soc Study Obes* **10**: 163–172.
- 28) Peng HY, Shibata U, Yoshitake Y, Saito S, Omi N. 2005. Energy balance and nutritional status in middle-aged Japanese women with a long-term habit of exercise. *J Jpn Soc Nutr Food Sci* **58**: 329–335.

- 29) Peng HY, Saito S, Hikihara Y, Ebine N, Yoshitake Y. 2005. Energy expenditure, body composition and maximal oxygen uptake in middle-aged Japanese women who have long-term habits of exercising. *Jpn J Phys Fitness Sport Med* **54**: 237–248 (in Japanese).
- 30) Hikihara Y, Saitoh S, Yoshitake Y. 2005. Validity of methods to measure total energy expenditure of baseball players in Japanese high school. *J Jpn Soc Nutr Food Sci* **54**: 363–372 (in Japanese).
- 31) Adachi M, Sasayama K, Hikihara Y, Okishima K, Mizuuchi H, Sunami Y, Shiomi M, Nishimuta M, Kikunaga S, Tanaka H, Saitoh S, Yoshitake Y. 2007. Assessing daily physical activity in elementary school students used by accelerometer: a validation study against doubly labeled water method. *Jpn J Phys Fitness Sports Med* **56**: 347–356 (in Japanese).
- 32) Yamamoto S, Ishikawa-Takata K, Bessyo K, Tanimoto M, Miyachi M, Tanaka S, Totani M, Tabata I. 2008. Basal metabolic rate and physical activity level in bodybuilders. *Jpn J Nutr* **66**: 195–200 (in Japanese).
- 33) Yamada Y, Yokoyama K, Noriyasu R, Osaki T, Adachi T, Itoi A, Naito Y, Morimoto T, Kimura M, Oda S. 2009. Light-intensity activities are important for estimating physical activity energy expenditure using uniaxial and triaxial accelerometers. *Eur J Appl Physiol* **105**: 141–152.
- 34) Baarends EM, Schols AM, Pannemans DL, Westerterp KR, Wouters EF. 1997. Total free living energy expenditure in patients with severe chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* **155**: 549–554.
- 35) Sawaya AL, Saltzman E, Fuss P, Young VR, Roberts SB. 1995. Dietary energy requirements of young and older women determined by using the doubly labeled water method. *Am J Clin Nutr* **62**: 338–344.
- 36) Rothenberg E, Bosaeus I, Lernfelt B, Landahl S, Steen B. 1998. Energy intake and expenditure: validation of a diet history by heart rate monitoring, activity diary and doubly labeled water. *Eur J Clin Nutr* **52**: 832–838.
- 37) Reilly JJ, Lord A, Bunker VW, Prentice AM, Coward WA, Thomas AJ, Briggs RS. 1993. Energy balance in healthy elderly women. *Br J Nutr* **69**: 21–27.
- 38) Bonnefoy M, Normand S, Pachiardi C, Lacour JR, Laville M, Kostka T. 2001. Simultaneous validation of ten physical activity questionnaires in older men: a doubly labeled water study. *J Am Geriatr Soc* **49**: 28–35.
- 39) Blanc S, Schoeller DA, Bauer D, Danielson ME, Tylavsky F, Simonsick EM, Harris TB, Kritchevsky SB, Everhart JE. 2004. Energy requirements in the eighth decade of life. *Am J Clin Nutr* **79**: 303–310.
- 40) Manini TM, Everhart JE, Patel KV, Schoeller DA, Colbert LH, Visser M, Tylavsky F, Bauer DC, Goodpaster BH, Harris TB. 2006. Daily activity energy expenditure and mortality among older adults. *JAMA* **296**: 171–179.
- 41) Rothenberg EM, Bosaeus IG, Steen BC. 2003. Energy expenditure at age 73 and 78—A five year follow-up. *Acta Diabetol* **40**: S134–138.
- 42) Fuller NJ, Sawyer MB, Coward WA, Paxton P, Elia M. 1996. Components of total energy expenditure in free-living elderly men (over 75 years of age): measurement, predictability and relationship to quality-of-life indices. *Br J Nutr* **75**: 161–173.
- 43) Rothenberg EM, Bosaeus IG, Westerterp KR, Steen BC. 2000. Resting energy expenditure, activity energy expenditure and total energy expenditure at age 91–96 years. *Br J Nutr* **84**: 319–324.
- 44) Wren RE, Blume H, Mazariegos M, Solomons N, Alvarez JO, Goran MI. 1997. Body composition, resting metabolic rate, and energy requirements of short- and normal-stature, low-income Guatemalan children. *Am J Clin Nutr* **66**: 406–412.
- 45) Fontvieille AM, Harper IT, Ferraro RT, Spraul M, Ravussin E. 1993. Daily energy expenditure by five-year-old children, measured by doubly labeled water. *J Pediatr* **123**: 200–207.
- 46) Bunt JC, Salbe AD, Harper IT, Hanson RL, Tataranni PA. 2003. Weight, adiposity, and physical activity as determinants of an insulin sensitivity index in Pima Indian children. *Diabetes Care* **26**: 2524–2530.
- 47) Franks PW, Ravussin E, Hanson RL, Harper IT, Allison DB, Knowler WC, Tataranni PA, Salbe AD. 2005. Habitual physical activity in children: the role of genes and the environment. *Am J Clin Nutr* **82**: 901–908.
- 48) Hoos MB, Plasqui G, Gerver WJ, Westerterp KR. 2003. Physical activity level measured by doubly labeled water and accelerometry in children. *Eur J Appl Physiol* **89**: 624–626.
- 49) Livingstone MB, Coward WA, Prentice AM, Davies PS, Strain JJ, McKenna PG, Mahoney CA, White JA, Stewart CM, Kerr MJ. 1992. Daily energy expenditure in free-living children: comparison of heart-rate monitoring with the doubly labeled water (2H2(18)O) method. *Am J Clin Nutr* **56**: 343–352.
- 50) Dugas LR, Ebersole K, Schoeller D, Yanovski JA, Barquera S, Rivera J, Durazo-Arzivu R, Luke A. 2008. Very low levels of energy expenditure among pre-adolescent Mexican-American girls. *Int J Pediatr Obes* **3**: 123–126.
- 51) Luke A, Roizen NJ, Sutton M, Schoeller DA. 1994. Energy expenditure in children with Down syndrome: correcting metabolic rate for movement. *J Pediatr* **125**: 829–838.
- 52) Ramirez-Marrero FA, Smith BA, Sherman WM, Kirby TE. 2005. Comparison of methods to estimate physical activity and energy expenditure in African American children. *Int J Sports Med* **26**: 363–371.
- 53) Treuth MS, Figueroa-Colon R, Hunter GR, Weinsier RL, Butte NF, Goran MI. 1998. Energy expenditure and physical fitness in overweight vs non-overweight prepubertal girls. *Int J Obes Relat Metab Disord* **22**: 440–447.
- 54) Treuth MS, Butte NF, Wong WW. 2000. Effects of familial predisposition to obesity on energy expenditure in multiethnic prepubertal girls. *Am J Clin Nutr* **71**: 893–900.
- 55) Maffei C, Pinelli L, Zaffanello M, Schena F, Iacumin P, Schutz Y. 1995. Daily energy expenditure in free-living conditions in obese and non-obese children: comparison of doubly labelled water (2H2(18)O) method and heart-rate monitoring. *Int J Obes Relat Metab Disord* **19**: 671–677.
- 56) Spadano JL, Bandini LG, Must A, Dallal GE, Dietz WH. 2005. Longitudinal changes in energy expenditure in girls from late childhood through midadolescence. *Am J Clin Nutr* **81**: 1102–1109.
- 57) Anderson SE, Bandini LG, Dietz WH, Must A. 2004. Relationship between temperament, nonresting energy expenditure, body composition, and physical activity in girls. *Int J Obes Relat Metab Disord* **28**: 300–306.
- 58) DeLany JP, Bray GA, Harsha DW, Volaufova J. 2006. Energy expenditure and substrate oxidation predict changes in body fat in children. *Am J Clin Nutr* **84**: 862–870.
- 59) DeLany JP, Bray GA, Harsha DW, Volaufova J. 2002. Energy expenditure in preadolescent African American

- and white boys and girls: the Baton Rouge Children's Study. *Am J Clin Nutr* **75**: 705–713.
- 60) Perks SM, Roemmich JN, Sandow-Pajewski M, Clark PA, Thomas E, Weltman A, Patrie J, Rogol AD. 2000. Alterations in growth and body composition during puberty. IV. Energy intake estimated by the youth-adolescent food-frequency questionnaire: validation by the doubly labeled water method. *Am J Clin Nutr* **72**: 1455–1460.
- 61) DeLany JP, Bray GA, Harsha DW, Volaufova J. 2004. Energy expenditure in African American and white boys and girls in a 2-y follow-up of the Baton Rouge Children's Study. *Am J Clin Nutr* **79**: 268–273.
- 62) Bandini LG, Schoeller DA, Dietz WH. 1990. Energy expenditure in obese and nonobese adolescents. *Pediatr Res* **27**: 198–203.
- 63) Bratteby LE, Sandhagen B, Fan H, Enghardt H, Samuelson G. 1998. Total energy expenditure and physical activity as assessed by the doubly labeled water method in Swedish adolescents in whom energy intake was underestimated by 7-d diet records. *Am J Clin Nutr* **67**: 905–911.
- 64) Arvidsson D, Slinde E, Hulthen L. 2005. Physical activity questionnaire for adolescents validated against doubly labeled water. *Eur J Clin Nutr* **59**: 376–383.
- 65) Slinde E, Arvidsson D, Sjöberg A, Rossander-Hulthén L. 2003. Minnesota leisure time activity questionnaire and doubly labeled water in adolescents. *Med Sci Sports Exerc* **35**: 1923–1928.
- 66) Ekelund U, Aman J, Yngve A, Renman C, Westerterp K, Sjöström M. 2002. Physical activity but not energy expenditure is reduced in obese adolescents: a case-control study. *Am J Clin Nutr* **76**: 935–941.
- 67) Butte NF, Wong WW, Hopkinson JM, Heinz CJ, Mehta NR, Smith EO. 2000. Energy requirements derived from total energy expenditure and energy deposition during the first 2 y of life. *Am J Clin Nutr* **72**: 1558–1569.
- 68) Tennefors C, Coward WA, Hernell O, Wright A, Forsum E. 2003. Total energy expenditure and physical activity level in healthy young Swedish children 9 or 14 months of age. *Eur J Clin Nutr* **57**: 647–653.
- 69) Davies PS, Gregory J, White A. 1995. Physical activity and body fatness in pre-school children. *Int J Obes Relat Metab Disord* **19**: 6–10.
- 70) Atkin LM, Davies PS. 2000. Diet composition and body composition in preschool children. *Am J Clin Nutr* **72**: 15–21.
- 71) Reilly JJ, Jackson DM, Montgomery C, Kelly LA, Slater C, Grant S, Paton JY. 2004. Total energy expenditure and physical activity in young Scottish children: mixed longitudinal study. *Lancet* **363**: 211–212.
- 72) Salbe AD, Weyer C, Harper I, Lindsay RS, Ravussin E, Tataranni PA. 2002. Assessing risk factors for obesity between childhood and adolescence: II. Energy metabolism and physical activity. *Pediatrics* **110**: 307–314.
- 73) Hernández-Triana M, Salazar G, Díaz E, Sánchez V, Basabe B, González S, Díaz ME. 2002. Total energy expenditure by the doubly-labeled water method in rural preschool children in Cuba. *Food Nutr Bull* **23**: 76–81.
- 74) Montgomery C, Reilly JJ, Jackson DM, Kelly LA, Slater C, Paton JY, Grant S. 2004. Relation between physical activity and energy expenditure in a representative sample of young children. *Am J Clin Nutr* **80**: 591–596.
- 75) Hoos MB, Gerver WJ, Kester AD, Westerterp KR. 2003. Physical activity levels in children and adolescents. *Int J Obes Relat Metab Disord* **27**: 605–609.
- 76) FAO. 2004. Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. FAO Food and Nutrition Technical Report Series No. 1. FAO, Rome.
- 77) Butte NF, King JC. 2005. Energy requirements during pregnancy and lactation. *Public Health Nutr* **8**: 1010–1027.
- 78) Goldberg GR, Prentice AM, Coward WA, Davies HL, Murgatroyd PR, Sawyer MB, Ashford J, Black AE. 1991. Longitudinal assessment of the components of energy balance in well-nourished lactating women. *Am J Clin Nutr* **54**: 788–798.
- 79) Forsum E, Kabir N, Sadurskis A, Westerterp K. 1992. Total energy expenditure of healthy Swedish women during pregnancy and lactation. *Am J Clin Nutr* **56**: 334–342.
- 80) Goldberg GR, Prentice AM, Coward WA, Davies HL, Murgatroyd PR, Wensing C, Black AE, Harding M, Sawyer M. 1993. Longitudinal assessment of energy expenditure in pregnancy by the doubly labeled water method. *Am J Clin Nutr* **57**: 494–505.
- 81) Kopp-Hoolihan LE, van Loan MD, Wong WW, King JC. 1999. Longitudinal assessment of energy balance in well-nourished, pregnant women. *Am J Clin Nutr* **69**: 697–704.
- 82) Butte NF, Wong WW, Treuth MS, Ellis KJ, O'Brian Smith E. 2004. Energy requirements during pregnancy based on total energy expenditure and energy deposition. *Am J Clin Nutr* **79**: 1078–1087.
- 83) Takimoto H, Sugiyama T, Fukuoka H, Kato N, Yoshiike N. 2006. Maternal weight gain ranges for optimal fetal growth in Japanese women. *Int J Gynecol Obstet* **92**: 272–278.
- 84) Butte NF, Wong WW, Hopkinson JM. 2001. Energy requirements of lactating women derived from doubly labeled water and milk energy output. *J Nutr* **131**: 53–58.
- 85) Suzuki K, Sasaki A, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Jpn J Nutr Diet* **62**: 369–372 (in Japanese).
- 86) Hirose J, Endo M, Nagao S, Mizushima K, Narita H, Shibata K. 2008. Amount of breast milk sucked by Japanese breast feeding infants. *J Jpn Soc Breastfeed Res* **2**: 23–28.
- 87) Yamawaki N, Yamada M, Kan-no T, Kojima T, Kaneko T, Yonekubo A. 2005. Macronutrient, mineral and trace element composition of breast milk from Japanese women. *J Trace Elements Med Biol* **19**: 171–181.
- 88) Tanaka S, Ohkawara K, Ishikawa-Takata K, Morita A, Watanabe S. 2008. Accuracy of predictive equations for basal metabolic rate and contribution of abdominal fat distribution to basal metabolic rate in obese Japanese people. *Anti-Aging Med* **5**: 17–21.
- 89) Takahashi E, Higuchi M, Hosokawa Y, Tabata I. 2007. Basal metabolic rate and body composition of Japanese young adult females. *Jpn J Nutr Diet* **65**: 241–247 (in Japanese).
- 90) Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR Jr, Schmitz KH, Emplaincourt PO, Jacobs DR Jr, Leon AS. 2000. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* **32**: S498–516.
- 91) Ohkawara K, Tanaka S, Ishikawa-Takata K, Tabata I. 2008. Twenty-four-hour analysis of elevated energy expenditure after physical activity in a metabolic chamber: models of daily total energy expenditure. *Am J Clin Nutr* **87**: 1268–1276.

Dietary Reference Intakes for Japanese 2010: Protein

Yasuhiro KIDO¹, Fujiko SHIZUKA², Yoshiharu SHIMOMURA³ and Takashi SUGIYAMA⁴

¹Laboratory of Nutrition Science, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606–8522, Japan

²Department of Human Life Sciences, Nagano Prefectural College, Miwa, Nagano 380–8525, Japan

³Laboratory of Nutritional Biochemistry, Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464–8601, Japan

⁴Departments of Obstetrics and Gynecology, Graduate School of Medicine, Mie University, Edobashi, Tsu, Mie 514–8507, Japan

(Received October 26, 2012)

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 depend-

ing 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

E-mail: kido@kpu.ac.jp

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. Accord-

ing 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)= $B \times 1,000 \div 365 \times C \div 100 \div A$.

EAR (g/d)= $(D \div E \times 100 + F \div G \times 100) \times A$, RDA (g/d)=EAR $\times 1.25$.

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 \div efficiency of protein utilization for maintenance + the protein storage requirement \div efficiency of protein utilization for growth.

The EAR (g/d) was considered as being equal to the EAR (g/kg body weight/d) \times 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) = Potassium 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 cal-

culations. 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:

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

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 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).

$$0-5 \text{ mo: } 12.6 (g/L) \times 0.78 (L/d) \times 100/70 = 14.0$$

$$6-8 \text{ mo: } 10.6 (g/L) \times 0.60 (L/d) \times 100/70 + 6.1 (g/d) = 15.2$$

$$9-11 \text{ mo: } 9.2 (g/L) \times 0.45 (L/d) \times 100/70 + 17.9 (g/d) = 23.8$$

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.

$$\text{Protein storage (g/d)} = \text{potassium accumulated (mmol/d)} \div 2.15 \times 6.25$$

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 $12.6 \text{ g/L} \times 0.78 \text{ L/d} \div 0.70 = 14.04 \text{ g/d}$, and adopted as 15 g/d according to the rounding off process employed. The additional requirement for lactating women (RDA)

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

- Munro HN. 1951. Carbohydrate and fat as factors in protein utilization and metabolism. *Physiol Rev* **31**: 449–488.
- Kishi K, Inoue G, Yoshimura Y, Yamamoto S, Yamamoto T. 1983. Quantitative interrelationship between effects of nitrogen and energy intakes on egg protein utilization in young men. *Tokushima J Exp Med* **30**: 17–24.
- Pellett P, Young V. 1992. The effect 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 Interactions (Scrimshaw NS, Schürch B, eds), p 81–136. United Nations University, Tokyo.
- Millward DJ, Bowtell JL, Pacy P, Rennie MJ. 1994. Physical activity, protein metabolism and protein requirements. *Proc Nutr Soc* **53**: 223–240.
- Young VR, Munro HN, Matthews DE, Bier DM. 1983. Relationship of energy metabolism to protein metabolism. In: New Aspects of Clinical Nutrition (Kleinberger G, Deutsch E, eds), Proceedings of 4th Congress of the European Society Parenteral and Enteral Nutrition (ESPEN), Vienna, 1982, p 43–73. S. Karger Medical and Scientific Publishers, Basel.
- Calloway D. 1982. Energy-protein relationships. In: Protein Quality in Humans: Assessment and In Vitro Estimation (Bodwell CE, Adkins JS, Hopkins DT, eds), p 148–168. Avi Publishing Company, Westport.
- Kido Y, Tsukahara T, Rokutan K, Shizuka F, Ohnaka M, Kishi K. 1997. Japanese dietary protein allowance is sufficient for moderate physical exercise in young men. *J Nutr Sci Vitaminol* **43**: 59–71.
- Kido Y, Tsukahara T, Rokutan K, Kishi K. 1997. Recommended daily exercise for Japanese does not increase the protein requirement in sedentary young men. *J Nutr Sci Vitaminol* **43**: 505–514.
- 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**: 109–127.
- FAO/WHO. 1973. Energy and Protein Requirements. Technical Report Series 522. WHO, Geneva.
- FAO/WHO/UNU. 1985. Energy and Protein Requirements. Technical Report Series 724. WHO, Geneva.
- WHO/FAO/UNU. 2007. Protein and Amino Acid Requirements in Human Nutrition. Technical Reports Series 935. WHO, Geneva.
- Bourges H, Lopez-Castro B. 1982. Protein requirements of young adult men fed a Mexican rural diet. *Arch Latinoam Nutr* **32**: 630–649.
- Egana JI, Uauy R, Cassorla X, Barrera G, Yanez E. 1992. Sweet lupin protein quality in young men. *J Nutr* **122**: 2341–2347.
- Huang PC, Lin CP. 1982. Protein requirements of young Chinese male adults on ordinary Chinese mixed diet and egg diet at ordinary levels of energy intake. *J Nutr* **112**: 897–907.
- 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**: 1673–1687.
- Inoue G, Takahashi T, Kishi K, Komatsu T, Niiyama Y. 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 (Torun B, Young VR, Rand WM, eds), p 77–87. United Nations University, Tokyo.
- Kaneko K, Ishikawa K, Setoguchi K, Koike G. 1988. Utilization and requirement of dietary protein taking into account the dermal and miscellaneous nitrogen losses in Japanese women. *J Nutr Sci Vitaminol* **34**: 459–467.
- Komatsu T, Kishi K, Yamamoto T, Inoue G. 1983. Nitrogen requirement of amino acid mixture with maintenance energy in young men. *J Nutr Sci Vitaminol* **29**: 169–185.
- Scrimshaw NS, Wayler AH, Murray E, Steinke FH, Rand WM, Young VR. 1983. Nitrogen balance response in young men given one of two isolated soy proteins or milk proteins. *J Nutr* **113**: 2492–2497.
- Tontisirin K, Sirichakawal PP, Valyasevi A. 1981. Protein requirements of adult Thai males. In: Protein-Energy Requirements of Developing Countries: Evaluation of New Data (Torun B, Young VR, Rand WM, eds), p 88–97. United Nations University, Tokyo.
- 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.
- Wayler A, Queiroz E, Scrimshaw NS, Steinke FH, Rand WM, Young VR. 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.
- Yanez E, Uauy R, Ballester D, Barrera G, Chavez N, Guzman E, Saitua MT, Zacarias I. 1982. Capacity of the Chilean mixed diet to meet the protein and energy requirements of young adult males. *Br J Nutr* **47**: 1–10.
- Young VR, Taylor YS, Rand WM, Scrimshaw NS. 1973. Protein requirements of man: efficiency of egg protein utilization at maintenance and submaintenance levels in young men. *J Nutr* **103**: 1164–1174.
- 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-

- teins. *J Nutr* **105**: 534–542.
- 27) Young VR, Puig M, Queiroz E, Scrimshaw NS, Rand WM. 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.
 - 28) Higaki H, Tsukahara M, Kido Y, Oguri S, Inoue G, Kishi. 1989. Utilization and requirement of habitual mixed dietary protein in Japanese. *J Nutr Sci Vitaminol* **43**: 192 (in Japanese).
 - 29) Cheng AH, Gomez A, Bergan JG, Lee TC, Monckeberg E, Chichester CO. 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.
 - 30) Gersovitz M, Motil K, Munro HN, Scrimshaw NS, Young VR. 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.
 - 31) Campbell WW, Crim MC, Dallal GE, Young VR, Evans WJ. 1994. Increased protein requirements in elderly people: new data and retrospective reassessments. *Am J Clin Nutr* **60**: 501–509.
 - 32) Castaneda C, Charnley JM, Evans WJ, Crim MC. 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**: 30–39.
 - 33) Ebisawa H, Ohzeki T, Ichikawa M, Fujita Y. 1992. Protein intake for maintenance of nitrogen balance in the elderly. *Reports of the Research Committee of Essential Amino Acids (Japan)* **136**: 9–12.
 - 34) 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.
 - 35) Intengan CL, Roxas BV, Loyola A, Carlos E. 1981. Protein requirements of Filipino children 20 to 29 months old consuming local diets. In: Protein-Energy Requirements of Developing Countries: Evaluation of Newdata (Torun B, Young VR, Rand WM, eds), p 172–181. United Nations University, Tokyo.
 - 36) Torun B, Cabrera-Santiago MI, Viteri FE. 1981. Protein requirements of pre-school children: Milk and soybean protein isolate. In: Protein-Energy Requirements of Developing Countries: Evaluation of Newdata (Torun B, Young VR, Rand WM, eds), p 182–190. United Nations University, Tokyo.
 - 37) Egana MJI FA, 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 (Rand WM, Uauy R, Scrimshaw NS, eds), p 249–257. United Nations University, Tokyo.
 - 38) Intengan C. 1984. Protein requirements of Filipino children 20–29 months old consuming local diets. In: Protein-Energy Requirements of Developing Countries: Results of International (Torun B, Young VR, Rand WM, eds), p 258–264. United Nations University, Tokyo.
 - 39) Gattas V, Barrera GA, Riumallo JS, Uauy R. 1990. Protein-energy requirements of prepubertal school-age boys determined by using the nitrogen-balance response to a mixed-protein diet. *Am J Clin Nutr* **52**: 1037–1042.
 - 40) Gattas V, Barrera GA, Riumallo JS, Uauy R. 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.
 - 41) Howat PM, Korslund MK, Abernathy RP, Ritchey SJ. 1975. Sweat nitrogen losses by and nitrogen balance of preadolescent girls consuming three levels of dietary protein. *Am J Clin Nutr* **28**: 879–882.
 - 42) Korslund MK, Leung EY, Meiners CR, Crews MG, Taper J, Abernathy RP, Ritchey SJ. 1976. The effects of sweat nitrogen losses in evaluating protein utilization by pre-adolescent children. *Am J Clin Nutr* **29**: 600–603.
 - 43) Viteri FE, Martinez C. 1984. 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 (Torun B, Young VR, Rand WM, eds), p 164–168. United Nations University, Tokyo.
 - 44) Torun B, Viteri FE. 1984. Obligatory nitrogen losses and factorial calculations of protein requirements of pre-school children. In: Protein-Energy Requirements of Developing Countries: Evaluation of Newdata (Torun B, Young VR, Rand WM, eds), p 159–163. United Nations University, Tokyo.
 - 45) Fomon SJ, Haschke F, Ziegler EE, Nelson SE. 1982. Body composition of reference children from birth to age 10 years. *Am J Clin Nutr* **35**: 1169–1175.
 - 46) Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. 2000. Body composition during the first 2 years of life: an updated reference. *Pediatr Res* **47**: 578–585.
 - 47) Ellis KJ, Shypailo RJ, Abrams SA, Wong WW. 2000. The reference child and adolescent models of body composition. A contemporary comparison. *Ann NY Acad Sci* **904**: 374–382.
 - 48) Takai T, Kuhara Y, Ose T, Koushi T. 1968. Observation on the breast milk and powdered formula fed ad libitum (Part 2). *Acta Paediatrica Japonica* **72**: 1583–1584 (in Japanese).
 - 49) Allen J, Keller R, Archer P, Neville M. 1991. Studies in human lactation: milk composition and daily secretion rates of macronutrients in the first year of lactation. *Am J Clin Nutr* **54**: 69–80.
 - 50) Nommsen LA, Lovelady CA, Heinig MJ, Lonnerdal B, Dewey KG. 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.
 - 51) Yoneyama K. 1998. Growth of breast-fed infants and intake of nutrients from breast-milk. *J Child Health* **57**: 49–57 (in Japanese).
 - 52) Kitamura K, Ochiai F, Shimizu Y, Tateoka Y, Tsukamoto H, Hotta K. 2002. Change of the macronutrient concentration in breast milk. *Jpn J Mater Health* **43**: 493–499 (in Japanese).
 - 53) Suzuki K, Sasaki S, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Jpn J Nutr Diet* **62**: 369–372.
 - 54) Hirose J, Endo M, Nagao S, Mizushima K, Narita H, Shibata K. 2008. Amount of breast milk sucked by Japanese breast feeding infants. *J Jpn Soc Breastfeed Res* **2**: 23–28.
 - 55) Yamamoto Y, Komekubo M, Iida K, Takahashi D, Tsuchiya F. 1981. The composition of Japanese breast milk. I. *J Child Health* **40**: 468–475 (in Japanese).
 - 56) Idota T, Sakurai T, Ishiyama Y, Murakami Y, Kubota J, Ii N, Sakamoto T, Doki R, Shimoda K, Asai Y. 1991. The latest survey for the composition of human milk