研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kasuga M.	Dietary Reference Intakes for Japanese. Preface.	J Nutr Sci Vitaminol	59, suppl	S1	2013
Tokudome S.	Dietary Reference Intakes for Japanese. Foreword.	J Nutr Sci Vitaminol	59, suppl	S2	2013
Tsuboyama-Kasaoka N, Tsubota-Utsugi M, Imai E, Nakade M, Kasuga M.	Historical overview of the establishment of Dietary Reference Intakes for Japanese.	J Nutr Sci Vitaminol.	59, suppl	S6-S8	2013
Sasaki S.	Dietary Reference Intakes for Japanese 2010: basic theories for the development.	J Nutr Sci Vitaminol	59, suppl	S9-S17	2013
Sasaki S.	Dietary Reference Intakes for Japanese 2010: basic concepts for application.	J Nutr Sci Vitaminol	59, suppl	S18-S25	2013
Tabata I, Ebine N, Kawashima Y, Ishikawa-Takata K, Tanaka S, Higuchi M, Yoshitake Y.	Dietary Reference Intakes for Japanese 2010: energy.	J Nutr Sci Vitaminol	59, suppl	S26-S35	2013
Kido Y, Shizuka F, Shimomura Y, Sugiyama T.	Dietary Reference Intakes for Japanese 2010: protein.	J Nutr Sci Vitaminol	59, suppl	S36-S43	2013
Ezaki O, Miyake Y, Sato S, Iso H.	Dietary Reference Intakes for Japanese 2010: fat.	J Nutr Sci Vitaminol	59, suppl	S44-S52	2013
Yamada K, Tsuboyama-Kasaoka N, Goda T, Saito K, Yamanouchi T, Yokoyama T, Chonan O, Imai E, Nakade M, Aoe S.	Dietary reference intakes for Japanese 2010: carbohydrates.	J Nutr Sci Vitaminol	59, suppl	S53-S56	2013
Tanaka K, Terao J, Shidoji Y, Tamai H, Imai E, Okano T.	Dietary Reference Intakes for Japanese 2010: fat-soluble vitamins.	J Nutr Sci Vitaminol	59, suppl	S57-S66	2013
Shibata K, Fukuwatari T, Imai E, Hayakawa T, Watanabe F, Takimoto H , Watanabe T, Umegaki K.	Dietary Reference Intakes for Japanese 2010: water-soluble vitamins.	J Nutr Sci Vitaminol	59, suppl	S67-S82	2013
Uenishi K, Ishimi Y, Nakamura K, Kodama H, Esashi T.	Dietary Reference Intakes for Japanese 2010: macrominerals.	J Nutr Sci Vitaminol	59, suppl	S83-S90	2013
Yoshida M, Kikunaga S, Yamauchi J, Tsubota-Utsugi M, Kodama H, Morita A, Esashi T.	Dietary Reference Intakes for Japanese 2010: microminerals.	J Nutr Sci Vitaminol	59, suppl	S91-S102	2013
Morita A, Yoshiike N, Takimoto H, Tsubota-Utsugi M, Kodama H, Shimizu T, Sugiyama T, Tabata I, Tamai H, Tsutsumi C, Hirahara F, Kuzuya M, Gomi I, Nakamura K, Yoshida H, Nakamura M.	Dietary Reference Intakes for Japanese 2010: lifestage.	J Nutr Sci Vitaminol	59, suppl	S103-S109	2013
Nagae A, Kuwabara A, Tozawa K, Kumode M, Takeuchi Y, Tanaka K.	Enteral nutrition and antibiotic use increase the risk for vitamin K deficiency in patients with severe motor and intellectual disabilities.	e-SPEN Journal,			in press
小林奈穂, 村山伸子, 稲村雪子, 久保田恵, 神田知子, 高橋孝子, 石田裕美.	給食施設における「日本人の食事摂取基準」の活用の現状（第一報）―病院および介護老人保健施設を対象とした質問紙調査―.	栄養学雑誌	70 記念号		印刷中

小林奈穂, 村山伸子, 稲村雪子, 久保田恵, 神田知子, 高橋孝子, 金光秀子, 辻ひろみ, 石田裕美.	給食施設における「日本人の食事摂取基準」の活用の現状（第二報）－高齢者施設を対象としたインタビュー調査―.	栄養学雑誌	70 記念号		印刷中
孫田みなみ, 笠岡（坪山）宜代, 瀧沢あす香, 坪田（宇津木）恵, 今井絵理, 岡純 .	政府が策定する食事指針・ガイドにおける食事摂取基準の活用状況.	栄養学雑誌			印刷中
Murakami K, Sasaki S, Okubo H, the Freshmen in Dietetic Courses Study II Group.	Characteristics of under- and over-reporters of energy intake among young Japanese Women.	J Nutr Sci Vitaminol	58	253-62	2012
Tokudome S, Nishi N, Tanaka H.	Towards a better National Health and Nutrition Survey in Japan.	Lancet	379	e44	2012
Shibata K, Imai E, Sano M, Fukuwatari T.	The urinary excretory ratio of nicotinamide catabolites was associated with the conversion ratio of tryptophan to nicotinamide in growing rats fed a niacin-free 20% casein diet.	Biosci Biotechnol Biochem	76	186-188	2012
Miyazaki A, Sano M, Fukuwatari T, Shibata K.	Effects of ethanol consumption on the B-group vitamin contents of liver, blood and urine in rats.	Br J Nutr	108	1034-1041	2012
Yoshida M, Fukuwatari T, Sakai J, Tsuji T, Shibata K.	Correlation between Mineral Intake and Urinary Excretion in Free-living Japanese Young Women.	Food Nutr Sci	3	123-128	2012
善方裕美, 渡辺優奈, 上西一弘他.	妊娠初期の骨密度とライフスタイル, 栄養摂取状態についての検討－SKY Study (Saitama, Kobe, Yokohama Pregnant Cohort Study) 第 1 報－.	Osteoporosis Japan	20	514-516	2012
神田知子, 高橋孝子, 久保田恵, 小林奈穂, 村山伸子, 齊藤陽子, 増田利隆, 河野美穂, 石田裕美.	栄養管理報告書を用いた特定給食施設における食事摂取基準の活用に関する調査.	栄養学雑誌	70	140-151	2012
吉田宗弘.	クロムはヒトの栄養にとって必須の微量元素だろうか？	日本衛生学雑誌	67	485-491	2012

ISSN 0301-4800

JOURNAL OF NUTRITIONAL SCIENCE AND VITAMINOLOGY

2013
Volume 59
Supplement

Dietary Reference Intakes for Japanese 2010

JNSVA5 59(Supplement) S1–S109 (2013)

Edited by
JAPAN SOCIETY OF NUTRITION & FOOD SCIENCE
AND THE VITAMIN SOCIETY OF JAPAN

Published by
CENTER FOR ACADEMIC PUBLICATIONS JAPAN

Dietary Reference Intakes for Japanese 2010

Editors

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Research Group of Health and Labour Sciences Research Grants "Research on the Application and Revision of the Dietary Reference Intakes for Japanese"

Preface

Preparing a Revised Version of the Dietary Reference Intakes

The 2010 version of Dietary Reference Intakes for Japanese (DRIs-J) has been prepared on the basis of the concept of DRIs in-line with the policy adopted for the DRIs-J 2005 version, which recommended that the criteria created be as evidence-based as possible.

The preparatory process accounted for as many as 40 working group-based conferences involving more than 50 researchers, who considered all studies of interest available to date, including domestic, international, and those studies and documents that served as the basis for the earlier version of DRIs. The 1,300 studies have been cited in the current DRIs-J 2010.

The following concept provided the basis for revising the existing DRIs. Generally, health disturbances associated with energy and nutritional intake are evaluated in terms of deficiency/insufficiency and excess, which may have implications for prophylaxis of lifestyle-related diseases. Therefore, the existing criteria for energy and nutritional intake, i.e., the DRIs, were re-formulated to address such issues. However, optimal energy and nutritional intake varies from individual to individual and within individuals and does not readily lend itself to calculation, thus calling for a probabilistic approach to its estimation.

In the current DRIs-J 2010, this approach allowed reference values to be estimated for energy as well as for 34 different nutrients. Beyond these estimates, the DRIs-J 2010 included recommendations on nutritional guidance, i.e., a description of the theoretical concept of the DRIs as a basis for "improvement of diet" and "management of food services," as well as associated considerations and a description of the theoretical principle adopted for the DRIs-J 2010. Furthermore, while providing estimates, the nutritional needs of individuals at each stage of their life have been carefully considered, with emphasized focus on infants, children, pregnant and lactating women, and the elderly; these were the stages that were given special attention during developing DRIs and when recommending DRIs.

Our future tasks include accumulating relevant high-quality evidence from Japanese and DRI-based studies, while characterizing the nutritional needs of individuals at different stages of their life and sorting the health issues associated with each of these stages.

Finally, only if the rationale for the indices used, scientific basis for the estimated values, and the process that led to the revision of DRIs have been fully appreciated can the DRIs be used meaningfully. Thus, it is not intended that the estimated reference values compiled in the DRIs are to be blindly adhered to, but that they serve as flexible criteria.

August 23, 2012

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Chairman, Expert Committee for "Dietary Reference Intakes for Japanese 2010"
President, National Center for Global Health and Medicine

Foreword

Preface to the English Version of the Dietary Reference Intakes for Japanese (DRIs-J) 2010

In order to prevent nutritional deficiencies, the Ministry of Health and Welfare, Japan first launched the Recommended Dietary Allowances for the Japanese in 1970 and has made periodic revisions every 5 years up to its 6th edition in 1999. The 7th version was issued in 2004 as the Dietary Reference Intakes for Japanese (DRIs-J) 2005. The current DRIs-J 2010 (for April 2010–March 2014) were established in 2009 by the Ministry of Health, Labour and Welfare (MHLW) on the basis of the Health Promotion Law.

The project to revise DRIs-J 2010 began in 2008. More than 50 scientists in Japan with proven expertise in the field of nutrition and physical activity were asked to participate in this program by the MHLW. In order to update the DRIs-J 2010 on a scientific basis, more than 1,300 articles were reviewed.

To avoid adverse effects of deficient/insufficient and excess and/or imbalanced consumption of energy and nutrients, the newly-edited DRIs-J 2010 incorporate 6 reference values based on sex, age group (life stage), and physical activity level–1 value for energy and 5 values for 34 nutrients–for healthy individuals and groups, including those with certain mild illnesses, such as hypertension, diabetes, or hyperlipidemia. However, the DRIs-J do not incorporate any dietary instructions/restrictions or prescribed diets.

The reference value for energy is the estimated energy requirement (EER), and the 5 reference values for the 34 nutrients include 3 for deficiencies–estimated average requirement (EAR), recommended dietary allowance (RDA), and adequate intake (AI), 1 for adverse effects–tolerable upper intake level (UL), and 1 for primary prevention of lifestyle-related diseases–tentative dietary goal for preventing lifestyle-related diseases (DG).

The 34 nutrients include major nutrients (protein, fat [total fats, saturated fatty acids, n-6 and n-3 polyunsaturated fatty acids, and cholesterol], carbohydrates [carbohydrate, dietary fiber], vitamins [fat-soluble vitamins: A, D, E, and K; water-soluble vitamins: B_1, B_2, niacin, B_6, B_{12}, folate, pantothenic acid, biotin and C]), and minerals (macrominerals: sodium, potassium, calcium, magnesium and phosphorus; microminerals: iron, zinc, copper, manganese, iodine, selenium, chromium and molybdenum).

The National Institute of Health and Nutrition proposed publication of the English version of the DRIs-J 2010 and all edited articles, which were prepared by the members involved in the research group for Research on the Application and Revision of the DRIs for Japanese as part of Comprehensive Research on Lifestyle-related Diseases including Cardiovascular Diseases and Diabetes Mellitus with Health and Labour Sciences Research Grants under the auspices of the MHLW. The articles provide compact descriptions of the DRIs-J 2010, including information on the historical overview of the establishment of the DRIs, basic theories for the development, basic concepts for their application, the DRI values for energy, protein, fat, carbohydrates, water-soluble vitamins, fat-soluble vitamins, macrominerals, microminerals, and the DRIs-J according to the life stage.

We sincerely hope this publication will be informative and useful for health professionals/staff engaged, particularly, in developing, planning, and implementing DRIs for the assessment of diet/nutrition and for the management of food services to individuals and groups. May it serve to promote/maintain health, prevent lifestyle-related diseases, including non-communicable diseases, and enhance the quality of life or well-being through diet, nutrition, and physical activity among the people of Asian Pacific areas/countries and worldwide.

August 16, 2012

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J Nutr Sci Vitaminol, **59**, S6–S8, 2013

Historical Overview of the Establishment of Dietary Reference Intakes for Japanese

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(Received October 26, 2012)

Summary Although nutritional standards for Japanese were published by national organizations until the 1940s, the Recommended Dietary Allowances (RDAs) for Japanese was officially established in 1969 by the Ministry of Health and Welfare (presently Ministry of Health, Labour and Welfare). These RDAs were revised every five years until 2005, when they were established as Dietary Reference Intakes for Japanese (DRIs-J). The nutrients included in RDAs and DRIs-J were changed according to the health condition and eating habits of Japanese. The current version, DRIs-J 2010, comprises reference values for energy and 34 nutrients.

Key Words dietary reference intakes, Recommended Dietary Allowances, history, Ministry of Health, Labour and Welfare

Historical Overview

Many nutrients are presently recognized to play an important role in human nutrition not only because they are essential for growth and maintenance of health, but also because they play an important role in the reduction of risk of noncommunicable diseases. The values of nutrient intakes that make allowance for individual variation in requirements and provide a margin of safety above the minimal requirement to prevent deficiencies have traditionally formed the basis for the establishment of the Recommended Dietary Allowances (RDAs).

Preliminary values for nutrient requirements for Japanese were first described in 1926 in the book *Nutrition* by Dr. Tadasu Saiki (*1*), the founder of the National Institute of Nutrition (presently National Institute of Health and Nutrition) in Japan. The National Institute of Nutrition played a key role in conducting basic scientific studies and developing nutrient requirements for Japanese. In responce to food shortage resulting from World War II, some national organizations created nutritional standards independently for Japanese until around 1945. Since then nutritional standards for Japanese have been developed by the Prime Minister's Office (presently Cabinet Office, government of Japan) and the Science and Technology Agency (presently Ministry of Education, Culture, Sports, Science and Technology) to promote growth, to maintain health and physical strength, and to improve work efficiency.

From 1969, the Ministry of Health and Welfare became the presiding ministry to create RDAs in Japan (*2*). The RDAs used for the time period 1970–1975 were officially established by six committees. As shown in Table 1, RDAs was subsequently revised every five years until 2005 for the purpose of improving physique and corresponding to changes in population structure, economy or dietary habits (*2–8*). The concept of Dietary Reference Intakes was first introduced in the 6th revision of the RDAs (2000–2005) (*8*). In order to more comprehensively follow the approach used in devising the 6th revision of the RDAs, the 7th revision was established as the "Dietary Reference Intakes for Japanese (DRIs-J) 2005" by the Ministry of Health, Labour and Welfare (MHLW) (*9*). These DRIs-J were based on a systematic review of the evidence. The current version, "DRIs-J 2010," was created based on the Health Promotion Law by the MHLW (*10*).

DRIs-J expanded on the basic theories of the US/Canadian DRIs in order to create DRIs that are specific to the Japanese population. The DRIs-J were designed not only to prevent energy or nutrient deficiencies that may be caused by insufficient intake of energy or nutrients, but also for the primary prevention of lifestyle-related diseases caused by excess and/or imbalanced consumption of energy and nutrients. DRIs-J consists of six reference values (one for energy and five for nutrients) for the prevention of deficiencies, adverse effects by excess intake, and lifestyle-related diseases. In addition, the recommended dietary intake level is shown as a range rather than a singular value.

Historical Changes in Values for Energy and Nutrients

In 1926, Dr. Saiki proposed the concept used as the basis of future Estimated Average Requirement (EAR), Adequate Intake (AI) or Estimated Energy Requirement (EER), and he calculated the energy requirement for Japanese. Since that time, national organizations decided to

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Table 1. History of the development of Dietary Recommendations in Japan by Ministry of Health, Labour and Welfare.

Versions	Periods of use	Date recommendations were made	Contents
RDAs 1st (*2*)	Apr. 1970–Mar. 1975	Aug. 1969	Energy+10 Nutrients
RDAs 1st revision (*3*)	Apr. 1975–Mar. 1980	Mar. 1975	Energy+9 Nutrients
RDAs 2nd revision (*4*)	Apr. 1980–Mar. 1985	Aug. 1979	Energy+12 Nutrients
RDAs 3rd revision (*5*)	Apr. 1985–Mar. 1990	Aug. 1984	Energy+13 Nutrients
RDAs 4th revision (*6*)	Apr. 1990–Mar. 1995	Sep. 1989	Energy+15 Nutrients
RDAs 5th revision (*7*)	Apr. 1995–Mar. 2000	Mar. 1994	Energy+16 Nutrients
RDAs 6th revision —DRIs— (*8*)[1]	Apr. 2000–Mar. 2005	Jun. 1999	Energy+28 Nutrients
DRIs-J 2005 (*9*)	Apr. 2005–Mar. 2010	Oct. 2004	Energy+34 Nutrients
DRIs-J 2010 (*10*)	Apr. 2010–Mar. 2015	May 2009	Energy+34 Nutrients

RDAs, Recommended Dietary Allowances; DRIs, Dietary Reference Intakes.
[1] The concept of DRIs was introduced in the RDAs 6th revision.

include values for selected nutrients in the nutritional standards, based on the accumulation of new evidence from the scientific literature. Table 2 shows the historical changes to the established energy and nutrients that are included in the dietary recommendations in Japan by MHLW. Reference values for energy, protein, vitamin A, vitamin D, vitamin B_1, vitamin B_2, vitamin C, calcium and iron were included in all versions of the RDAs from the 1st to the current DRIs-J 2010. Although the 1st version of RDAs only included 10 nutrients (*2*), the current DRIs-J 2010 provides recommendations for 34 nutrients (*10*). Changes to nutrient reference values for the RDAs and DRIs-J are established based on changes in the health condition and/or dietary habits of Japanese at the time of revision. In particular, it was important that the nutritional problem in Japan expanded to include not only nutrient deficiency and improvement of physical strength but also excess and/or imbalanced consumption of energy and nutrients, lack of exercise, increase of overweight/obesity and chronic disease. In order to correspond to these problems, not only the results of an experimental studies but also epidemiological studies were added to evidence for DRIs-J creation.

Selection criteria for inclusion of nutrients in DRIs-J are 1) nutrients that are essential for life and the maintenance and/or improvement of health, and 2) nutrient intake values that are backed by scientific evidence or have achieved global consensus. Nutrient values that could not be established due to insufficient evidence are not included.

This paper describes an overview of the history and establishment of DRIs in Japan. Future revisions of DRIs-J must take into account the health condition and eating habits of Japanese in order to determine the kinds of nutrients that should be included.

REFERENCES

1) Saiki T. 1926. Nutrition. Eiyousha, Tokyo.
2) Ministry of Health and Welfare (Council of Nutrition). 1969. Recommended Dietary Allowance for the Japanese. Ministry of Health and Welfare, Tokyo.
3) Ministry of Health and Welfare (Council of Nutrition). 1975. Recommended Dietary Allowance for the Japanese (1st revision). Ministry of Health and Welfare, Tokyo.
4) Ministry of Health and Welfare (Section of Nutrition, Council for Public Health). 1979. Recommended Dietary Allowance for the Japanese (2nd revision). Ministry of Health and Welfare, Tokyo.
5) Ministry of Health and Welfare (Section of Nutrition, Council for Public Health). 1984. Recommended Dietary Allowance for the Japanese (3rd revision). Ministry of Health and Welfare, Tokyo.
6) Ministry of Health and Welfare (Section of Nutrition, Council for Public Health). 1989. Recommended Dietary Allowance for the Japanese (4th revision). Ministry of Health and Welfare, Tokyo.
7) Ministry of Health and Welfare (Section of Nutrition, Council for Public Health). 1994. Recommended Dietary Allowance for the Japanese (5th revision). Ministry of Health and Welfare, Tokyo.
8) Ministry of Health and Welfare (Section of Health Promotion, Council for Public Health). 1999. Recommended Dietary Allowance for the Japanese (6th revision). Ministry of Health and Welfare, Tokyo.
9) Ministry of Health, Labour and Welfare (Expert Committee for "Dietary Reference Intakes for Japanese"). 2004. Dietary Reference Intakes for Japanese, 2005. Ministry of Health, Labour and Welfare, Tokyo.
10) Ministry of Health, Labour and Welfare (Expert Committee for "Dietary Reference Intakes for Japanese"). 2009. Dietary Reference Intakes for Japanese, 2010 Ministry of Health, Labour and Welfare, Tokyo.

Table 2. Historical changes to the established energy and nutrients included in the Dietary Recommendations in Japan.

Versions		RDAs							DRIs-J	
		1st	1st revision	2nd revision	3rd revision	4th revision	5th revision	6th revision —DRIs—[1]	2005	2010
Energy		RDA	RDA	RDA	RDA	RDA	RDA	RDA	EER	EER
Protein		RDA	RDA	RDA	RDA	RDA	RDA	RDA	EAR, RDA, DG	EAR, RDA
Fat	Total fat	—	—	RDA	RDA	RDA	RDA	RDA	DG	DG
	Saturated fatty acids	—	—	—	—	—	—	—	DG	DG
	n-6 fatty acids	—	—	—	—	—	—	—	AI, DG	AI, DG
	n-3 fatty acids	—	—	—	—	—	—	—	AI, DG	AI, DG
	Cholesterol	—	—	—	—	—	—	—	DG	DG
Carbohydrates	Carbohydrates	—	—	—	—	—	—	—	DG	DG
	Dietary fibers	—	—	—	—	—	target amount	target amount	AI, DG	DG
Fat-soluble vitamins	Vitamin A	RDA	RDA	RDA	RDA	RDA	RDA	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Vitamin D	RDA	RDA	RDA	RDA	RDA	RDA	RDA, UL	AI, UL	AI, UL
	Vitamin E	—	—	—	—	target amount	target amount	RDA, UL	AI, UL	AI, UL
	Vitamin K	—	—	—	—	—	—	RDA, UL	AI	AI
Water-soluble vitamins	Vitamin B_1	RDA	RDA	RDA	RDA	RDA	RDA	RDA	EAR, RDA	EAR, RDA
	Vitamin B_2	RDA	RDA	RDA	RDA	RDA	RDA	RDA	EAR, RDA	EAR, RDA
	Niacin	RDA (nicotinic acid)	RDA (nicotinic acid)	RDA	RDA	RDA	RDA	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Vitamin B_6	—	—	—	—	—	—	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Vitamin B_{12}	—	—	—	—	—	—	RDA	EAR, RDA	EAR, RDA
	Folate	—	—	—	—	—	—	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Pantothenic acid	—	—	—	—	—	—	RDA	AI	AI
	Biotin	—	—	—	—	—	—	RDA	AI	AI
	Vitamin C	RDA	RDA	RDA	RDA	RDA	RDA	RDA	EAR, RDA	EAR, RDA
Macrominerals	Sodium	RDA (sodium chloride)	—	target amount	target amount	target amount	target amount	—	EAR, DG	EAR, DG
	Potassium	—	—	—	target amount	target amount	target amount	RDA	AI, DG	AI, DG
	Calcium	RDA	RDA	RDA	RDA	RDA	RDA	RDA, UL	AI, DG, UL	EAR, RDA, UL
	Magnesium	—	—	—	—	target amount	target amount	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Phosphorus	—	—	target amount	target amount	target amount	target amount	RDA, UL	AI, UL	AI, UL
Microminerals	Iron	RDA	RDA	RDA	RDA	RDA	RDA	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Zinc	—	—	—	—	—	—	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Copper	—	—	—	—	—	—	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Manganese	—	—	—	—	—	—	RDA, UL	AI, UL	AI, UL
	Iodine	—	—	—	—	—	—	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Selenium	—	—	—	—	—	—	RDA, UL	EAR, RDA, UL	EAR, RDA, UL
	Chromium	—	—	—	—	—	—	RDA, UL	EAR, RDA	EAR, RDA
	Molybdenum	—	—	—	—	—	—	RDA, UL	EAR, RDA, UL	EAR, RDA, UL

RDA, Recommended Dietary Allowance; DRIs-J, Dietary Reference Intakes for Japanese; EAR, estimated average requirement; AI, adequate intake; EER, estimated energy requirement; UL, tolerable upper intake level; DG, tentative dietary goal for preventing lifestyle-related diseases.
Persons≧1 y old.
[1] The concept of DRIs was introduced in the RDAs 6th revision.

J Nutr Sci Vitaminol, **59**, S9–S17, 2013

Dietary Reference Intakes for Japanese 2010: Basic Theories for the Development

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(Received October 26, 2012)

Summary The Dietary Reference Intakes for Japanese (DRIs-J) 2010 was developed to provide reference values for the intake of energy and 34 nutrients for health maintenance and promotion and primary prevention of lifestyle-related diseases in healthy individuals and groups. The DRIs-J 2010, which follows the main concepts of the DRIs-J 2005, the prior version, provides the values for energy requirements as expressed by the estimated energy requirement (EER) and the values for nutrient intake as expressed by 5, the estimated average requirement (EAR), recommended dietary allowance (RDA), adequate intake (AI), tolerable upper intake level (UL), and tentative dietary goal for preventing lifestyle-related diseases (DG). On account of 3 factors—optimal intake varies among individuals, intake cannot be measured precisely, and the DRIs are aimed at maintaining health and preventing disease over the long term rather than addressing acute health effects in the short term—the DRIs were determined using the probability approach to provide the appropriate values for habitual rather than short-term intake. Each value of the DRIs used in the DRI-J 2010 is provided for 13 age groups (the values for energy and protein are provided for 14 groups), with separate values provided for women who are pregnant or lactating and for men and women. The EER is provided for 3 physical activity levels and the EAR, RDA, AI, and UL for 19, 18, 10, and 16 nutrients, respectively. The basic concepts behind the DRIs-J 2010 are almost same as those behind the DRIs of the United States and Canada with the unique exception that the DRIs-J 2010 also includes the DGs, dietary goals that were independently determined after consideration of the average body size, disease prevalence, and dietary habits of the Japanese population and the cumulative evidence regarding Japanese and East Asian populations. The DRIs-J 2010 has been used in practice since 2010 and is expected to be used until 2014. This review briefly describes the basic theories in its development.

Key Words dietary reference intakes, development, theory, Japan

Introduction

Released every 5 y by the Ministry of Health, Labour, and Welfare of Japan, the Dietary Reference Intakes for Japanese (DRIs-J) are the core values used in developing national nutritional guidelines for the Japanese population. The most recent version, the DRIs-J 2010, contains practically the same values as those contained in the Report from the Expert Committee for "Dietary Reference Intakes for Japanese," which was released in 2009. Until fiscal year 2004, Japan had been using the recommended dietary allowance (RDA) as an index with some small modifications in accordance with changing needs in each period. In 2005, Japan began using the DRIs, as reflected in the development of the DRIs-J 2005, with which the DRIs-J 2010 largely accords. This review briefly describes the basic theories used in the development of the DRIs-J 2010, which is undoubtedly fundamental in understanding its proper use. This brief review consists of the following 3 sections: (1) the criteria used in the selection of nutrient and energy values, (2) the determination of the each of the DRIs and (3) the basic parameters used in designing the DRIs.

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Selection Criteria

The selection criteria for each nutrient included in the DRIs were the following: (1) the nutrient is essential for human life and the maintenance and improvement of health, (2) the required intake of the nutrient can be quantitatively defined, and (3) the required intake can be determined with a sufficient level of scientific reliability. Nutrients found to be closely associated with the development of lifestyle-related diseases in the Japanese population were also selected. Based on these criteria, 34 nutrients were selected for inclusion in the DRIs-J 2010. Energy was also included as an essential dietary factor for maintenance of human life. Quantitative values were established according to sex, age group, and pregnancy/lactation status.

Individual Values of the DRIs

1. Energy

For adults, a certain fixed energy intake is necessary to maintain body weight. Insufficient energy intake leads to weight loss, leanness, and protein–energy mal-

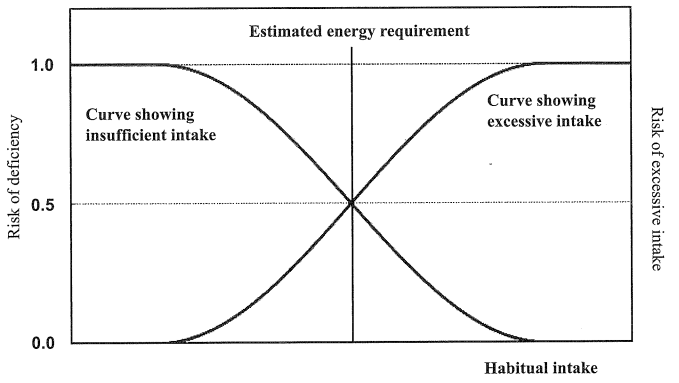

Fig. 1. Theoretical model for understanding estimated energy requirement. Left and right vertical axes show probability of insufficient and excessive intake for individuals, respectively.

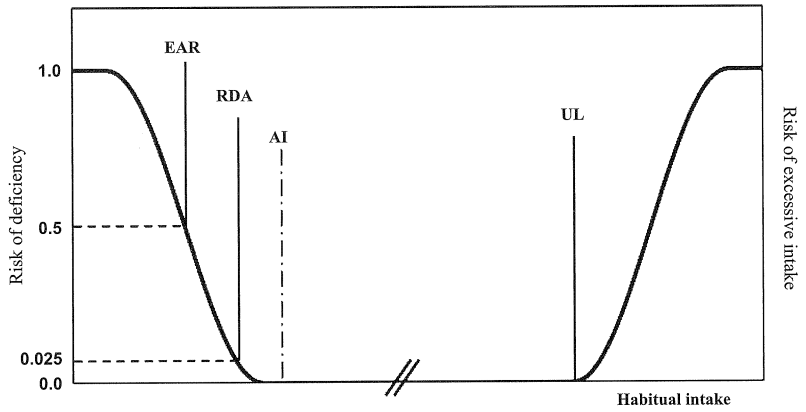

Fig. 2. Theoretical model for understanding EAR, RDA, AI, and UL for nutrients. EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.

nutrition, while excessive intake leads to body weight gain and obesity. Energy intake is optimized when intake equals expenditure (i.e., when energy balance is achieved), resulting in no weight change. The energy requirement that expresses optimal intake is established mainly using values obtained using the doubly labeled water method to assess samples of the Japanese population and the reference of values of populations of other countries. As it is impossible to measure an individual's required intake accurately; the energy value is an estimated value, and thus referred to as the *estimated* energy requirement (EER). The EER is established based on sex, age group, and physical activity level (PAL). The EER is recommended for use in practical settings in place of the true energy requirement because the latter is not possible to determine precisely. An energy intake close to the EER results in a high probability of body weight maintenance, whereas as intake above or below EER results in a high probability of body weight gain or loss, respectively, as illustrated in Fig. 1. By applying this concept to a group, the probability can be converted into the percentage of a population with excessive or insufficient energy intake of energy. PAL is categorized into 3 levels (low, moderate, and high).

2. *Nutrients*

2-1. Basic concept. The EAR was established only for evaluating insufficient nutrient intake, not ensuring adequate or optimal intake, and thus cannot be the only value used in practice. The recommended dietary allowance (RDA) was thus established for use in a practical setting, while the adequate intake (AI) was established for nutrients for which neither the EAR nor RDA can be established. As is discussed later, the AI is more similar to the RDA than the estimated average requirement (EAR) in its application. All 3 DRIs are used for evaluating nutrient deficiency. For those nutrients for which excessive intake has been reported to pose a health hazard, the tolerable upper intake level (UL) was established. However, the UL cannot be determined for several nutrients that may pose a health hazard because of insufficient data for value determination. Figure 2 illustrates a theoretical model of the EAR, RDA, AI, and UL. Applying this figure to a group gives the percentage of individuals with health problems due to insufficient or excessive intake.

Several nutrients are included because of their role in the primary prevention of lifestyle-related diseases. However, both the quantity and the quality of research into the values for these nutrients for this purpose has been insufficient (*1*). For this reason, the index established for this purpose is referred to as the *tentative* dietary goal for preventing lifestyle-related diseases (DG).

Table 1. Basic concepts of indices and characteristics of nutrients.

Objective	Prevention of deficiency	Prevention of health problems due to excessive intake	Primary prevention of lifestyle-related diseases
Indices	EAR, RDA, AI	UL	DG
Main methods, laboratory studies, epidemiologic studies for establishing evidence	Laboratory studies, epidemiologic studies (including intervention studies)	Case reports	Epidemiologic studies (including intervention studies)
Importance of certain nutrients regarding targeted health problems	Important	Important	Not consistently important due to existence of many other related environmental factors
Typical period associated with health problems	Several months	Several months	Several years to several decades
Number of reports of target health problems	Very few to many	Very few to few	Many
Possibility of developing target health problems from typical food intake	Yes	Very little	Yes
Possibility of developing target health problems even with intake of supplements and fortified foods	Yes (supplements include only a limited number of nutrients)	Yes (particular attention is needed)	Yes (supplements include only a limited number of nutrients)
Strength of need to consider established values	Consider when possible (depending on needs)	Must be considered	Consider along with various related factors
Possibility of developing target health problems at established intake	Low possibility when intake is approximate to or above RDA or AI	Very low possibility when intake is below UL but not 0%	Possible because related factors may also contribute development of problems

EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level; DG, tentative dietary goal for preventing lifestyle-related diseases.

The characteristics of and concepts related to these DRIs are summarized in Table 1 (2). From an application point of view, DRIs related to insufficient and excessive intake should be given the highest priority; only when these DRIs have been found reliable should primary prevention of lifestyle-related diseases be considered. Table 2 shows the list of nutrients and each of the DRIs established for individuals aged 1 y and above. For infants aged 0 to 11 mo, DRIs were established for 30 nutrients excluding saturated fatty acid, cholesterol, carbohydrate, and dietary fiber.

2-2. EAR. The EAR is defined as the estimated average requirement of an entire defined population (e.g., Japanese men aged 30 to 49 y) based on the distribution of the required intake as measured in a sample population. In other words, it is defined as the intake that satisfies the requirement for 50% (and at the same time does not satisfy that of 50%) of individuals in a certain population. Intake equal to the EAR does not necessarily suggest development of classical nutrient deficiency. The definition of deficiency varies among nutrients.

2-3. RDA. The RDA is defined as the intake that satisfies the requirement of nearly all (97 to 98%) individuals of a certain population. The RDA is theoretically calculated using the standard deviation (SD) of the distribution of the required intake as observed in an experimental study from which the EAR was determined using the following formula:

$$RDA = EAR \times (1 + 2 \times SD)$$

However, because experimental studies can rarely successively determine the SD, an estimated value is generally used instead. The RDA can also be determined using the coefficient of variation (CV) of the EAR and the following formula:

$$RDA = EAR \times (1 + 2 \times CV)$$

The CVs used in the DRIs are shown in Table 3.

Table 2. Nutrients listed and indices used in the Dietary Reference Intakes for individuals aged 1 y and over.[1]

Nutrient			EAR	RDA	AI	UL	DG
Group	Sub-group	Nutrient					
Protein			✓	✓	—	—	—
Fat		Total fat	—	—	—	—	✓
		Saturated fatty acids	—	—	—	—	✓
		n-6 fatty acids	—	—	✓	—	✓
		n-3 fatty acids	—	—	✓	—	✓
		Cholesterol	—	—	—	—	✓
Carbohydrates		Carbohydrates	—	—	—	—	✓
		Dietary fiber	—	—	—	—	✓
Vitamin	Fat-soluble vitamins	Vitamin A	✓	✓	—	✓	—
		Vitamin D	—	—	✓	✓	—
		Vitamin E	—	—	✓	✓	—
		Vitamin K	—	—	✓	—	—
	Water-soluble vitamins	Vitamin B_1	✓	✓	—	—	—
		Vitamin B_2	✓	✓	—	—	—
		Niacin	✓	✓	—	✓	—
		Vitamin B_6	✓	✓	—	✓	—
		Vitamin B_{12}	✓	✓	—	—	—
		Folic acid	✓	✓	—	✓[2]	—
		Pantothenic acid	—	—	✓	—	—
		Biotin	—	—	✓	—	—
		Vitamin C	✓	✓	—	—	—
Mineral	Macrominerals	Sodium	✓	—	—	—	✓
		Potassium	—	—	✓	—	✓
		Calcium	✓	✓	—	✓	—
		Magnesium	✓	✓	—	✓[2]	—
		Phosphorus	—	—	✓	✓	—
	Microminerals	Iron	✓	✓	—	✓	—
		Zinc	✓	✓	—	✓	—
		Copper	✓	✓	—	✓	—
		Manganese	—	—	✓	✓	—
		Iodine	✓	✓	—	✓	—
		Selenium	✓	✓	—	✓	—
		Chromium	✓	✓	—	—	—
		Molybdenum	✓	✓	—	✓	—

EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level; DG, tentative dietary goal for preventing lifestyle-related diseases.

[1] Included when DRIs were defined only for certain age groups.

[2] Defined as intake other than that from typical foods.

Table 3. Coefficient of variation used to estimate the recommended dietary allowance from the estimated average requirement.

Coefficient of variation	Coefficient used for calculating recommended dietary allowance	Nutrients
10%	1.2	Vitamin B_1, vitamin B_2, niacin, vitamin B_6, vitamin B_{12}, folic acid, vitamin C, calcium, magnesium, iron for adolescents aged 15 to 17 y, zinc, selenium, chromium, molybdenum
12.5%	1.25	Protein
15%	1.3	Copper
20%	1.4	Vitamin A, iron for children aged 6 mo to 14 y, iodine

2-4. AI. The AI is defined as the intake sufficient to maintain the health of and prevent the nutrient deficiency of almost all members of a population. The AI is used only when both the EAR and RDA are unavailable. Determination of the AI is mainly based on epidemiologic observations of the nutritional intake of a healthy population and the following 3 concepts:

1) For nutrients for which insufficient intake is unlikely, the AI is estimated from the results of simultaneous assessment of health status by the presence of biomarkers and other factors and nutrient intake. When almost no insufficiency is observed, the median intake value is used as the AI.

2) For nutrients for which biomarker and others are unavailable but the representative nutrient distribution of the Japanese population is available, the median intake value is used as the AI.

3) For infants, the AI is determined by multiplying the volume of typical milk intake and the typical nutrient content of breast milk.

2-5. UL. The UL is defined as the upper limit of habitual intake that is considered to pose no risk of health problems. Theoretically, the UL is the no observed adverse effect level (NOAEL), the maximum intake determined to result in no adverse effects in human studies. Due to limited data regarding the NOAEL in humans and the fact that the studies upon which it is based were of isolated groups, the UL is given as the NOAEL divided by an uncertainty factor (UF) varying from 1 to 5 according to conditions. When the lowest observed adverse effect level (LOAEL), the minimum intake known to cause adverse effects based on studies of particular groups with excessive intake or use of supplements, is known, the NOAEL is determined by dividing the LOAEL by 10.

Adverse effects due to excessive intake in humans are rarely reported, and ethical considerations prohibit conducting human studies into determination of the NOAEL and LOAEL. Therefore, both the NOAEL and LOAEL are estimated based on data collected from animal or, in some cases, in-vitro studies. When only the LOAEL is available, the NOAEL is estimated by dividing the LOAEL by a UF of 10, estimated based on animal studies. When neither the scientific basis nor a consensus of professionals is sufficient for determining the UF, an appropriate UF is selected within a range of 1 to 5 when human data are available and a UF of 10 when only animal data are available. The UFs used in the DRIs are shown in Table 4. It should be noted that determination of the UL slightly differs among nutrients.

Table 4. Uncertainty factor used for calculation of tolerable upper intake level.

UF	Nutrients
1	Vitamin E, copper, manganese, iodine (infants)
1.2	Vitamin D (adults), calcium, phosphorus
1.5	Vitamin A (pregnant women), zinc, iodine (adults)
1.8	Vitamin D (infants)
2	Molybdenum
3	Folic acid, selenium
5	Vitamin A (adults), niacin, vitamin B_6
10	Vitamin A (infants)
30	Iron

2-6. DG. A DG is given as preferable intake for primary prevention of lifestyle-related diseases by reducing the risk of their development and that of their biological markers. A DG is determined based on epidemiologic studies and reference to the results of experimental studies. However, the relationship between nutritional intake and risk of developing lifestyle-related diseases is continuous in nature. No remarkable threshold exists, making it difficult to propose an optimum intake range or threshold.

In the DRIs-J 2010, the diseases for which DGs were established were limited to cardiovascular diseases (e.g., hypertension, dyslipidemia, stroke, and myocardial infarction) and cancer (especially stomach cancer). As such, the DGs pertain to intake of fats (fatty acids), cholesterol, carbohydrates, dietary fiber, sodium (salt), and potassium. The major strategy for prevention of osteoporosis and bone fracture, a strongly desirable goal, is maintenance of bone mass. Of the nutrients related to bone health, among which calcium and vitamin D appear in the DRIs-J 2010, a DG was not given for calcium because the EAR and RDA were determined using bone mass as a marker of deficiency of calcium intake, nor was a DG given for vitamin D because of insufficient consensus regarding the determination of the AI of vitamin D, specifically the use of plasma 25-hydroxyvi-

Table 5. Basic and specific dietary goals for selected nutrients.

Basic goal	Specific goal	Nutrients
Modify intake to approximate DG	Increase intake	Dietary fiber, *n*-3 fatty acids, potassium
	Decrease intake	Cholesterol, sodium
DG is given as a range and goal is modifying to come within range		Total fat, saturated fatty acids, carbohydrates
EAR, RDA, or AI is given and only a UL is given for DG		*n*-6 fatty acids

EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases.

tamin D level. The EAR and RDA of vitamin C were determined with some consideration of the prevention of cardiovascular disease. Since the vitamin C requirement has the character of a DG, a DG for vitamin C was not given considering the calculation process. DGs for saturated fatty acids, *n*-6 fatty acids, and carbohydrates were determined using percentage of energy rather than weight of intake per day (e.g., grams per day) as a unit in consideration of the importance of the energy balance of these nutrients. The goal in determining several DGs was bringing habitual intake toward an upper or lower intake level, while the goal in determining other DGs was to bring or keep habitual intake within a certain intake range. The relationships among the types of DGs and nutrients are shown in Table 5.

Basic Parameters Used in Designing the DRIs

1. Age group

Table 6 shows the manner in which segments of a population were classified into different age groups for determination of the DRIs. As in the DRIs-J 2005, infants were generally divided into 2 groups—aged 0 to 5 and 6 to 11 mo—and further divided into 3 groups for determination of energy and protein intake—aged 0 to 5, 6 to 8, and 9 to 11 mo. Children and adolescents were defined as those aged from 1 to 17 y and adults as those aged 18 y and above. For nutrients for which special consideration of the intake of the elderly was necessary, those aged 70 y and above were defined as elderly.

2. Reference body size

The DRIs are expressed only as single representative values of intake for each sex and age group without consideration of body size (body height and weight) within each group. In other words, all the values were determined based on assumption of a typical body size for each sex and age group. For all age groups of individuals aged 1 y and above, typical body size is based on the median height and weight of each sex and age group as reported by the 2005 and 2006 National Health and Nutrition Survey (NHNS) in Japan (*3*, *4*). For infants aged 0 to 11 mo, typical body size is based on the median values of each sex and age group reported by the 2000 National Growth Survey in Infancy and Childhood (*5*). Table 6 lists the values obtained.

3. Nutrient intakes used to establish AIs and DGs

In certain instances, the nutrient intake of a population must be measured to establish AIs and DGs. In the DRIs-J 2010, the median and percentile of sex- and age-group-specific intake reported in the 2005 and 2006 NHNS (*3*, *4*) were used as reference values. The age group classification of children aged 6 to 11 y differed between the DRIs-J 2010 and the National Health and Dietary Assessment such that the former included 3 groups (6 to 7, 8 to 9, and 10 to 11 y) and the latter 2 groups (6 to 8 and 9 to 11 y). Hence, the mean value of children aged 6 to 8 y, the average of the mean values of children aged 6 to 8 y and aged 9 to 11 y, and the mean value of children aged 9 to 11 y as reported in the 2005 and 2006 NHNS were to determine the DRIs for the age groups 6 to 7 y, 8 to 9 y, and 10 to 11 y, respectively.

It is well known that the accuracy of almost all

Table 6. Reference values of body size based on body height and body weight.[1]

Sex	Males		Females[2]	
Age	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)
0–5 mo	61.5	6.4	60.0	5.9
6–11 mo	71.5	8.8	69.9	8.2
6–8 mo	69.7	8.5	68.1	7.8
9–11 mo	73.2	9.1	71.6	8.5
1–2 y	85.0	11.7	84.0	11.0
3–5 y	103.4	16.2	103.2	16.2
6–7 y	120.0	22.0	118.6	22.0
8–9 y	130.0	27.5	130.2	27.2
10–11 y	142.9	35.5	141.4	34.5
12–14 y	159.6	48.0	155.0	46.0
15–17 y	170.0	58.4	157.0	50.6
18–29 y	171.4	63.0	158.0	50.6
30–49 y	170.5	68.5	158.0	53.0
50–69 y	165.7	65.0	153.0	53.6
≥70 y	161.0	59.7	147.5	49.0

[1] Median of each age group as reported in the 2005 and 2006 National Health and Nutrition Survey in Japan was used for all age groups of individuals aged 1 y old and over. Median height and weight as shown in the growth percentile curve for each month in the 2000 National Growth Survey in Infancy and Childhood was used for infants aged under 1 y.

[2] Excluding pregnant women.

dietary assessments, including those conducted using the dietary record method, suffer from under-reporting (*6*). One Japanese study reported an average under-reporting rate of 16% in men and 20% in women (*7*). However, the extent of under-reporting in the 2005 and 2006 NHNS (*3*, *4*). upon whose data the DRIs-J 2010 were largely based, is unknown. A theory and practical means of resolving this problem have not been proposed in either Western countries or Japan. Therefore, the data obtained from the surveys (*3*, *4*) were used without any adjustment for possible under-reporting. Table 7 lists the nutrients for which intake data were used to determine the AIs or DGs.

4. Integration of research results

Determination of the DRIs was performed in accordance with reference to systematic reviews and the results of high-quality studies to the greatest extent possible. Because a value must have been determined using results from more than one study, the guidelines shown in Table 8 were used for integration of research results.

5. Consideration of intervention studies using supplements

Supplementation of several nutrients at extremely high doses that cannot be obtained from typically ingested foods is thought to prevent lifestyle-related diseases. Any intervention studies using supplements to examine this claim were consulted in determining the DRIs and included as references. However, as there have also been reports of unfavorable health effects (*8*) after certain favorable results have been reported, a conservative standpoint was used when considering the suitability of additional intake from non-usual sources, such as supplements. The results of studies that examined intake levels unachievable by consumption of typical foods were not considered in the determination of the DGs.

6. Extrapolation methods

6-1. Basic concepts. The data used to establish 5 DRIs (EAR, RDA, AI, UL, and DG) were obtained for a limited range of sex and age groups. Therefore, establishing the DRIs for each sex and age group required extrapolation of available data from one group to other groups. As the reference values for the EAR and AI are often based on the daily intake (weight/day) while the reference values for the UL are given per kg of body weight, different extrapolation methods were used. The EAR for each sex and age group was established by extrapolating from the EAR reference values. The RDA for each sex and age group was established by multiplying the EAR by the coefficient shown in Table 3. The sex- and age-group-specific AI was calculated by extrapolation from the reference AI value.

6-2. EAR and AI. It is difficult to develop a method of extrapolation that accounts for the characteristics of each nutrient. Because the efficiency of energy metabolism highly correlates with body surface area, a formula estimating body surface area from body height and/or body weight has been widely used to determine energy metabolism. Among the formulae developed to estimate body surface area from body height and/or weight (*9*), a formula developed in 1947 using the weight ratio to the 0.75 power was used in determining the DRIs (*10*). Recent studies have reported that this method is useful for estimating the organ weights of various animals, including the cardiovascular and respiratory organ weights of mammals (*11*). Based on these reports, extrapolation is performed as follows when EAR and AI reference values per day (weight/day) and a representa-

Table 7. Nutrients for which intake data were available to compute adequate intakes and dietary goals.

Index	Nutrients
AI	*n*-6 fatty acid, *n*-3 fatty acid, vitamin D, vitamin E, pantothenic acid, biotin[1], phosphorus, manganese[1]
DG	Total fat, saturated fatty acid, *n*-3 fatty acid, sodium, potassium

AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases.
[1] Data obtained from sources other than the 2005 and 2006 National Health and Nutrition Survey in Japan were used as references.

Table 8. Methods used to integrate study results.

Extent of similarity or difference among study results	Availability or lack of studies using Japanese subjects	Integration concept of study results
Relatively similar	Relative availability	Use of studies with priority
	Relative lack	Use of all studies with equal priority and the mean of the values reported
Relatively different	Availability of relatively high-quality studies	Use of studies with priority
	Availability of relatively low-quality studies	Use of selected high-quality studies and the mean of the values reported
	Lack of studies	

Table 9. Growth factors used in determination of EAR and AI for children and adolescents aged 1 y and over.

Age group	Growth factor
Males and females 1–2 y	0.30
Males and females 3–14 y	0.15
Males 15–17 y	0.15
Females 15–17 y	0
Males and females 18 y and over	0

EAR, estimated average requirement; AI, adequate intake.

tive value (median or mean) of body weight of a given group are available:

$$X=X_0\times(W/W_0)^{0.75}\times(1+G),$$

where X=EAR or AI (intake per day) of a specific age group, X_0=reference value of EAR or AI (intake per day), W=reference body weight of the specific age group, W_0=median or mean of body weight of group that provided EAR or AI reference value, and G=growth factor (see Table 9).

In several studies, the EAR or AI reference value is given per kg of body weight. In such cases, extrapolation is performed as follows:

$$X=X_0\times W\times(1+G),$$

where X=EAR or AI (intake per day) of a specific age group, X_0=reference value of EAR or AI (intake per day), W=reference body weight of age group, and G=growth factor (see Table 9).

For children, the following growth factor values must also be taken into account: (*1*) the additional intake of a nutrient required for growth and (*2*) the quantity of the nutrient accumulated in the body during growth. To obtain these values, the values used by the FAO, WHO, and UNU (*12*) and the United States and Canada in their DRIs (*9*) were modified for each age group of the Japanese population (Table 9). For infants aged 6 to 11 mo, the following 2 methods were considered: (*1*) extrapolation based on the value for infants aged 0 to 5 mo and (*2*) use of the median value of infants aged 0 to 5 mo and children aged 1 to 2 y. For extrapolation of the DRI values to infants aged 0 to 5 mo, the following formula has been proposed (*9*):

DRI for infants aged 0 to 5 mo
=reference weight of infants aged 6 to 11 mo/(reference weight of infants aged 0 to 5 mo)$^{0.75}$

As infants aged 0 to 5 mo are in the growth stage, determination of their DRIs must consider allowances for growth factors, which the formula given above fails to do. When the value of the reference weight is substituted in the formula, the expressions for boys and girls are $(8.8/6.4)^{0.75}$ and $(8.2/5.9)^{0.75}$, yielding values of 1.27 and 1.28, respectively. As use of these formulae produces extrapolated values that are slightly different for boys and girls, the mean of these values is used to determine the AI for both sexes.

6-3. UL. As is the case for the EARs and AIs, none of methods used to extrapolate the ULs produce values that are sufficiently reliable. For age groups for which data are insufficient, 1 of 2 methods is generally used to establish the value. When the UL reference value is given as a quantity in terms of kg of body weight, the UL is extrapolated as follows:

$$X=X_0\times W,$$

where X=UL (intake per day) of a specific age group, X_0=UL reference value (intake per day), and W=reference body weight of the specific age group.

When the UL reference value is given as a quantity per day, the UL is extrapolated as follows:

$$X=X_0\times(W/W_0),$$

where X=UL (intake per day) of a specific age group, X_0=UL reference value (intake per day), W=reference body weight of the specific age group, W_0=median or mean of body weight of group that provided UL reference value.

7. Methods of rounding values

For the sake of convenience and reliability, EAR, RDA, AI, UL, and DG values are routinely rounded off according to the rules shown in Table 10. For all age groups of children and adults, a single rule was applied for each nutrient. Values for infant and additional values for pregnant and lactating women were rounded to the same number of digits as those used for other sex and age classes. After rounding values, they were smoothed when necessary to remove an excessive difference from neighboring age groups.

Table 10. Methods used for rounding values.

Approximate median value	Method of rounding
0.5	Nearest 0.1
1	Nearest 0.1
5	Nearest 0.5
10	Nearest whole number
50	Nearest 5
100	Nearest 10
500	Nearest 50
1,000	Nearest 100
5,000	Nearest 500

When reference value of UL was given as a quantity per day, the extrapolation equation used was the following: $X=X_0\times(W/W_0)$, where X=UL (intake per day) of a specific age group, X_0=reference value of UL (intake per day), W=reference body weight of the specific age group, W_0=median or mean of body weight of group that provided reference value of UL.

Discussion

This review briefly described the theory used in determining the DRIs-J 2010, whose understanding is indispensable in the appropriate use of the values contained in this report. The theory is similar to those used in determining the DRIs in the United States and Canada. However, the DRIs-J 2010 adopted the concept of prevention of chronic diseases using DGs. This is unique and seems to be important because control and prevention of major chronic diseases, i.e., lifestyle-related diseases, is the most important issue in most of devel-

oped countries. However, the scientific basis behind this concept is insufficient, requiring its modification based on scientific evidence accumulated in the future. Continued effort to establish the most appropriate DRIs for the Japanese population should be strongly encouraged with an eye toward future revision of the DRIs.

REFERENCES

1) Trumbo PR. 2008. Challenges with using chronic disease endpoints in setting dietary reference intakes. *Nutr Rev* **66**: 459–464.
2) Sasaki S. 2005. Chapter 8 Understanding the DRIs with epidemiologic concepts, p 217–240. *In*: Easy-to-understand Evidence-based Nutrition and Nutritional Epidemiology, Dobunshoin, Tokyo (in Japanese).
3) Ministry of Health, Labour and Welfare, Japan. 2007. National Health and Nutrition Survey Japan (2005).
4) Ministry of Health, Labour and Welfare, Japan. 2009. National Health and Nutrition Survey Japan (2006).
5) Kato N, Okuno A, Takaishi M. 2001. Results on 2000 national growth survey in infancy and childhood. *Shoni Hoken Kenkyu* (*J Child Health*) **60**: 707–720 (in Japanese).
6) Livingstone MB, Black AE. 2003. Markers of the validity of reported energy intake. *J Nutr* **133** (Suppl 3): S895–920.
7) 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.
8) Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. 2005. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* **142**: 37–46.
9) Food and Nutrition Board, Institute of Medicine. 1998. The B vitamins and choline: overview and methods. *In*: Dietary Reference Intakes: For Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline (Institute of Medicine, ed.), p 27–40. National Academy Press, Washington DC.
10) Kleiber M. 1947. Body size and metabolic rate. *Physiol Rev* **27**: 511–541.
11) West GB, Brown JH, Enquist BJ. 1997. A general model for the origin of algometric scaling laws in biology. *Science* **276**: 122–126.
12) FAO/WHO/UNU. 1985. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Technical Report Series, No. 724. WHO, Geneva.

J Nutr Sci Vitaminol, 59, S18–S25, 2013

Dietary Reference Intakes for Japanese 2010: Basic Concepts for Application

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(Received October 26, 2012)

Summary The Dietary Reference Intakes for Japanese (DRIs-J) 2010 is not merely as 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. This review briefly describes the basic concepts in the application of the DRIs-J 2010. It consists of two sections considering the purposes of use in the Dietary Reference Intakes (DRIs) in Japan: (1) the basic concepts in their application and related issues and (2) the methods of their application. The latter is further divided into 3 sections each describing a goal in the application of the DRIs: (1) improvement of diet for an individual, (2) improvement of diet for a group, and (3) management of food services. A major challenge in the application of the DRIs is that compared to research into determination of the intake of energy and nutrients for development of the DRIs, research into application of the DRIs has been extremely scarce in Japan. 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.

Key Words dietary reference intakes, application, Japan

Introduction

This review briefly describes the basic concepts in the application of the Dietary Reference Intakes for Japanese (DRIs-J) 2010. Although the use of standardized concepts for DRIs has been proposed in the United States and Europe, universal concepts have not yet been established (*1–3*). As body size, major health problems, and nutritional intake all differ between Japanese and Western populations, country-specific conceptualization of the DRIs is needed.

Basic Concepts in DRI Application and Related Issues

1. Target individuals and groups

The targets of the DRIs are healthy individuals and groups mainly composed of healthy individuals, as well as individuals not receiving dietary education or undergoing dietary therapy or restriction and individuals with low levels of risk factors, such as high blood pressure, dyslipidemia, or hyperglycemia. In cases in which dietary education, therapy, or restriction is recommended to an individual or a group for treatment or prevention of a disease, disease-specific guidelines should be referred to and the DRIs-J 2010 should be used as a supplemental reference. Several studies have reported differences between the estimated average requirements (EARs) and the nutritional requirements of healthy individuals and certain groups, including the elderly (i.e., those needing nursing care) and the disabled (*4–6*). However, as evidence regarding these differences has not yet sufficiently accumulated, it is still unclear whether the values developed for healthy subjects are applicable to these groups.

2. Sources of intake

With some exceptions, the primary sources of energy and nutrients are foods eaten as meals, including fortified foods, and dietary supplements taken for health improvement and not for treatment of disease.

3. Duration of intake

The DRIs are standards for "habitual" intake expressed as "intake per day." Thus, they apply to long-term rather than short-term (e.g., single-day) intake. This is due to the fact that health problems addressed by the DRIs are caused by habitual inadequate intake. The period needed to develop health problems due to inadequate intake depends on the nutrient(s) involved and the type of health problems. For example, serum vitamin B_1 level decreases greatly 2 wk after eliminating vitamin B_1 from the diet, and various symptoms caused by its deficiency emerge within 4 wk (*7*). This illustrates the necessity of dietary management of vitamin B_1 within a period shorter than 1 mo. On the other hand, excessive intake of sodium (salt) is correlated with hypertension due to aging (*8*), indicating the importance of the dietary management of sodium over several decades.

Due to the characteristics of nutrient intake, in particular its day-to-day variability, it is difficult to define the habitual intake of a particular nutrient. According to previous observations (*9–12*), the period required for

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Table 1. Priority of application of DRIs-J 2010 energy and nutrient intake values.

Energy/nutrient	Nutrients (examples)	Notes
1. Energy	—	Including alcohol
2. Protein	Protein	—
3. Fat	Fat	% energy (%E)
4. Nutrients listed in food composition table[1] (nutrients for which both EAR and RDA or AI has been established)	Vitamin A, vitamin B_1, vitamin B_2, vitamin C, calcium, iron	Nutrients for which critical deficiency has been observed and for which prevention of deficiency is important. Requires consideration of relatively short-term intake.
5. Nutrients listed in food composition table[1] (nutrients for which a DG has been established)	Saturated fatty acids, dietary fiber, sodium, potassium	Nutrients important in primary prevention of lifestyle-related diseases. Requires consideration of relatively long-term intake.
6. Nutrients not listed in food composition table[1]	—	Usually low priority except for particular groups or groups with particular food habits.

[1] Table appears in *Standard Tables of Food Composition in Japan*, 5th Revised and Enlarged Edition.
DRIs-J, Dietary Reference Intakes for Japanese; EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases.

assessing or managing habitual intake is approximately 1 mo, with some exceptions for nutrients with great day-to-day variability in intake.

4. Priority of goals and nutrients in nutritional management (Table 1)

Reliability and priority in application are not same among energy and nutrients. Maintaining adequate energy balance between intake and expenditure is fundamental in nutritional management. Nutrients are categorized into 2 types depending on the purpose of intake: avoidance of both insufficient and excessive intake (while considering natural growth in infants and children) and primary prevention of lifestyle-related diseases. As the former should be given priority, EARs, recommended daily allowances (RDAs), adequate intakes (AIs), and tolerable upper intake level (ULs) should be determined prior to determining tentative dietary goals for preventing lifestyle-related diseases (DGs). DGs should only be considered when maintenance of health status is assured. Priority is also low for nutritional management of nutrients without confirmed deficiency in humans and for nutrients for which intake cannot be measured or estimated. However, the order of priority is not fixed and may need to be changed, depending on the characteristics of the individuals or groups that are being assessed and the goals of the DRIs.

5. Points for application based on each of the DRIs

5-1. Estimated energy requirement. In nutritional management, the estimated energy requirement (EER) of an individual must be considered to determine the energy per serving. The EER is determined by measurement of energy expenditure using the doubly labeled water method. Physical activity level (PAL) is estimated using the following formula, which is based on measurement of energy expenditure and basal metabolic rate (BMR):

$$PAL = EER/BMR$$

However, as the EER is immeasurable from an application point of view, it is estimated from BMR and PAL with consideration of sex and age class using the following formula:

$$EER = BMR \times PAL$$

Nevertheless, the BMR is not always easy to measure, and the estimation error of PAL tends to be large. It is therefore not always practical to estimate energy requirements using the BMR and PAL.

Several formulae have been proposed to estimate the BMR based on individual characteristics, including sex, age, height, and weight, such as the Harris-Benedict equation (*13*); an equation developed by the Food and Agricultural Organization (FAO), the World Health Organization (WHO), and the United Nations University (UNU) (*14*); and the NIHN equation for the Japanese population (*15*). However, equations developed for Western populations have been found to overestimate the EER for the Japanese population (*16*, *17*). Thus, when using these equations for estimating an individual's energy requirement, their reliability and applicability must be fully considered, in addition to the estimation error of PAL.

The true energy requirement has been found to have a standard deviation of 200 kcal/d among male adults and 160 kcal/d among female adults (*18*). Because of this wide variation in true energy requirement at an individual level and several other factors, determination of energy balance (i.e., balance between energy intake and expenditure) should be based on evaluation of body weight and body mass index (BMI), both of which are relatively easy to measure accurately, instead of comparison of EER with energy intake as evaluated by

Table 2. Differences between nutrient definitions in DRIs-J 2010 and *Standard Tables of Food Composition in Japan*, 5th Revised and Enlarged Edition.

Nutrient	Difference		Notes when intake or serving size is estimated from food composition table[1] for use in DRIs-J 2010
	DRIs-J 2010	Food composition table[1]	
Vitamin E	Only α-tocopherol is reported.	α-, β-, γ-, and δ-tocopherol are reported individually.	Only α-tocopherol should be used.
Niacin	Niacin equivalents (=niacin [mg]+1/60 tryptophan [mg]) is used.	Nicotinic acid equivalent is used (niacin synthesized in the body from tryptophan is not included).	Niacin (mg) + 1/60 tryptophan (mg) should be used. Since tryptophan concentration in food is roughly 1/100 that of protein, its value approaches the value of niacin (mg)+1/6,000 protein (mg), and can be rewritten as niacin (mg)+1/6 protein (g).

[1] Reference *27*).

dietary assessment.

5-2. EAR and RDA. Since use of the EAR poses a 50% probability of insufficient intake, dietary intervention is needed when the intake of several or many members in a group is below the EAR. The RDA is the intake level that poses a nearly 0% of deficiency in an individual or the individuals in a group. Therefore, if the intake of individuals or a group approaches or is above the RDA, it can be assumed that they face nearly no risk of deficiency. However, users of the DRIs-J 2010 should understand the purpose and definition of each DRI and the characteristics of each nutrient because the application method differs according to the purpose.

5-3. AI. The AI is determined when the EAR is not available. Although there is very low risk of deficiency when the intake of a nutrient is above the AI, it is not possible to identify the existence of deficiency or its risk when intake is below the AI.

5-4. UL. The UL indicates a threshold intake above which a risk of health problems exists. Since UL values are theoretically and empirically difficult to establish, most are based on a few reports of accidental overdose, indicating the insufficiency of scientific evidence for determining ULs. Therefore, individuals should use ULs as values to avoid approaching rather than to avoid exceeding, and not use them in primary prevention of lifestyle-related diseases.

5-5. DG. A DG is established for primary prevention of lifestyle-related diseases. As diet is one of many causes of lifestyle-related diseases, it is not correct to strictly maintain DG simply for their primary prevention. For example, excessive intake of sodium (salt) is just one of several factors increasing the risk of hypertension (*19*). Compared to health problems due to insufficient or excessive intake, lifestyle-related diseases are considered outcomes of lifestyle factors, including dietary habits, sustained over very long periods. In view of this consideration, long-term (lifetime) management is more important than strict short-term management.

6. Dietary assessment

6-1. Relationship to application. Evaluation of energy and nutrient intake is performed for comparison of an intake value with its corresponding DRI value. However, due to the various problems discussed below, especially measurement errors in dietary assessment, users of the DRIs-J 2010 must pay careful attention to the means of standardization and endeavor to maintain accuracy in both assessment and interpretation of the values.

6-2. Under- and over-reporting. Of the several methods used for dietary assessment, most are based on self-reporting by subjects, inevitably leading to reporting errors. Of under- and over-reporting, the most significant reporting errors, under-reporting occurs more frequently. Under-reporting of energy in particular requires careful attention. In research, the level of measurement error differs, depending on the assessment method used and subject characteristics. Among Japanese adults, males under-report their energy intake by 11% on average and females by 15% (*20*).

Under-reporting may have a highly negative effect on the interpretation of a dietary assessment. For example, the excessive energy intake of a man who gains 5 kg in a year is 96 kcal/d (i.e., 7,000×5/365), assuming that 1 kg of body weight is equal to approximately 7,000 kcal (*21*, *22*). The measurement error due to under-reporting by 13% would be 260 kcal/d for a man whose total energy intake is 2,000 kcal/d, a value much larger than the 96 kcal/d. This example shows that under-reporting makes it almost impossible to compare a value obtained by dietary assessment with the EER. Furthermore, under- and over-reporting are strongly affected by the degree of obesity (*23*). Comparing intake estimated from analysis of 24-h urinary excretion of nitrogen (a biomarker of protein intake), potassium, and sodium and the corresponding self-reported intake of Japanese subjects, one study found a clear relationship between the degree of reporting error and the degree of obesity in terms of BMI (*24*).

6-3. Day-to-day variation. It is widely known that day-to-day variations exist in energy and nutritional intakes (*8*). Nevertheless, determination of intake dis-

Table 3. Basic concepts in applying DRIs-J 2010 for dietary improvement of individuals.

Purpose	Indices	Dietary assessment	Planning for and application of dietary improvement
Assessment of energy balance	Change in BMI and/or body weight	Balance is negative when BMI is below 18.5 and positive when BMI is over 25.0.	Planning should aim to maintain BMI within normal range.
		Evaluation of change by measurement of body weight change.	Note: Measurement should be performed at least twice within a certain period and plans reviewed and revised based on the results.
Assessment of insufficient nutrient intake	EAR, AI	Determination of percentage of individuals with intake below EAR.	Planning should aim to minimize the number of individuals with intake below EAR.
		When using AI, compare AI and measured intake to ensure that intake is not below AI.	When intake is approximate to or above RDA or AI, planning should aim to maintain intake. Note: Measurement of intake below AI does not indicate the probability of inadequacy.
Assessment of excessive nutrient intake	UL	Estimation of possibility of excessive intake by comparing measured intake and UL.	When intake is above UL, planning should aim to reduce intake below UL. Note: Intake above UL should be avoided. When excessive intake is reported, plans should be reviewed, revised, and implemented promptly.
Assessment of risk of primary prevention of lifestyle-related disease	DG	Comparison of measured intake and DG. However, assessment should be done with comprehensive consideration of existence and degree of other nutrition-related and non-nutrition-related factors of target lifestyle-related disease.	Planning should aim to maintain intake within a range of DG. Note: Assessment of target nutrient should be conducted with comprehensive consideration of (1) the existence and degree of other nutrition-related and non-nutrition-related factors contributing to the target lifestyle-related disease and (2) the sustainability of a plan over the long term, as lifestyle-related diseases develop over the course of the lifespan.

DRIs-J, Dietary Reference Intakes for Japanese; BMI, body mass index; EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level; DG, tentative dietary goal for preventing lifestyle-related diseases.

tributions without consideration of day-to-day variations is required, as the DRIs do not consider variations despite the fact that the degree of day-to-day variation in energy and nutrient intake differs among individuals and groups (*9–12*). A further challenge is that due to difficulties in study methodology, actual day-to-day variation in the Japanese remains poorly investigated.

Day-to-day variation also poses difficulty in assessing the intake distribution of a group. Because of day-to-day variation, a distribution curve obtained from assessment of a nutrient over a limited number of days is narrower than that obtained from assessment of habitual intake. Therefore, the observed percentage of individuals with deficient/insufficient or excessive intake depends on the number of days examined in a dietary assessment (*25*). Moreover, seasonal variation as a component of day-to-day variation must be considered. The intake of several nutrients, including vitamin C, has been found to have clear seasonal variation in Japanese populations (*7, 11, 24–26*).

6-4. Food composition table. A food composition table is used to calculate nutrient intakes in a dietary assessment and those of the menu of a food service. However, the definitions of the nutrients slightly differ between the DRIs and the food composition table (*27*) (Table 2).

7. Assessment of body size, clinical symptoms, and results of clinical examinations

Body weight and BMI are the most important and practical indices used in planning and evaluating dietary interventions. When evaluating the results of dietary interventions, change in body weight is a more practical index than change in BMI. In an intervention for weight decrease or increase, body weight should be measured and recorded every 4 wk and be followed up for more than 16 wk (*28*). Besides body size, abdominal girth, body fat percentage, and other indices may be used, depending on the purpose of the intervention.

Clinical symptoms and the results of clinical examinations may also be used as indices of insufficient or excessive intake of nutrients. For iron, hemoglobin concentration in blood and menstrual blood loss may be

Table 4. Basic concepts in applying DRIs-J 2010 for dietary improvement of groups.

Purpose	Indices	Dietary assessment	Planning for and application of dietary improvement
Assessment of energy balance	Change in BMI and/or body weight	Balance is negative when BMI is below 18.5 and positive when BMI is over 25.0.	Planning should aim to maintain BMI within normal range.
		Evaluation of change by measurement of body weight change.	Note: Measurement should be performed at least twice within a certain period and plans reviewed and revised based on results.
Assessment of insufficient nutrient intake	EAR, AI	Determination of percentage of individuals with intake below EAR.	Planning should aim to minimize number of individuals with intake below EAR.
		When using AI, compare AI and measured intake to ensure that intake is not below AI using distribution of measured intake.	When using AI, planning should aim to increase mean group intake to approximate AI. Note: It is difficult to compare percentage of individuals with intake below EAR and the percentage with intake below AI because the percentages have different meanings.
Assessment of excessive nutrient intake	UL	Calculation of percentage of individuals at risk of excessive intake using distribution of measured intake and UL.	Planning should aim to reduce intake of all individuals below UL. Note: Intake above UL should be avoided. When excessive intake is reported, plans should be reviewed, revised, and implemented promptly.
Assessment of risk of primary prevention of lifestyle-related disease	DG	Calculation of percentage of individuals with intake outside range of DG using measured intake and DG.	Planning should aim to increase number of individuals with intake within or approximates the range of DG. Note: Assessment of target nutrient should be conducted with comprehensive consideration of (*1*) the existence and degree of other nutrition-related and non-nutrition-related factors contributing to the target lifestyle-related disease and (*2*) the sustainability of a plan over the long term, as lifestyle-related diseases develop over the course of the lifespan.

used as markers (*29*, *30*). However, their careful interpretation is required because clinical symptoms and the results of clinical examinations are affected by other factors besides the levels of a target nutrient.

Methods of Application

The DRIs are used for many purposes but mainly for *dietary improvement* and *management of food services*. Theories of application of dietary improvement, which consists of assessment of dietary intake, preparation based on assessment, and practice, differ between individuals and groups, and should therefore be described separately. The term *management of food service* refers to dietary planning for a particular group and an on-going meal service. The DRIs, which are the fundamental data sources used to establish dietary guidelines and recommendations, do not necessarily need to be achieved immediately for any purpose.

1. Dietary improvement of individuals

1-1. Basic concepts. Table 3 shows the basic concept in application of the DRIs to the dietary improvement of individuals. This concept is based on the concepts proposed in the DRIs of the United States and Canada (*1*, *2*, *31*) and the application patterns of the DRIs in Japan.

1-2. Dietary assessment (Table 3). For assessment of insufficient or excessive intake of energy, BMI or body weight change should be used. The Japan Society for the Study of Obesity defines a normal BMI as a value between 18.5 and 25.0 (*32*). However, even if an individual is within this range, increase or decrease in body weight suggests a positive or negative energy balance, respectively, and thus requires careful assessment.

When evaluating sufficiency of nutrient intake, either the EAR and RDA is used or, if both are unavailable, the AI. Probability of inadequacy is estimated using measured intake, the EAR, and the RDA. There is nearly no risk of inadequacy when intake is close to or above the RDA. When intake is above the EAR but below the RDA, increasing intake up to the RDA is recommended. However, decisions regarding the intake of a particular nutrient should be made with consideration of the intake of other nutrients. When intake is below the EAR, increasing intake is strongly recommended. Assessment

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 sustainably of a particular plan over many years, as prevention of lifestyle-related diseases is a lifelong 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 ±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, B_1, B_2, 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 B_1 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 Koshu 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, 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): 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, otassium 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, Iitoi 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.

J Nutr Sci Vitaminol, 59, S26–S35, 2013

Dietary Reference Intakes for Japanese 2010: Energy

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(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 (kcal/d)=basal metabolic rate (BMR) (kcal/d)×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

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Table 1. Basal metabolic rate of the Japanese population.

Sex	Males			Females		
Age	Reference BMR (kcal/kg weight/d)	Reference weight (kg)	BMR (kcal/d)	Reference BMR (kcal/kg weight/d)	Reference weight (kg)	BMR (kcal/d)
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:

BMR (kcal/d)
=Reference BMR (kcal/kg body weight/d)×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=total energy expenditure (kcal/d)/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 (kcal/d)=BMR (kcal/d)×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 l.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 l : 2 : 1. As shown in Table 2, the mean±standard deviation (SD) for the PAL of all subjects was l.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 l.69, leading the reference PAL for elderly subjects to be set as l.70. However, 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 (kcal/d)=BMR (kcal/d)×PAL+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 l.36, 1.47, 1.57, 1.59, 1.63, 1.66, and l.76 for ages l 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.

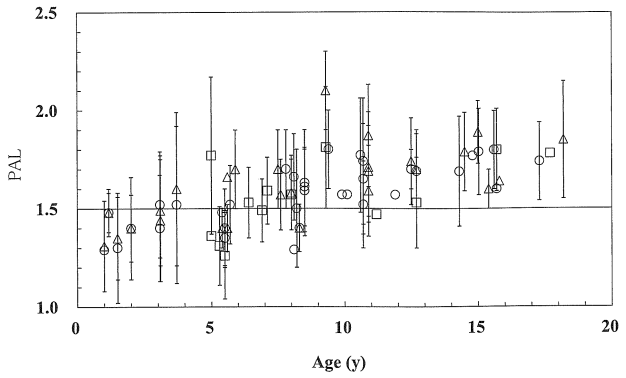

Fig. 1. PAL of children. The data presented for all age groups were taken only from studies that measured basal metabolic rate except for those for children aged 3 to 5 y, for whom data were also taken from studies that estimated basal metabolic rate, and children aged 1 to 2 y, for whom data were also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

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.

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:

EER (kcal/d)
=total energy expenditure (kcal/d)+energy deposition (kcal/d).

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*):

Total energy expenditure (kcal/d)
=92.8×reference weight (kg)−152.0.

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, attention 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*).

Total energy expenditure (kcal/d)
=82.6×body weight (kg)−29.0.

9. Additional values for pregnant women

The EER of pregnant women is calculated as follows:

EER (kcal/d)
=EER before pregnancy (kcal/d)+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:

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

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

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

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

Application

Concept of reference basal metabolic rate

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

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

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

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

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

Relationship between reference BMR and fat-free mass

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

Measurement errors in the EER

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

Physical activity level

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

MET value×1.1≒Af.

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

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

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

each sex and age group are shown in Table 6.

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 Nut* **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 PJH. 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, Pachiaudi 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 Diaetol* **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) Ramírez-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) Maffeis 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 F, Hulthen L. 2005. Physical activity questionnaire for adolescents validated against doubly labelled water. *Eur J Clin Nutr* **59**: 376–383.

65) Slinde F, 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 trance 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.

J Nutr Sci Vitaminol, 59, S36–S43, 2013

Dietary Reference Intakes for Japanese 2010: Protein

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(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 depending on the nature of the protein. Body proteins finally degrade into amino acids, some of which are form urea and are excreted. Therefore, protein has to be supplied from food even in adults. For growing children, increased quantity of dietary protein is required for construction and accumulation of newly synthesized tissues.

1-2. Energy intake

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

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

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

1-3. Lifestyle

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

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

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

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

1-4. Estimation of variability

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

2. Determining DRIs

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

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

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

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

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

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

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

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

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

Males

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

Females

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

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

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

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

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

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

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

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

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

Table 2. Protein storage during pregnancy.

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

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

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

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

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

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

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

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

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

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

AI (g/d)=12.6 (g/L)×0.78 (L/d)=9.83

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2-2. Tolerable upper intake level (UL)

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

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

The DRIs for protein are summarized in Table 3.

REFERENCES

1) Munro HN. 1951. Carbohydrate and fat as factors in protein utilization and metabolism. *Physiol Rev* **31**: 449–488.
2) Kishi K, Inoue G, Yoshimura Y, 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.
3) 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.
4) Millward DJ, Bowtell JL, Pacy P, Rennie MJ. 1994. Physical activity, protein metabolism and protein requirements. *Proc Nutr Soc* **53**: 223–240.
5) 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 Sociery Parenteral and Enteral Nutrition (ESPEN), Vienna, 1982, p 43–73. S. Karger Medical and Scientific Publishers, Basel.
6) 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.
7) 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.
8) 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.
9) Rand WM, Pellett PL, Young VR. 2003. Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am J Clin Nutr* **77**: 109–127.
10) FAO/WHO. 1973. Energy and Protein Requirements. Technical Report Series 522. WHO, Geneva.
11) FAO/WHO/UNU. 1985. Energy and Protein Requirements. Technical Report Series 724. WHO, Geneva.
12) WHO/FAO/UNU. 2007. Protein and Amino Acid Requirements in Human Nutrition. Technical Reports Series 935. WHO, Genava.
13) Bourges H, Lopez-Castro B. 1982. Protein requirements of young adult men fed a Mexican rural diet. *Arch Latinoam Nutr* **32**: 630–649.
14) Egana JI, Uauy R, Cassorla X, Barrera G, Yanez E. 1992. Sweet lupin protein quality in young men. *J Nutr* **122**: 2341–2347.
15) 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.
16) 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.
17) 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.
18) 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.
19) 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.
20) 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.
21) 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.
22) 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.
23) 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.
24) 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.
25) 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.
26) Young VR, Fajardo L, Murray E, Rand WM, Scrimshaw NS. 1975. Protein requirements of man: comparative nitrogen balance response within the submaintenance-to-maintenance range of intakes of wheat and beef pro-

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 F, 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 requierments 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 monthes old consuming local diets. *In*: Protein-Energy Requirements of Developing Countries: Results fo 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 preadolescent 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 preschool 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

obtained from Japanese mothers. Part 1. The contents of gross components and minerals. *Jpn J Pediatr Gastroenterol Nutr* **5**: 145–158 (in Japanese).

57) Yoneyama K, Goto I, Nagata H. 1995. Changes in the concentrations of nutrient components of human milk during lactation. *Jpn J Public Health* **42**: 472–481.

58) Isomura K. 2007. Analysis of the breast milk—Based on the latest Japanese breast milk—. *Obstet Gynecol Pract* **56**: 305–313 (in Japanese).

59) Dewey KG, Lonnerdal B. 1983. Milk and nutrient intake of breast-fed infants from 1 to 6 months: relation to growth and fatness. *J Pediatr Gastroenterol Nutr* **2**: 497–506.

60) Butte NF, Garza C, Smith EO, Nichols BL. 1984. Human milk intake and growth in exclusively breast-fed infants. *J Pediatr* **104**: 187–195.

61) Nakano T, Kato K, Kobayashi N, Shimatani M, Ishi K, Takimoto H, Totani M. 2003. Nutrient intake from baby foods infant formula and cow's milk—results from a nation-wide infant's dietary survey. *J Child Health* **62**: 630–639 (in Japanese).

62) Hokama T. 1998. Study on iron intakes from complementary food in an Okinawan village, Japan (Part 2). *Journal of Child Health* **57**: 45–48.

63) King JC, Calloway DH, Margen S. 1973. Nitrogen retention, total body 40 K and weight gain in teenage pregnant girls. *J Nutr* **103**: 772–785.

64) Takimoto H, Sugiyama T, Fukuoka H, Kato N, Yoshiike N. 2006. Maternal weight gain ranges for optimal fetal growth in Japanese women. *Int J Gynaecol Obstet* **92**: 272–278.

65) Pipe NG, Smith T, Halliday D, Edmonds CJ, Williams C, Coltart TM. 1979. Changes in fat, fat-free mass and body water in human normal pregnancy. *Br J Obstet Gynaecol* **86**: 929–940.

66) Forsum E, Sadurskis A, Wager J. 1988. Resting metabolic rate and body composition of healthy Swedish women during pregnancy. *Am J Clin Nutr* **47**: 942–947.

67) Butte N, Ellis K, Wong W, Hopkinson JM, Smith EO. 2003. Composition of gestational weight gain impacts maternal fat retention and infant birth weight. *Am J Obstet Gynecol* **189**: 1423–1432.

68) Metges CC, Barth CA. 2000. Metabolic consequences of a high dietary-protein intake in adulthood: assessment of the available evidence. *J Nutr* **130**: 886–889.

69) Klein CJ, Stanek GS, Wiles CE 3rd. 1998. Overfeeding macronutrients to critically ill adults: metabolic complications. *J Am Diet Assoc* **98**: 795–806.

J Nutr Sci Vitaminol, 59, S44–S52, 2013

Dietary Reference Intakes for Japanese 2010: Fat

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(Received October 26, 2012)

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

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

Background Information

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

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

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

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

Determining DRIs

1. Total fat

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

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

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

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

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

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

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

AI, adequate intake.

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

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

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

2. Saturated fat

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

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

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

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

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

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

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

3. Monounsaturated fat

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

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

4. n-6 fatty acids

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

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

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

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

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

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

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

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

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

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

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

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

5. n-3 fatty acids

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

6. Dietary cholesterol

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

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

7. Trans fatty acids

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

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

fatty acids leads to an increase in blood LDL-cholesterol and a decrease in HDL-cholesterol concentration, resulting in an increase in the LDL-cholesterol/HDL-cholesterol and total cholesterol/HDL-cholesterol ratios in a dose-dependent manner (*81*). High intake of trans fatty acids has also been associated with increased risk of CHD in a dose-dependent manner (*11*). However, it is unclear whether the incidence of CHD is significantly higher among average Japanese adults, who consume a low amount of trans fatty acids, than it is among Japanese adults who consume no trans fatty acids at all. Nevertheless, it is conceivable that in individuals with multiple risk factors for CHD, such as smoking, hypertension, diabetes mellitus, and dyslipidemia, increased intake of trans fatty acids may promote atherosclerosis to a greater degree than in individuals without these risk factors. Increased intake of trans fatty acids may increase the incidence of several diseases, such as CHD, obesity, and allergies and result in lower birth weight and increased risk of fetal loss, especially in individuals with other risk factors. Therefore, it is recommended that we eat less trans fatty acids at all ages.

REFERENCES

1) Mensink RP, Zock PL, Kester AD, Katan MB. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* **77**: 1146–1155.
2) Astrup A, Ryan L, Grunwald GK, Storgaard M, Saris W, Melanson E, Hill JO. 2000. The role of dietary fat in body fatness: evidence from a preliminary meta-analysis of ad libitum low-fat dietary intervention studies. *Br J Nutr* **83** (Suppl 1): S25–32.
3) Yu-Poth S, Zhao G, Etherton T, Naglak M, Jonnalagadda S, Kris-Etherton PM. 1999. Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. *Am J Clin Nutr* **69**: 632–646.
4) Cornier MA, Donahoo WT, Pereira R, Gurevich I, Westergren R, Enerback S, Eckel PJ, Goalstone ML, Hill JO, Eckel RH, Draznin B. 2005. Insulin sensitivity determines the effectiveness of dietary macronutrient composition on weight loss in obese women. *Obes Res* **13**: 703–709.
5) Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. 2007. Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. *JAMA* **297**: 2092–2102.
6) Samaha FF, Foster GD, Makris AP. 2007. Low-carbohydrate diets, obesity, and metabolic risk factors for cardiovascular disease. *Curr Atheroscler Rep* **9**: 441–447.
7) Iso H, Sato S, Kitamura A, Naito Y, Shimamoto T, Komachi Y. 2003. Fat and protein intakes and risk of intraparenchymal hemorrhage among middle-aged Japanese. *Am J Epidemiol* **157**: 32–39.
8) McGee D, Reed D, Stemmerman G, Rhoads G, Yano K, Feinleib M. 1985. The relationship of dietary fat and cholesterol to mortality in 10 years: the Honolulu Heart Program. *Int J Epidemiol* **14**: 97–105.
9) Takeya Y, Popper JS, Shimizu Y, Kato H, Rhoads GG, Kagan A. 1984. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: incidence of stroke in Japan and Hawaii. *Stroke* **15**: 15–23.
10) Seino F, Date C, Nakayama T, Yoshiike N, Yokoyama T, Yamaguchi M, Tanaka H. 1997. Dietary lipids and incidence of cerebral infarction in a Japanese rural community. *J Nutr Sci Vitaminol* **43**: 83–99.
11) Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC. 2005. Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the nurses' health study. *Am J Epidemiol* **161**: 672–679.
12) Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willett WC. 1996. Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. *BMJ* **313**: 84–90.
13) Jakobsen MU, Overvad K, Dyerberg J, Schroll M, Heitmann BL. 2004. Dietary fat and risk of coronary heart disease: possible effect modification by gender and age. *Am J Epidemiol* **160**: 141–149.
14) Xu J, Eilat-Adar S, Loria C, Goldbourt U, Howard BV, Fabsitz RR, Zephier EM, Mattil C, Lee ET. 2006. Dietary fat intake and risk of coronary heart disease: the Strong Heart Study. *Am J Clin Nutr* **84**: 894–902.
15) Blankenhorn DH, Johnson RL, Mack WJ, el Zein HA, Vailas LI. 1990. The influence of diet on the appearance of new lesions in human coronary arteries. *JAMA* **263**: 1646–1652.
16) Turpeinen O, Karvonen MJ, Pekkarinen M, Miettinen M, Elosuo R, Paavilainen E. 1979. Dietary prevention of coronary heart disease: the Finnish Mental Hospital Study. *Int J Epidemiol* **8**: 99–118.
17) Watts GF, Jackson P, Mandalia S, Brunt JN, Lewis ES, Coltart DJ, Lewis B. 1994. Nutrient intake and progression of coronary artery disease. *Am J Cardiol* **73**: 328–332.
18) Ezaki O, Sakono M, Miyake Y, Ito H. 2007. Concept of reference intake of saturated fatty acids in the Japanese population. *J Jpn Soc Nutr Food Sci* **60**: 19–52.
19) Meyer KA, Kushi LH, Jacobs DR Jr, Folsom AR. 2001. Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care* **24**: 1528–1535.
20) Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC. 2001. Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr* **73**: 1019–1026.
21) Van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB. 2002. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care* **25**: 417–424.
22) Feskens EJ, Loeber JG, Kromhout D. 1994. Diet and physical activity as determinants of hyperinsulinemia: the Zutphen Elderly Study. *Am J Epidemiol* **140**: 350–360.
23) Maron DJ, Fair JM, Haskell WL. 1991. Saturated fat intake and insulin resistance in men with coronary artery disease. The Stanford Coronary Risk Intervention Project Investigators and Staff. *Circulation* **84**: 2020–2027.
24) Marshall JA, Bessesen DH, Hamman RF. 1997. High saturated fat and low starch and fibre are associated with hyperinsulinaemia in a non-diabetic population: the San Luis Valley Diabetes Study. *Diabetologia* **40**: 430–438.
25) Pérez-Jiménez F, López-Miranda J, Pinillos MD, Gómez P, Paz-Rojas E, Montilla P, Marín C, Velasco MJ, Blanco-Molina A, Jiménez Perepérez JA, Ordovás JM. 2001. A Mediterranean and a high-carbohydrate diet improve glucose metabolism in healthy young persons. *Diabetologia* **44**: 2038–2043.
26) Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivel-

lese AA, Tapsell LC, Nalsen C, Berglund L, Louheranta A, Rasmussen BM, Calvert GD, Maffetone A, Pedersen E, Gustafsson IB, Storlien LH; KANWU Study. 2001. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* **44**: 312–319.

27) Klag MJ, Ford DE, Mead LA, He J, Whelton PK, Liang, KY, Levine DM. 1993. Serum cholesterol in young men and subsequent cardiovascular disease. *N Engl J Med* **328**: 313–318.

28) Gerhard GT, Ahmann A, Meeuws K, McMurry MP, Duell PB, Connor WE. 2004. Effects of a low-fat diet compared with those of a high-monounsaturated fat diet on body weight, plasma lipids and lipoproteins, and glycemic control in type 2 diabetes. *Am J Clin Nutr* **80**: 668–673.

29) Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, Keys MH, Kromhout D, Nedeljkovic S, Punsar S, Srccareccia F, Toshima H. 1986. The diet and 15-year death rate in the seven countries study. *Am J Epidemiol* **124**: 903–915.

30) Esrey KL, Joseph L, Grover SA. 1996. Relationship between dietary intake and coronary heart disease mortality: lipid research clinics prevalence follow-up study. *J Clin Epidemiol* **49**: 211–216.

31) Posner M, Cobb JL, Belanger AJ, Cupples A, D'Agostino RB, Stokes J 3rd. 1991. Dietary lipid predictors of coronary heart disease in men. The Framingham Study. *Arch Intern Med* **151**: 1181–1187.

32) Pearce ML, Dayton S. 1971. Incidence of cancer in men on a diet high in polyunsaturated fat. *Lancet* **1**: 464–467.

33) Kimura Y, Kono S, Toyomura K, Nagano J, Mizoue T, Moore MA, Mibu R, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Yasunami Y, Maekawa T, Takenaka K, Ichimiya H, Imaizumi N. 2007. Meat, fish and fat intake in relation to subsite-specific risk of colorectal cancer: The Fukuoka Colorectal Cancer Study. *Cancer Sci* **98**: 590–597.

34) Zock PL, Katan MB. 1998. Linoleic acid intake and cancer risk: a review and meta-analysis. *Am J Clin Nutr* **68**: 142–153.

35) Lewis RA, Austen KF. 1984. The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions, and pharmacology. *J Clin Invest* **73**: 889–897.

36) Miyake Y, Sasaki S, Arakawa M, Tanaka K, Murakami K, Ohya Y. 2008. Fatty acid intake and asthma symptoms in Japanese children: the Ryukyus Child Health Study. *Clin Exp Allergy* **38**: 1644–1650.

37) Hu FB, Stampfer MJ, Manson JE, Rimm EB, Wolk A, Colditz GA, Hennekens CH, Willett WC. 1999. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *Am J Clin Nutr* **69**: 890–897.

38) Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, Rimm EB. 2005. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation* **111**: 157–164.

39) Bjerve KS. 1989. n-3 fatty acid deficiency in man. *J Intern Med Suppl* **731**: 171–175.

40) De Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. 1999. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* **99**: 779–785.

41) Singh RB, Dubnov G, Niaz MA, Ghosh S, Singh R, Rastogi SS, Manor O, Pella D, Berry EM. 2002. Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial. *Lancet* **360**: 1455–1461.

42) Folsom AR, Demissie Z. 2004. Fish intake, marine omega-3 fatty acids, and mortality in a cohort of postmenopausal women. *Am J Epidemiol* **160**: 1005–1010.

43) Albert CM, Oh K, Whang W, Manson JE, Chae CU, Stampfer MJ, Willett WC, Hu FB. 2005. Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. *Circulation* **112**: 3232–3238.

44) Ezaki O, Takahashi M, Shigematsu T, Shimamura K, Kimura J, Ezaki H, Gotoh T. 1999. Long-term effects of dietary alpha-linolenic acid from perilla oil on serum fatty acids composition and on the risk factors of coronary heart disease in Japanese elderly subjects. *J Nutr Sci Vitaminol* **45**: 759–772.

45) Brouwer IA, Katan MB, Zock PL. 2004. Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. *J Nutr* **134**: 919–922.

46) Ezaki O, Sato S, Sakono M, Miyake Y, Mito N, Umezawa M. 2006. Concept of reference intake of n-3 polyunsaturated fatty acids in the Japanese population. *J Jpn Soc Nutr Food Sci* **59**: 123–158.

47) Mozaffarian D, Rimm EB. 2006. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA* **296**: 1885–1899.

48) Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S. 2006. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. *Circulation* **113**: 195–202.

49) Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K; Japan EPA Lipid Intervention Study (JELIS) Investigators. 2007. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* **369**: 1090–1098.

50) Yamagishi K, Iso H, Date C, Fukui M, Wakai K, Kikuchi S, Inaba Y, Tanabe N, Tamakoshi A. 2008. Fish, omega-3 polyunsaturated fatty acids, and mortality from cardiovascular diseases in a nationwide community-based cohort of Japanese men and women the JACC (Japan Collaborative Cohort Study for Evaluation of Cancer Risk) Study. *J Am Coll Cardiol* **52**: 988–996.

51) Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, Lucci D, Nicolosi GL, Porcu M, Tognoni G. 2008. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* **372**: 1223–1230.

52) He K, Rimm EB, Merchant A, Rosner BA, Stampfer MJ, Willett WC, Ascherio A. 2002. Fish consumption and risk of stroke in men. *JAMA* **288**: 3130–3136.

53) Iso H, Rexrode KM, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Hennekens CH, Willett WC. 2001. Intake of fish and omega-3 fatty acids and risk of stroke in women. *JAMA* **285**: 304–312.

54) Virtanen JK, Siscovick DS, Longstreth WT Jr, Kuller LH, Mozaffarian D. 2008. Fish consumption and risk of subclinical brain abnormalities on MRI in older adults. *Neurology* **71**: 439–446.

55) Tanaka K, Ishikawa Y, Yokoyama M, Origasa H, Matsuzaki M, Saito Y, Matsuzawa Y, Sasaki J, Oikawa S, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K; JELIS Investigators, Japan. 2008. Reduction in the recurrence of stroke by eicosapentaenoic acid for hypercholesterolemic patients: subanalysis of the JELIS trial. *Stroke* **39**: 2052–2058.
56) Chua B, Flood V, Rochtchina E, Wang JJ, Smith W, Mitchell P. 2006. Dietary fatty acids and the 5-year incidence of age-related maculopathy. *Arch Ophthalmol* **124**: 981–986.
57) SanGiovanni JP, Chew EY, Clemons TE, Davis MD, Ferris FL 3rd, Gensler GR, Kurinij N, Lindblad AS, Milton RC, Seddon JM, Sperduto RD. 2007. The relationship of dietary lipid intake and age-related macular degeneration in a case-control study: AREDS Report No. 20. *Arch Ophthalmol* **125**: 671–679.
58) Seddon JM, George S, Rosner B. 2006. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration. *Arch Ophthalmol* **124**: 995–1001.
59) Miyake Y, Sasaki S, Tanaka K, Ohya Y, Miyamoto S, Matsunaga I, Yoshida T, Hirota Y, Oda H. 2007. Fish and fat intake and prevalence of allergic rhinitis in Japanese females: the Osaka Maternal and Child Health Study. *J Am Coll Nutr* **26**: 279–287.
60) Hogstrom M, Nordstrom P, Nordstrom A. 2007. n-3 Fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO2 Study. *Am J Clin Nutr* **85**: 803–807.
61) Beydoun MA, Kaufman JS, Sloane PD, Heiss G, Ibrahim J. 2008. n-3 Fatty acids, hypertension and risk of cognitive decline among older adults in the Atherosclerosis Risk in Communities (ARIC) study. *Public Health Nutr* **11**: 17–29.
62) Van Gelder BM, Tijhuis M, Kalmijn S, Kromhout D. 2007. Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study. *Am J Clin Nutr* **85**: 1142–1147.
63) Iso H, Jacobs DR Jr, Wentworth D, Neaton JD, Cohen JD. 1989. Serum cholesterol levels and six-year mortality from stroke in 350,977 men screened for the multiple risk factor intervention trial. *N Engl J Med* **320**: 904–910.
64) Konishi M, Iso H, Komachi Y, Iida M, Shimamoto T, Jacobs DR Jr, Terao A, Baba S, Sankai T, Ito M. 1993. Associations of serum total cholesterol, different types of stroke, and stenosis distribution of cerebral arteries. The Akita Pathology Study. *Stroke* **24**: 954–964.
65) Tanaka H, Ueda Y, Hayashi M, Date C, Baba T, Yamashita H, Shoji H, Tanaka Y, Owada K, Detels R. 1982. Risk factors for cerebral hemorrhage and cerebral infarction in a Japanese rural community. *Stroke* **13**: 62–73.
66) Sauvaget C, Nagano J, Hayashi M, Yamada M. 2004. Animal protein, animal fat, and cholesterol intakes and risk of cerebral infarction mortality in the adult health study. *Stroke* **35**: 1531–1537.
67) Atkins D, Psaty BM, Koepsell TD, Longstreth WT Jr, Larson EB. 1993. Cholesterol reduction and the risk for stroke in men. A meta-analysis of randomized, controlled trials. *Ann Intern Med* **119**: 136–145.
68) Djousse L, Gaziano JM. 2008. Egg consumption in relation to cardiovascular disease and mortality: the Physicians' Health Study. *Am J Clin Nutr* **87**: 964–969.
69) Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC. 1997. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* **337**: 1491–1499.
70) Hu FB, Stampfer MJ, Rimm EB, Manson JE, Ascherio A, Colditz GA, Rosner BA, Spiegelman D, Speizer FE, Sacks FM, Hennekens CH, Willett WC. 1999. A prospective study of egg consumption and risk of cardiovascular disease in men and women. *JAMA* **281**: 1387–1394.
71) Nakamura Y, Okamura T, Tamaki S, Kadowaki T, Hayakawa T, Kita Y, Okayama A, Ueshima H. 2004. Egg consumption, serum cholesterol, and cause-specific and all-cause mortality: the National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged, 1980 (NIPPON DATA80). *Am J Clin Nutr* **80**: 58–63.
72) Nakamura Y, Iso H, Kita Y, Ueshima H, Okada K, Konishi M, Inoue M, Tsugane S. 2006. Egg consumption, serum total cholesterol concentrations and coronary heart disease incidence: Japan Public Health Center-based prospective study. *Br J Nutr* **96**: 921–928.
73) Lucenteforte E, Talamini R, Montella M, Dal Maso L, Tavani A, Deandrea S, Pelucchi C, Greggi S, Zucchetto A, Barbone F, Parpinel M, Franceschi S, La Vecchia C, Negri E. 2008. Macronutrients, fatty acids and cholesterol intake and endometrial cancer. *Ann Oncol* **19**: 168–172.
74) Pan Y, Ugnat AM, Mao Y, Wen SW, Johnson KC. 2004. A case-control study of diet and the risk of ovarian cancer. *Cancer Epidemiol Biomarkers Prev* **13**: 1521–1527.
75) Ezaki O, Sakono M, Miyake Y, Mito N. 2005. Concepts for reference intake of cholesterol in the Japanese population. *J Jpn Soc Nutr Food Sci* **58**: 69–83.
76) Mozaffarian D. 2006. Trans fatty acids-effects on systemic inflammation and endothelial function. *Atheroscler Suppl* **7**: 29–32.
77) Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC. 1994. Trans-fatty acids intake and risk of myocardial infarction. *Circulation* **89**: 94–101.
78) Jakobsen MU, Overvad K, Dyerberg J, Heitmann BL. 2008. Intake of ruminant trans fatty acids and risk of coronary heart disease. *Int J Epidemiol* **37**: 173–182.
79) Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D, Virtamo J. 1997. Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol* **145**: 876–887.
80) Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. 1993. Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet* **341**: 581–585.
81) Hunter JE. 2006. Dietary trans fatty acids: review of recent human studies and food industry responses. *Lipids* **41**: 967–992.

J Nutr Sci Vitaminol, 59, S53–S56, 2013

Dietary Reference Intakes for Japanese 2010: Carbohydrates

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(Received October 26, 2012)

Summary The Dietary Reference Intakes (DRIs) of carbohydrates and dietary fiber were determined for Japanese. The estimated average requirement (EAR) and recommended dietary allowance (RDA) for carbohydrates were not determined because of insufficient data. The tentative dietary goal for preventing lifestyle-related diseases (DG) for children aged 1 y and above was determined for carbohydrates (% energy). In addition, the DG for adults aged 18 y and above was determined for dietary fiber. Dietary fiber intake is associated with myocardial infarction; therefore, the DG was determined on the basis of the results of a meta-analysis and the median dietary fiber intake of Japanese. The DG for alcohol was not determined because of insufficient data.

Key Words carbohydrate, dietary fibers, alcohol, lifestyle-related diseases

Introduction

A carbohydrate comprises either a monosaccharide or its polymer (*1*). Carbohydrates play an important nutritional role as an energy source; digestible carbohydrates (i.e., sugars and starches) contain approximately 4 kcal of energy/g. Although there is no internationally standardized definition, dietary fiber is usually considered an indigestible component in the diet, many of which are carbohydrates. Indigestible carbohydrates are fermented by intestinal bacteria, theoretically providing 0–2 kcal/g (*2*). Dietary fiber is an important nutrient, not as an energy source, but because of its relationship with lifestyle-related diseases attributable to physiological functioning.

Alcohol was included in this chapter considering that it has several effects on health and affects nutritional status and energy production.

Carbohydrates

Basic concept

The primary role of carbohydrates is to supply glucose to tissues that can ordinarily only use glucose as an energy source, such as the brain, nervous tissue, red blood cells, renal tubules, the testes, and oxygen-deficient skeletal muscle. It is estimated that the daily glucose requirement of these tissues is at least 100 g/d (*3*); however, this value is not the true minimal glucose requirement, because gluconeogenesis occurs in the liver. According to the National Health and Nutrition Survey in Japan (*4*, *5*), almost all Japanese consume the minimum requirement.

The dietary goal for preventing lifestyle-related diseases (DG) for carbohydrates was determined as the difference between the energy derived from proteins and lipids and the estimated energy requirement (EER), provided that sufficient proteins and a suitable amount of lipids are being ingested. Thus, the DG of carbohydrates is expressed as a percentage of energy. Since the indigestible carbohydrates in ordinary diets have almost no energy, they are considered to be carbohydrates. Furthermore, the energy derived from carbohydrates is not strongly influenced if the energy derived from ordinary amounts of alcohol consumption is included (*6*). However, this does not mean that alcohol can be used as a substitute for carbohydrates.

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Table 1. Dietary Reference Intakes for carbohydrates (% energy).[1]

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

DG, tentative dietary goal for preventing lifestyle-related diseases.
[1] Including energy derived from alcohol.

Table 2. Dietary Reference Intakes for dietary fibers (g/d).

Sex	Males	Females
Age	DG	DG
0–5 mo	—	—
6–11 mo	—	—
1–2 y	—	—
3–5 y	—	—
6–7 y	—	—
8–9 y	—	—
10–11 y	—	—
12–14 y	—	—
15–17 y	—	—
18–29 y	≥19	≥17
30–49 y	≥19	≥17
50–69 y	≥19	≥17
≥70 y	≥19	≥17
Pregnant women (amount to be added)		—
Lactating women (amount to be added)		—

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

Determining the Dietary Reference Intakes
DG (Tentative dietary goal for preventing lifestyle-related diseases)

Adults/children. The DG for carbohydrates was determined for children aged 1 y and above. The DG was determined according to the intake of carbohydrates (60–72% energy), assuming that the subject is consuming their EER (physical activity level II), lipids within the DG, and the recommended dietary allowance (RDA) of protein. Although a lack of sufficient evidence, considering cases in which a person's protein intake is greater than the RDA and that EER differs with respect to physical activity level, the DGs for adults and children were set at 50–70% of energy intake.

DRIs values for carbohydrates are listed in Table 1.

Dietary fiber

Basic concept

Dietary fiber intake is associated with various lifestyle-related diseases. Many studies report negative relationships between dietary fiber intake and the incidence of myocardial infarction, myocardial infarction-related deaths (*7*), the incidence of diabetes (*8*), blood pressure (*9*), and low-density lipoprotein cholesterol (*10*). There are also many reports showing a correlation between dietary fiber intake and obesity (*11*, *12*). However, the associations between dietary fiber intake and cancer and its effect on bowel habits (e.g., constipation) are not well identified (*13*, *14*).

The lifestyle-related disease with the clearest connection to dietary fiber intake is myocardial infarction (*7*). Therefore, the DG was determined on the basis of the results of a meta-analysis (*7*) as well as the current intake levels of dietary fiber in Japanese.

Determining the Dietary Reference Intakes
Tentative dietary goal for preventing lifestyle-related diseases

Adults. The results of a meta-analysis of the correlation between dietary fiber intake and myocardial infarction revealed that the mortality rate decreases with a daily intake level of at least 24 g/d and increases with a daily intake level less than 12 g/d (*7*). According to the National Health and Nutrition Surveys Japan in 2005 and 2006 (*4*, *5*), the median dietary fiber intakes of male and female adults are 12.3–16.3 and 11.8–16.1 g/d, respectively.

The DG for dietary fiber was determined on the basis of the intermediate value (i.e., 18 g/d) between the 2 values indicated in the meta-analysis (*7*) although a lack of scientific basis. Furthermore, taking into account the age and body weight of the research subjects and the difference in standard body weight between Japanese men and women, the DG was determined to be 19 and 17 g/d for men and women, respectively.

DRIs values for dietary fiber are listed in Table 2.

Alcohol

Basic concept

In Japan, 7.1 kcal/g is used as the amount of available energy from alcohol (ethanol) (*15*, *16*). However, the energy utilization efficiency of alcohol varies according

to a variety of conditions including alcohol consumption levels, the ability to metabolize alcohol, dietary intake levels, and physical condition.

The range of "moderate alcohol consumption" (*17*) is thought to be in the order of 20 g/d pure alcohol equivalent. In this range, there would be no problem using 7.1 kcal/g to calculate the amount of energy from the perspective of maintaining body weight.

Epidemiological studies show that alcohol intake is correlated with death and the incidence of cardiovascular disease, cancer, and other lifestyle-related diseases (*18–21*). Western and Japanese have very different genetic backgrounds with respect to the metabolic enzymes of alcohol (*22*). Thus, it is possible that the health effects of alcohol in Japanese are different from those in Western people. The exact level of alcoholic intake that affects the total mortality rate is still controversial among cohort studies in Japan. Some studies report that the risk of mortality is lowest among subjects who consume less than 21 g alcohol/d (*23*), while others report that the risk is only high with a consumption of more than 43 g/d (*24*). Furthermore, other reports indicate that the risk increases gradually with increasing alcohol consumption (*25*). However, in all cases, it is clear that heavy alcohol consumption increases the risk of mortality.

REFERENCES

1) Cummings JH, Stephen AM. 2007. Carbohydrate terminology and classification. *Eur J Clin Nutr* **61** (Suppl 1): S5–18.
2) Oku T, Yamada K, Kanaya K. 2002. Estimation of available energy of dietary fiber in various food materials. *Nippon Shokumotsuseni Kenkyû-kai* (*J Jpn Assoc Dietary Fiber Res*) **6**: 81–86 (in Japanese).
3) Food and Nutrition Board, Institute of Medicine. 2005. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, and Amino Acids (Macronutrients). National Academies Press, Washington DC.
4) Ministry of Health, Labour and Welfare, Japan. 2007. National Health and Nutrition Survey Japan (2005). Tokyo (in Japanese).
5) Ministry of Health, Labour and Welfare, Japan. 2008. National Health and Nutrition Survey Japan, (2006) Tokyo (in Japanese).
6) Suter PM. 1997. How much do alcohol calories count? *J Am Coll Nutr* **16**: 105–106.
7) Pereira MA, O'Reilly E, Augustsson K, Fraser GE, Goldbourt U, Heitmann BL, Hallmans G, Knekt P, Liu S, Pietinen P, Spiegelman D, Stevens J, Virtamo J, Willett WC, Ascherio A. 2004. Dietary fiber and risk of coronary heart disease: a pooled analysis of cohort studies. *Arch Intern Med* **164**: 370–376.
8) Schulze MB, Schulz M, Heidemann C, Schienkiewitz A, Hoffmann K, Boeing H. 2007. Fiber and magnesium intake and incidence of type 2 diabetes: a prospective study and meta-analysis. *Arch Intern Med* **167**: 956–965.
9) Whelton SP, Hyre AD, Pedersen B, Yi Y, Whelton PK, He J. 2005. Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. *J Hypertens* **23**: 475–481.
10) Brown L, Rosner B, Willett WW, Sacks FM. 1999. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr* **69**: 30–42.
11) Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. 2003. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am J Clin Nutr* **78**: 920–927.
12) Murakami K, Sasaki S, Okubo H, Takahashi Y, Hosoi Y, Itabashi M. 2007. Dietary fiber intake, dietary glycemic index and load, and body mass index: a cross-sectional study of 3931 Japanese women aged 18–20 years. *Eur J Clin Nutr* **61**: 986–995.
13) Bingham SA, Day NE, Luben R, Ferrari P, Slimani N, Norat T, Clavel-Chapelon F, Kesse E, Nieters A, Boeing H, Tjonneland A, Overvad K, Martinez C, Dorronsoro M, Gonzalez CA, Key TJ, Trichopoulou A, Naska A, Vineis P, Tumino R, Krogh V, Bueno-de-Mesquita HB, Peeters PH, Berglund G, Hallmans G, Lund E, Skeie G, Kaaks R, Riboli E. 2003. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. *Lancet* **361**: 1496–1501.
14) Park Y, Hunter DJ, Spiegelman D, Bergkvist L, Berrino F, van den Brandt PA, Buring JE, Colditz GA, Freudenheim JL, Fuchs CS, Giovannucci E, Goldbohm RA, Graham S, Harnack L, Hartman AM, Jacobs DR Jr, Kato I, Krogh V, Leitzmann MF, McCullough ML, Miller AB, Pietinen P, Rohan TE, Schatzkin A, Willett WC, Wolk A, Zeleniuch-Jacquotte A, Zhang SM, Smith-Warner SA. 2005. Dietary fiber intake and risk of colorectal cancer: a pooled analysis of prospective cohort studies. *JAMA* **294**: 2849–2857.
15) Atwater WO, Benedict FG. 1993. An experimental inquiry regarding the nutritive value of alcohol. 1902. *Obes Res* **1**: 228–244.
16) Ministry of Education, Culture, Sports, Science and Technology, Japan. 2005. Standard Tables of Food Composition in Japan, Fifth revised and enlarged ed. National Printing Bureau, Tokyo (in Japanese).
17) The Planning Committee for the Health Japan 21. 2000. National Health Promotion Movement in the 21st Century (Health Japan 21). Ministry of Health, Labour and Welfare, Japan, Tokyo (in Japanese).
18) Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Cogliano V. 2007. Carcinogenicity of alcoholic beverages. *Lancet Oncol* **8**: 292–293.
19) Bagnardi V, Blangiardo M, La Vecchia C, Corrao G. 2001. Alcohol consumption and the risk of cancer: a meta-analysis. *Alcohol Res Health* **25**: 263–270.
20) Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K. 2000. Alcohol and coronary heart disease: a meta-analysis. *Addiction* **95**: 1505–1523.
21) Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L, de Gaetano G. 2006. Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. *Arch Intern Med* **166**: 2437–2445.
22) Higuchi S, Matsushita S, Masaki T, Yokoyama A, Kimura M, Suzuki G, Mochizuki H. 2004. Influence of genetic variations of ethanol-metabolizing enzymes on phenotypes of alcohol-related disorders. *Ann NY Acad Sci* **1025**: 472–480.
23) Tsugane S, Fahey MT, Sasaki S, Baba S. 1999. Alcohol consumption and all-cause and cancer mortality among

middle-aged Japanese men: seven-year follow-up of the JPHC study Cohort I. Japan Public Health Center. *Am J Epidemiol* **150**: 1201–1207.

24) Kono S, Ikeda M, Tokudome S, Nishizumi M, Kuratsune M. 1986. Alcohol and mortality: a cohort study of male Japanese physicians. *Int J Epidemiol* **15**: 527–532.

25) Nakaya N, Kurashima K, Yamaguchi J, Ohkubo T, Nishino Y, Tsubono Y, Shibuya D, Fukudo S, Fukao A, Tsuji I, Hisamichi S. 2004. Alcohol consumption and mortality in Japan: the Miyagi Cohort Study. *J Epidemiol* **14** (Suppl 1): S18–25.

J Nutr Sci Vitaminol, 59, S57–S66, 2013

Dietary Reference Intakes for Japanese 2010: Fat-Soluble Vitamins

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(Received October 26, 2012)

Summary We have determined the Dietary Reference Intakes for fat-soluble vitamins (vitamin A, vitamin D, vitamin E, and vitamin K) for the Japanese. Regarding vitamin A, the estimated average requirement (EAR) and the recommended dietary allowance (RDA) were defined for those aged 1 y old and over. For vitamin D, vitamin E, and vitamin K, the EAR or RDA was not adopted, because of the insufficient data available. Thus, the adequate intake (AI) was determined for those vitamins based on the food surveillance data and biomarkers for each vitamin. The AI for vitamin D was decided as the median intake of vitamin D in the population with a circulating 25-hydroxy vitamin D level which was high enough for bone health. The basis for the AI for vitamin E was the median intake of α-tocopherol in the healthy population considering the lack of unfavorable health consequences attributable to its deficiency. The AI for vitamin K was determined as the vitamin K intake, required to avoid blood coagulation abnormalities. The tolerable upper intake level (UL) was determined for vitamin A, vitamin D and vitamin E, but not for vitamin K, since no adverse effects have been reported even with its high dosage.

Key Words vitamin A, vitamin D, vitamin E, vitamin K

Vitamin A

Background information

Compounds with potent vitamin A activity in vivo after oral intake include retinol; retinal; carotenoids; and 50 different types of provitamin A carotenoids, including β-carotene, α-carotene, and β-cryptoxanthin. The retinol equivalent (RE) is the vitamin A unit used in Dietary Reference Intakes for Japanese (DRIs-J) 2010, the most current Dietary Reference Intakes (DRIs) for the Japanese. Retinoic acid, a hormone binding to the nuclear receptor, is responsible for the majority of vitamin A activity in vivo, but is not converted to retinal or retinol in vivo, and its content in food is relatively low. Retinylester provitamin A carotenoids are the main forms of vitamin A contained in animal and plant foods, respectively. Retinylester hydrolase in the intestinal brush border catalyzes the hydrolysis of retinylester to retinol, which is then absorbed at a rate that ranges from 70% to 90% (*1*, *2*). Cleavage of carotenoids yields 2 molecules of vitamin A (retinal) from β-carotene (*3*) and 1 molecule from other provitamin A carotenoids.

In the DRIs-J 2010, the absorption rate of β-carotene is 1/6 of its total value, which is in accordance with rate in the DRIs for the United States and Canada (*4*). Assuming that the conversion rate of β-carotene to retinol is 50%, the bioavailability of β-carotene as vitamin A is 1/12 (1/6×1/2), such that 12 μg of food-derived β-carotene would correspond to 1 μg in RE units. Thus, the following formula can be used to convert the value of food-derived vitamin A-related compounds into RE units:

Retinol equivalent (μg RE)
=retinol (μg)+β-carotene (μg)×1/12
+α-carotene (μg)×1/24+β-cryptoxantin (μg)
×1/24+other provitamin A carotenoids (μg)
×1/24.

A word of caution is indicated when calculating the value for oil-solubilized β-carotene, as its bioavailability as a form of vitamin A is 1/2 of its total value, such that 2 μg of fat-solubilized β-carotene would correspond to 1 μg of retinol.

Determining DRIs

Classical vitamin A deficiency leads to corneal xerosis in infants and possibly to blindness and to night blindness in adults. Other deficiency signs include growth retardation; skeletal and neurological development defects; disturbed growth and differentiation of epi-

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thelial cells; dryness, thickening, and keratinization of the skin; immunodeficiency; and susceptibility to infection (5). Due to the abundant storage of vitamin A in the liver, inadequate intake does not lead to decreased plasma retinol concentration unless hepatic vitamin A storage is below 20 μg/g (6, 7). Thus, plasma retinol concentration cannot be used as an index of vitamin A status. Theoretically, hepatic vitamin A storage is the best index, but its measurement is highly invasive and not applicable to humans. Thus, the vitamin A intake required to maintain minimal hepatic vitamin A storage has been used for estimating the Estimated Average Requirement (EAR) for vitamin A.

Compartment analysis assuming the existence of 3 compartments—serum, liver, and other tissues—has shown that the daily disposal rate of vitamin A is approximately 2% (8, 9). Using this percentage, the daily disposal amount (DDA), daily disposal rate (DDR), body storage (BS) according to body weight (BW), and hepatic storage (HS) of vitamin A can be calculated as follows:

DDA (μg/d)=BS (μg)×DDR (2%/d (10)).

BS/BW (μg/kg BW)
=HS (≥20 μg/g)×liver weight/BW (21 g/kg BW)
×10/9,

where 90% of the body storage of vitamin A is in the liver (10, 11).

DDA/BW (μg/[kg BW·d])
=BS (≥20 μg/g×21 g/kg×10/9)×DDR (2/100)
=9.3 μg/kg BW.

Thus, the amount of vitamin A intake required to compensate for its daily elimination, thereby ensuring that hepatic storage of vitamin A is maintained and vitamin A deficiency is avoided, is estimated to be 9.3 μg RE/kg BW/d.

EAR and Recommended Dietary Allowance (RDA) for adults

The EAR for vitamin A for those aged 18 y and above, as calculated by multiplication of the reference value of 9.3 μg RE/kg BW/d and the reference BW, is 550 to 600 μg RE/d for males and 450 to 500 μg RE/d for females. Assuming the inter-individual variability in vitamin A requirement to be 20% (4), multiplication of these EAR values by 1.4 yields an RDA of 800 to 850 μg RE/d for males and 650 to 700 μg RE/d for females.

EAR and RDA for children

The RDA for children aged 6 to 17 y was determined by extrapolation from the EAR for adults aged 18 to 29 y by the 0.75th power of the BW ratio, which represents the ratio of body surface area (4). Extrapolation of the adult EAR to preschool children based on BW ratio may yield values that maintain plasma retinol levels below 20 μg/100 mL, and thus render children susceptible to corneal xerosis (12). Therefore, the RDA for children aged less than 5 y must be at least 200 μg RE/d to avoid this unfavorable outcome; therefore, for children aged less than 5 y, the DDA was calculated as follows, assuming the ratio of liver weight/BW to be 42 g/kg BW (10):

DDA/BW (μg/kg BW/d)
=BS (≥20 μg/g×42 g/kg×10/9)×DDR (2/100)
=18.7 μg/kg BW.

Using the value obtained, the EAR for children aged 1 to 5 y was calculated as follows:

EAR=18.7 μg/kg BW/d×reference BW×(1+growth factor)
=EAR×1.4.

Adequate Intake of infants aged 0 to 5 mo

Vitamin A concentration in breast milk is highest during the first 10 d after delivery, after which it gradually decreases (13, 14). Based on the values for average vitamin A concentration (411 μg RE/L) (14) and daily milk intake (0.78 L/d) (15, 16), vitamin A intake in breast milk-fed infants aged 0 to 5 mo was estimated at 320 μg RE/d. Thus, adequate intake (AI) for this age group was determined to be 300 μg/d. The level of provitamin A carotenoids was not taken into account because its availability is unknown.

AI of infants 6 to 11 mo

Based on extrapolation from the AI for infants aged 0 to 5 mo, the AI for infants aged 6 to 11 mo was determined to be 400 μg RE/d. The level of provitamin A carotenoids was not taken into account because its availability is unknown.

Amount to be added during pregnancy

The amount of vitamin A transported to the fetus through the placenta must be taken into account when estimating the vitamin A requirement for pregnant women. At the late-stage of a fetus, the amount of vitamin A deposited in the fetal liver was 1,800 μg (17, 18) so that the total amount of vitamin A transported to the fetus during pregnancy is estimated at 3,600 μg. Using this value, the EAR value for the additional amount of vitamin A required during the late stage was determined to be 60 μg RE/d, which, assuming an inter-individual variability of 20% (4), yielded an RDA value of 80 μg RE/d during the late-stage. The additional amount required during the early- and mid-stage was not determined.

Amount to be added during lactation

Based on measurement of the amount of vitamin A secreted in breast milk, the EAR value for the additional amount of vitamin A required during lactation was estimated at 300 μg RE/d, which, assuming an inter-individual variability of 20%, yielded an RDA value of 450 μg RE/d (4).

Tolerable upper intake level

An elevated plasma level of retinoic acid is considered responsible for most clinical signs (19) and symptoms of vitamin A intoxication, such as headache. Based on reported fetal abnormalities due to excessive intake of vitamin A, (20, 21) the no observable adverse effect level (NOAEL) during pregnancy was estimated at 4,500 μg RE/d, which, assuming an uncertainty factor of 1.5 and taking the additional amount into account, yielded an upper level (UL) of 3,000 μg RE/d.

Based on research into hepatotoxicity caused by the excessive vitamin A deposition (22), the NOAEL in adults was estimated at 13,500 μg RE/d, which, assuming an uncertainty factor of 5, yielded a UL of 2,700 μg RE/d. Based on clinical observation of increased intracranial pressure in infants caused by excessive vitamin

Table 1. DRIs for vitamin A (μg RE/d).[1]

Sex	Males				Females			
Age	EAR[2]	RDA[2]	AI[3]	UL[3]	EAR[2]	RDA[2]	AI[3]	UL[3]
0–5 mo	—	—	300	600	—	—	300	600
6–11 mo	—	—	400	600	—	—	400	600
1–2 y	300	400	—	600	250	350	—	600
3–5 y	300	450	—	700	300	450	—	700
6–7 y	300	450	—	900	300	400	—	900
8–9 y	350	500	—	1,200	350	500	—	1,200
10–11 y	450	600	—	1,500	400	550	—	1,500
12–14 y	550	750	—	2,000	500	700	—	2,000
15–17 y	650	900	—	2,500	450	650	—	2,500
18–29 y	600	850	—	2,700	450	650	—	2,700
30–49 y	600	850	—	2,700	500	700	—	2,700
50–69 y	600	850	—	2,700	500	700	—	2,700
≥70 y	550	800	—	2,700	450	650	—	2,700
Pregnant women (amount to be added)								
Early-stage					+0	+0	—	—
Mid-stage					+0	+0	—	—
Late-stage					+60	+80	—	—
Lactating women (amount to be added)					+300	+450	—	—

DRIs, Dietary Reference Intakes; RE, retinol equivalents; EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.

[1] Retinol equivalent (μg RE)=retinol (μg)+β-carotene (μg)×1/12+α-carotene (μg)×1/24+β-cryptoxanthin (μg)×1/24 +other provitamin A carotenoids (μg)×1/24.

[2] Including provitamin A carotenoids.

[3] Excluding provitamin A carotenoids.

A intake (*23*), the NOAEL in infants was estimated at 6,000 μg RE/d, which, assuming an uncertainty factor of 10, yielded a UL of 600 μg RE/d.

The UL for children aged 1 to 17 y was determined by extrapolation from the UL for adults based on the ratio of body surface area. For safety reasons, the values for men were applied to women. Extrapolation to infants aged 1 to 2 y old yielded a UL of 500 μg RE/d, which is lower than that for infants aged 6 to 11 mo (600 μg RE/d). Thus, the UL for infants aged 1 to 2 y old was revised to 600 μg RE/d. Although a recent study found that ingesting approximately 1,500 μg RE/d of retinol for 30 y doubled the fracture risk in the elderly (*24*), data from other studies contradicted this finding. Thus, determination of a separate UL for vitamin A for the elderly was not considered in developing the most recent DRIs. Moreover, as excessive intake of β-carotene has not been reported to be associated with the unfavorable consequences of vitamin A intoxication described above, the level of provitamin A carotenoids was also not included in the estimation of UL.

Remarks regarding carotenoids

Due to the strict regulation of their conversion into vitamin A, provitamin A carotenoids, when ingested orally, cannot cause vitamin A intoxication. Unconverted provitamin A carotenoids, as well as carotenoids that are not metabolized to vitamin A are stored in vivo as they are. Beneficial actions have been reported with ingestion of these carotenoids, including anti-oxidant activity and immune potentiation and photoprotection of skin by anti-oxidation. Regarding the benefits of specific carotenoids, prevention of prostate cancer by lycopene, improvement in age-related macular degeneration by lutein and zeaxanthin, and the maintenance of retinal pigment by lutein and zeaxanthin have also been reported. Although the results of cohort studies suggest that higher intake of carotenoids is associated with lower incidence of lung cancer (*25*), supplementary intervention has been reported to be ineffective or even harmful in the prevention of cancer, especially lung cancer (*26–29*). Thus, further research into the efficacy and safety of carotenoids is required. In developing the current DRIs, the carotenoids were not separately considered because their deficiency has not been reported.

DRI values for vitamin A are listed in Table 1.

Vitamin D

Background information

Vitamin D_2 and vitamin D_3 are naturally occurring compounds with potent vitamin D activity. The indices for the DRI of vitamin D is based on the summation of the values of these 2 compounds. The human body obtains vitamin D from 2 sources. One is exposure to ultraviolet irradiation, which converts pro-vitamin D_3

(7-dehydrocholesterol) in the skin to pre-vitamin D_3, which in turn is converted into vitamin D_3 by thermal isomerization. The other is dietary intake of vitamin D_2 and vitamin D_3 from such sources as mushrooms and fish; good sources for vitamin D_2 and vitamin D_3, respectively. The current DRIs do not discriminate between vitamin D_2 and D_3 intake because the compounds have similar characteristics and a similar molecular weight and exert an almost equal level of biological activity.

Vitamin D is first metabolized to 25-hydroxy vitamin D (25OHD) before being metabolized to 1α,25-dihydroxy vitamin D ($1\alpha,25(OH)_2D$), its active form. Major actions of vitamin D include enhancing the absorption of calcium and phosphate in the intestine and kidneys and stimulating bone formation and growth. Circulating 25OHD level is the best index of vitamin D status. As vitamin D deficiency and resultant hypocalcemia cause elevated levels of serum parathyroid hormone (PTH), serum concentration of PTH can also be a good index of vitamin D deficiency (*30*).

Adequate intake

Evidence for determining AI

Vitamin D deficiency impairs calcium absorption from the intestine and kidney, thus decreases calcium availability, resulting in rickets in children and osteomalacia in adults. In adults, especially the elderly, even so-called "vitamin D insufficiency," which is milder than vitamin D deficiency, can result in increased secretion of PTH, increased bone resorption, and decreased bone mineral density. Therefore, the basis for determining the vitamin D requirement is maintenance of a serum 25OHD level sufficiently high to maintain normal calcium availability and avoid elevation of serum PTH level. Due to limitations on the data available, AI was determined as the median intake of vitamin D in a population in which the required circulating 25OHD level is maintained.

AI for adults

In a study conducted in the northern United States, an area in which residents receive limited sunshine exposure, serum PTH level after vitamin D administration decreased in those with a serum 25OHD level below 50 nmol/L but not in those with a level above 50 nmol/L (*31*). In a study in Niigata, those with a 25OHD level less than 50 nmol/L had higher serum PTH levels and a higher prevalence of low bone mineral density (*32*). Based on consideration of these results, maintenance of a circulating 25OHD level of at least 50 nmol/L is considered necessary to avoid elevation of serum PTH level and decrease in bone mineral density. In the study conducted in the northern United States, serum PTH level exhibited seasonal variation, reaching a nadir between August and October and a peak between March and May. However, this variation was not observed in those taking 5.5 μg/d or more of vitamin D (*33*), leading to the conclusion that taking at least 5.5 μg/d of vitamin D can prevent elevation of PTH in those living in areas in which they have limited sunshine exposure.

In 7 studies that examined Japanese women (*34–39*) aged 50 to 69 y, the average 25OHD level was found to exceed 50 nmol/L. In contrast, in several studies that examined women aged 18 to 29 y (*32, 34*) and women aged 30 to 49 y (*34*), the average level was found to be below 50 nmol/L. Based on these findings and the findings from US studies, the median vitamin D intake of adults aged 50 to 69 y was determined to be an appropriate basis for determining the adult AI. As the 2005 and 2006 National Health and Nutritional Survey (NHNS) (*40, 41*) found that the median intake of vitamin D in adults aged 50 to 69 y was 5.5 μg/d, the AI was set as 5.5 μg/d. Due to lack of data for those aged 18 to 29 y, 30 to 49 y, and above 70 y, as well as lack of data for males, AI for both males and females in these age groups was also set at 5.5 μg/d.

AI for children

As the findings regarding the relationship between vitamin D intake and plasma 25OHD concentration in children have been inconsistent, they were considered unsuitable as the basis for determining the vitamin D AI for children. Thus, the median vitamin D intake, as reported in the 2005 and 2006 NHNS (*40, 41*), was used as the basis for determining the AI.

AI for infants

In an epidemiological study conducted in Kyoto, 22% of neonates were found to have craniotabes, a mineralization defect of bone, likely due to vitamin D deficiency (*42*). The incidence of craniotabes exhibited seasonal variation, with a peak and nadir between January and May and between July and November, respectively. Circulating 25OHD level was found to be below 25 nmol/L in 37% of all neonates diagnosed with craniotabes at 1 mo after birth. In breast milk-fed neonates, serum concentration of 25OHD was found to be less than 25 nmol/L in 57% of subjects and below 12.5 nmol/L in 17%. In contrast, none of the formula or mixed-fed infants were found to have an inadequate serum 25OHD level. It should be noted that neonates born in a vitamin D-deficient state may not recover to a vitamin D-sufficient state within a short period, and that the serum 25OHD level of breast milk-fed infants was found to decrease further during the winter months (*43*), indicating that the vitamin D delivered from breast milk may have been unsatisfactory. The vitamin D AI for infants was determined to be 2.5 μg/d by multiplying 0.78 L/d (*15, 16*), the average daily milk intake, by 3.05 μg/L (*44*), the vitamin D concentration in breast milk as reported in the *Standard Tables of Food Composition in Japan*, 5th Revised and Enlarged Edition.

However, this AI value is appropriate only for infants with adequate sun exposure, defined as 2 h/wk to the face or 30 min/wk to the face and extremities. Breast-milk-fed infants with little sun exposure are at higher risk of developing rickets. Considering that previous research found that no infants developed rickets after supplementation with 2.5 μg/d of vitamin D for 6 mo and assuming that infants receive an average of 2.38 μg/d of vitamin D from breast milk, it follows that a daily intake of 4.88 μg/d of vitamin D is satisfactory for avoiding rickets. Based on these data, the AI of vitamin D for infants aged 0 to 5 mo with limited sun exposure was determined to be 5 μg/d. Recently, however, a

Table 2. DRIs for vitamin D (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo[1]	—	—	2.5 (5.0)	25	—	—	2.5 (5.0)	25
6–11 mo[1]	—	—	5.0 (5.0)	25	—	—	5.0 (5.0)	25
1–2 y	—	—	2.5	25	—	—	2.5	25
3–5 y	—	—	2.5	30	—	—	2.5	30
6–7 y	—	—	2.5	30	—	—	2.5	30
8–9 y	—	—	3.0	35	—	—	3.0	35
10–11 y	—	—	3.5	35	—	—	3.5	35
12–14 y	—	—	3.5	45	—	—	3.5	45
15–17 y	—	—	4.5	50	—	—	4.5	50
18–29 y	—	—	5.5	50	—	—	5.5	50
30–49 y	—	—	5.5	50	—	—	5.5	50
50–69 y	—	—	5.5	50	—	—	5.5	50
≥70 y	—	—	5.5	50	—	—	5.5	50
Pregnant women (amount to be added)					—	—	+1.5	—
Lactating women (amount to be added)					—	—	+2.5	—

[1] Adequate intakes for an infant who is exposed to appropriate sunlight. The value in parentheses is adequate intakes for those with less sunlight exposure.

study using a novel, highly accurate procedure found the average vitamin D concentration in breast milk to be only 0.6 μg/L (*14*). If this value is employed, the average vitamin D intake of breast-milk-fed infants would be only 0.47 μg/d. Such discrepancies indicate the need for further research into this value (*45*, *46*).

AI for infants aged 6 to 11 mo

The AI of vitamin D for infants aged 6 to 11 mo with adequate sun exposure was determined to be 5 μg/d. This value was also applied to infants aged 6 to 11 mo with limited sun exposure due to lack of evidence for determining the AI.

Additional amount during pregnancy

In a study of pregnant women with limited sun exposure, an inadequate serum 25OHD concentration was observed in those with an average vitamin D intake of less than 5.3 μg/d but not in those an average (*47*) vitamin D intake higher than 7 μg/d (*48*). As these findings indicate that pregnant women require at least 7 μg/d of vitamin D, the additional amount of vitamin D required for pregnant women was determined to be 1.5 μg/d.

Additional amount during lactation

Based on the findings described above, the additional amount of vitamin D required for lactating women was determined to be 2.5 μg/d.

Tolerable upper untake level

Basic considerations

Prolonged intake of excessive quantities of vitamin D can lead to unfavorable outcomes, such as hypercalcemia, renal dysfunction, soft tissue calcification, and growth retardation. As an increased serum 25OHD level itself does not directly cause health problems, the presence of hypercalcemia rather than of a high serum 25OHD level is considered an appropriate indicator for determining the UL.

UL for adults

In an intervention study administering doses of vitamin D for 3 mo, serum calcium concentration was found to exceed the reference value in some subjects receiving 95 μg/d of vitamin D but not in those receiving 60 μg/d of vitamin D (*49*). Thus, the lowest observed adverse effect level (LOAEL) and NOAEL were determined to be 95 μg/d and 60 μg/d, respectively. The latter value was divided by an uncertainty factor of 1.2 yielding a UL for adults of 50 μg/d. Since neither administration of 45 μg/d of vitamin D to elderly subjects for 3 mo (*50*) nor administration of 50 μg/d to pregnant and lactating subjects (*51*) was found to be associated with hypercalcemia, stratification by sex or age group was not performed, and a UL of 50 μg/d was applied to all adult groups.

UL for infants

Based on a study that observed no growth retardation in infants administered an average of 44 μg/d of vitamin D for 6 mo, the NOAEL for infants was determined to be 44 μg/d (*52*), which, assuming an uncertainty factor of 1.8, yielded a UL of 25 μg/d.

UL for children

As data were unavailable for this age group, the UL for children was determined by extrapolating the UL values for adults (50 μg/d) and infants (25 μg/d) based on the reference body weight. Sex differences were not considered.

DRI values for vitamin D are listed in Table 2.

Vitamin E

Background information

Vitamin E is composed of 8 analogues: α-, β-, γ- and

Table 3. DRIs for vitamin E (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	3.0	—	—	—	3.0	—
6–11 mo	—	—	3.5	—	—	—	3.5	—
1–2 y	—	—	3.5	150	—	—	3.5	150
3–5 y	—	—	4.5	200	—	—	4.5	200
6–7 y	—	—	5.0	300	—	—	5.0	300
8–9 y	—	—	6.0	350	—	—	5.5	350
10–11 y	—	—	6.5	450	—	—	6.0	450
12–14 y	—	—	7.0	600	—	—	7.0	600
15–17 y	—	—	8.0	750	—	—	7.0	650
18–29 y	—	—	7.0	800	—	—	6.5	650
30–49 y	—	—	7.0	900	—	—	6.5	700
50–69 y	—	—	7.0	850	—	—	6.5	700
≥70 y	—	—	7.0	750	—	—	6.5	650
Pregnant women (amount to be added)					—	—	+0.0	—
Lactating women (amount to be added)					—	—	+3.0	—

[1] Computation was made on α-tocopherol, not including vitamins E other than α-tocopherol.

δ-forms, of tocopherol and tocotrienol. After intestinal absorption, vitamin E is packaged into chylomicron, transformed into chylomicron remnant by lipoprotein lipase, and transported to the liver. Of the 8 analogues, only α-tocopherol is preferentially bound to α-tocopherol binding protein, whereas the other analogues are metabolized in the liver. Alpha-tocopherol is then formed into very low-density lipoprotein (VLDL), converted into low-density lipoprotein (LDL), and distributed to various tissues (*53*). Due to these metabolic processes, α-tocopherol constitutes the predominant vitamin E analogues present in the blood and various tissues. Based on these facts, only α-tocopherol was considered when determining the current DRI for vitamin E.

Determining DRI

Basis for determining AI

Erythrocytes are susceptible to hemolysis by hydrogen peroxide when the circulating α-tocopherol level is between 6 and 12 μmol/L (*54*), but resistant to it when the serum α-tocopherol level is higher than 14 μmol/L (*55*). Although the data from an intervention study that administered graded doses of vitamin E to vitamin E-deficient subjects are available (*56*), they were not considered appropriate for estimating the EAR and RDA because they were collected many years ago. Several studies that simultaneously studied vitamin E intake and serum α-tocopherol level consistently reported that the average serum α-tocopherol level exceeded 22 μmol/L in all study populations (*40*, *41*, *57–59*). Average vitamin E intake in these studies ranged from 5.6 to 11.1 mg/d, a range that encompasses the 2005 and 2006 NHNS values (*40*, *41*) of an average vitamin E intake of 7.0 mg/d in men and 6.5 mg/d in women. As these findings indicate that the median intake of the Japanese likely yields an adequate vitamin E status, the AI was determined to be the 2005 and 2006 NHNS median values stratified by sex and age group (*40*, *41*).

AI for adults

As described above, AI was determined to be the 2005 and 2006 NHNS median values for those aged 18 to 29 y stratified by sex and age group, specifically 7.0 mg/d for men and 6.5 mg/d for women, as these values are expected to yield a blood α-tocopherol level exceeding 12 μmol/L (*40*, *41*). As aging has not been reported to be associated with compromised absorption or utilization of vitamin E, the same values were applied to the elderly.

AI for children

The 2005 and 2006 NHNS median values for children stratified by sex and age group were used as the basis for determining the AI for children, as they had been for adults.

AI for infants aged 0 to 5 mo

The AI for infants aged 0 to 5 mo was determined to be 3.0 mg/d by multiplying the average α-tocopherol concentration in breast milk (3.5 to 4.0 mg/L) (*14*, *60*) by the average milk intake (0.78 L/d) (*15*, *16*).

AI for infants aged 6 to 11 mo

The AI for infants aged 6 to 11 mo old was determined to be 3.5 mg/d by extrapolation from the adult value by the 0.75th power of the BW ratio.

AI during pregnancy

The AI for pregnant women was determined to be the same as that for non-pregnant women because vitamin E deficiency during pregnancy has not been reported.

Additional amount during lactation

Since the average α-tocopherol content provided in breast milk is approximately 3.0 mg/d (*14*, *60*), the AI

during lactation was determined to be 3 mg/d.

Tolerable upper intake level

The basis for determining the UL for vitamin E is its possible effect on bleeding tendency. Based on the finding that supplementation with 800 mg/d of α-tocopherol for 28 d did not increase bleeding tendency in healthy males (average body weight, 62.2 kg) (*61*), the NOAEL was determined to be 800 mg/d. Assuming an uncertainty factor of 1.0 and considering that no data regarding LOAEL are available, the sex- and age-group stratified UL was calculated by correcting the 800 mg/d value by BW ratio. Because few data are available regarding the UL for infants aged 0 to 11 mo and because typical feeding with breast milk or baby food does not cause excessive intake, the UL was not determined for this age group.

Additional remarks

Although numerous intervention studies have examined the effect of vitamin E supplementation on the risk of coronary heart diseases, the findings have been inconsistent (*62–65*).

DRI values for vitamin E are listed in Table 3.

Vitamin K

Basic considerations

Naturally occurring vitamin K consists of phylloquinones (PKs; vitamin K_1) and menaquinones (MKs; vitamin K_2). Menaquinones are further subdivided into 11 analogues depending on the number of isoprene units (4–14) in the prenyl side chain. Among the menaquinones, of nutritional importance are menaquinone-4 (MK-4), which is ubiquitously present in animal foods, and menaquinone-7 (MK-7), which is abundantly present in natto, a traditional Japanese food made from soybeans fermented with *Bacillus subtilis*. At present, data are scarce for determining the relative biological activity of these analogues, and no corrections have been made for PK and MK-4 with similar molecular weights. MK-7, which has a much larger molecular weight, can be converted into its MK-4 equivalent using the following formula:

MK-4 equivalent (mg)=MK-7 (mg)×444.7/649.

The sum of the quantity of PK, MK-4, and MK-7 as corrected above was employed in determining the DRI for vitamin K. Although long-chain MKs are produced by intestinal bacteria and MK-4 is also produced by enzymatic conversion from PK, their contribution was not considered sufficiently large to contribute to fulfilling this requirement. Although antibiotic treatment can impair vitamin K status by decreasing the production of MKs by intestinal flora and decreasing vitamin K utilization by inhibiting the enzymatic activity of vitamin K epoxide reductase (*66*), antibiotic treatment itself does not cause vitamin K deficiency if average vitamin K intake is maintained (*67*).

The principal biological action of vitamin K is activation of prothrombin and other serum coagulation factors, thereby enhancing blood coagulation. Other actions include the modulation of bone formation by activation of osteocalcin, a bone matrix protein, and inhibition of arterial calcification by activation of matrix gla protein (MGP), another vitamin-K-dependent matrix protein.

Determining DRI

Evidence for determining AI

Since delayed blood coagulation is the only clinically manifested abnormality attributable to vitamin K deficiency, the intake necessary to maintain normal serum coagulation was considered an appropriate basis for determining the AI for vitamin K. In Japan, however, coagulation abnormalities due to vitamin K deficiency are rarely observed in healthy subjects. An intervention study of young vitamin K-deficient male volunteers weighing 72 kg found that administration of 40 and 32 μg/d of vitamin resulted in a decrease in serum PK level and an elevation in undercarboxylated prothrombin, a serum marker for vitamin K deficiency, respectively, but that administration of 82 μg/d of vitamin K returned these levels to normal values (*68*). Based on these findings, the vitamin K requirement for healthy adults was determined to be approximately 1 μg/[kg·d].

Recent studies have suggested that skeletal vitamin K deficiency is a risk factor for fracture (*69, 70*), indicating that a much higher vitamin K intake is necessary for skeletal action. Although a recent meta-analysis found that vitamin K administration significantly reduced fracture incidence, it employed a high dosage (45 mg/d) of MK-4, which is considered to be pharmacological rather than nutritional (*71*). Based on the findings of previous research, a vitamin K intake of approximately 1.0 μg/[kg·d] was determined to be satisfactory to avoid even mild deficiency, and thus set as the AI for vitamin K.

AI for adults

As described above, a vitamin K intake of 82 μg/d in those weighing 72 kg was found sufficient to avoid deficiency (*68*). Extrapolation of this value by the 0.75th power of the BW ratio was used as the basis for determining the adult AI. Although the elderly may be more susceptible to vitamin K deficiency due to various factors such as impaired intestinal absorption of vitamin K, at present, the data are scarce, and thus the AI for the elderly was the same as that for those aged 50 to 69 y.

AI for children

The AI for children was determined by extrapolating the AI for adults by the 0.75th power of the BW ratio.

AI for infants aged 0 to 5 mo

Neonates are susceptible to vitamin K deficiency for various reasons, such as poor transplacental vitamin K transport (*72*), low vitamin K content in the breast milk (*14, 73*), or low production of vitamin K in the intestinal flora (*74*). As neonatal vitamin K deficiency is known to cause neonatal melena, a form of gastrointestinal bleeding, and intracranial bleeding, vitamin K is orally administered just after birth for their prevention. The AI of 4.0 μg/d for this age group was determined by multiplying the average milk intake (0.78 L/d) by the average vitamin K content of milk (5.17 μg/L) and assuming oral administration of vitamin K just after birth in the clinical setting.

Table 4. DRIs for Vitamin K (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	4	—	—	—	4	—
6–11 mo	—	—	7	—	—	—	7	—
1–2 y	—	—	25	—	—	—	25	—
3–5 y	—	—	30	—	—	—	30	—
6–7 y	—	—	40	—	—	—	40	—
8–9 y	—	—	45	—	—	—	45	—
10–11 y	—	—	55	—	—	—	55	—
12–14 y	—	—	70	—	—	—	65	—
15–17 y	—	—	80	—	—	—	60	—
18–29 y	—	—	75	—	—	—	60	—
30–49 y	—	—	75	—	—	—	65	—
50–69 y	—	—	75	—	—	—	65	—
≥70 y	—	—	75	—	—	—	65	—
Pregnant women (amount to be added)					—	—	+0	—
Lactating women (amount to be added)					—	—	+0	—

AI for infants aged 6 to 11 mo

The AI was determined to be 7 μg/d by considering the amount of vitamin K received from sources other than breast milk.

Additional amount during pregnancy

Increased requirements for vitamin K or alterations in circulating vitamin K levels in pregnant women have not been reported. Because of poor transplacental transport, vitamin K intake in pregnant women is unlikely to affect vitamin K status in the fetuses or neonates. Thus, no additional amount required for pregnant women was determined.

Additional amount during lactation

Since lactating women have not been reported to be at higher risk for vitamin K deficiency, no additional amount required for lactating women was determined.

Tolerable upper intake level

Although menadione, a vitamin K metabolite, can cause toxicity, no toxicity has been reported regarding PKs and MKs. As 45 mg/d of MK-4 is clinically administered to many patients in Japan with osteoporosis with no reports of serious adverse events, the UL for vitamin K was not determined.

Other remarks

Due to the abundant vitamin K content of natto, its intake is contraindicated in patients treated with warfarin. In contrast, patients undergoing long-term antibiotic treatment or experiencing chronic obstruction of the biliary tract or impaired fat absorption are at higher risk of vitamin K deficiency.

DRI values for vitamin K are listed in Table 4.

REFERENCES

1) Moise AR, Noy N, Palczewski K, Blaner WS. 2007. Delivery of retinoid-based therapies to target tissues. *Biochemistry* **46**: 4449–4458.
2) Debier C, Larondelle Y. 2005. Vitamins A and E: metabolism, roles and transfer to offspring. *Br J Nutr* **93**: 153–174.
3) During A, Harrison EH. 2004. Intestinal absorption and metabolism of carotenoids: insights from cell culture. *Arch Biochem Biophys* **430**: 77–88.
4) Food and Nutrition Board, Institute of Medicine. 2002. *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 82–161. National Academy Press, Washington DC.
5) Sigmundsdottir H, Butcher EC. 2008. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nat Immunol* **9**: 981–987.
6) Sauberlich HE, Hodges RE, Wallace DL, Kolder H, Canham JE, Hood J, Raica N Jr, Lowry LK. 1974. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. *Vitam Horm* **32**: 251–275.
7) Ahmad SM, Haskell MJ, Raqib R, Stephensen CB. 2008. Men with low vitamin A stores respond adequately to primary yellow fever and secondary tetanus toxoid vaccination. *J Nutr* **138**: 2276–2283.
8) Cifelli CJ, Green JB, Green MH. 2007. Use of model-based compartmental analysis to study vitamin A kinetics and metabolism. *Vitam Horm* **75**: 161–195.
9) Furr HC, Green MH, Haskell M, Mokhtar N, Nestel P, Newton S, Ribaya-Mercado JD, Tang G, Tanumihardjo S, Wasantwisut E. 2005. Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans. *Public Health Nutr* **8**: 596–607.
10) Cifelli CJ, Green JB, Wang Z, Yin S, Russell RM, Tang G, Green MH. 2008. Kinetic analysis shows that vitamin A disposal rate in humans is positively correlated with

vitamin A stores. *J Nutr* **138**: 971–977.
11) Raica N Jr, Scott J, Lowry L, Sauberlich HE. 1972. Vitamin A concentration in human tissues collected from five areas in the United States. *Am J Clin Nutr* **25**: 291–296.
12) Joint FAO/WHO Expert Group. 2004. Vitamin A Human Vitamin and Mineral Requirements, 2nd ed, p 17–44. WHO/FAO, Geneva.
13) Canfield LM, Clandinin MT, Davies DP, Fernandez MC, Jackson J, Hawkes J, Goldman WJ, Pramuk K, Reyes H, Sablan B, Sonobe T, Bo X. 2003. Multinational study of major breast milk carotenoids of healthy mothers. *Eur J Nutr* **42**: 133–141.
14) Kamao M, Tsugawa N, Suhara Y, Wada A, Mori T, Murata K, Nishino R, Ukita T, Uenishi K, Tanaka K, Okano T. 2007. Quantification of fat-soluble vitamins in human breast milk by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* **859**: 192–200.
15) Suzuki K, Sasaki S, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Jpn J Nutr Diet* **62**: 369–372 (in Japanese).
16) 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 Breastfeeding Res* **2**: 23–28 (in Japanese).
17) Montreewasuwat N, Olson JA. 1979. Serum and liver concentrations of vitamin A in Thai fetuses as a function of gestational age. *Am J Clin Nutr* **32**: 601–606.
18) Strobel M, Tinz J, Biesalski HK. 2007. The importance of beta-carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. *Eur J Nutr* **46** (Suppl 1): I1–20.
19) Penniston KL, Tanumihardjo SA. 2006. The acute and chronic toxic effects of vitamin A. *Am J Clin Nutr* **83**: 191–201.
20) Azais-Braesco V, Pascal G. 2000. Vitamin A in pregnancy: requirements and safety limits. *Am J Clin Nutr* **71** (5 Suppl): 1325S–1333S.
21) Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. 1995. Teratogenicity of high vitamin A intake. *N Engl J Med* **333**: 1369–1373.
22) Minuk GY, Kelly JK, Hwang WS. 1988. Vitamin A hepatotoxicity in multiple family members. *Hepatology* **8**: 272–275.
23) Persson B, Tunell R, Ekengren K. 1965. Chronic vitamin a intoxication during the first half year of life; Description of 5 cases. *Acta Paediatr Scand* **54**: 49–60.
24) Michaelsson K, Lithell H, Vessby B, Melhus H. 2003. Serum retinol levels and the risk of fracture. *N Engl J Med* **348**: 287–294.
25) Männistö S, Smith-Warner SA, Spiegelman D, Albanes D, Anderson K, van den Brandt PA, Cerhan JR, Colditz G, Feskanich D, Freudenheim JL, Giovannucci E, Goldbohm RA, Graham S, Miller AB, Rohan TE, Virtamo J, Willett WC, Hunter DJ. 2004. Dietary carotenoids and risk of lung cancer in a pooled analysis of seven cohort studies. *Cancer Epidemiol Biomarkers Prev* **13**: 40–48.
26) The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. 1994. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* **330**: 1029–1035.
27) Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, Hartman AM, Palmgren J, Freedman LS, Haapakoski J, Barrett MJ, Pietinen P, Malila N, Tala E, Liippo K, Salomaa ER, Tangrea JA, Teppo L, Askin FB, Taskinen E, Erozan Y, Greenwald P, Huttunen JK. 1996. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* **88**: 1560–1570.
28) Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S. 1996. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* **334**: 1150–1155.
29) Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. 1996. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* **334**: 1145–1149.
30) Holick MF. 2004. Vitamin D. *In*: Nutrition and Bone Health (Holick MF, Dawson-Hughes B, eds), p 403–440. Humana Press, Totowa, NJ.
31) Malabanan A, Veronikis IE, Holick MF. 1998. Redefining vitamin D insufficiency. *Lancet* **351**: 805–806.
32) Nakamura K, Tsugawa N, Saito T, Ishikawa M, Tsuchiya Y, Hyodo K, Maruyama K, Oshiki R, Kobayashi R, Nashimoto M, Yoshihara A, Ozaki R, Okano T, Yamamoto M. 2008. Vitamin D status, bone mass, and bone metabolism in home-dwelling postmenopausal Japanese women: Yokogoshi Study. *Bone* **42**: 271–277.
33) Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B. 1989. Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. *N Engl J Med* **321**: 1777–1783.
34) Nakamura K, Nashimoto M, Tsuchiya Y, Obata A, Miyanishi K, Yamamoto M. 2001. Vitamin D insufficiency in Japanese female college students: a preliminary report. *Int J Vitam Nutr Res* **71**: 302–305.
35) Nakamura K, Nashimoto M, Matsuyama S, Yamamoto M. 2001. Low serum concentrations of 25-hydroxyvitamin D in young adult Japanese women: a cross sectional study. *Nutrition* **17**: 921–925.
36) Nakamura K, Nashimoto M, Hori Y, Muto K, Yamamoto M. 1999. Serum 25-hydroxyvitamin D levels in active women of middle and advanced age in a rural community in Japan. *Nutrition* **15**: 870–873.
37) Nakamura K, Nashimoto M, Yamamoto M. 2000. Summer/winter differences in the serum 25-hydroxyvitamin D_3 and parathyroid hormone levels of Japanese women. *Int J Biometeorol* **44**: 186–189.
38) Nakamura K, Nashimoto M, Hori Y, Yamamoto M. 2000. Serum 25-hydroxyvitamin D concentrations and related dietary factors in peri- and postmenopausal Japanese women. *Am J Clin Nutr* **71**: 1161–1165.
39) Nakamura K, Nashimoto M, Yamamoto M. 2001. Are the serum 25-hydroxyvitamin D concentrations in winter associated with forearm bone mineral density in healthy elderly Japanese women? *Int J Vitam Nutr Res* **71**: 25–29.
40) Ministry of Health Labour and Welfare, Japan. 2007. National Health and Nutritional Survey Japan (2005). Tokyo.
41) Ministry of Health Labour and Welfare, Japan. 2008. National Health and Nutritional Survey Japan (2006). Tokyo.

42) Yorifuji J, Yorifuji T, Tachibana K, Nagai S, Kawai M, Momoi T, Nagasaka H, Hatayama H, Nakahata T. 2008. Craniotabes in normal newborns: the earliest sign of subclinical vitamin D deficiency. *J Clin Endocrinol Metab* **93**: 1784–1788.
43) Nakao H. 1988. Nutritional significance of human milk vitamin D in neonatal period. *Kobe J Med Sci* **34**: 121–128.
44) Ministry of Education, Culture, Sports, Science and Technology, Japan. 2005. Standard Tables of Food Composition in Japan, Fifth revised and enlarged ed, 2004. Tokyo.
45) Specker BL, Valanis B, Hertzberg V, Edwards N, Tsang RC. 1985. Sunshine exposure and serum 25-hydroxyvitamin D concentrations in exclusively breast-fed infants. *J Pediatr* **107**: 372–376.
46) Specker BL, Ho ML, Oestreich A, Yin TA, Shui QM, Chen XC, Tsang RC. 1992. Prospective study of vitamin D supplementation and rickets in China. *J Pediatr* **120**: 733–739.
47) MacLennan WJ, Hamilton JC, Darmady JM. 1980. The effects of season and stage of pregnancy on plasma 25-hydroxy-vitamin D concentrations in pregnant women. *Postgrad Med J* **56**: 75–79.
48) Henriksen C, Brunvand L, Stoltenberg C, Trygg K, Haug E, Pedersen JI. 1995. Diet and vitamin D status among pregnant Pakistani women in Oslo. *Eur J Clin Nutr* **49**: 211–218.
49) Narang NK, Gupta RC, Jain MK. 1984. Role of vitamin D in pulmonary tuberculosis. *J Assoc Physicians India* **32**: 185–188.
50) Honkanen R, Alhava E, Parviainen M, Talasniemi S, Monkkonen R. 1990. The necessity and safety of calcium and vitamin D in the elderly. *J Am Geriatr Soc* **38**: 862–866.
51) Ala-Houhala M, Koskinen T, Terho A, Koivula T, Visakorpi J. 1986. Maternal compared with infant vitamin D supplementation. *Arch Dis Child* **61**: 1159–1163.
52) Fomon SJ, Younoszai MK, Thomas LN. 1966. Influence of vitamin D on linear growth of normal full-term infants. *J Nutr* **88**: 345–350.
53) Traber MG, Arai H. 1999. Molecular mechanisms of vitamin E transport. *Annu Rev Nutr* **19**: 343–355.
54) Horwitt MK, Century B, Zeman AA. 1963. Erythrocyte survival time and reticulocyte levels after tocopherol depletion in man. *Am J Clin Nutr* **12**: 99–106.
55) Farrell PM, Bieri JG, Fratantoni JF, Wood RE, di Sant'Agnese PA. 1977. The occurrence and effects of human vitamin E deficiency. A study in patients with cystic fibrosis. *J Clin Invest* **60**: 233–241.
56) Horwitt MK. 1960. Vitamin E and lipid metabolism in man. *Am J Clin Nutr* **8**: 451–461.
57) Sasaki S, Ushio F, Amano K, Morihara M, Todoriki O, Uehara Y, Toyooka E. 2000. Serum biomarker-based validation of a self-administered diet history questionnaire for Japanese subjects. *J Nutr Sci Vitaminol* **46**: 285–296.
58) Hiraoka M. 2001. Nutritional status of vitamin A, E, C, B1, B2, B6, nicotinic acid, B12, folate, and beta-carotene in young women. *J Nutr Sci Vitaminol* **47**: 20–27.
59) Maruyama C, Imamura K, Oshima S, Suzukawa M, Egami S, Tonomoto M, Baba N, Harada M, Ayaori M, Inakuma T, Ishikawa T. 2001. Effects of tomato juice consumption on plasma and lipoprotein carotenoid concentrations and the susceptibility of low density lipoprotein to oxidative modification. *J Nutr Sci Vitaminol* **47**: 213–221.
60) Sakurai T, Furukawa M, Asoh M, Kanno T, Kojima T, Yonekubo A. 2005. Fat-soluble and water-soluble vitamin contents of breast milk from Japanese women. *J Nutr Sci Vitaminol* **51**: 239–247.
61) Morinobu T, Ban R, Yoshikawa S, Murata T, Tamai H. 2002. The safety of high-dose vitamin E supplementation in healthy Japanese male adults. *J Nutr Sci Vitaminol* **48**: 6–9.
62) Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. 2005. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* **142**: 37–46.
63) Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. 2007. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* **297**: 842–857.
64) Asleh R, Blum S, Kalet-Litman S, Alshiek J, Miller-Lotan R, Asaf R, Rock W, Aviram M, Milman U, Shapira C, Abassi Z, Levy AP. 2008. Correction of HDL dysfunction in individuals with diabetes and the haptoglobin 2–2 genotype. *Diabetes* **57**: 2794–2800.
65) Milman U, Blum S, Shapira C, Aronson D, Miller-Lotan R, Anbinder Y, Alshiek J, Bennett L, Kostenko M, Landau M, Keidar S, Levy Y, Khemlin A, Radan A, Levy AP. 2008. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2–2 genotype: a prospective double-blinded clinical trial. *Arterioscler Thromb Vasc Biol* **28**: 341–347.
66) Frick PG, Riedler G, Brogli H. 1967. Dose response and minimal daily requirement for vitamin K in man. *J Appl Physiol* **23**: 387–389.
67) Suttie JW. 1995. The importance of menaquinones in human nutrition. *Annu Rev Nutr* **15**: 399–417.
68) Suttie JW, Mummah-Schendel LL, Shah DV, Lyle BJ, Greger JL. 1988. Vitamin K deficiency from dietary vitamin K restriction in humans. *Am J Clin Nutr* **47**: 475–480.
69) Feskanich D, Weber P, Willett WC, Rockett H, Booth SL, Colditz GA. 1999. Vitamin K intake and hip fractures in women: a prospective study. *Am J Clin Nutr* **69**: 74–79.
70) Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, Wilson PW, Ordovas J, Schaefer EJ, Dawson-Hughes B, Kiel DP. 2000. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr* **71**: 1201–1208.
71) Cockayne S, Adamson J, Lanham-New S, Shearer MJ, Gilbody S, Torgerson DJ. 2006. Vitamin K and the prevention of fractures: systematic review and meta-analysis of randomized controlled trials. *Arch Intern Med* **166**: 1256–1261.
72) Shearer MJ, Rahim S, Barkhan P, Stimmler L. 1982. Plasma vitamin K1 in mothers and their newborn babies. *Lancet* **2**: 460–463.
73) Kojima T, Asoh M, Yamawaki N, Kanno T, Hasegawa H, Yonekubo A. 2004. Vitamin K concentrations in the maternal milk of Japanese women. *Acta Paediatr* **93**: 457–463.
74) Greer FR, Mummah-Schendel LL, Marshall S, Suttie JW. 1988. Vitamin K1 (phylloquinone) and vitamin K2 (menaquinone) status in newborns during the first week of life. *Pediatrics* **81**: 137–140.

J Nutr Sci Vitaminol, 59, S67–S82, 2013

Dietary Reference Intakes for Japanese 2010: Water-Soluble Vitamins

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(Received October 26, 2012)

Summary A potential approach for determining the estimated average requirement (EAR) is based on the observation that a water-soluble vitamin or its catabolite(s) can be detected in urine. In this approach, the urinary excretion of a water-soluble vitamin or its catabolite(s) increase when the intake exceeds the requirement. This approach is applied to vitamin B_1, vitamin B_2 and niacin. A second approach is to determine the blood concentration. In this case, the requirement is indicated by a value rather than a threshold level. The second approach is applied to vitamin B_6, vitamin B_{12}, folate, and vitamin C. The recommended dietary allowance (RDA) was calculated by multiplying the EAR by 1.2. For pantothenic acid and biotin, there were insufficient data for determining the EAR. Thus, adequate intakes were set based on food surveillance data.

Key Words water-soluble vitamins, DRI, urine, blood, requirement

Vitamin B_1

Background information

The chemical name of vitamin B_1 is thiamin, and the active form is thiamin diphosphate (TDP). Severe thiamin deficiency results in a nerve and heart disease, termed beriberi. Less severe deficiency results in nonspecific symptoms such as malaise, loss of weight, irritability, and confusion.

In foods, thiamin exists mainly as a TDP-protein complex. Thus, the absorption of thiamin in the digestive tract involves 2 stages: (1) the release of TDP from the complex by the action of proteases and (2) the release of thiamin from TDP by the action of phosphatases and pyrophosphatases. There are 2 mechanisms of absorption. At low luminal concentrations (<2 μmol/L), the process is carrier-mediated; at higher concentrations (e.g., a 2.5 mg dose for humans) passive diffusion also occurs.

Most of the thiamin in serum is bound to protein, mainly albumin. Thiamin is taken up by blood cells and body tissues via active transport. Intracellular thiamin occurs predominantly (80%) as TDP, most of which is bound to proteins. The relative availability of dietary vitamin B_1 to free thiamin in a typical Japanese diet is around 60% (*1*, *2*).

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Determining DRIs

Evidence for determining the estimated average requirement (EAR)

Orally administered thiamin is rapidly converted to TDP in the body tissues. Thereafter, excess thiamin is excreted as free form in the urine. Urinary excretion of thiamin has been shown sharply to increase at a concentration >0.35 mg thiamin/1,000 kcal/d (*3*). Based on this evidence, the EAR of thiamin ($C_{12}H_{17}N_4OS$, molecular weight 265.3) was determined. It should be noted that the Standard Tables of Food Composition in Japan give the content of vitamin B_1 as the value of thiamin hydrochloride ($C_{12}H_{17}ClN_4OS{\cdot}HCl$, molecular weight 337.3). Thus, the EAR of vitamin B_1 becomes 0.45 mg thiamin hydrochloride/1,000 kcal/d. The recommended dietary allowance (RDA) is set by assuming a coefficient of variation of 10%. Thus the RDA becomes 0.54 mg thiamin hydrochloride/1,000 kcal/d.

Table 1. DRIs for vitamin B_1 (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.1	—	—	—	0.1	—
6–11 mo	—	—	0.3	—	—	—	0.3	—
1–2 y	0.5	0.5	—	—	0.4	0.5	—	—
3–5 y	0.6	0.7	—	—	0.6	0.7	—	—
6–7 y	0.7	0.8	—	—	0.7	0.8	—	—
8–9 y	0.8	1.0	—	—	0.8	1.0	—	—
10–11 y	1.0	1.2	—	—	0.9	1.1	—	—
12–14 y	1.1	1.4	—	—	1.0	1.2	—	—
15–17 y	1.2	1.5	—	—	1.0	1.2	—	—
18–29 y	1.2	1.4	—	—	0.9	1.1	—	—
30–49 y	1.2	1.4	—	—	0.9	1.1	—	—
50–69 y	1.1	1.3	—	—	0.9	1.1	—	—
≥70 y	1.0	1.2	—	—	0.8	0.9	—	—
Pregnant women (amount to be added)								
Early-stage					+0.0	+0.0	—	—
Mid-stage					+0.1	+0.1	—	—
Late-stage					+0.2	+0.2	—	—
Lactating women (amount to be added)					+0.2	+0.2	—	—

DRIs, Dietary Reference Intakes; EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.
[1] Calculated by using PAL II of the EER.

For example, the RDAs for 18- to 29-y-old males and females are 1.4 mg/d and 1.1 mg/d, respectively, assuming a physical activity level (PAL) II, i.e., within the estimated energy requirement (EER).

Life stages

0–5 mo. The mean concentration of thiamin hydrochloride in breast milk is 0.13 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily vitamin B_1 intake of about 0.1 mg/d. This value was set as the adequate intake (AI).

6–11 mo. The AI for infants aged 6–11 mo is calculated using the average of the values from the following 2 expressions: Expression 1, AI for infant boy or girl aged 6–11 mo (extrapolated AI from infants)=AI for infants (0–5 mo)×(average reference infant boy or girl body weight of 6–11 mo/average reference infant boy or girl body weight of 0–5 mo)$^{0.75}$; Expression 2, AI for infant boy or girl aged 6–11 mo (extrapolated AI from adults)=RDA×(average reference infant boy or girl body weight of 6–11 mo/average reference male or female weight of 18–29 y old)$^{0.75}$×(1+growth factor). Thus, the AI of infants aged 6–11 mo is 0.3 mg/d.

Pregnant women. The additional amounts are calculated based on the assumption that the requirement for vitamin B_1 increases according to energy expenditure. In other words, the additional EAR and RDA for pregnant women are calculated by multiplying the EAR or RDA by the additional energy expenditure resulting from pregnancy.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. But, the availability of dietary vitamin B_1 is low compared with the free form of vitamin B_1. The relative availability of dietary vitamin B_1 to free thiamin in a typical Japanese diet is around 60% (*1, 2*). Thus, the EAR is divided by 0.6. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

Chronic intake of thiamin (50 mg/kg body weight/d) has been reported to cause severe toxicity symptoms (*9*). For example, intake of 10 g of thiamin hydrochloride for 2.5 wk daily resulted in headaches, irritability, insomnia, pulsus celer, weakness, contact dermatitis, and itchiness. These symptoms disappeared in 2 d when the intake was discontinued (*10*). Nevertheless, there is insufficient evidence for determining the tolerable upper intake level (UL).

The Dietary Reference Intakes (DRIs) for vitamin B_1 are summarized in Table 1.

Vitamin B_2

Background information

The chemical name of vitamin B_2 is riboflavin, and the active forms are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Riboflavin deficiency results in angular cheilitis, glossitis (magenta tongue), seborrheic dermatitis, and other disorders.

Table 2. DRIs for vitamin B_2 (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.3	—	—	—	0.3	—
6–11 mo	—	—	0.4	—	—	—	0.4	—
1–2 y	0.5	0.6	—	—	0.5	0.5	—	—
3–5 y	0.7	0.8	—	—	0.6	0.8	—	—
6–7 y	0.8	0.9	—	—	0.7	0.9	—	—
8–9 y	0.9	1.1	—	—	0.9	1.0	—	—
10–11 y	1.1	1.4	—	—	1.0	1.2	—	—
12–14 y	1.3	1.5	—	—	1.1	1.4	—	—
15–17 y	1.4	1.7	—	—	1.1	1.4	—	—
18–29 y	1.3	1.6	—	—	1.0	1.2	—	—
30–49 y	1.3	1.6	—	—	1.0	1.2	—	—
50–69 y	1.2	1.5	—	—	1.0	1.2	—	—
≥70 y	1.1	1.3	—	—	0.9	1.0	—	—
Pregnant women (amount to be added)								
Early-stage					+0.0	+0.0	—	—
Mid-stage					+0.1	+0.2	—	—
Late-stage					+0.2	+0.3	—	—
Lactating women (amount to be added)					+0.3	+0.4	—	—

[1] Calculated by using PAL II of the EER.

In foods, riboflavin exists mainly as a complex of FMN or FAD, non-covalently bound to related enzyme proteins. During digestion, FAD and FMN are firstly liberated in acidic conditions, and are then hydrolyzed by pyrophosphatase and phosphatase. Finally, riboflavin is released and absorbed from the small intestine (*11*). The absorbed riboflavin is incorporated into the body tissues, and used for FAD synthesis. In the rat liver, for example, about 90% of riboflavin exists as FAD, about 10% as FMN, and the remaining 1% as riboflavin.

In the blood, riboflavin exists mainly in the form of FAD, with ~10% FMN and ~4% riboflavin. A large portion of riboflavin is associated with immunoglobulins, but some is bound to albumin (*12*). The absorbed riboflavin is incorporated into the body tissues, and converted mainly to FAD via FMN.

Excess riboflavin is rapidly excreted in the urine, primarily as free riboflavin.

Determining DRIs

Evidence for determining the EAR

Usually only a small amount of riboflavin is excreted in the urine; the level of excretion varies according to the intake of vitamin B_2. If the body requirement is met, urinary excretion shows a rapid increase. A gradual increase in the intake of free riboflavin to ≥1.1 mg/d was shown to result in a rapid rise in urinary excretion by healthy males and females (*13*, *14*). Based on these results, and the involvement of vitamin B_2 in energy metabolism, EAR was determined as the energy intake/d, i.e., 0.50 mg riboflavin/1,000 kcal/d. For example, the EARs for 18- to 29-y-old males and females are 1.3 mg/d and 1.0 mg/d, respectively, assuming a PAL II, i.e., within the EER.

Life stages

0–5 mo. For infants of 0–5 mo, breast milk is the sole source of vitamin B_2. The mean concentration of riboflavin in breast milk is 0.40 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily vitamin B_2 intake of about 0.3 mg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 0.4 mg/d.

Pregnant women. The additional amounts are calculated based on the assumption that the requirement for vitamin B_2 increases according to energy expenditure. In other words, the additional EAR and RDA for pregnant women are calculated by multiplying the EAR or RDA by the additional energy expenditure resulting from pregnancy.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. The mean concentration of riboflavin in breast milk is 0.40 mg/L (*4–6*) and the average secretion of breast milk is 0.78 L/d (*7*, *8*). Thus, the additional EAR becomes 0.3 mg/d. The additional RDA is calculated by multiplying the additional EAR by

1.2.

Tolerable upper intake level

Chronic use of riboflavin has not been reported to cause severe toxicity. For example, a daily intake of 400 mg of riboflavin for 3 mo (*15*), supplemental oral intake of up to 60 mg riboflavin, or single intravenous injection of 11.6 mg riboflavin (*16*) caused no deleterious effects. This may be attributed to rapid excretion of riboflavin in the urine, and also to limited solubility and reduced absorption at higher doses. Stripp demonstrated limited absorption of 50–500 mg of riboflavin, and consequently no adverse effects (*17*). Zempleni et al. reported that the maximum absorbable amount of riboflavin in a single dose was 27 mg (*16*). Moreover, there are no data indicating that riboflavin administration during pregnancy is potentially dangerous. Thus, there is no evidence for determining the UL.

The DRIs for vitamin B_2 are summarized in Table 2.

Niacin

Background information

The main compounds showing niacin activity are nicotinic acid, nicotinamide, and tryptophan. The DRIs for niacin are expressed in niacin equivalent (NE).

The Standard Tables of Food Composition in Japan, (*18*) list niacin as the sum of nicotinic acid and nicotinamide, and do not include nicotinamide biosynthesized from tryptophan. Therefore, to calculate NE in a diet, the amount of nicotinamide biosynthesized from dietary tryptophan should be added to the amount of niacin. The conversion ratio for tryptophan to nicotinamide is set at 1/60 on a weight basis. The NE is calculated using the following formula:

Niacin equivalent (mg NE)

=niacin intake (mg)+(1/60) tryptophan intake (mg)

Most protein contains approximately 1% of tryptophan, and therefore the amount of nicotinamide biosynthesized from tryptophan (mg) is estimated as the amount of protein (g) divided by 6.

In living cells, niacin exists mainly as the cofactor NAD(P), which binds weakly to enzyme proteins. During cooking and processing of animal and plant foods, NAD(P) is hydrolyzed to nicotinamide and nicotinic acid, respectively. Any remaining NAD(P) is hydrolyzed to nicotinamide in the gastrointestinal tract. Nicotinamide and nicotinic acid are absorbed in the small intestine. Most nicotinic acid binds to complex carbohydrates in cereal grains, and is therefore less digestible (*19*). The relative availability of dietary niacin to free nicotinamide is approximately 60% in a typical Japanese diet (*1*, *2*).

Determining DRIs

Evidence for determining the EAR

The conversion ratio of tryptophan to nicotinamide is set at 1/60 on a weight basis (*20*, *21*). Niacin relates to energy metabolism, and therefore the EAR for niacin is expressed as mg NE/1,000 kcal. Human studies show that NE intake correlates well with urinary nicotinamide metabolite N^1-methylnicotinamide, and that a urinary N^1-methylnicotinamide of 1.0 mg/d reflects clinical niacin deficiency (*20*, *22–25*). Analysis of previous studies shows that the niacin intake equivalent to a urinary N^1-methylnicotinamide of 1.0 mg/d is 4.8 mg NE/1,000 kcal. This value was set as the EAR for subjects aged 1–69 y. The RDA is determined as 5.8 mg NE/1,000 kcal, calculated by multiplying the EAR by 1.2. Based on niacin intake and urinary nicotinamide metabolite data, niacin activity in older subjects is considered to be the same as that in younger subjects. Thus, the EAR and RDA were set at 4.8 mg NE/1,000 kcal and 5.8 mg NE/1,000 kcal, respectively, for adults >70 y old. To express the EAR and RDA in mg NE/d, each value is multiplied by the estimated energy requirement corresponding to a subject's sex, age, and physical activity.

Life stages

0–5 mo. The mean nicotinamide concentration in breast milk is 2.0 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily nicotinamide intake of ~1.6 mg/d. The AI for infants aged 0–5 mo was set at 2 mg/d. Nicotinamide is unlikely to be biosynthesized from tryptophan at this stage, and therefore the AI is expressed in mg/d.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. The average of the obtained values for each sex is 3.1 mg NE/d. Thus, the AI for infants aged 6–11 mo becomes 3 mg NE/d.

Pregnant women. The additional amounts are set based on the assumption that the requirement for niacin increases according to energy expenditure. There is no evidence for setting the EAR by factorial method. Thus, the EAR and RDA for niacin are expressed as mg NE/1,000 kcal. However, the amount of nicotinamide biosynthesized from tryptophan increases during pregnancy, and this compensates for the increase in niacin requirement (*16*). Thus, pregnant women do not require additional niacin intake.

Lactating women. The conversion rate of tryptophan to nicotinamide returns to a normal level after delivery (*26*), and therefore lactating women require additional niacin intake to compensate for the loss of niacin to breast milk. Daily niacin secretion to milk of 1.6 mg/d is adjusted by the relative availability of dietary niacin to free nicotinamide 60% (*1*, *2*). Thus, the additional EAR for lactating women was set at 3 mg NE/d (rounded up from 2.6 mg NE/d). The additional RDA was set at 3 mg NE/d, calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

Nicotinic acid and nicotinamide are often used in niacin supplements and fortified foods. The UL for niacin therefore takes into account the nicotinic acid and nicotinamide taken from supplements and fortified foods. The large doses of nicotinamide and nicotinic acid used to treat patients with type I diabetes and hypercholesterolemia, respectively, may cause gastrointestinal effects such as dyspepsia, diarrhea, and constipation, and also

Table 3. DRIs for niacin (mgNE/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL[2]	EAR	RDA	AI	UL[2]
0–5 mo[3]	—	—	2	—	—	—	2	—
6–11 mo	—	—	3	—	—	—	3	—
1–2 y	5	6	—	60 (15)	4	5	—	60 (15)
3–5 y	6	7	—	80 (20)	6	7	—	80 (20)
6–7 y	7	9	—	100 (30)	7	8	—	100 (30)
8–9 y	9	10	—	150 (35)	8	10	—	150 (35)
10–11 y	11	13	—	200 (45)	10	12	—	150 (45)
12–14 y	12	14	—	250 (60)	11	13	—	250 (60)
15–17 y	13	16	—	300 (70)	11	13	—	250 (65)
18–29 y	13	15	—	300 (80)	9	11	—	250 (65)
30–49 y	13	15	—	350 (85)	10	12	—	250 (65)
50–69 y	12	14	—	350 (80)	9	11	—	250 (65)
≥70 y	11	13	—	300 (75)	8	10	—	250 (60)
Pregnant women (amount to be added)					+0	+0	—	—
Lactating women (amount to be added)					+3	+3	—	—

[1] NE=niacin equivalents (mgNE)=niacin intake (mg)+1/60 of tryptophan intake (mg). Calculated by using PAL II of the EER.
[2] The ULs were the amounts of nicotinamide (mg) and mg of nicotinic acid in parentheses. Values were calculated using reference body weight.
[3] Values were expressed as mg/d.

hepatotoxic symptoms such as dysfunction and fulminant hepatitis. According to previous reports (*26–30*), the no observed adverse effect levels (NOAELs) for nicotinamide and nicotinic acid were set at 25 mg/kg body weight and 6.25 mg/kg body weight, respectively. The NOAELs were divided by an uncertainty factor of 5, and the obtained values of 5 mg/kg body weight and 1.25 mg/kg body weight were set as the ULs for nicotinamide and nicotinic acid, respectively. A pharmacological dose of nicotinic acid has the transient vasodilatory effect of flushing (reddening of the skin), but no adverse health effects. Thus, it is not appropriate to use flushing for setting a UL for nicotinic acid.

The DRIs for niacin are summarized in Table 3.

Vitamin B_6

Background information

The chemical substances possessing vitamin B_6 activity are pyridoxine, pyridoxal, and pyridoxamine and their respective phosphorylated forms. The functional form is pyridoxal 5′-phosphate (PLP). Vitamin B_6 deficiency results in seborrheic dermatitis, epileptiform convulsions, and microcytic anemia. In foods, vitamin B_6 exists mainly as a complex of PLP or pyridoxamine 5′-phosphate (PMP), associated with protein. During digestion, PLP and PMP are released and hydrolyzed by phosphatase, after which pyridoxal and pyridoxamine are released and absorbed. Plants possess pyridoxine 5′β-glucoside (PNG), which, if ingested, is partially hydrolyzed to pyridoxine and absorbed. The bioavailability of vitamin B_6 in humans is estimated to be 50% (*31*). The bioavailability in typical American foods is estimated to be 75% (*32*), while that in a typical rice-based Japanese diet is 73% (*1*).

In serum, PLP and pyridoxal are the dominant B_6 vitamers. PLP is bound to protein, predominantly albumin. Erythrocytes possess pyridoxal kinase and pyridoxamine 5′-phosphate/pyridoxine 5′-phosphate oxidase, and therefore PLP can be synthesized from pyridoxal and PMP. Pyridoxal is incorporated into the body tissues and converted to PLP.

Pyridoxal is metabolized in the liver to 4-pyridoxic acid, and excreted in the urine.

Determining DRIs

Evidence for determining the EAR

Vitamin B_6 is involved in the catabolism of amino acids and formation of bioactive amines, including some neurotransmitters such as γ-aminobutyric acid. The plasma PLP concentration has been reported to reflect the body store of vitamin B_6 (*33*). A low plasma PLP concentration was shown to be associated with electroencephalographic changes in young, non-pregnant women (*34*). Furthermore, a plasma PLP concentration of 30 nmol/L was required to alleviate vitamin B_6 deficiency-induced disorders (*35*). The EAR for vitamin B_6 is based on the amount of vitamin B_6 that can maintain a plasma PLP level of 30 nmol/L. The vitamin B_6 requirement increases as the protein intake increases, and the plasma PLP concentration correlates well with vitamin

Table 4. DRIs for vitamin B_6 (mg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL[2]	EAR	RDA	AI	UL[2]
0–5 mo	—	—	0.2	—	—	—	0.2	—
6–11 mo	—	—	0.3	—	—	—	0.3	—
1–2 y	0.4	0.5	—	10	0.4	0.5	—	10
3–5 y	0.5	0.6	—	15	0.5	0.6	—	15
6–7 y	0.7	0.8	—	20	0.6	0.7	—	20
8–9 y	0.8	0.9	—	25	0.8	0.9	—	25
10–11 y	0.9	1.0	—	30	0.9	1.0	—	30
12–14 y	1.0	1.3	—	40	1.0	1.3	—	40
15–17 y	1.1	1.4	—	50	1.0	1.3	—	45
18–29 y	1.1	1.4	—	55	1.0	1.1	—	45
30–49 y	1.1	1.4	—	60	1.0	1.1	—	45
50–69 y	1.1	1.4	—	55	1.0	1.1	—	45
≥70 y	1.1	1.4	—	50	1.0	1.1	—	40
Pregnant women (amount to be added)					+0.7	+0.8	—	—
Lactating women (amount to be added)					+0.3	+0.3	—	—

[1] Calculated by using recommended dietary allowance of protein (except for additional amount for pregnant and lactating women).
[2] Quantity as pyridoxine, not indicating values in dietary vitamin B_6.

B_6 intake per protein intake (*36*). Thus, 0.014 mg pyridoxine/g protein was estimated as the concentration required to maintain a plasma PLP concentration of 30 nmol/L. Based on the bioavailability of vitamin B_6 in a typical rice-based Japanese diet (*1*), the EAR becomes 0.019 mg pyridoxine/g protein. The RDA is calculated by multiplying the EAR by 1.2, to give 0.023 mg pyridoxine/g protein. To obtain the daily requirement of vitamin B_6, the EAR of vitamin B_6 is multiplied to a RDA of protein. For example, the EAR for 18- to 29-y-old males and females are 1.1 mg pyridoxine/d and 1.0 mg pyridoxine/d, assuming that RDAs of protein is 60 g/d and 50 g/d, respectively.

Life stages

0–5 mo. For infants of 0–5 mo, breast milk is the sole source of vitamin B_6. The mean concentration of pyridoxine in breast milk is 0.25 mg/L (*4–6, 37*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily vitamin B_6 intake of about 0.2 mg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 0.3 mg/d.

Pregnant women. The plasma PLP concentration has been reported to decrease during pregnancy (*38*). However, during the last stage, it must be maintained at 30 nmol/L. Thus, the additional amount is set at 0.5 mg/d (*36*). The additional EAR during pregnancy is set at 0.7 mg/d including a bioavailability of 73%. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. The additional EAR for pregnant women is calculated based on the mean concentration of vitamin B_6 in breast milk (0.25 mg/L) (*8*), the average secretion (0.78 L/d) of breast milk (*7, 8*), and a bioavailability of 73%, i.e., 0.3 mg/d. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

A continuously high intake of pyridoxine for several months was shown to result in sensory neuropathy (*39*). This symptom was used as a criterion for estimating the UL for pyridoxine. By contrast, administration of 100–300 mg pyridoxine/d over a period of 4 mo did not cause sensory neuropathy in 24 patients with carpal tunnel syndrome (*40*). Based on these data, the NOAEL was set at 300 mg/d. Assuming an uncertainty factor of 5, the UL for pyridoxine was set at 60 mg/d, namely 0.8 mg/kg body weight. The UL for each age group was obtained by multiplying the UL by the respective weight.

The DRIs for vitamin B_6 are summarized in Table 4.

Vitamin B_{12}

Background information

Vitamin B_{12} (B_{12}) belongs to the corrinoids, which are compounds having in common a corrin nucleus. There are various B_{12} compounds with different upper ligands; in particular, methylcobalamin and 5′-deoxya-

Table 5. DRIs for vitamin B_{12} (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.4	—	—	—	0.4	—
6–11 mo	—	—	0.6	—	—	—	0.6	—
1–2 y	0.8	0.9	—	—	0.8	0.9	—	—
3–5 y	0.9	1.1	—	—	0.9	1.1	—	—
6–7 y	1.1	1.4	—	—	1.1	1.4	—	—
8–9 y	1.3	1.6	—	—	1.3	1.6	—	—
10–11 y	1.6	1.9	—	—	1.6	1.9	—	—
12–14 y	2.0	2.4	—	—	2.0	2.4	—	—
15–17 y	2.0	2.4	—	—	2.0	2.4	—	—
18–29 y	2.0	2.4	—	—	2.0	2.4	—	—
30–49 y	2.0	2.4	—	—	2.0	2.4	—	—
50–69 y	2.0	2.4	—	—	2.0	2.4	—	—
≥70 y	2.0	2.4	—	—	2.0	2.4	—	—
Pregnant women (amount to be added)					+0.3	+0.4	—	—
Lactating women (amount to be added)					+0.7	+0.8	—	—

denosylcobalamin function as B_{12} coenzymes. The DRIs for B_{12} were set as cyanocobalamin (molecular weight 1,355.4).

Humans possess a complex process for gastrointestinal absorption of dietary B_{12} (*41*). B_{12} released from food protein is first bound to haptocorrin (salivary B_{12}-binding protein) in the stomach. After proteolysis of the haptocorrin–B_{12} complex by pancreatic proteases in the duodenum, the released B_{12} binds to intrinsic factor (IF, gastric B_{12}-binding protein) in the proximal ileum. The IF–B_{12} complex can enter mucosal cells in the distal ileum by receptor-mediated endocytosis.

The bioavailability of dietary B_{12} is highly dependent on this IF-mediated absorption system. Under physiological conditions, 50% of dietary B_{12} is assumed to be absorbed by healthy adults (*42*). The IF-mediated B_{12} absorption system becomes saturated at a dietary concentration of about 2 μg of B_{12} (*43*). Ingestion of a large quantity of B_{12} from certain foods results in a significant decrease in the absorption rate of B_{12}.

Substantial amounts of B_{12} are excreted in bile (average excretion of 2.5 μg/d) (*44*). Approximately 50% of biliary B_{12} is re-absorbed by the intestine, with the remainder excreted in the feces.

Determining DRIs

Evidence for determining the EAR

It is not possible to determine the EAR of B_{12} for healthy adults, because of the saturable IF-mediated B_{12} gastrointestinal absorption system and/or substantial amounts of enterohepatic B_{12} circulation. Thus, the EAR for adults was estimated based on clinical data (the amount of B_{12} required for maintenance of adequate hematological status and serum B_{12} level) from B_{12}-deficient patients with pernicious anemia, following intramuscular injection with varying concentrations (0.1–10 μg/d) of B_{12} (*45*, *46*). The data suggest an average intramuscular requirement of 1.5 μg/d for maintenance of adequate hematological status. B_{12}-deficient patients with pernicious anemia cannot reabsorb B_{12} (0.5 μg/d) from the bile, because of the lack of an IF-mediated B_{12} absorption system (*42*). Thus, under normal physiological conditions, an average intake of 1.0 μg/d is required to compensate for the estimated extra losses of biliary B_{12} (0.5 μg/d) from the average intramuscular requirement (1.5 μg/d). We adjusted this value with a 50% absorption rate of dietary B_{12}, to obtain an EAR (2.0 μg/d) for healthy adults. The RDA was calculated as 2.4 μg/d, by multiplying the EAR by 1.2.

The EAR for children was calculated from the EAR for adults (2.0 μg/d), using the following equation for body surface area at each age: [(reference weight at each age/reference weight of 18- to 29-y-olds)$^{0.75}$×(1+growth factor)].

The EARs and DRIs for >50-y-olds were set at identical values to those for 18- to 49-y-olds, because of the lack of detailed information concerning the decrease in B_{12} absorption in elderly persons.

Life stages

0–5 mo. The mean concentration of B_{12} in breast milk is 0.45 μg/L (*5*, *6*, *47*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily B_{12} intake of 0.35 μg/d. The AI was rounded up to 0.4 μg/d.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 0.6 μg/d (rounded down from 0.61 μg/d).

Table 6. DRIs for folate (μg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL[2]	EAR	RDA	AI	UL[2]
0–5 mo	—	—	40	—	—	—	40	—
6–11 mo	—	—	65	—	—	—	65	—
1–2 y	80	100	—	300	80	100	—	300
3–5 y	90	110	—	400	90	110	—	400
6–7 y	110	140	—	600	110	140	—	600
8–9 y	130	160	—	700	130	160	—	700
10–11 y	160	190	—	900	160	190	—	900
12–14 y	200	240	—	1,200	200	240	—	1,200
15–17 y	200	240	—	1,300	200	240	—	1,300
18–29 y	200	240	—	1,300	200	240	—	1,300
30–49 y	200	240	—	1,400	200	240	—	1,400
50–69 y	200	240	—	1,400	200	240	—	1,400
≥70 y	200	240	—	1,300	200	240	—	1,300
Pregnant women (amount to be added)					+200	+240	—	—
Lactating women (amount to be added)					+80	+100	—	—

[1] Women planning pregnancy or possibly pregnant are advised to take 400 μg/d of supplemental pteroyl monoglutamate to reduce risks for fetal NTDs.
[2] ULs were estimated as pteroyl monoglutamates.

Pregnant women. Based on the liver B_{12} content of infants, the human fetus is estimated to accumulate 0.1–0.2 μg/d of B_{12} (*48*, *49*). Using the median (0.15 μg/d) of the fetal deposition and the 50% absorption rate for dietary B_{12} in healthy adults, the additional EAR for pregnant women becomes 0.3 μg/d. The additional RDA is calculated as 0.4 μg/d (rounded up from 0.36 μg/d) by multiplying the additional EAR by 1.2.

Lactating women. Using the average values for breast milk B_{12} concentration and secretion, and the 50% absorption rate for dietary B_{12} in healthy adults (0.45 μg/L×0.78 L/d÷0.5), the additional EAR for lactating women becomes 0.7 μg/d (rounded up from 0.702 μg/d). The additional RDA is calculated as 0.8 μg/d (rounded down from 0.84 μg/d) by multiplying the additional EAR by 1.2.

Tolerable upper intake level

Oral administration of substantial amounts (>500 μg) of B_{12} was shown to result in only about 1% absorption in the intestine (*50*). Even when a mega dose (2.5 mg) of B_{12} was administrated parenterally, no harmful effect of the excess intake was observed (*51*). Thus, in the present study, we did not determine the UL for B_{12}.

The DRIs for vitamin B_{12} are summarized in Table 5.

Folate

Background information

In its narrowest sense, folate is referred to as pteroylmonoglutamate. In broader terms, it includes coenzyme species in their reduced form, and also single-carbon compounds and their polyglutamate forms. The Standard Tables of Food Composition (*18*) list food folates, and also their DRIs, in their broader terms, as equivalents of pteroylmonoglutamate.

Cellular folate is mostly bound to enzyme proteins in their single-carbon polyglutamate coenzyme form. In comparison with monoglutamates, these polyglutamates readily lose their activities during heat processing (*52*). Most of the folate coenzymes are released through cooking and digestion by gastric acid. Following digestion by intestinal enzymes, they are converted to 5-methyltetrahydrofolate, and absorbed through the surface cells of the small intestine.

The relative bioavailability of food folate varies considerably (25–81%) (*53–55*). In a bioavailability study of wheat bread, the bioavailability was estimated to be 50% (*2*, *54*).

Determining DRIs

Evidence for determining the EAR

Red blood cell folate (≥300 nmol/L) and plasma total homocysteine (<14 μmol/L) concentrations were applied as biomarkers to reflect middle- to long-term folate nutritional status (*54*, *56–59*). The EAR for adults (18–49 y) was estimated as 200 μg/d. The RDA was calculated as 240 μg/d, by multiplying the EAR by 1.2. The EAR for children was calculated from the EAR for adults (200 μg/d), using the following equation for body surface area at each age: [(reference weight at each age/reference weight of 18- to 29-y-olds)$^{0.75}$×(1+growth factor)]. The values were rounded to the nearest 10 μg. For adults aged ≥50 y, folate bioavailability was estimated to be equivalent to that of younger adults (*60*),

and therefore the same values were applied.

Life stages

0–5 mo. The mean concentration of folate in breast milk is 54 μg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily folate intake of folate of about 40 μg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 65 μg/d.

Pregnant women. Macrocytic anemia in pregnancy recovers naturally after delivery (*61*), indicating a considerable increase in demand for folate during pregnancy. The addition of 100 μg/d of pteroylmonoglutamate to a diet adequate in food folate has been reported to result in adequate levels of red cell folate (*62, 63*). Thus, this value was set as the additional EAR (200 μg/d=100/bioavailability rate 0.5). The additional RDA was calculated by multiplying the additional EAR by 1.2.

Lactating women. The additional amount is calculated based on the assumption that the excreted amount in breast milk is supplemented. Thus, the additional EAR is calculated using the following formula: (breast milk consumption×breast milk content)÷folate bioavailability, which becomes (0.78 L×54 μg/L)÷0.5. The additional RDA is calculated by multiplying the additional EAR by 1.2.

Tolerable upper intake level

In the United States, there have been reports of adverse health effects resulting from elevated serum folate, caused by intake of folic acid-supplemented foods (*64*). These adverse effects may be induced by dihydropteroylmonoglutamate derived from pteroylmonoglutamate, which inhibits the activities of thymidylate synthase, phosphoribosylaminoimidazolecarboxamide transformylase, and 5,10-methylenetetrahydrogenase (*65–67*). Thus, excess pteroylmonoglutamate may inhibit the single-carbon transfer pathways of folate metabolism.

In order to develop the upper limit of folate intake, we considered the US and Canadian DRIs. It has been reported that women of reproductive age who were given 0.36–5 mg/d of folic acid during preconception to 3-mo gestation suffered no serious side-effects (*68–74*). Based on this finding, the adverse effect level was estimated to be 5 mg/d, equivalent to 80 μg/kg body weight/d. The UL was estimated as 27 μg/kg body weight/d, by dividing by an uncertainty factor of 3.

Additional concerns regarding women of reproductive age

Fetal neural tube defects (NTDs) are disorders of the closure of the neural tube (which occurs approximately 28 d after conception), and are clinically diagnosed as anencephaly, spina bifida, and myelomeningocele. Abundant evidence suggests that preconceptual intake of pteroylmonoglutamate decreases fetal NTD risk (*68–74*). Genetic polymorphisms of enzymes related to folate metabolism (e.g., methylene tetrahydrofolate reductase) may be associated with NTD risk (*75–80*). Other congenital disorders that can be avoided by administering folic acid are cleft lip/palate (*81, 82*) and congenital heart disease (*83*). Thus, adequate maternal folate status is essential for the prevention of NTDs. In order to estimate the minimum effective dose for risk reduction of NTDs, the lowest reported preconception dose (0.36 mg/d) was applied. This value was rounded up to 0.4 mg/d (400 μg/d), i.e., a dietary folate equivalent of 800 μg/d.

Association between cardiovascular disease and folate

Higher folate intake is associated with decreased risk of strokes or heart disease. Several randomized controlled trials have investigated the preventive effect of folic acid, but with inconsistent results (*84–88*). Thus, we did not determine any specific values for modifying DRI values.

The DRIs for folate are summarized in Table 6.

Pantothenic acid

Background information

Pantothenic acid exists mainly as the coenzyme A (CoA) derivatives, acetyl CoA and acyl CoA. Additionally, some pantothenic acid, such as phosphopantetheine, binds to enzyme proteins in living cells. Most CoA and phosphopantetheine derivatives separate from proteins during cooking and processing of food, and also under the acidic conditions of the stomach. Free CoA and phosphopantetheine derivatives are digested to release pantothenic acid, which is absorbed in the intestine. The relative availability of dietary pantothenic acid to free pantothenic acid is approximately 70% in a typical Japanese diet (*1, 2*).

Determining DRIs

Evidence for determining the AI

There is no evidence for setting an EAR for pantothenic acid, because deficiency of this vitamin has not been reported to occur in humans. Thus, we estimated the AIs based on food surveillance data. According to the National Health and Nutrition Survey 2005 and 2006, (*89, 90*), the median dietary pantothenic acid intake for adults and adolescents is 3–7 mg/d. In another dietary assessment study, the mean pantothenic acid intake of young Japanese females was reported to be 4.6 mg/d (*91*). There is no evidence that such intake levels cause pantothenic acid deficiency. Thus, the AIs were set at the median dietary pantothenic acid intake determined in the National Health and Nutrition Survey Japan 2005 and 2006, corresponding to a subject's sex and age. The AIs for elderly subjects were set at the same median value, because there are no data indicating specific consideration for pantothenic acid nutrition in the elderly.

Life stages

0–5 mo. The mean pantothenic acid concentration in breast milk is 5.0 mg/L (*6, 47*). The average intake of breast milk is 0.78 L/d (*7, 8*), representing a daily pantothenic acid intake of 3.9 mg/d. The AI was rounded up to 4 mg/d.

6–11 mo. To set the AI for infants aged 6–11 mo,

Table 7. DRIs for pantothenic acid (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	4	—	—	—	4	—
6–11 mo	—	—	5	—	—	—	5	—
1–2 y	—	—	3	—	—	—	3	—
3–5 y	—	—	4	—	—	—	4	—
6–7 y	—	—	5	—	—	—	5	—
8–9 y	—	—	6	—	—	—	5	—
10–11 y	—	—	7	—	—	—	6	—
12–14 y	—	—	7	—	—	—	6	—
15–17 y	—	—	7	—	—	—	5	—
18–29 y	—	—	5	—	—	—	5	—
30–49 y	—	—	5	—	—	—	5	—
50–69 y	—	—	6	—	—	—	5	—
≥70 y	—	—	6	—	—	—	5	—
Pregnant women (amount to be added)					—	—	+1	—
Lactating women (amount to be added)					—	—	+1	—

the extrapolated values are calculated from the AI for infants aged 0–5 mo, using the weight ratio method. The average of the obtained values for each sex is 5.0 mg/d. Thus, the AI for infants aged 6–11 mo was set at 5 mg/d.

Pregnant women. There is no evidence for determining the amount of additional pantothenic acid for pregnant women by factorial method. Moreover, there is no indication that the pantothenic acid requirement increases with the increase in energy requirement during pregnancy. Thus, the pantothenic acid intake for pregnant women is estimated using the median of dietary pantothenic acid intake determined in the National Health and Nutrition Survey Japan 2005 and 2006 (*89*, *90*). The additional AI for pregnant women was set at 1 mg/d.

Lactating women. The additional water-soluble vitamin intake for lactating women is determined based on the assumption that the excreted amount in breast milk is supplemented, with adjustment according to relative bioavailability. However, for pantothenic acid, the estimated AIs are in excess of the pantothenic acid requirement. Thus, the pantothenic acid intakes for lactating and non-lactating women are estimated using the median dietary pantothenic acid intake determined in the National Health and Nutrition Survey Japan 2005 and 2006 (*89*, *90*). The additional AI for lactating women was set at 1 mg/d.

Tolerable upper intake level

A pharmacological dose of pantothenic acid, administered over a 3-mo period in combination with nicotinamide, ascorbic acid, and pyridoxine, was reported to cause adverse effects such as nausea, poor appetite, and abdominal pain in children (*92*). However, there are no reports that a pharmacological dose of pantothenic acid causes any adverse health effects. Thus, in the present study, no UL for pantothenic acid was set.

The DRIs for pantothenic acid are summarized in Table 7.

Biotin

Background information

Biotin is involved in gluconeogenesis, amino acid catabolism, and fatty acid synthesis. Biotin deficiency is known as "egg white injury," and is characterized by symptoms such as dermatitis, alopecia, and nervous irritability in humans and experimental animals. Biotin is also essential for reproduction. Maternal biotin deficiency during gestation results in congenital malformations such as cleft palate, micromelia, and micrognathia in mammalian fetuses.

Determining DRIs

Evidence for determining the AI

Biotin in foods exists not only in a free form, but also in a protein-bound form. Biotin generally binds to the lysine in proteins, and is converted to the free form during cooking and processing. In the digestive tract, intestinal hydrolysis of protein-bound biotin yields biotinyl oligopeptide and biocytin, which are cleaved to free biotin by biotinidase prior to absorption. Free biotin is mainly absorbed from the small intestine. There are no reports concerning the bioavailability of biotin in foods. However, the proportions of free biotin and protein-bound biotin are likely to vary substantially, even within food groups. The bioavailability of biotin in a typical Japanese meal is reported to be about 80% (*1*).

There are no data on which to base an EAR for adults. It has been reported that the average daily biotin intake for Americans is 35.5 μg. A number of studies have

Table 8. DRIs for biotin (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	4	—	—	—	4	—
6–11 mo	—	—	10	—	—	—	10	—
1–2 y	—	—	20	—	—	—	20	—
3–5 y	—	—	25	—	—	—	25	—
6–7 y	—	—	30	—	—	—	30	—
8–9 y	—	—	35	—	—	—	35	—
10–11 y	—	—	40	—	—	—	40	—
12–14 y	—	—	50	—	—	—	50	—
15–17 y	—	—	50	—	—	—	50	—
18–29 y	—	—	50	—	—	—	50	—
30–49 y	—	—	50	—	—	—	50	—
50–69 y	—	—	50	—	—	—	50	—
≥70 y	—	—	50	—	—	—	50	—
Pregnant women (amount to be added)					—	—	+2	—
Lactating women (amount to be added)					—	—	+5	—

determined the average daily biotin intake for Japanese as 45.1 μg, 60.7 μg, and 70.1 μg (*93–97*). Thus, the AI were set based on the average dietary biotin intake for adult males and females, i.e., 50 μg/d.

The AI for children is calculated from the AI for adults (50 μg/d), using the following equation: AI for 18- to 29-y-olds×(reference body weight for children/reference body weight for 18- to 29-y-olds)$^{0.75}$×(1+growth factor).

Few studies have investigated biotin requirements in the elderly. There are no data indicating that the biotin requirements of healthy subjects aged ≥70 y differ from those of young adults. Thus, the AI for subjects aged ≥70 y is the same as that for adults aged 18–29 y.

There were insufficient data to enable differences in requirements to be discerned between males and females of all age groups.

Life stages

0–5 mo. The mean biotin content of breast milk is 5 μg/L (*5*, *6*, *47*, *98*). The average intake of milk is 0.78 L/d) (*7*, *8*), representing a daily biotin intake of ~3.9 μg/d. The AI was rounded up to 4 μg/d.

6–11 mo. The AI for infants aged 6–11 mo is calculated from the average of values extrapolated from the AI for infants aged 0–5 mo and the AI for adults aged 18–29 y. This gives a value of 10.4 μg/d (14.9 μg/d for males and 16.6 μg/d for females). The AI was rounded down to 10 μg/d.

Pregnant women. Pregnant women have been demonstrated to exhibit reduced biotin concentration in the serum, and also reduced biotin excretion in the urine. By contrast, urinary excretion of organic acids such as 3-hydroxyisovaleric acid increases during late pregnancy (*99*). These findings indicate that pregnancy increases biotin requirements. However, there are no data on the additional amount required by pregnant women. Thus, the additional AI for pregnant women is calculated using the following formula: AI of biotin for infants aged 0–5 mo×average additional amount of energy for pregnant women/average additional amount of energy for male and female infants aged 0–5 mo. The additional AI for pregnant women was set at 2 μg/d.

Lactating women. The additional amount of biotin required during lactation should be calculated from the difference in biotin requirements for lactating and nonlactating women of a similar age. However, no such data are available. Thus, the increased requirement during lactation is based on the estimated biotin concentration in breast milk and the average milk secretion (0.78 L/d), adjusted by the bioavailability (*1*) (5 μg/L×0.78 L/d/0.8=4.875 μg/d). The additional AI for lactating women was set at 5 μg/d.

Tolerable upper intake level

There was insufficient evidence for determining the UL for healthy individuals. No adverse effects are associated with excess biotin intake, even in patients with biotin-responsive inborn errors of metabolism (*100*).

The DRIs for biotin are summarized in Table 8.

Vitamin C

Background information

Vitamin C refers to ascorbic acid and its oxidized form, dehydroascorbic acid, which exerts a biological effect through immediate reduction into ascorbic acid in the body (*101*). Severe vitamin C deficiency results in scurvy, which may be preventable by an ascorbic acid intake of 6–12 mg/d (*102*). Intake of a higher dose of vitamin C exerts an antioxidant effect, thereby helping

Table 9. DRIs for vitamin C (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	40	—	—	—	40	—
6–11 mo	—	—	40	—	—	—	40	—
1–2 y	35	40	—	—	35	40	—	—
3–5 y	40	45	—	—	40	45	—	—
6–7 y	45	55	—	—	45	55	—	—
8–9 y	55	65	—	—	55	65	—	—
10–11 y	65	80	—	—	65	80	—	—
12–14 y	85	100	—	—	85	100	—	—
15–17 y	85	100	—	—	85	100	—	—
18–29 y	85	100	—	—	85	100	—	—
30–49 y	85	100	—	—	85	100	—	—
50–69 y	85	100	—	—	85	100	—	—
≥70 y	85	100	—	—	85	100	—	—
Pregnant women (amount to be added)					+10	+10	—	—
Lactating women (amount to be added)					+40	+50	—	—

to prevent cardiovascular disease (*103*).

Ascorbic acid is readily absorbed by the intestine at a dose of <200 mg/d. Absorption is reduced at higher doses, and is <50% at a dose of >1 g/d (*104*). Vitamin C is reused within the body and excreted from the kidneys as unmetabolized ascorbic acid; the plasma is saturated at a dose of approximately 400 mg/d (*105*, *106*).

Determining DRIs

Evidence for determining the EAR

Optimal antioxidant activity in plasma, and prevention of cardiovascular disease, is achieved at a plasma ascorbic acid concentration of 50 μmol/L (*103*). This can be maintained by an ascorbic acid intake of approximately 85 mg/d (*107*), which is recognized as the EAR. The RDA is calculated by multiplying the EAR by 1.2, to give 100 mg/d. In a vitamin C depletion–repletion study, excretion of unmetabolized ascorbic acid into the urine was not detectable at an intake of 50–60 mg/d, but was detectable at an intake of 100 mg/d, where leukocyte vitamin C as an indicative of body store was saturated (*105*, *106*). This finding supports an RDA value of 100 mg/d. Levine et al. (*106*) did not consider differences in requirement according to sex.

Life stages

0–5 mo. The mean concentration of vitamin C in breast milk is 50 mg/L (*4–6*). The average intake of breast milk is 0.78 L/d (*7*, *8*), representing a daily vitamin C intake of about 40 mg/d. This value was set as the AI.

6–11 mo. To set the AI for infants aged 6–11 mo, the extrapolated values are calculated from the AI for infants aged 0–5 mo and the EAR for adults, using the weight ratio method described for vitamin B_1. The means of these extrapolated values are determined for each sex. Thus, the AI for infants aged 6–11 mo becomes 40 mg/d.

Pregnant women. The additional amounts are calculated based on the intake of vitamin C required to prevent infant scurvy. Thus, the additional EAR becomes 10 mg/d. The additional RDA is set by assuming a coefficient of variation of 10%.

Lactating women. The additional amounts are calculated based on the assumption that the excreted amount in breast milk is supplemented. The additional RDA is set by assuming a coefficient of variation of 10%.

Elderly. Vitamin C requirement appears to be higher in elderly subjects (aged 60–96 y old) than in younger subjects (aged 15–65 y old) (*107*). However, it is difficult to determine the required intake for the elderly subjects, because of insufficient data.

Tolerable upper intake level

Vitamin C is safe for healthy subjects, because excess intake results in a lower absorption rate from the intestine, and enhanced excretion in the urine following absorption (*105*, *106*, *108*). However, for patients with renal dysfunction, intake of several grams of vitamin C may increase the risk of kidney stones (*109*, *110*). Acute gastrointestinal intolerance was observed following excess intake; for example, intake of 3–4 g/d induced diarrhea (*111*). There are insufficient data with which to determine the UL. Absorption of vitamin C is saturated at high doses. By contrast, intake of ≥1 g/d from supplements is not advised (*102*, *105*, *106*).

Special consideration for smokers

There is evidence that smokers require more vita-

min C than do nonsmokers (*107*, *112*). This is also the case for passive smokers (*113*, *114*). Thus, smokers would require more vitamin C than nonsmokers, while they should recognize that smoking cessation is a basic countermeasure.

The DRIs for vitamin C are summarized in Table 9.

REFERENCES

1) Fukuwatari T, Shibata K. 2008. Relative availability of B-group vitamins in a test diet to free vitamins. *Nippon Kaseigaku Zassi (J Home Economic Jpn)* **59**: 403–410 (in Japanese).
2) Fukuwatari T, Shibata T. 2009. Relative availability of water-soluble vitamins in a white bread diet to free vitamin. *Nippon Kaseigaku Zassi (J Home Economic Jpn)* **60**: 57–63 (in Japanese).
3) FAO/WHO. 1967. WHO Technical Report Series No. 362. FAO Nutrition Meeting Report Series No. 41. Requirements of vitamin A, thiamine, riboflavin and niacin. Reports of a Joint FAO/WHO Expert Group. Rome, 6–17 September 1965. p 30–38. Geneva.
4) Idota T, Sugawara M, Yakabe T, Sato N, Maeda T. 1996. The latest survey for the composition of human milk obtained from Japanese mothers. Part X. Content of water-soluble vitamins. *Nippon Shôni Eiyô Shôkaki-byô Gakkai Zassi (Jpn J Pediatric Gastroenterol Nutr)* **10**: 11–20 (in Japanese).
5) Sakurai T, Furukawa M, Asoh M, Kanno T, Kojima T, Yonekubo A. 2005. Fat-soluble and water-soluble vitamin contents of breast milk from Japanese women. *J Nutr Sci Vitaminol* **51**: 239–247.
6) Shibata K, Endo M, Yamauchi M, Hirose J, Fukuwatari T. 2009. Distribution of the water-soluble vitamin content of Japanese breast milk. *Nippon Eiyô Shokuryô Gakkaishi (J Jpn Soc Nutr Food Sci)* **62**: 179–184 (in Japanese).
7) Suzuki K, Sasaki S, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Eiyô-gaku Zasshi (Jpn J Nutr Diet)* **62**: 369–372 (in Japanese).
8) Hirose J, Endo M, Nagao S, Mizushima K, Narita H, Shibata K. 2008. Amount of breast milk sucked by Japanese breast feeding infants. *Nippon Bonyu-Hoiku-gaku Zassi (J Jpn Soc Breastfeeding Res)* **2**: 23–28 (in Japanese).
9) Iber FL, Blass JP, Brin M, Leevy CM. 1982. Thiamin in the elderly—relation to alcoholism and to neurological degenerative disease. *Am J Clin Nutr* **36**: 1067–1082.
10) Mills CA. 1941. Thiamine overdosage and toxicity. *J Am Med Assoc* **116**: 2101.
11) Merrill AH Jr, Lambeth JD, Edmondson DE, McCormick DB. 1981. Formation and mode of action of flavoproteins. *Annu Rev Nutr* **1**: 281–317.
12) Innis WS, McCormick DB, Merrill AH, Jr. 1985. Variations in riboflavin binding by human plasma: identification of immunoglobulins as the major proteins responsible. *Biochem Med* **34**: 151–165.
13) Horwitt MK, Harvey CC, Hills OW, Liebert E. 1950. Correlation of urinary excretion of riboflavin with dietary intake and symptoms of ariboflavinosis. *J Nutr* **41**: 247–264.
14) Davis M, Oldham H, Roberts L. 1946. Riboflavin excretions of young women on diets containing varying levels of the B vitamins. *J Nutr* **32**: 143–161.
15) Schoenen JLM, Bastings E. 1994. High-dose riboflavin as a prophylactic treatment of migraine: results of an open pilot study. *Cephalalgia* **14**: 328–329.
16) Zempleni J, Galloway JR, McCormick DB. 1996. Pharmacokinetics of orally and intravenously administered riboflavin in healthy humans. *Am J Clin Nutr* **63**: 54–66.
17) Stripp B. 1965. Intestinal absorption of riboflavin by man. *Acta Pharmacol Toxicol (Copenh)* **22**: 353–362.
18) Ministry of Education, Culture, Sports, Science and Technology, Japan. 2005. Standard Tables of Food Composition in Japan, Fifth revised and enlargement ed, 2004. Tokyo (in Japanese).
19) Carter EG, Carpenter KJ. 1982. The bioavailability for humans of bound niacin from wheat bran. *Am J Clin Nutr* **36**: 855–861.
20) Horwitt MK, Harper AE, Henderson LM. 1981. Niacin-tryptophan relationships for evaluating niacin equivalents. *Am J Clin Nutr* **34**: 423–427.
21) Fukuwatari T, Ohta M, Kimtjra N, Sasaki R, Shibata K. 2004. Conversion ratio of tryptophan to niacin in Japanese women fed a purified diet conforming to the Japanese Dietary Reference Intakes. *J Nutr Sci Vitaminol* **50**: 385–391.
22) Goldsmith G, Sarett H, Register U, Gibbens J. 1952. Studies of niacin requirement in man. I. Experimental pellagra in subjects on corn diets low in niacin and tryptophan. *J Clin Invest* **31**: 533–542.
23) Goldsmith G, Rosenthal H, Gibbens J, Unglaub W. 1955. Studies of niacin requirement in man. II. Requirement on wheat and corn diets low in tryptophan. *J Nutr* **56**: 371–386.
24) Horwitt M, Harvey C, Rothwell W, Cutler J, Haffron D. 1956. Tryptophan-niacin relationships in man: Studies with diets deficient in riboflavin and niacin, together with observations on the excretion of nitrogen and niacin metabolites. *J Nutr* **60**: 1–43.
25) Jacob RA, Swendseid ME, McKee RW, Fu CS, Clemens RA. 1989. Biochemical markers for assessment of niacin status in young men: urinary and blood levels of niacin metabolites. *J Nutr* **119**: 591–598.
26) Fukuwatari T, Murakami M, Ohta M, Kimura N, Jin-No Y, Sasaki R, Shibata K. 2004. Changes in the urinary excretion of the metabolites of the tryptophan-niacin pathway during pregnancy in Japanese women and rats. *J Nutr Sci Vitaminol* **50**: 392–398.
27) Winter SL, Boyer JL. 1973. Hepatic toxicity from large doses of vitamin B3 (nicotinamide). *N Engl J Med* **289**: 1180–1182.
28) Rader JI, Calvert RJ, Hathcock JN. 1992. Hepatic toxicity of unmodified and time-release preparations of niacin. *Am J Med* **92**: 77–81.
29) McKenney JM, Proctor JD, Harris S, Chinchili VM. 1994. A comparison of the efficacy and toxic effects of sustained- vs immediate-release niacin in hypercholesterolemic patients. *JAMA* **271**: 672–677.
30) Pozzilli P, Visalli N, Signore A, Baroni MG, Buzzetti R, Cavallo MG, Boccuni ML, Fava D, Gragnoli C, Andreani D, Lucentini L, Matteoli MC, Crinò A, Cicconetti CA, Teodonio C, Paci E, Amoretti R, Pisano L, Pennafina MG, Santopadre G, Marozzi G, Multari G, Suppa MA, Campea L, De Mattia GC, Cassone Faldetta M, Marietti G, Perrone F, Greco AV, Ghirlanda, G. 1995. Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). *Diabetologia* **38**: 848–852.
31) Gregory JF 3rd. 1997. Bioavailability of vitamin B-6. *Eur J Clin Nutr* **51** (Suppl 1): S43–48.

32) Tarr JB, Tamura T, Stokstad EL. 1981. Availability of vitamin B_6 and pantothenate in an average American diet in man. *Am J Clin Nutr* **34**: 1328–1337.

33) Lui A, Lumeng L, Aronoff GR, Li TK. 1985. Relationship between body store of vitamin B_6 and plasma pyridoxal-P clearance: metabolic balance studies in humans. *J Lab Clin Med* **106**: 491–497.

34) Kretsch MJ, Sauberlich HE, Newbrun E. 1991. Electroencephalographic changes and periodontal status during short-term vitamin B-6 depletion of young, nonpregnant women. *Am J Clin Nutr* **53**: 1266–1274.

35) Leklem JE. 1990. Vitamin B-6: a status report. *J Nutr* **120** (Suppl 11): 1503–1507.

36) Food and Nutritional Board, Institute of Medicine. 1998. Vitamin B6. *In*: Dietary Reference Intake, p 150–195. National Academy Press, Washington DC.

37) Isa Y, Kaitou A, Hayakawa T, Sasaki S, Shinzawa K, Suzuki K, Totani M, Tsuge H. 2004. The vitamin B_6 content in milk of Japanese women. *Vitamins* **78**: 437–440 (in Japanese).

38) Reinken L, Dapunt O. 1978. Vitamin B_6 nutriture during pregnancy. *Int J Vitam Nutr Res* **48**: 341–347.

39) Schaumburg H, Kaplan J, Windebank A, Vick N, Rasmus S, Pleasure D, Brown MJ. 1983. Sensory neuropathy from pyridoxine abuse. A new megavitamin syndrome. *N Engl J Med* **309**: 445–448.

40) Del TA, Bernstein A, Chinn K. 1985. Carpal tunnel syndrome and vitamin B_6 therapy. *In*: Vitamin B_6: Its Role in Health and Disease. Current Topics in Nutrition and Disease (Reynolds RD, Leklem JE, eds). Alan R Liss, New York.

41) Watanabe F. 2007. Vitamin B_{12} sources and bioavailability. *Exp Biol Med (Maywood)* **232**: 1266–1274.

42) Food and Nutrition Board, Institute of Medicine. 1998. The B vitamins and choline: overview and methods. *In*: Dietary Reference Intakes: For Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline, p 306–356. National Academy Press, Washington DC.

43) Scott JM. 1997. Bioavailability of vitamin B_{12}. *Eur J Clin Nutr* **51**: S49–53.

44) el Kholty S, Gueant JL, Bressler L, Djalali M, Boissel P, Gerard P, Nicolas JP. 1991. Portal and biliary phases of enterohepatic circulation of corrinoids in humans. *Gastroenterology* **101**: 1399–1408.

45) Darby WJ, Bridgforth EB, Le Brocquy J, Clark SL Jr, De Oliveira JD, Kevany J, McGanity WJ, Perez C. 1958. Vitamin B12 requirement of adult man. *Am J Med* **25**: 726–732.

46) Bastrup-Madsen P, Helleberg-Rasmussen I, Norregaard S, Halver B, Hansen T. 1983. Long term therapy of pernicious anaemia with the depot cobalamin preparation betolvex. *Scand J Haematol* **31**: 57–62.

47) Watanabe T, Taniguchi A, Shoji K, Inakuma T, Fukui T, Watanabe F, Miyamoto E, Hashizume N, Sasaki A, Totani M, Nishimuta M, Shibata K. 2005. The concentrations of water-soluble vitamins in breast milk of Japanese women. *Vitamins* **79**: 573–581 (in Japanese).

48) Loria A, Vaz-Pinto A, Arroyo P, Ramirez-Mateos C, Sanchez-Medal L. 1977. Nutritional anemia. VI. Fetal hepatic storage of metabolites in the second half of pregnancy. *J Pediatr* **91**: 569–573.

49) Vaz Pinto A, Torras V, Sandoval JF, Dillman E, Mateos CR, Cordova MS. 1975. Folic acid and vitamin B_{12} determination in fetal liver. *Am J Clin Nutr* **28**: 1085–1086.

50) Berlin H, Berlin R, Brante G. 1968. Oral treatment of pernicious anemia with high doses of vitamin B_{12} without intrinsic factor. *Acta Med Scand* **184**: 247–258.

51) Mangiarotti G, Canavese C, Salomone M, Thea A, Pacitti A, Gaido M, Calitri V, Pelizza D, Canavero W, Vercellone A. 1986. Hypervitaminosis B_{12} in maintenance hemodialysis patients receiving massive supplementation of vitamin B_{12}. *Int J Artif Organs* **9**: 417–420.

52) Gregory JF 3rd. 1989. Chemical and nutritional aspects of folate research: analytical procedures, methods of folate synthesis, stability, and bioavailability of dietary folates. *Adv Food Nutr Res* **33**: 1–101.

53) Konings EJ, Troost FJ, Castenmiller JJ, Roomans HH, Van Den Brandt PA, Saris WH. 2002. Intestinal absorption of different types of folate in healthy subjects with an ileostomy. *Br J Nutr* **88**: 235–242.

54) Sauberlich HE, Kretsch MJ, Skala JH, Johnson HL, Taylor PC. 1987. Folate requirement and metabolism in nonpregnant women. *Am J Clin Nutr* **46**: 1016–1028.

55) Tamura T, Stokstad EL. 1973. The availability of food folate in man. *Br J Haematol* **25**: 513–532.

56) O'Keefe CA, Bailey LB, Thomas EA, Hofler SA, Davis BA, Cerda JJ, Gregory JF 3rd. 1995. Controlled dietary folate affects folate status in nonpregnant women. *J Nutr* **125**: 2717–2725.

57) Milne DB, Johnson LK, Mahalko JR, Sandstead HH. 1983. Folate status of adult males living in a metabolic unit: possible relationships with iron nutriture. *Am J Clin Nutr* **37**: 768–773.

58) Cuskelly GJ, McNulty H, Scott JM. 1996. Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. *Lancet* **347**: 657–659.

59) Brouwer IA, van Dusseldorp M, West CE, Meyboom S, Thomas CM, Duran M, van het Hof KH, Eskes TK, Hautvast JG, Steegers-Theunissen RP. 1999. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. *J Nutr* **129**: 1135–1139.

60) Wolfe JM, Bailey LB, Herrlinger-Garcia K, Theriaque DW, Gregory JF 3rd, Kauwell GP. 2003. Folate catabolite excretion is responsive to changes in dietary folate intake in elderly women. *Am J Clin Nutr* **77**: 919–923.

61) McPartlin J, Halligan A, Scott JM, Darling M, Weir DG. 1993. Accelerated folate breakdown in pregnancy. *Lancet* **341**: 148–149.

62) Chanarin I, Rothman D, Ward A, Perry J. 1968. Folate status and requirement in pregnancy. *Br Med J* **2**: 390–394.

63) Daly S, Mills JL, Molloy AM, Conley M, Lee YJ, Kirke PN, Weir DG, Scott JM. 1997. Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet* **350**: 1666–1669.

64) Smith AD. 2007. Folic acid fortification: the good, the bad, and the puzzle of vitamin B-12. *Am J Clin Nutr* **85**: 3–5.

65) Allegra CJ, Drake JC, Jolivet J, Chabner BA. 1985. Inhibition of phosphoribosylaminoimidazolecarboxamide transformylase by methotrexate and dihydrofolic acid polyglutamates. *Proc Natl Acad Sci USA* **82**: 4881–4885.

66) Matthews RG, Baugh CM. 1980. Interactions of pig liver methylenetetrahydrofolate reductase with methylenetetrahydropteroylpolyglutamate substrates and with dihydropteroylpolyglutamate inhibitors. *Biochem-*

istry **19**: 2040–2045.
67) Dolnick BJ, Cheng YC. 1978. Human thymidylate synthetase. II. Derivatives of pteroylmono- and -polyglutamates as substrates and inhibitors. *J Biol Chem* **253**: 3563–3567.
68) Berry RJ, Li Z, Erickson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong LY, Gindler J, Hong SX, Correa A. 1999. Prevention of neural-tube defects with folic acid in China. China-U.S. Collaborative Project for Neural Tube Defect Prevention. *N Engl J Med* **341**: 1485–1490.
69) Mulinare J, Cordero JF, Erickson JD, Berry RJ. 1988. Periconceptional use of multivitamins and the occurrence of neural tube defects. *JAMA* **260**: 3141–3145.
70) Milunsky A, Jick H, Jick SS, Bruell CL, MacLaughlin DS, Rothman KJ, Willett W. 1989. Multivitamin/folic acid supplementation in early pregnancy reduces the prevalence of neural tube defects. *JAMA* **262**: 2847–2852.
71) Laurence KM, James N, Miller MH, Tennant GB, Campbell H. 1981. Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects. *Br Med J (Clin Res Ed)* **282**: 1509–1511.
72) Smithells RW, Nevin NC, Seller MJ, Sheppard S, Harris R, Read AP, Fielding DW, Walker S, Schorah CJ, Wild J. 1983. Further experience of vitamin supplementation for prevention of neural tube defect recurrences. *Lancet* **1**: 1027–1031.
73) Vergel RG, Sanchez LR, Heredero BL, Rodriguez PL, Martinez AJ. 1990. Primary prevention of neural tube defects with folic acid supplementation: Cuban experience. *Prenat Diagn* **10**: 149–152.
74) Czeizel AE, Dudas I. 1992. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* **327**: 1832–1835.
75) Akar N, Akar E, Deda G, Arsan S. 2000. Spina bifida and common mutations at the homocysteine metabolism pathway. *Clin Genet* **57**: 230–231.
76) Botto LD, Yang Q. 2000. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. *Am J Epidemiol* **151**: 862–877.
77) Cunha AL, Hirata MH, Kim CA, Guerra-Shinohara EM, Nonoyama K, Hirata RD. 2002. Metabolic effects of C677T and A1298C mutations at the MTHFR gene in Brazilian children with neural tube defects. *Clin Chim Acta* **318**: 139–143.
78) de Franchis R, Buoninconti A, Mandato C, Pepe A, Sperandeo MP, Del Gado R, Capra V, Salvaggio E, Andria G, Mastroiacovo P. 1998. The C677T mutation of the 5,10-methylenetetrahydrofolate reductase gene is a moderate risk factor for spina bifida in Italy. *J Med Genet* **35**: 1009–1013.
79) De Marco P, Calevo MG, Moroni A, Arata L, Merello E, Cama A, Finnell RH, Andreussi L, Capra V. 2001. Polymorphisms in genes involved in folate metabolism as risk factors for NTDs. *Eur J Pediatr Surg* **11**(Suppl 1): S14–17.
80) Martinez de Villarreal LE, Delgado-Enciso I, Valdez-Leal R, Ortiz-Lopez R, Rojas-Martinez A, Limon-Benavides C, Sanchez-Pena MA, Ancer-Rodriguez J, Barrera-Saldana HA, Villarreal-Perez JZ. 2001. Folate levels and N(5),N(10)-methylenetetrahydrofolate reductase genotype (MTHFR) in mothers of offspring with neural tube defects: a case-control study. *Arch Med Res* **32**: 277–282.
81) van Rooij IA, Ocke MC, Straatman H, Zielhuis GA, Merkus HM, Steegers-Theunissen RP. 2004. Periconceptional folate intake by supplement and food reduces the risk of nonsyndromic cleft lip with or without cleft palate. *Prev Med* **39**: 689–694.
82) Shaw GM, Rozen R, Finnell RH, Todoroff K, Lammer EJ. 1998. Infant C677T mutation in MTHFR, maternal periconceptional vitamin use, and cleft lip. *Am J Med Genet* **80**: 196–198.
83) Junker R, Kotthoff S, Vielhaber H, Halimeh S, Kosch A, Koch HG, Kassenbohmer R, Heineking B, Nowak-Gottl U. 2001. Infant methylenetetrahydrofolate reductase 677TT genotype is a risk factor for congenital heart disease. *Cardiovasc Res* **51**: 251–254.
84) Voutilainen S, Rissanen TH, Virtanen J, Lakka TA, Salonen JT. 2001. Low dietary folate intake is associated with an excess incidence of acute coronary events: The Kuopio Ischemic Heart Disease Risk Factor Study. *Circulation* **103**: 2674–2680.
85) Ishihara J, Iso H, Inoue M, Iwasaki M, Okada K, Kita Y, Kokubo Y, Okayama A, Tsugane S. 2008. Intake of folate, vitamin B_6 and vitamin B_{12} and the risk of CHD: the Japan Public Health Center-Based Prospective Study Cohort I. *J Am Coll Nutr* **27**: 127–136.
86) Weng LC, Yeh WT, Bai CH, Chen HJ, Chuang SY, Chang HY, Lin BF, Chen KJ, Pan WH. 2008. Is ischemic stroke risk related to folate status or other nutrients correlated with folate intake? *Stroke* **39**: 3152–3158.
87) Wang X, Qin X, Demirtas H, Li J, Mao G, Huo Y, Sun N, Liu L, Xu X. 2007. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* **369**: 1876–1882.
88) Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH, Stampfer M. 2004. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the Vitamin Intervention for Stroke Prevention (VISP) randomized controlled trial. *JAMA* **291**: 565–575.
89) Ministry of Health, Labour and Welfare, Japan. 2007. National Health and Nutrition Survey Japan (2005). Tokyo (in Japanese).
90) Ministry of Health, Labour and Welfare, Japan. 2008. National Health and Nutrition Survey Japan (2006). Tokyo (in Japanese).
91) Kimura N, Fukuwatari T, Sasaki R, Hayakawa F, Shibata K. 2003. Vitamin intake in Japanese women college students. *J Nutr Sci Vitaminol* **49**: 149–155.
92) Haslam RH, Dalby JT, Rademaker AW. 1984. Effects of megavitamin therapy on children with attention deficit disorders. *Pediatrics* **74**: 103–111.
93) Iyenga GV, Wolfe WR, Tanner JT, Morris ER. 2000. Content of minor and trace elements, and organic nutrients in representative mixed total diet composites from the USA. *Sci Total Environ* **256**: 215–226.
94) Saitoh Y, Ushio F. 2004. Estimate of the daily dietary intake of biotin, vitamin B6 and niacin from the 1999 Tokyo total diet study. *Eiyô-gaku Zasshi (Jpn J Nutr Diet)* **62**: 165–169 (in Japanese).
95) Watanabe T, Taniguchi A. 2006. Study on the estimate of dietary intake of biotin by total diet study. *Nippon Rinsho Eiyô-Gakkai Zasshi (J Jpn Soc Clin Nutr)* **27**: 304–312 (in Japanese).
96) Murakami T, Yamano T, Nakama A, Mori Y. 2008. Estimation of dietary intake of biotin and its measurement

uncertainty using total diet samples in Osaka, Japan. *J AOAC Int* **91**: 1402–1408.

97) Watanabe T, Taniguchi A. 2009. Estimation of dietary intake of biotin from the Japanese diet. *Vitamins* **83**: 461–468 (in Japanese).

98) Hirano M, Honma K, Daimatsu T, Hayakawa K, Oizumi J, Zaima K, Kanke Y. 1992. Longitudinal variations of biotin content in human milk. *Int J Vitam Nutr Res* **62**: 281–282.

99) Mock DM, Quirk JG, Mock NI. 2002. Marginal biotin deficiency during normal pregnancy. *Am J Clin Nutr* **75**: 295–299.

100) Roth KS, Yang W, Foremann JW, Rothman R, Segal S. 1980. Holocarboxylase synthetase deficiency: a biotin-responsive organic acidemia. *J Pediatr* **96**: 845–849.

101) Tsujimura M, Higasa S, Aono K, Seki T. 2006. Vitamin C activity of L-dehydroascorbic acid in human: time-dependent vitamin C urinary excretion after the oral load. *Vitamins* **80**: 281–285 (in Japanese).

102) Hodges RE, Hood J, Canham JE, Sauberlich HE, Baker EM. 1971. Clinical manifestations of ascorbic acid deficiency in man. *Am J Clin Nutr* **24**: 432–443.

103) Gey K. 1998. Vitamins E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *Biofactors* **7**: 113–174.

104) Levine M, Rumsey SC, Daruwala R, Park JB, Wang Y. 1999. Criteria and recommendations for vitamin C intake. *JAMA* **281**: 1415–1423.

105) Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, Park JB, Lazarev A, Graumlich JF, King J, Cantilena LR. 1996. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA* **93**: 3704–3709.

106) Levine M, Wang Y, Paaatty SJ, Morrow J. 2001. A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci USA* **98**: 9842–9846.

107) Brubacher D, Moser U, Jordan P. 2000. Vitamin C concentrations in plasma as a function of intake: a meta-analysis. *Int J Vit Nutr Res* **70**: 226–237.

108) Blanchard J, Tozer TN, Rowland M. 1997. Pharmacokinetic perspectives on megadoses of ascorbic acid. *Am J Clin Nutr* **66**: 1165–1171.

109) Traxer O, Huet B, Poindexter J, Pak CY, Pearle MS. 2003. Effect of ascorbic acid consumption on urinary stone risk factors. *J Urol* **170**: 397–401.

110) Massey LK, Liebman M, Kynast-Gales SA. 2005. Ascorbate increases human oxaluria and kidney stone risk. *J Nutr* **135**: 1673–1677.

111) Cameron E, Campbell A. 1974. The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. *Chem Biol Interact* **9**: 285–315.

112) Kallner AB, Hartmann D, Hornig DH. 1981. On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. *Am J Clin Nutr* **34**: 1347–1355.

113) Tribble DL, Giuliano LJ, Fortmann SP. 1993. Reduced plasma ascorbic acid concentrations in nonsmokers regularly exposed to environmental tobacco smoke. *Am J Clin Nutr* **58**: 886–890.

114) Preston AM, Rodriguez C, Rivera CE, Sahai H. 2003. Influence of environmental tobacco smoke on vitamin C status in children. *Am J Clin Nutr* **77**: 167–172.

J Nutr Sci Vitaminol, **59**, S83–S90, 2013

Dietary Reference Intakes for Japanese 2010: Macrominerals

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(Received October 26, 2012)

Summary Dietary Reference Intakes of five macrominerals (sodium, potassium, calcium, magnesium and phosphate) were determined for Japanese. The estimated average requirement (EAR) and the recommended dietary allowance (RDA) for adults ages 18 y and older were determined in calcium and magnesium. In sodium, the EAR was determined. The RDA was not determined because the values were much lower than normal intake levels. Furthermore the dietary goal for preventing lifestyle-related diseases (DG) was determined based on preventing hypertension. In potassium, the value that is considered appropriate to maintain in vivo potassium balance was used as the adequate intake, the DG was established from a standpoint of prevention of hypertension. In calcium, the EAR and RDA were determined by the factorial method. In phosphate, the AI was determined based on the intake level of the National Health and Nutrition Surveys. The tolerable upper intake level (UL) for adults was determined in calcium, phosphate and magnesium, but the UL of magnesium was applied from a source other than ordinary food.

Key Words sodium, potassium, calcium, magnesium, phosphate

Sodium

Background information

Sodium, the main cation contained in extracellular fluid, is necessary to maintain extracellular fluid volume, plasma osmolality, and acid-base balance. Sodium is mostly consumed in the form of sodium chloride (NaCl), commonly referred to as salt. The largest portion of ingested sodium is absorbed from the small intestine and the majority of absorbed sodium is excreted in the urine via the kidneys. If sodium intake increases, the amount of urinary excretion will increase, and if intake decreases, the amount of urinary excretion will decrease.

A NaCl equivalent is calculated as follows from the molecular weight of salt and sodium:

NaCl equivalent=sodium (g)×58.5/23
=sodium (g)×2.54.

If kidney functioning is normal, sodium balance will be maintained by the re-absorption of sodium in the kidneys, thereby preventing sodium deficiency. Endogenous loss of sodium is calculated as the sum of the sodium excreted in the urine, feces, dermal tissue, and other tissues when sodium intake is 0 mg/d.

Determining the Dietary Reference Intakes (DRIs)

Based on the belief that the amount of endogenous sodium loss is equal to the amount of sodium required, the estimated average requirement (EAR) was established with the goal of compensating for endogenous loss. However, the values are less than 1% of the value of intake distribution, determined by the National Health and Nutrition Survey (*1*, *2*). Therefore, the meaning in practical use does not presume to provide the average required quantity. Since it has no meaning when utilizing the amount recommended, it was not calculated.

For infants aged 0 to 5 mo, the adequate intake (AI) was calculated using the average concentration of sodium in breast milk (135 mg/L) (*3*, *4*) and average volume of breast milk secreted per day (0.78 L/d) (*5*, *6*). For infants aged 6 to 11 mo, the AI was calculated using the average consumption of sodium from breast milk (*3*, *4*, *7*, *8*) and complementary food (*9*). The dietary goal for preventing lifestyle-related diseases (DG) for sodium was established by epidemiology research that considered the relationship between high blood pressure (*10*, *11*) and cancer (*12*) and sodium ingestion, changes in sodium intake in the Japanese (*1*, *2*), and the desirable level of sodium established in many Western countries. In adults, the target to attain over 5 y was calculated to be less than 9 mg/d for men and less than 7.5 mg/d for women. In children aged 1 to 11 y, the value was calculated by extrapolation from the value for adults aged 18 to 29 y by the 0.75th power of the weight ratio. The

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Table 1. DRIs for sodium (mg/d, the value in parentheses is equivalent to table salt [g/d]).

Sex	Males			Females		
Age	EAR	AI	DG	EAR	AI	DG
0–5 mo	—	100 (0.3)	—	—	100 (0.3)	—
6–11 mo	—	600 (1.5)	—	—	600 (1.5)	—
1–2 y	—	—	(<4.0)	—	—	(<4.0)
3–5 y	—	—	(<5.0)	—	—	(<5.0)
6–7 y	—	—	(<6.0)	—	—	(<6.0)
8–9 y	—	—	(<7.0)	—	—	(<7.0)
10–11 y	—	—	(<8.0)	—	—	(<7.5)
12–14 y	—	—	(<9.0)	—	—	(<7.5)
15–17 y	—	—	(<9.0)	—	—	(<7.5)
18–29 y	600 (1.5)	—	(<9.0)	600 (1.5)	—	(<7.5)
30–49 y	600 (1.5)	—	(<9.0)	600 (1.5)	—	(<7.5)
50–69 y	600 (1.5)	—	(<9.0)	600 (1.5)	—	(<7.5)
≥70 y	600 (1.5)	—	(<9.0)	600 (1.5)	—	(<7.5)
Pregnant women (amount to be added)				—	—	—
Lactating women (amount to be added)				—	—	—

DRIs, Dietary Reference Intakes; EAR, estimated average requirement; AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases.

value for adults aged 18 to 29 y was applied to adolescents aged 12 to 17 y.

DRIs for sodium are summarized in Table 1.

Potassium

Background information

As the main cation contained in intracellular fluid, potassium is an important factor in determining the osmotic pressure of aqueous humors and maintaining acid-base balance, and participates in nerve transmission, muscle contraction, and vascular tone. In healthy individuals, potassium deficiency is rarely observed, typically afflicting only those experiencing diarrhea or heavy perspiration or taking diuretics. Average sodium intake in Japan is high compared with that of many countries (*1*, *2*). As the urinary excretion of sodium is related to potassium intake, it is believed that increasing ingestion of potassium is important for the Japanese.

Determining DRIs

Based on the National Health and Nutrition Survey data, the AI was determined to compensate for endogenous potassium loss and maintenance of potassium balance at the present intake level (*1*, *2*). In research conducted in other countries, an intake of 1,600 mg was found adequate to maintain potassium balance (*13*). The current intake of the Japanese was found to exceed this value (*1*, *2*), reaching an AI of 2,500 mg for men, which is not an unrealizable value, nor is 2,000 mg for women in consideration of the difference in energy intake.

Based on the AI of adults aged 18 to 29 y, it was extrapolated by the 0.75th power of the weight ratio in consideration of the growth factor. The AI for infants aged 0 to 5 mo infants was calculated using the average concentration of potassium in breast milk (*3*, *4*) and the average volume of breast milk secreted per day (*5*, *6*). The AI for infants aged 6 to 11 mo was calculated using the average consumption of potassium from breast milk (*7*, *8*) and complementary food (*8*). Since it is supplied with normal meals, the additional amount required for pregnant women was not determined. The additional amount required for lactating women was calculated as follows:

Additional amount of potassium required for lactating women
=average amount of potassium in breast milk (*3*, *4*)×the amount of milk (*5*, *6*).

If renal functioning is normal, the potassium intake from normal meals will not lead to excessive potassium levels, which can cause metabolic disorder. Therefore, the tolerable upper intake level (UL) was not determined.

The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (*14*) reported that an intake of 3,500 mg potassium/d is desirable to prevent high blood pressure. This value is supported from the viewpoint of primary prevention of lifestyle-related diseases, centering on prevention of high blood pressure. However, considering that the current median intake of adult Japanese is 2,384 mg for men and 2,215 mg for women (*1*, *2*), this intake may be difficult to realize. Aiming for its realization 5 y from now, it was considered appropriate to aim at the mean value of the current median intake and the value reported in *the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure* (*14*), and to calculate the DG

Table 2. DRIs for potassium (mg/d).

Sex	Males		Females	
Age	AI[1]	UL[2]	AI[1]	UL[2]
0–5 mo	400	—	400	—
6–11 mo	700	—	700	—
1–2 y	900	—	800	—
3–5 y	1,000	—	1,000	—
6–7 y	1,300	—	1,200	—
8–9 y	1,500	—	1,400	—
10–11 y	1,900	—	1,700	—
12–14 y	2,300	—	2,100	—
15–17 y	2,700	—	2,000	—
18–29 y	2,500	2,800	2,000	2,700
30–49 y	2,500	2,900	2,000	2,800
50–69 y	2,500	3,000	2,000	3,000
≥70 y	2,500	3,000	2,000	2,900
Pregnant women (amount to be added)			+0	—
Lactating women (amount to be added)			+400	—

UL, tolerable upper intake level.
[1] The value that is considered appropriate to maintain in vivo potassium balance was used as the adequate intake.
[2] The value was established from a standpoint of prevention of hypertension.

Table 3. EAR and RDA of calcium determined using the factorial method.

Sex	Age (y)	Reference body weight (kg)	Accumulation (A) (mg/d)	Urinary excretion (B) (mg/d)	Percutaneous loss (C) (mg/d)	A+B+C (mg/d)	Apparent absorption rate (D) (%)	EAR (E=(A+B+C)/D) (mg/d)	RDA (E×1.2) (mg/d)
Males	1–2	11.7	99	38	6	143	40	358	430
	3–5	16.2	114	48	8	171	35	487	585
	6–7	22.0	99	61	10	170	35	486	583
	8–9	27.5	103	72	12	187	35	534	641
	10–11	35.5	134	87	15	236	40	590	707
	12–14	48.0	242	109	18	370	45	821	986
	15–17	58.4	151	127	21	299	45	664	797
	18–29	63.0	38	134	22	195	30	648	778
	30–49	68.5	0	143	24	167	30	556	667
	50–69	65.0	0	137	23	160	27	593	712
	≥70	59.7	0	129	21	150	25	601	722
Females	1–2	11.0	95	36	6	137	40	343	412
	3–5	16.2	99	48	8	156	35	444	533
	6–7	22.0	86	61	10	157	35	449	539
	8–9	27.2	135	71	12	218	35	624	749
	10–11	34.5	171	85	14	271	45	601	722
	12–14	46.0	178	106	18	302	45	670	804
	15–17	50.6	89	114	19	222	40	555	665
	18–29	50.6	33	114	19	166	30	553	663
	30–49	53.0	0	118	20	138	25	550	660
	50–69	53.6	0	119	20	139	25	555	666
	≥70	49.0	0	111	19	130	25	519	622

RDA, recommended dietary allowance.

Table 4. DRIs for calcium (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	200	—	—	—	200	—
6–11 mo	—	—	250	—	—	—	250	—
1–2 y	350	400	—	—	350	400	—	—
3–5 y	500	600	—	—	450	550	—	—
6–7 y	500	600	—	—	450	550	—	—
8–9 y	550	650	—	—	600	750	—	—
10–11 y	600	700	—	—	600	700	—	—
12–14 y	800	1,000	—	—	650	800	—	—
15–17 y	650	800	—	—	550	650	—	—
18–29 y	650	800	—	2,300	550	650	—	2,300
30–49 y	550	650	—	2,300	550	650	—	2,300
50–69 y	600	700	—	2,300	550	650	—	2,300
≥70 y	600	700	—	2,300	500	600	—	2,300
Pregnant women (amount to be added)					+0	+0	—	—
Lactating women (amount to be added)					+0	+0	—	—

based on this view.

DRIs for potassium are summarized in Table 2.

Calcium

Background information

Calcium accounts for 1% to 2% of body weight, with more than 99% of total body calcium contained in the bones and teeth and the remaining 1% contained in blood, tissue fluid, and cells, where it plays a role in various bodily functions. The calcium concentration in the blood is controlled within a very narrow range. If the concentration decreases, parathyroid hormone will stimulate the absorption of calcium from bone, which undergoes repeated bone resorption (resorption of calcium from the bones) and bone formation (accumulation of the calcium in the bones). Bone mass increases during growth and begins to decrease in menopause or later and then continues to do so during the aging process (*15*, *16*). Since the primary means of prevention of bone fracture is increasing bone mass, the calcium requirement has the character of a DG.

Determining DRIs

The EAR was calculated using the factorial method, which considers the amount of calcium accumulated in the body (*17–27*), excreted by urine (*28–30*), lost via dermal tissue (*31*), and the apparent rate (*32–50*) (Table 3).

Assuming that infants aged 0 to 5 mo can obtain the required calcium from their mother's milk, the AI was calculated using the average concentration of calcium in breast milk (*3*, *4*, *8*) and the average volume of breast milk secreted per day (*5*, *6*). For infants aged 6 to 11 mo, the AI was calculated using the average consumption of calcium from breast milk (*3*, *4*, *7*, *8*), and complementary food (*9*).

It was assumed that determining the additional amount required for pregnant and lactating women was unnecessary. Although the metabolism of calcium changes during pregnancy and lactation, during which more calcium is taken into the body, the calcium accumulated in an embryo and in the mother's milk originates from the bones of the mother's body, and even if they supply calcium, they cannot prevent bone mass reduction in the mother's body. Furthermore, since calcium intake is excreted in the mother's urine, the bone mass reduction that occurs during pregnancy and lactation is recovered within 6 mo after breast feeding is terminated if the quantity required before pregnancy is being consumed, and thus ingesting any additional amount is unnecessary.

Because milk alkali syndrome, a type of hypercalcemia that occurs with excessive ingestion of calcium and alkaline chemicals, has been reported (*51–59*), the UL was calculated with high reliability based on case reports of the obstacles encountered by superfluous ingestion of calcium. The UL was determined using the lowest observed adverse effect level (LOAEL) of calcium that causes milk alkali syndrome, which is 2.8 g, and dividing it by an uncertainty factor of 1.2, which yields a UL of 2.3 g.

DRIs for calcium are summarized in Table 4.

Magnesium

Background information

Magnesium contributes to the maintenance of bone health and various enzyme reactions. Approximately 25 g of magnesium exists in the adult body, and it exists in bone at levels of 50% to 60% (*60*). If magnesium is deficient, re-absorption of magnesium occurs from the kidneys, for which magnesium absorption increase from

Table 5. DRIs for magnesium (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL[1]	EAR	RDA	AI	UL[1]
0–5 mo	—	—	20	—	—	—	20	—
6–11 mo	—	—	60	—	—	—	60	—
1–2 y	60	70	—	—	60	70	—	—
3–5 y	80	100	—	—	80	100	—	—
6–7 y	110	130	—	—	110	130	—	—
8–9 y	140	170	—	—	140	160	—	—
10–11 y	180	210	—	—	170	210	—	—
12–14 y	240	290	—	—	230	280	—	—
15–17 y	290	350	—	—	250	300	—	—
18–29 y	280	340	—	—	230	270	—	—
30–49 y	310	370	—	—	240	290	—	—
50–69 y	290	350	—	—	240	290	—	—
≥70 y	270	320	—	—	220	260	—	—
Pregnant women (amount to be added)					+30	+40	—	—
Lactating women (amount to be added)					+0	+0	—	—

[1] When the nutrient is obtained from ordinary food, no upper threshold is set. When the nutrient is obtained from a source other than ordinary food, the upper threshold is set at 350 mg/d for adults and 5 mg/kg weight/d for children.

the bone will be used. At an average intake of approximately 300 to 350 mg, magnesium is absorbed from the intestinal tract at a rate of approximately 30% to 50% (*61*), with the rate increasing with lower intake.

Magnesium deficiency causes hypercalcemia, muscular convulsions, and coronary-artery spasms (*62*). Moreover, no fixed view exists, although it is suggested that insufficient magnesium over a long period raises the risk of lifestyle-related diseases, such as osteoporosis, cardiac disease, and diabetes (*60*). Although adverse effects are not caused by ingestion from meals, diarrhea may be caused by superfluous ingestion from supplements.

Determining DRIs

The EAR was calculated on the basis of results obtained by a previous study of magnesium balance (*63*). The research for Japanese was thought to be important, and 4.5 mg was made into the EAR per an adult's body weight. The EAR value of 4.5 mg was adopted as the recommended dietary allowance (RDA) after multiplying it by the reference body weight, applying a factor of 1.2, and assuming a coefficient of variation of 10%.

The results of an American balance test examining 12 boys and 13 girls aged 9 to 14 y using a stable magnesium isotope determined the EAR to be 5 mg (*33*). This value was subsequently adopted as the RDA after multiplying it by the reference body weight and applying a factor of 1.2, as had been applied to the adult EAR. The AI for infants aged 0 to 5 mo was calculated using the average concentration of magnesium in breast milk (*3*, *4*) and the average volume of breast milk secreted per day (*5*, *6*). The AI for infants aged 6 to 11 mo was calculated using the average consumption of magnesium from breast milk (*3*, *4*, *7*, *8*) and complementary food (*9*). The additional amount required for pregnant women was calculated using the results of a magnesium balance study of pregnant woman (*64*). Because neither calcium balance nor the amount of magnesium excreted in urine changes during lactation (*65*, *66*), it was assumed that determining the additional amount required during lactation was unnecessary.

The first-stage undesirable effect of superfluous ingestion of magnesium from sources other than food is diarrhea. Many individuals may experience mild transient diarrhea even without increased magnesium intake. Therefore, it is thought that it becomes the clearest index for the existence of development of symptoms of diarrhea to determine the UL. In addition, the report supposes that undesirable health effects of superfluous ingestion of magnesium from typical food sources were not found. Therefore, the UL from intake of typical foods was not determined.

DRIs for magnesium are summarized in Table 5.

Phosphorus

Background information

Phosphorus is indispensable to energy metabolism, which depends on phosphorylation in the cell. Even when phosphorus loss due to cooking is taken into consideration, the quantity of phosphorus ingested from food every day is always sufficient. The possibility of excessive ingestion of phosphorus is regarded as questionable, particularly as various orthophosphates are widely used as food additives.

Determining DRIs

Due to the lack of evidence in determining the pre-

Table 6. DRIs for phosphorus (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	120	—	—	—	120	—
6–11 mo	—	—	260	—	—	—	260	—
1–2 y	—	—	600	—	—	—	600	—
3–5 y	—	—	800	—	—	—	700	—
6–7 y	—	—	900	—	—	—	900	—
8–9 y	—	—	1,100	—	—	—	1,000	—
10–11 y	—	—	1,200	—	—	—	1,100	—
12–14 y	—	—	1,200	—	—	—	1,100	—
15–17 y	—	—	1,200	—	—	—	1,000	—
18–29 y	—	—	1,000	3,000	—	—	900	3,000
30–49 y	—	—	1,000	3,000	—	—	900	3,000
50–69 y	—	—	1,000	3,000	—	—	900	3,000
≥70 y	—	—	1,000	3,000	—	—	900	3,000
Pregnant women (amount to be added)					—	—	+0	—
Lactating women (amount to be added)					—	—	+0	—

sumed EAR and RDA, the AI for phosphorus was determined using the median intake reported in the National Health and Nutrition Survey (*1*, *2*) and the DRIs for the United States and Canada (*67*). The AI for infants aged 0 to 5 mo was calculated using the average concentration of phosphorus in breast milk (*3*, *4*) and the average volume of breast milk secreted per day (*5*, *6*). The AI for infants aged 6 to 11 mo was calculated using average consumption of phosphorus from breast milk (*3*, *4*, *7*, *8*) and complementary food (*9*). The additional amount for pregnant and lactating women was not calculated. It is known that serum inorganic phosphorus level increases in accordance with increases in phosphorus intake. The no observable adverse effect level (NOAEL) is considered to be an intake in the case where serum inorganic phosphorus serves as a normal upper limit. We set the uncertainty factor to 1.2, and calculated UL.

DRIs for phosphorus are summarized in Table 6.

Dr. Takatoshi Esashi who is one of the authors passed away on March 26, 2012. He was a leader of the working group for minerals in the decision of DRIs for Japanese, 2010. We would like to offer our respectful condolences on his death.

REFERENCES

1) Ministry of Health, Labour and Welfare, Japan. 2007. National Health and Nutrition Survey Japan (2005). Tokyo.
2) Ministry of Health, Labour and Welfare, Japan. 2008. National Health and Nutrition Survey Japan (2006). Tokyo.
3) 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 Elem Med Biol* **19**: 171–181.
4) Idota T. 2007. The components of Japanese mother's milk. The survey for the composition of human milk obtained from Japanese mothers—the goal of infant formula—. *Obstet Gynecol Pract* **56**: 315–325.
5) Suzuki K, Sasaki S, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Jpn J Nutr Diet* **62**: 369–372 (in Japanese).
6) 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 Breastfeeding Res* **2**: 23–28 (in Japanese).
7) Yoneyama K. 1998. Growth of breast-fed infants and intake of nutrients from breast-milk. *Journal of Child Health* **57**: 49–57 (in Japanese).
8) Yoneyama K, Goto I, Nagata H. 1995. Changes in the concentrations of nutrient components of human milk during lactation. *Jpn J Public Health* **42**: 472–481 (in Japanese).
9) Nakao T, Idota T, Nakajima I. 2003. The survey of nutrient intake from weaning foods in Japan. *J Child Health* **62**: 630–639 (in Japanese).
10) 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.
11) He FJ, Macgregor GA. 2009. A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *J Hum Hypertens* **23**: 363–384.
12) World Cancer Research Fund/American Institute for Cancer Research. 2007. Food, Nutrition, Physical Activity and the Prevention of Cancer, a Global Perspective. AICR, Washington DC.
13) Sebastian A, McSherry E, Morris RC Jr. 1971. Renal potassium wasting in renal tubular acidosis (RTA): its occurrence in type 1 and 2 RTA despite sustained correction of systemic acidosis. *J Clin Invest* **50**: 667–678.
14) Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

1997. The sixth report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI). *Arch Inetrn Med* **157**: 2413–2446.
15) Weaver CM. 2006. Calcium. *In*: Present Knowledge in Nutrition, 9th ed (Bowman BA, Russell RM, eds), Vol I, p 373–382. ILSI Press, Washington DC.
16) Dawson-Hughes B. 2006. Osteoporosis. *In*: Present Knowledge in Nutrition, 9th ed (Bowman BA, Russel RM, eds), Vol II, p 687–697. ILSI Press, Washington DC.
17) van der Sluis IM, de Ridder MA, Boot AM, Krenning EP, de Muinck Keizer-Schrama SM. 2002. Reference data for bone density and body composition measured with dual energy x-ray absorptiometry in white children and young adults. *Arch Dis Child* **87**: 341–347.
18) Bachrach LK, Hastie T, Wang MC, Narasimhan B, Marcus R. 1999. Bone mineral acquisition in healthy Asia, Hispanic, Black, and Caucasian youth: a longitudinal study. *J Clin Endocrinol Metab* **84**: 4702–4712.
19) Maynard LM, Guo SS, Chumlea WC, Roche AF, Wisemandle WA, Zeller CM, Towne B, Siervogel RM. 1998. Total-body and regional bone mineral content and areal bone mineral density in children aged 8–18 y: the Fels longitudinal study. *Am J Clin Nutr* **68**: 1111–1117.
20) Kalkwarf HJ, Zemel BS, Gilsanz V, Lappe JM, Horlick M, Oberfield S, Mahboubi S, Fan B, Frederick MM, Winer K, Shepherd JA. 2007. The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. *J Clin Endocrinol Metab* **92**: 2087–2099.
21) Molgaad C, Thomasen BL, Michaelsen KF. 1999. Whole body bone mineral accretion in healthy children and adolescents. *Arch Dis Child* **81**: 10–15.
22) Zhu K, Zhang Q, Foo LH, Trube A, Ma G, Hu X, Du X, Cowell CT, Fraser DR, Greenfield H. 2006. Growth, bone mass, and vitamin D status of Chinese adolescent girls 3 y after withdrawal of milk supplementation. *Am J Clin Nutr* **83**: 714–721.
23) Abrams SA, Copeland KC, Gunn SK, Gundberg CM, Klein KO, Ellis KJ. 2000. Calcium absorption, bone mass accumulation, and kinetics increase during early pubertal development in girls. *J Clin Endocrinol Metab* **85**: 1805–1809.
24) Martin AD, Bailey DA, McKay HA, Whiting S. 1997. Bone mineral and calcium accretion during puberty. *Am J Clin Nutr* **66**: 611–615.
25) Whiting SJ, Vatanparast H, Baxter-Jones A, Faulkner RA, Mirwald R, Bailey DA. 2004. Factors that affect bone mineral accrual in the adolescent growth spurt. *J Nutr* **134**: 696–700S.
26) Nishiyama S, Kiwaki K, Inomoto T, Seino Y. 1999. Bone mineral density of the lumber spine and total body mass in Japanese children and adolescents. *Pediatrics International* **103**: 1131–1138 (in Japanese).
27) 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.
28) Schaafsma G. 1992. The scientific basis of recommended dietary allowance for calcium. *J Int Med* **231**: 187–194.
29) Uenishi K, Ishida H, Kamei A, Shiraki M, Ezawa I, Goto S, Fukuoka H, Hosoi T, Orimo H. 2000. Calcium requirement in young Japanese female. *Osteoporosis Jpn* **8**: 217–219 (in Japanese).
30) Uenishi K, Ishida H, Kamei A, Shiraki M, Ezawa I, Goto S, Fukuoka H, Hosoi T, Orimo H. 2001. Calcium requirement estimated by balance study in elderly Japanese people. *Osteoporosis Int* **12**: 858–863.
31) Charles P, Eriksen EF, Hasling C, Sondergard K, Mosekilde L. 1991. Dermal, intestinal, and renal obligatory losses of calcium: relation to skeletal calcium loss. *Am J Clin Nutr* **54** (Suppl): S266–273.
32) Abrame SA, Wen J, Stuff JE. 1997. Absorption of calcium, zinc and iron from breast milk by 5- to 7-month-old infants. *Pediatr Res* **41**: 384–390.
33) Abrams SA, Grusak MA, Stuff J, O'Brien KO. 1997. Calcium and magnesium balance in 9–14-y-old children. *Am J Clin Nutr* **66**: 1172–1177.
34) Heaney RP, Recker RR, Hinders SM. 1988. Variability of calcium absorption. *Am J Clin Nutr* **47**: 262–264.
35) Abrams SA, Copeland KC, Gunn SK, Stuff JE, Clarke LL, Ellis KJ. 1999. Calcium absorption and kinetics are similar in 7- and 8-year-old Mexican-American and Caucasian girls despite hormonal differences. *J Nutr* **129**: 666–671.
36) Miller JZ, Smith DL, Flora L, Slemenda C, Jiang XY, Johnston CC Jr. 1998. Calcium absorption from calcium carbonate and a new form of calcium (CCM) in healthy male and female adolescents. *Am J Clin Nutr* **48**: 1291–1294.
37) Abrams SA, O'brien KO, Liang LK, Stuff JE. 1995. Differences in calcium absorption and kinetics between black and white girls aged 5–16 years. *J Bone Miner Res* **10**: 829–833.
38) Bryant RJ, Wastney ME, Martin BR, Wood O, McCabe GP, Morshidi M, Smith DL, Peacock M, Weaver CM. 2003. Racial differences in bone turnover and calcium metabolism in adolescent females. *J Clin Endocrinol Metab* **88**: 1043–1047.
39) Weaver CM, Martin BR, Plawecki KL, Peacock M, Wood OB, Smith DL, Wastney ME. 1995. Differences in calcium metabolism between adolescent and adult females. *Am J Clin Nutr* **61**: 577–581.
40) Uenishi K, Ishida H, Kamei A, Shiraki M, Ezawa I, Goto S, Fukuoka H, Hosoi T, Orimo H. 2003. Calcium balance in pregnant and lactating women. *Osteoporosis Jpn* **11**: 249–251 (in Japanese).
41) Heaney RP, Recker RR, Stegman MR, Moy AJ. 1989. Calcium absorption in women: relationships to calcium intake, estrogen status, and age. *J Bone Miner Res* **4**: 469–475.
42) Roughead ZK, Johnson LK, Lykken GI, Hunt JR. 2003. Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women. *J Nutr* **133**: 1020–1026.
43) Tahiri M, Tressol JC, Arnaud J, Bornet FR, Bouteloup-Demange C, Feillet-Coudray C, Brandolini M, Ducros V, Pepin D, Brouns F, Roussel AM, Rayssiguier Y, Coudray C. 2003. Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. *Am J Clin Nutr* **77**: 449–457.
44) Cifuentes M, Riedt CS, Brolin RE, Field MP, Sherrell RM, Shapses SA. 2004. Weight loss and calcium intake influence calcium absorption in overweight postmenopausal women. *Am J Clin Nutr* **80**: 123–130.
45) Lynch MF, Griffin IJ, Hawthorne KM, Chen Z, Hamzo M, Abrams SA. 2007. Calcium balance in 1–4-y-old children. *Am J Clin Nutr* **85**: 750–754.
46) Kohlenberg-Mueller K, Raschka L. 2003. Calcium bal-

ance in young adults on a vegan and lactovegetarian diet. *J Bone Miner Metab* **21**: 28–33.

47) Abrams SA, Griffin IJ, Hawthorne KM, Liang L. 2005. Height and height z-score are related to calcium absorption in five-to fifteen-year-old girls. *J Clin Endocrinol Metab* **90**: 5077–5081.

48) O'Brien KO, Abrams SA, Liang LK, Ellis KJ, Gagel RF. 1996. Increased efficiency of calcium absorption during short periods of inadequate calcium intake in girls. *Am J Clin Nutr* **63**: 579–583.

49) Weaver CM, McCabe LD, McCabe GP, Braun M, Martin BR, Dimeglio LA, Peacock M. 2008. Vitamin D status and calcium metabolism in adolescent black and white girls on range of controlled calcium intakes. *J Clin Endocrinol Metab* **93**: 3907–3914.

50) Moser-Veillon PB, Mangels AR, Vieira NE, Yergey AL, Patterson KY, Hill AD, Veillon C. 2001. Calcium fractional absorption and metabolism assessed using stable isotope differ between postpartum and never pregnant women. *J Nutr* **131**: 2295–2299.

51) Abrams SA, Silber TJ, Esteban NV, Vieira NE, Stuff JE, Meyers R, Majd M, Yergey AL. 1993. Mineral balance and bone turnover in adolescents with anorexia nervosa. *J Pediatr* **123**: 326–331.

52) Bullimore DW, Miloszewski KJ. 1987. Raised parathyroid hormone levels in the milk alkali syndrome: an appropriate response? *Postgrad Med J* **63**: 789–792.

53) Campbell SB, Macfarlane DJ, Fleming SJ, Khafagi FA. 1994. Increased skeletal uptake of Tc-99m methylene diphosphonate in milk-alkali syndrome. *Clin Nucl Med* **19**: 207–211.

54) Carroll PR, Clark OH. 1983. Milk alkali syndrome. Does it exist and can it be differentiated from primary hyperparathyroidism? *Ann Surg* **197**: 427–433.

55) Hart M, Windle J, McHale M, Grissom R. 1982. Milk-alkali syndrome and hypercalcemia: a case report. *Nebr Med J* **67**: 128–130.

56) Kallmeyer JC, Funston MR. 1983. The milk-alkali syndrome. A case report. *S Afr Med J* **64**: 287–288.

57) Kapsner P, Langsdorf L, Marcus R, Kraemer FB, Hoffman AR. 1986. Milk-alkali syndrome in patients treated with calcium carbonate after cardiac transplantation. *Arch Intern Med* **146**: 1965–1968.

58) Kleinman GE, Rodriquez H, Good MC, Caudle MR. 1991. Hypercalcemic crisis in pregnancy associated with excessive ingestion of calcium carbonate antacid (milk-alkali syndrome): successful treatment with hemodialysis. *Obstet Gynecol* **78**: 496–499.

59) Schuman CA, Jones HW 3rd. 1985. The 'milk-alkali' syndrome: two case reports with discussion of pathogenesis. *Q J Med* **55**: 119–126.

60) Fleet JC, Cashman KD. 2001. Magnesium. *In*: Present Knowledge in Nutrition (Bowman BA, Russell RM, eds), 8th ed, p 292–301. ILSI Press, Washington DC.

61) Schwartz R, Spencer H, Welsh JJ. 1984. Magnesium absorption in human subjects from leafy vegetables, intrinsically labeled with stable 26Mg. *Am J Clin Nutr* **39**: 571–576.

62) Food and Nutrition Board, Institute of Medicine. 1997. Magnesium. *In*: Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride (Institute of Medicine, ed), p 190–249. National Academies Press, Washington DC.

63) Nishimuta M, Kodama N, Morikuni E. 2004. Balances of calcium, magnesium and phosphorus in Japanese young adults. *J Nutr Sci Vitaminol* **50**: 19–25.

64) Seeling MS. 1980. Magnesium balance in pregnancy. *In*: Magnesium Deficiency in the Pathogenesis of Disease. Plenum Medical, New York.

65) Caddell JL, Saier FL, Thomason CA. 1975. Parenteral magnesium load tests in postpartum American women. *Am J Clin Nutr* **28**: 1099–1104.

66) Klein CJ, Moser-Veillon PB, Douglass LW, Ruben KA, Trocki O. 1995. Longitudinal study of urinary calcium, magnesium, and zinc excretion in lactating and nonlactating postpartum women. *Am J Clin Nutr* **61**: 779–786.

67) Food and Nutrition Board, Institute of Medicine. 1997. Phosphorus. *In*: Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride (Institute of Medicine, ed), p 146–189. National Academies Press, Washington DC.

J Nutr Sci Vitaminol, 59, S91–S102, 2013

Dietary Reference Intakes for Japanese 2010: Microminerals

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(Received October 26, 2012)

Summary The Dietary Reference Intakes (DRIs) of 8 microminerals (iron, zinc, copper, manganese, iodine, selenium, chromium and molybdenum) were determined for Japanese. The estimated average requirement (EAR) and the recommended dietary allowance (RDA) for adults ages 18 y and older were determined in seven microminerals other than for manganese. Due to lack of data with which to set the EAR for manganese, determination of the adequate intake (AI) of manganese was based on the average manganese intake of the Japanese population. Data with which to determine the EARs were obtained using the following methods: iron and zinc, use of a factorial modeling method; copper and selenium, determination of the relationship between biomarkers and intake; iodine, determination of thyroid iodine accumulation and turnover; and chromium and molybdenum, performance of a balance test. The EARs and RDAs of iron, zinc, copper, iodine and selenium for children and adolescents aged 1 to 17 y were also determined. Based on the average micromineral concentration in the milk of Japanese women and the average intake of breast milk in Japanese infants, the AI for infants was determined for 8 microminerals. The tolerable upper intake level (ULs) of adults were determined for all microminerals except chromium, for which there are insufficient data. The ULs for iron, iodine and selenium for children and adolescents were also determined.

Key Words chromium, copper, iodine, iron, manganese, molybdenum, selenium, zinc

Iron

Background information

Iron functions as a component of a number of proteins, including hemoglobin and several enzymes. Iron deficiency induces anemia and decreases physical performance and cognitive functions. Women's iron status is highly influenced by menstrual iron loss. In Japan, approximately 25% of women aged 30 through 39 y have been diagnosed with anemia, defined as a hemoglobin level lower than 12.0 g/dL (*1*).

Determining the Dietary Reference Intakes (DRIs)

The estimated average requirement (EAR) for iron was determined using a factorial modeling method in which the factors were basal iron loss (mostly via fecal loss), menstrual iron loss, iron storage with growth (mostly via increase in hemoglobin mass), increased iron requirement with pregnancy or lactation, and extent of dietary iron absorption. The average basal iron loss was estimated to be 0.96 mg/d, as determined by a study of 41 persons in 4 groups of a mean body weight of 68.6 kg (*2*), and this value extrapolated to each sex and age group using the 0.75th power of a weight ratio. The average menstrual iron loss was estimated to be 0.46 mg/d for girls aged 10 to 17 y and 0.55 mg/d for women aged 18 y and older based on the average menstrual blood loss of Japanese women (*3*, *4*). The iron storage with growth for each sex and age group (0.09 to 0.46 mg/d) was estimated based on blood volume and hemoglobin concentration by age group (*5*, *6*), iron content in hemoglobin (3.39 mg/g) (*7*), increase in tissue iron (non-storage iron), and increase in storage iron (*8*). The average of increased iron requirements due to pregnancy (0.32, 2.68, and 3.64 mg/d for the early, mid, and late stages of pregnancy, respectively) were calculated based on fetal and placental iron storage (*9*) and increase in hemoglobin mass caused by erythrocyte

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Table 1. Dietary Reference Intakes for iron (mg/d).[1]

Sex	Males				Females					
Age	EAR	RDA	AI	UL	Non-menstruating women		Menstruating women		AI	UL
					EAR	RDA	EAR	RDA		
0–5 mo	—	—	0.5	—	—	—	—	—	0.5	—
6–11 mo	3.5	5.0	—	—	3.5	4.5	—	—	—	—
1–2 y	3.0	4.0	—	25	3.0	4.5	—	—	—	20
3–5 y	4.0	5.5	—	25	4.0	5.5	—	—	—	25
6–7 y	4.5	6.5	—	30	4.5	6.5	—	—	—	30
8–9 y	6.0	8.5	—	35	5.5	8.0	—	—	—	35
10–11 y	7.0	10.0	—	35	6.5	9.5	9.5	13.5	—	35
12–14 y	8.0	11.0	—	50	7.0	10.0	10.0	14.0	—	45
15–17 y	8.0	9.5	—	45	5.5	7.0	8.5	10.5	—	40
18–29 y	6.0	7.0	—	50	5.0	6.0	8.5	10.5	—	40
30–49 y	6.5	7.5	—	55	5.5	6.5	9.0	11.0	—	40
50–69 y	6.0	7.5	—	50	5.5	6.5	9.0	11.0	—	45
≥70 y	6.0	7.0	—	50	5.0	6.0	—	—	—	40
Pregnant women (amount to be added)										
Early-stage					+2.0	+2.5	—	—	—	—
Mid and late-stage					+12.5	+15.0	—	—	—	—
Lactating women (amount to be added)					+2.0	+2.5	—	—	—	—

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

[1] The values were set excluding those with menorrhagia (blood loss exceeding 80 mL/period).

mass expansion. The average iron requirement due to by lactation (0.33 mg/d) was calculated from the average iron concentration (0.426 mg/L) (*10*) and volume of secretion (0.78 L/d) (*11*, *12*) of breast milk in Japanese women.

In accordance with a value adopted by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) (*13*), the average percentage of dietary iron absorption by all ages is estimated to be 15% except for women during the mid and late stages of pregnancy, for whom it is estimated to be 25% (*14*). The EARs were calculated as follows: men and non-menstruating women aged 18 y and older, EAR=basal loss/absorption; menstruating women aged 18 y and older, EAR=(basal loss+menstrual loss)/absorption; boys and non-menstruating girls aged 6 mo to 17 y, EAR=(basal loss+accumulation with growth)/absorption; menstruating girls aged 10 to 17 y, EAR=(basal loss+menstrual loss+accumulation with growth)/absorption; pregnant and lactating women, additional EAR=increased demand induced by pregnancy or lactation/absorption. The recommended dietary allowances (RDAs) were determined as follows: children aged 6 mo to 14 y, EAR×1.4; aged 15 or older, EAR×1.2.

The adequate intake (AI) for infants aged 0 to 5 mo was calculated based on mean iron intake of infants fed breast milk as follows: AI=average iron concentration in breast milk in Japanese women (0.426 mg/L) (*10*)×average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*). The tolerable upper intake levels (ULs) for individuals aged 15 y or older was set at 0.8 mg/kg/d according to the provisional maximal tolerable intake reported by the WHO and FAO (*15*). The UL for toddlers aged 1 to 2 y was set at 2.0 mg/kg/d based on the lowest observed adverse effect level (LOAEL) for toddlers, which is 60 mg/kg/d (*16*), and an uncertainty factor of 30. The ULs for children aged 3 to 5 y, 6 to 7 y, 8 to 9 y, and 10 to 14 y were set at 1.6, 1.4, 1.2, and 1.0 mg/kg/d, respectively.

Table 1 summarizes the DRIs for iron. The EARs and RDAs in this table do not apply to women with hypermenorrhea, defined as menstrual blood loss over 80 mL per month.

Zinc

Background information

Zinc is an essential component of almost 100 specific enzymes, including alcohol dehydrogenase and RNA polymerases. Zinc deficiency may occur in patients receiving prolonged total parenteral nutrition (TPN) without zinc supplementation (*17*) or in infants fed breast milk with low zinc content (*18*), and manifests as several specific symptoms, including acrodermatitis enteropathica, hypogeusia, and chronic diarrhea.

Table 2. Dietary Reference Intakes for zinc (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	2	—	—	—	2	—
6–11 mo	—	—	3	—	—	—	3	—
1–2 y	4	5	—	—	4	5	—	—
3–5 y	5	6	—	—	5	6	—	—
6–7 y	6	7	—	—	6	7	—	—
8–9 y	7	8	—	—	7	8	—	—
10–11 y	8	10	—	—	8	10	—	—
12–14 y	9	11	—	—	8	9	—	—
15–17 y	11	13	—	—	7	9	—	—
18–29 y	10	12	—	40	7	9	—	35
30–49 y	10	12	—	45	8	9	—	35
50–69 y	10	12	—	45	8	9	—	35
≥70 y	9	11	—	40	7	9	—	30
Pregnant women (amount to be added)					+1	+2	—	—
Lactating women (amount to be added)					+3	+3	—	—

Determining DRIs

The EAR for zinc was determined using a factorial modeling method in which the factors were urinary zinc excretion, the sum of integumental and sweat zinc loss, zinc loss in semen or menstrual blood, endogenous zinc excretion via the intestine, and the extent of absorption of dietary zinc. The RDA for zinc was set equal to 120% of the EAR. As estimated according to the US/Canadian DRIs (*19*), urinary zinc excretion, the sum of integumental and sweat zinc loss, and zinc loss in semen or menstrual blood for adults of a reference body weight (men, 76 kg; women, 61 kg) were found to be the following: urinary zinc loss, 0.63 (men) and 0.44 mg/d (women); sum of integumental and sweat zinc loss, 0.54 (men) and 0.46 mg/d (women); zinc loss in semen, 0.10 mg/d; and zinc losses in menstrual blood, 0.10 mg/d. As a result, endogenous zinc losses via routes other than the intestine for men and women were determined to be 1.27 (0.63+0.54+0.10) mg/d and 1.00 (0.44+0.46+0.10) mg/d, respectively.

The results of several studies using a stable isotope (*20–26*) have shown that the relationship between endogenous zinc excretion via the intestine and the quantity of zinc absorbed in adults with a body weight of 76 kg can be calculated using the following equation: endogenous excretion via the intestine=0.628×(quantity absorbed+0.2784). Because total endogenous zinc excretion is the sum of endogenous excretion via the intestine and other routes, the relationship between total endogenous zinc excretion and quantity of zinc absorbed in adults with a body weight of 76 kg can be calculated using the following equations: men, total endogenous excretion=0.628×(quantity absorbed+0.2784+1.27); women, total endogenous excretion=0.628×(quantity absorbed+0.2784+1.00×(76/61)$^{0.75}$). The quantity of zinc intake necessary to achieve zinc balance, the state in which zinc absorption is equal to total endogenous excretion, has been calculated to be 4.16 mg/d for men and 3.92 mg/d for women. The relationship between zinc absorption and zinc intake is expressed by the following equation (*20–26*): quantity of absorbed zinc =1.113×(zinc intake)$^{0.5462}$. The EAR for zinc, defined as the minimal intake necessary to maintain zinc balance, for adults with a body weight of 76 kg was determined to be 11.18 mg/d for men and 10.03 mg/d for women. These values were extrapolated to the EAR for each age group of adults aged 18 y or older using the 0.75th power of a weight ratio. The EAR for adolescents aged 12 to 17 y was determined by extrapolation of the EAR for adults using the 0.75th power of a weight ratio and a growth factor.

In a study of Japanese children (mean body weight, 16.34 kg), the minimal intake necessary to maintain zinc balance was estimated to be 3.87 mg/d (*27*). Thus, the EAR for children with a body weight of 16.34 kg was calculated to be 4.06 mg/d, which is obtained by addition of 3.87 mg/d to the sum of integumental and sweat zinc loss (0.19 mg/d). The EAR for children aged 1 to 11 y was determined by extrapolation of 4.06 mg/d to each age group using the 0.75th power of a weight ratio and a growth factor. The additional EAR for pregnant women, which was determined by measurement of zinc storage during pregnancy (0.40 mg/d) (*28*) and extent of zinc absorption (27%) (*19*), was set at 1 mg/d. The additional EAR for lactating women, which was determined by measurement of average zinc content in Japanese breast milk (1.83 mg/L) (*29, 30*), average intake of breast milk in Japanese infants (0.78 L/d) (*11,*

Table 3. Dietary Reference Intakes for copper (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.3	—	—	—	0.3	—
6–11 mo	—	—	0.3	—	—	—	0.3	—
1–2 y	0.2	0.3	—	—	0.2	0.3	—	—
3–5 y	0.3	0.3	—	—	0.3	0.3	—	—
6–7 y	0.3	0.4	—	—	0.3	0.4	—	—
8–9 y	0.4	0.5	—	—	0.4	0.5	—	—
10–11 y	0.5	0.6	—	—	0.5	0.6	—	—
12–14 y	0.6	0.8	—	—	0.6	0.8	—	—
15–17 y	0.7	0.9	—	—	0.6	0.7	—	—
18–29 y	0.7	0.9	—	10	0.6	0.7	—	10
30–49 y	0.7	0.9	—	10	0.6	0.7	—	10
50–69 y	0.7	0.9	—	10	0.6	0.7	—	10
≥70 y	0.6	0.8	—	10	0.5	0.7	—	10
Pregnant women (amount to be added)					+0.1	+0.1	—	—
Lactating women (amount to be added)					+0.5	+0.6	—	—

12), and extent of zinc absorption by lactating women (53%) (*31*), was set at 3 mg/d.

Because there is no remarkable difference between the zinc intake from breast milk of US and Japanese infants, the AI for Japanese infants aged 0 to 5 mo was set at 2 mg/d in accordance with the US/Canadian DRIs (*19*). The AI for infants aged 6 to 11 mo was mean of the extrapolation of 2 mg/d using the 0.75th power of a weight ratio (2.6 mg/d) and the sum of zinc intake from complementary food and formula milk (3.1 mg/d) (*32*).

Based on the results of a study in which subjects were administered 50 mg/d of zinc supplements (*33*), the LOAEL of zinc was estimated to be 60 mg/d in women with a body weight of 61 kg. Based on this value and an uncertainty factor of 1.5, the UL for adults was set at 0.66 mg/kg/d. Since there are no available data, no ULs for infants, children, pregnancy and lactating women have been set.

Table 2 summarizes the DRIs for zinc. The values are expressed as integral values in consideration of limitations in the accuracy of EAR calculation.

Copper

Background information

Copper functions as a component of several metalloenzymes, including monoamine oxidase, ferroxidase (ceruloplasmin), cytochrome *c* oxidase, and superoxide dismutase (CuSOD). Since ferroxidase is an essential enzyme in heme synthesis, copper deficiency induces normocytic, hypochromic anemia. Simple copper deficiency in human is rare, but has been observed in infants with a low copper intake (*34*) or patients receiving prolonged TPN (*35*).

Determining DRIs

The EAR for copper in adults was determined using biomarkers of copper status. Biomarkers used were plasma copper, urinary copper, and salivary copper levels and plasma CuSOD activity. According to 2 reliable studies using a stable isotope (*36, 37*), the minimal intake to achieve saturation of these biomarkers is estimated to be 0.72 mg/d. Because the mean body weight of the subjects in these studies was 74.7 kg, the 0.72 mg/d was set as the EAR for adults with a body weight of 74.7 kg. Thus, the EAR for each sex and age group of adults aged 18 y and older was determined by extrapolation of 0.72 mg/d using the 0.75th power of a weight ratio, and the EAR for children and adolescents aged 1 to 17 y by extrapolation of 0.72 mg/d using the 0.75th power of a weight ratio and a growth factor. Based on copper storage (13.7 mg) (*38*) and the extent of dietary copper absorption (60%) (*39*) in a full-term fetus, the additional EAR for pregnant women was determined to be 0.08 (13.7÷280 d÷0.6) mg/d. Based on the average copper concentration (0.35 mg/L) (*40*) and average volume of secretion (0.78 L/d) (*11, 12*) of breast milk in Japanese women and an estimated copper absorption rate of 60%, (*39*) the additional EAR for lactating women was determined to be 0.455 (0.35×0.78÷0.6) mg/d, and the RDA set equal to 130% of the EAR.

Based on the average copper concentration in breast milk in Japanese women (0.35 mg/L) (*40*) and the average intake of breast milk by Japanese infants (0.78 L/d) (*11, 12*), the AI for infants aged 0 to 5 mo was determined to be 0.273 (0.35×0.78) mg/d. Based on the average copper concentration in breast milk in Japanese women more than 6 mo after a delivery (0.16 mg/L) (*40*), the average intake of breast milk (0.525 L/d) (*41, 42*), and the average copper intake from complementary foods (0.195 mg/d) (*32*), the AI for infants aged 6 to 11 mo was determined to be 0.279

Table 4. Dietary Reference Intakes for manganese (mg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.01	—	—	—	0.01	—
6–11 mo	—	—	0.5	—	—	—	0.5	—
1–2 y	—	—	1.5	—	—	—	1.5	—
3–5 y	—	—	1.5	—	—	—	1.5	—
6–7 y	—	—	2.0	—	—	—	2.0	—
8–9 y	—	—	2.5	—	—	—	2.5	—
10–11 y	—	—	3.0	—	—	—	3.0	—
12–14 y	—	—	4.0	—	—	—	3.5	—
15–17 y	—	—	4.5	—	—	—	3.5	—
18–29 y	—	—	4.0	11	—	—	3.5	11
30–49 y	—	—	4.0	11	—	—	3.5	11
50–69 y	—	—	4.0	11	—	—	3.5	11
≥70 y	—	—	4.0	11	—	—	3.5	11
Pregnant women (amount to be added)					—	—	+0	—
Lactating women (amount to be added)					—	—	+0	—

(0.16×0.525+0.195) mg/d. Based on estimation of the no observed adverse effect level (NOAEL) of copper (10 mg/d) by a case report from an ingestion study of copper supplements (*43*) and an uncertainty factor of 1.0, the UL for adults was set at 10 mg/d. Since there are no data available, ULs for children and adolescents have not been set.

Table 3 summarizes the DRIs for copper.

Manganese

Background information

Since there are several manganese metalloenzymes, including arginase, pyruvate carboxylase and manganese superoxide dismutase, manganese is considered an essential nutrient. In a human study, 5 of 7 young men fed a low manganese diet (≤0.11 mg/d) for 39 d manifested a skin abnormality diagnosed as miliaria crystallina that was successfully treated by manganese repletion (1.53 to 2.55 mg/d) (*44*). However, the possibility of dietary manganese deficiency is nearly 0% because plant foods, including cereals and beans, contain high levels of manganese.

Determining DRIs

Several manganese balance studies have been performed to estimate manganese requirements (*45, 46*). However, the USA/Canada DRIs concluded that a minimal requirement to maintain manganese balance could not be estimated from a short-term balance study (*47*). Accordingly, as there is insufficient information with which to set the EAR, the AI was set based on the average manganese intake of the Japanese population, which far exceeds the minimal requirement to maintain manganese balance. Based on a review of the manganese intake of the Japanese population, the average manganese intake of adults is estimated to be 3.7 mg/d (*48*). To account for the differences in male and female energy intake, the AI for adults aged 18 y and older was set at 4.0 mg/d for men and 3.5 mg/d for women. The AI for children and adolescents aged 1 to 17 y was determined by extrapolation of the AI using the 0.75th power of a weight ratio and a growth factor. Based on the average manganese concentration in breast milk in Japanese women (0.011 mg/L) (*40*) and the average intake of breast milk in Japanese infants (0.78 L/d) (*11, 12*), the AI for infants aged 0 to 5 mo was set at 0.086 (0.011×0.78) mg/d. Based on the average manganese concentration in breast milk in Japanese women, the average intake of breast milk (0.525 L/d) (*41, 42*), and the average manganese intake from complementary foods (0.44 mg/d) (*32*), the AI for infants aged 6 to 11 mo was set at 0.45 (0.011×0.525+0.44) mg/d.

The AI for women who are not pregnant/lactating (3.5 mg/d) far exceeds the AI for pregnant women in the USA/Canada DRIs (2.0 mg/d) (*47*). Accordingly, the AI for pregnant women was set at the same value as the AI for women who are not pregnant (3.5 mg/d). Based on the average manganese concentration in breast milk in Japanese women (0.011 mg/L) (*40*), the average intake of breast milk in Japanese infants (0.78 L/d) (*11, 12*), and the average extent of absorption of dietary manganese (about 5%) (*49*), manganese loss by lactation is estimated to be less than 0.3 (0.011×0.78÷0.05) mg/d, which is much lower than the AI for women who are not pregnant/lactating (3.5 mg/d). Therefore, the AI for lactating women was set at the same value of the AI for women who are not pregnant/lactating.

Based on the manganese intake of vegetarians (*47, 50*), the USA/Canada DRIs estimated the NOAEL of manganese to be 11 mg/d. Based on this value and an uncertainty factor of 1.0, the UL for manganese in

Table 5. Dietary Reference Intakes for iodine (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	100	250	—	—	100	250
6–11 mo	—	—	130	250	—	—	130	250
1–2 y	35	50	—	250	35	50	—	250
3–5 y	45	60	—	350	45	60	—	350
6–7 y	55	75	—	500	55	75	—	500
8–9 y	65	90	—	500	65	90	—	500
10–11 y	75	110	—	500	75	110	—	500
12–14 y	95	130	—	1,300	95	130	—	1,300
15–17 y	100	140	—	2,100	100	140	—	2,100
18–29 y	95	130	—	2,200	95	130	—	2,200
30–49 y	95	130	—	2,200	95	130	—	2,200
50–69 y	95	130	—	2,200	95	130	—	2,200
≥70 y	95	130	—	2,200	95	130	—	2,200
Pregnant women (amount to be added)					+75	+110	—	—
Lactating women (amount to be added)					+100	+140	—	—

adults was set at 11 mg/d. Since there are no data available, ULs for children and adolescents have not been set.

Table 4 summarizes the DRIs for manganese.

Iodine

Background information

Iodine is an essential component of thyroid hormone. As such, iodine deficiency induces mental retardation, hypothyroidism, goiter, cretinism, and varying degrees of other growth and development abnormalities.

Marine products contain iodine at high levels, in particular, *kombu* (a type of kelp) contains it at more than 2 mg/g dry weight. Since the Japanese routinely eat *kombu*, their average iodine intake is very much higher than that of other populations. Based on measurement of urinary iodine excretion (*51*, *52*), annual consumption of *kombu* (*53*), and chemical iodine analysis of duplicate diets (*54*, *55*), the average iodine intake of the Japanese, which has been found to be intermittently high, is estimated to be 1.5 mg/d.

Determining DRIs

Similar to the USA/Canada DRIs (*56*), the EAR for iodine was determined by measurement of thyroid iodine accumulation and turnover. Based on the results of 2 USA studies (*57*, *58*), the average accumulation of radioiodine by the thyroid gland is estimated to be 93.9 μg/d in adults. Thus, the EAR for adults aged 18 y and older was set at 95 μg/d, and the RDA set equal to 140% of the EAR. The EAR for children and adolescents aged 1 to 17 y was determined by extrapolation of the EAR for adults aged 18 to 29 y using the 0.75th power of a weight ratio and a growth factor.

The iodine content of Japanese breast milk varies markedly with iodine intake (*59*). When a woman's iodine intake is less than 1.5 mg/d or her *kombu* ingestion is restricted, the average iodine content in her breast milk is estimated to be 133 μg/L (*59*, *60*). Based on this average iodine concentration of breast milk and the average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*), the AI for infants aged 0 to 5 mo was set at 100 (133×0.78) μg/d. The AI for infants aged 6 to 11 mo (130 μg/d) was determined by extrapolation of this value using the 0.75th power of a weight ratio.

Based on the median value of iodine turnover in newborn infants (75 μg/d) (*61*), the additional EAR for pregnant women was set at 75 μg/d. Based on the average iodine content in breast milk in Japanese women (133 μg/L) (*59*, *60*), the average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*), and the extent of absorption of dietary iodine (100%), the additional EAR for lactating women was determined to be 100 (133×0.78) μg/d, and the RDA set equal to 140% of the EAR.

Initially, excessive iodine intake also induces hypothyroidism and goiter, a phenomenon referred to as the Wolff-Chaikoff effect. However, the Wolff-Chaikoff effect does not occur with continuous excessive iodine intake, a phenomenon referred to as "escape." Based on the results of an epidemiological study of subjects living in a coastal area of Hokkaido (*62*, *63*), which estimated the NOAEL of iodine for Japanese adults to be 3.3 mg/d, and an uncertainty factor of 1.5, the UL for iodine in adults was set at 2.2 mg/d. As this UL applies to continuous daily iodine intake, it is not necessary to restrict intermittent high iodine (up to about 5 mg/d) intake.

In a study of children aged 6 to 12 y, a significant increase in thyroid size was observed in subjects whose estimated iodine intake was more than 500 μg/d (*64*). Based on this observation, the UL for children aged 6

Table 6. Dietary Reference Intakes for selenium (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	15	—	—	—	15	—
6–11 mo	—	—	15	—	—	—	15	—
1–2 y	10	10	—	50	10	10	—	50
3–5 y	10	15	—	70	10	15	—	70
6–7 y	15	15	—	100	15	15	—	100
8–9 y	15	20	—	120	15	20	—	120
10–11 y	20	25	—	160	20	20	—	150
12–14 y	25	30	—	210	20	25	—	200
15–17 y	25	35	—	260	20	25	—	220
18–29 y	25	30	—	280	20	25	—	220
30–49 y	25	30	—	300	20	25	—	230
50–69 y	25	30	—	280	20	25	—	230
≥70 y	25	30	—	260	20	25	—	210
Pregnant women (amount to be added)					+5	+5	—	—
Lactating women (amount to be added)					+15	+20	—	—

to 11 y was set at 500 μg/d. The UL for children aged 1 to 5 y was determined by extrapolation of the UL for children aged 6 to 7 y using a weight ratio. The UL for adolescents aged 12 to 14 y was set as the mean of 2 values: the value of the extrapolation of the UL for children aged 10 to 11 y using a weight ratio and the value of the extrapolation of the UL for adults aged 18 to 29 y using a weight ratio. The UL for adolescents aged 15 to 17 y was determined by extrapolation of the UL for adults aged 18 to 29 y using a weight ratio.

Based on a case report of hypothyroidism in infants fed breast milk (*60*), the NOAEL of iodine for infants ages 0 through 5 mo is estimated to be 254 μg/d. Based on this value and an uncertainty factor of 1.0, the UL for infants aged 0 to 5 mo was set at 250 μg/d. Since the UL is 250 μg/d for both infants aged 0 through 5 mo and children aged 1 to 2 y, the UL for infants aged 6 to 11 mo was also set at 250 μg/d.

Excessive ingestion of iodine by pregnant or lactating women can cause hypothyroidism in their infants. In a case report of hypothyroidism in infants fed breast milk (*60*), the mothers' iodine intake from *kombu* was estimated to be 2.28 to 3.18 mg/d. If the iodine intake from foods other than *kombu* is taken into consideration, their total iodine intake would exceed the UL for women who are not pregnant. Accordingly, the UL for women who are not pregnant can be applied to pregnant and lactating women.

Table 5 summarizes the DRIs for iodine.

Selenium

Background information

Selenium functions as a form of selenocysteine residue in protein. Genome analysis has identified 25 selenium-containing proteins in humans, including glutathione peroxidase (GPX), iodothyronine deiodinase, and thioredoxin reductase. Keshan disease, an endemic form of fatal cardiomyopathy that has been observed in children living in a low-selenium area of China, has been firmly linked to selenium deficiency, with administration of selenium having been found to prevent it (*65*). Several clinical selenium-responsive syndromes have been observed in patients receiving prolonged TPN, among whom one patient with an extremely low plasma selenium concentration (9 ng/mL) developed muscle pain and tenderness in the thighs, resulting in an inability to walk (*66*), while another developed a cardiomyopathy and died after a cardiac arrest secondary to septic shock (*67*).

Determining DRIs

Synthesis of selenium-containing protein is strongly associated with selenium intake. The relationship between selenium intake and plasma GPX activity has been particularly well established. In the USA/Canada DRIs, the EAR for selenium was set based on determination of the minimal intake resulting in saturation in plasma GPX activity (45 μg/d for adults with a body weight of 76 kg) (*68*). However, the WHO concluded that selenium deficiency is prevented when 2/3 of the value of saturated plasma GPX activity is maintained (*69*). Based on the results of a Chinese study (*70*), the selenium intake necessary to maintain 2/3 of the value of saturated plasma GPX activity is estimated to be 24.2 μg/d for adults with a body weight of 60 kg. Accordingly, the EAR for selenium in adults aged 18 y and older was calculated by extrapolation of this value using the 0.75th power of a weight ratio. The EAR for children and adolescents aged 1 to 17 y was calculated by extrapolation of this value using the 0.75th power of a weight ratio and a growth factor.

Table 7. Dietary Reference Intakes for chromium (μg/d).[1]

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	0.8	—	—	—	0.8	—
6–11 mo	—	—	1.0	—	—	—	1.0	—
1–2 y	—	—	—	—	—	—	—	—
3–5 y	—	—	—	—	—	—	—	—
6–7 y	—	—	—	—	—	—	—	—
8–9 y	—	—	—	—	—	—	—	—
10–11 y	—	—	—	—	—	—	—	—
12–14 y	—	—	—	—	—	—	—	—
15–17 y	—	—	—	—	—	—	—	—
18–29 y	35	40	—	—	25	30	—	—
30–49 y	35	40	—	—	25	30	—	—
50–69 y	30	40	—	—	25	30	—	—
≥70 y	30	35	—	—	20	25	—	—
Pregnant women					—	—	—	—
Lactating women					—	—	—	—

[1] Computed using the estimated energy requirement for physical activity level II.

Based on average body selenium concentration (250 μg/kg) (*71*) and the sum of placenta and birth weight (3.5 kg), fetal and placental selenium storage is estimated to be approximately 900 μg (250×3.5) during pregnancy. Based on average blood selenium concentration (184 μg/L), the increased selenium requirement due to increase in blood volume (1.5 L) during pregnancy is estimated to be approximately 300 μg (*72*). Because absorption of dietary selenium is estimated to be about 90% (*73*), the additional EAR for pregnancy is estimated to be 4.8 ((900+300)÷280 d÷0.9) μg/d, and the RDA set equal to 120% of the EAR. Based on the average selenium content in the breast milk of Japanese women (17 μg/L) (*40*), the average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*), and the extent of absorption of dietary selenium (90%) (*73*), the additional EAR for lactating women was set at 15 (17×0.78÷0.9) μg/d, and the RDA set equal to 120% of the EAR.

Based on the average selenium concentration in the milk of Japanese women (17 μg/L) (*40*) and the average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*), the AI for infants aged 0 to 5 mo was set at 13.3 (17×0.78) μg/d. The AI for infants aged 6 to 11 mo was determined by extrapolation of 13.3 μg/d using the 0.75th power of a weight ratio.

Based on a Chinese report of chronic selenium intoxication, the NOAEL of selenium is estimated to be 13.3 μg/kg/d (*74*). However, an epidemiological study found that long-term supplementation of 200 μg/d of selenium increased the incidence of Type 2 diabetes in subjects with sufficient selenium intake (*75*), indicating that supplementation at this level causes adverse effects if intake through other sources is adequate. The average selenium intake of the Japanese population is estimated to be approximately 100 μg/d (*76*), which far exceeds the RDA of selenium. Thus, the UL of selenium was set at 300 (100+200) μg/d for men aged 30 to 49 y, whose mean body weight (68.5 kg) is the highest among the sex and age groups. The ULs for other sex and age groups, including children and adolescents, were determined by extrapolation of 300 μg/d using a weight ratio.

Table 6 summarizes the DRIs for selenium.

Chromium

Background information

Trivalent chromium is believed to enhance the action of insulin in the form of a chromium-binding oligopeptide. Patients receiving prolonged TPN without chromium supplementation have been observed to experience glucose intolerance together with several symptoms and disorders, including weight loss, peripheral neuropathy, and low respiratory quotient (*77*). Since these symptoms disappear with administration of trivalent chromium, their origin has been attributed to chromium deficiency.

Determining DRIs

As there is currently no means of determining the metabolic balance of chromium in adults, the USA/Canada DRIs set the AI for chromium based on a chromium intake study (*78*). Because no study has investigated chromium intake in Japan, the EAR was tentatively based on the results of a balance test of chromium in the elderly (*79*), in which a positive balance was observed in subjects whose average chromium intake was 12.8 μg/1,000 kcal. Accordingly, the EAR for adults aged 18 y and older was determined based on the an average chromium intake of 12.8 μg/1,000 kcal and the estimated energy requirement for physical activity level II, and the RDA for chromium set equal to 120% of the EAR. The EAR for children and adolescents aged 1 to 17 y has not been set due to the tentative nature of the

Table 8. Dietary Reference Intakes for molybdenum (μg/d).

Sex	Males				Females			
Age	EAR	RDA	AI	UL	EAR	RDA	AI	UL
0–5 mo	—	—	2	—	—	—	2	—
6–11 mo	—	—	3	—	—	—	3	—
1–2 y	—	—	—	—	—	—	—	—
3–5 y	—	—	—	—	—	—	—	—
6–7 y	—	—	—	—	—	—	—	—
8–9 y	—	—	—	—	—	—	—	—
10–11 y	—	—	—	—	—	—	—	—
12–14 y	—	—	—	—	—	—	—	—
15–17 y	—	—	—	—	—	—	—	—
18–29 y	20	25	—	550	20	20	—	450
30–49 y	25	30	—	600	20	25	—	500
50–69 y	20	25	—	600	20	25	—	500
≥70 y	20	25	—	550	20	20	—	450
Pregnant women					—	—	—	—
Lactating women (amount to be added)					+3	+3	—	—

adult EAR, nor has the EAR for either pregnant women or lactating women, the former due to lack of data and the latter due to an inability to measure absorption of dietary chromium.

Based on the median chromium concentration in milk in Japanese women (1.0 μg/L) (*80*) and the average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*), the AI for infants aged 0 to 5 mo was set at 0.78 μg/d. The AI for infants aged 6 to 11 mo was determined by extrapolation of 0.78 μg/d using the 0.75th power of a weight ratio.

The UL for chromium has not been set because the quantitative relationship between trivalent chromium intake and the possible adverse effects of excessive trivalent chromium intake has been insufficiently established.

Table 7 summarizes the DRIs for chromium.

Molybdenum

Background information

Molybdenum functions as a cofactor for a limited number of enzymes, including xanthine oxidase, aldehyde oxidase, and sulfite oxidase in mammals, and is believed to be an essential trace element in animal nutrition. Human nutritional deficiency of molybdenum was observed in a patient subjected to prolonged TPN (*81*), who manifested clinical symptoms suggestive of sulfite oxidase deficiency. Other symptoms, including irritability, leading to coma, tachycardia, tachypnea, and night blindness, have been reported.

Determining DRIs

The EAR for molybdenum was based on the results of a human balance test of 4 American male subjects (mean body weight, 76.4 kg), all of whom showed a positive balance and no manifestation of any disorder when they ingested 22 μg/d of molybdenum for 102 d (*82*). Based on estimation of integumental and sweat molybdenum loss (3 μg/d) (*83*), the EAR for adults with a body weight of 76.4 kg was calculated to be 25 μg/d. The EAR for adults aged 18 y and older was calculated by extrapolation of 25 μg/d using the 0.75th power of a weight ratio. Since the EAR for adults is based on 1 study of only 4 subjects, the EAR for children and adolescents aged 1 to 17 y has not been set, nor has the additional EAR for pregnant women due to lack of data. Based on the average molybdenum content of the milk of Japanese women (3 μg/L) (*80*, *84*), the average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*), and the extent of absorption of dietary molybdenum (93%) (*85*), the additional EAR for lactating women was set at 3 μg/d (3×0.78÷0.93), and the RDA for molybdenum set equal to 120% of the EAR.

Based on the average molybdenum content of the milk of Japanese women (3 μg/L) (*80*, *84*) and the average intake of breast milk in Japanese infants (0.78 L/d) (*11*, *12*), the AI for infants aged 0 to 5 mo was set at 3 (3×0.78) μg/d. The AI for infants aged 6 to 11 mo was determined by extrapolation of 2.34 μg/d using the 0.75th power of a weight ratio.

Due to the lack of data regarding the dose-dependent adverse effects of excessive molybdenum intake in humans, the UL for molybdenum is based on the NOAEL of molybdenum for rats (900 μg/kg/d) (*86*). Based on the NOAEL and an uncertainty factor of 100, the UL for adults aged 18 y and older was set at 9 μg/kg/d. Due to lack of data, ULs for children and adolescents have not been set.

Table 8 summarizes the DRIs for molybdenum.

Dr. Takatoshi Esashi, who is one of the authors, passed away on March 26, 2012. He was a leader of the working group for minerals in the decision of DRIs for Japanese, 2010. We would like to offer our respectful condolences on his death.

REFERENCES

1) Ministry of Health, Labour and Welfare, Japan. 2011. Outline of the National Health and Nutritional Survey of Japan in 2008, p 195. Dai-ichi Shuppan, Tokyo (in Japanese).
2) Green R, Charlton R, Seftel H, Bothwell T, Mayet F, Adams B, Finch C, Layrisse M. 1968. Body iron excretion in man: a collaborative study. *Am J Med* **45**: 336–353.
3) Yokoi K. 2003. Numerical methods for estimating iron requirements from population data. *Biol Trace Elem Res* **95**: 155–172.
4) Nogami Y. 1966. Studies on the amount of menstruation. *Nippon Funin Gakkai Zasshi* (*Jap J Fert Ster*) **11**: 189–203 (in Japanese).
5) Hawkins WW. 1964. Iron, copper and cobalt. *In*: Nutrition: A Comprehensive Treatise (Beaton GH, McHenry EW, eds), p 309–372. Academic Press, New York.
6) Beaton GH, Corey PN, Steele C. 1989. Conceptual and methodological issues regarding the epidemiology of iron deficiency and their implications for studies of the functional consequences of iron deficiency. *Am J Clin Nutr* **50**: 575–585.
7) Smith NJ, Rios E. 1974. Iron metabolism and iron deficiency in infancy and childhood. *Adv Pediatr* **21**: 239–280.
8) Dallman PR. 1986. Iron deficiency in the weanling: a nutritional problem on the way to resolution. *Acta Paediatr Scand* **323**: 59–67.
9) Bothwell TH, Charlton RW. 1981. Iron Deficiency in Women, p 7–9. The Nutrition Foundation, Washington DC.
10) Hirai Y, Kawakata N, Satoh K, Ikeda Y, Hisayasu S, Orimo H, Yoshino Y. 1990. Concentrations of lactoferrin and iron in human milk at different stages of lactation. *J Nutr Sci Vitaminol* **36**: 531–544.
11) Suzuki K, Sasaki S, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Eiyo-gaku Zasshi* (*Jpn J Nutr Diet*) **62**: 369–372 (in Japanese).
12) Hirose J, Endo M, Nagao S, Mizushima K, Narita H. 2008. Amount of breast milk sucked by Japanese breast feeding infants. *J Jpn Soc Breastfeeding Res* **2**: 23–28 (in Japanese).
13) FAO/WHO Expert Consultation. 1988. Requirements of Vitamin A, Iron, Folate and Vitamin B12. FAO Food and Nutrition Series No. 23, p 33–50. Food and Agriculture Organization of the United Nations, Rome.
14) Barrett JF, Whittaker PG, Williams JG, Lind T. 1994. Absorption of non-haem iron from food during normal pregnancy. *BMJ* **309**: 79–82.
15) FAO/WHO. 1983. Evaluation of certain food additives and contaminants (27th report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696.
16) Food and Drug Administration. 1997. Federal Register 62. 2217–2250, January 15, 1997. Iron-containing supplements and drugs: label warning statements and unit-dose packaging requirements. Downloaded from http://vm.cfsan.fda.gov/~lrd/fr970115.html.
17) Okada A, Takagi Y, Itakura T, Satani M, Manabe H. 1976. Skin lesions during intravenous hyperalimentation: zinc deficiency. *Surgery* **80**: 629–635.
18) Iwata H, Fujisawa S, Takeuchi M, Morishima T, Kubota A. 1990. Two brother cases of acquired acrodermatitis enteropathica caused by mother's milk with low levels of zinc. *Hifuka no Rinsho* **32**: 951–955 (in Japanese).
19) Food and Nutrition Board, Institute of Medicine. 2001. Zinc. *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 442–501. National Academies Press, Washington DC.
20) Jackson MJ, Jones DA, Edwards RH, Swainbank IG, Coleman ML. 1984. Zinc homeostasis in man: Studies using a new stable-dilution technique. *Br J Nutr* **51**: 199–208.
21) Hunt JR, Mullen LK, Lykken GI. 1992. Zinc retention from an experimental diet based on the U.S. F.D.A. total diet study. *Nutr Res* **112**: 1335–1344.
22) Lee DY, Prasad AS, Hydrick-Adair C, Brewer G, Johnson PE. 1993. Homeostasis of zinc in marginal human zinc deficiency: Role of absorption and endogenous excretion of zinc. *J Lab Clin Med* **122**: 549–556.
23) Taylor CM, Bacon JR, Aggett PJ, Bremner I. 1991. Homeostatic regulation of zinc absorption and endogenous losses in zinc-deprived men. *Am J Clin Nutr* **53**: 755–763.
24) Turnlund JR, King JC, Keyes WR, Gong B, Michel MC. 1984. A stable isotope study of zinc absorption in young men: Effects of phytate and alpha-cellulose. *Am J Clin Nutr* **40**: 1071–1077.
25) Wada L, Turnlund JR, King JC. 1985. Zinc utilization in young men fed adequate and low zinc intakes. *J Nutr* **115**: 1345–1354.
26) Turnlund JR, Durkin N, Costa F, Margen S. 1986. Stable isotope studies of zinc absorption and retention in young and elderly men. *J Nutr* **116**: 1239–1247.
27) Suzuki K, Goto S, Kanke Y, Kitano T, Koishi H. 1983. The zinc and copper balance of the preadolescent children. *Nippon Eiyô Shokuryô Gakkaishi* (*J Jpn Soc Nutr Food Sci*) **36**: 231–237 (in Japanese).
28) Swanson CA, King JC. 1987. Zinc and pregnancy outcome. *Am J Clin Nutr* **46**: 763–771.
29) Higashi A, Ikeda T, Uehara I, Matsuda I. 1982. Zinc and copper contents in breast milk of Japanese women. *Tohoku J Exp Med* **137**: 41–47.
30) Ohtake M, Tamura T. 1993. Changes in zinc and copper concentrations in breast milk and blood of Japanese women during lactation. *J Nutr Sci Vitaminol* **39**: 189–200.
31) Sian L, Krebs NF, Westcott JE, Fengliang L, Tong L, Miller LV, Sonko B, Hambidge M. 2002. Zinc homeostasis during lactation in a population with a low zinc intake. *Am J Clin Nutr* **75**: 99–103.
32) Nakano T, Kato K, Kobayashi N, Shimatani M, Ishii K, Takimoto H, Totani M. 2003. Nutrient intake from baby foods, infant formula and cow's milk. Results from a nation-wide infant's dietary survey. *Shoni Hoken Kenkyu* (*J Jpn Soc Child Health*) **62**: 630–639 (in Japanese).
33) Black MR, Medeiros DM, Brunett E, Welke R. 1988. Zinc supplements and serum lipids in young adult white males. *Am J Clin Nutr* **47**: 970–975.
34) Shaw JCL. 1992. Copper deficiency in term and preterm infants. *In*: Nutritional Anemias (Fomon SJ, Zlotkin S, eds), p 105–117. Vevey/Raven Press, New York.
35) Fujita M, Itakura T, Takagi Y, Okada M. 1989. Copper

deficiency during total parenteral nutrition: Clinical analysis of three cases. *J Parent Enter Nutr* **13**: 421–425.
36) Turnlund JR, Keen CL, Smith RG. 1990. Copper status and urinary and salivary copper in young men at three levels of dietary copper. *Am J Clin Nutr* **51**: 658–664.
37) Turnlund JR, Scott KC, Peiffer GL, Jang AM, Keyes WR, Keen CL, Sakanashi TM. 1997. Copper status of young men consuming a low-copper diet. *Am J Clin Nutr* **65**: 72–78.
38) Widdowson EM, Dickerson JWT. 1964. Chemical composition of the body. *In*: Mineral Metabolism: An Advanced Treatise (Comar CL, Bronner F, eds), Vol II, Part A, p 1–248. Academic Press, New York.
39) Turnlund JR, Keyes WR, Peiffer GL, Scott KC. 1998. Copper absorption, excretion, and retention by young men consuming low dietary copper determined by using the stable isotope 65Cu. *Am J Clin Nutr* **67**: 1219–1225.
40) 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 Elem Med Biol* **19**: 171–181.
41) Yoneyama K. 1998. Growth of breast-fed infants and intake of nutrients from breast-milk. *Shoni Hoken Kenkyu (J Jpn Soc Child Health)* **57**: 49–57 (in Japanese).
42) Yoneyama K, Goto I, Nagata H. 1995. Changes in the concentrations of nutrient components of human milk during lactation. *Nippon Koshu Eisei Zasshi (Jpn J Public Health)* **42**: 472–481 (in Japanese).
43) Pratt WB, Omdahl JL, Sorenson JR. 1985. Lack of effects of copper gluconate supplementation. *Am J Clin Nutr* **42**: 681–682.
44) Friedman BJ, Freeland-Graves JH, Bales CW, Behmardi F, Shorey-Kutschke RL, Willis RA, Crosby JB, Trickett PC, Houston SD. 1987. Manganese balance and clinical observation in young men fed manganese-deficient diet. *J Nutr* **117**: 133–143.
45) Freeland-Graves JH, Behmardi F, Bales CW, Dougherty V, Lin PH, Crosby JB, Trickett PC. 1988. Metabolic balance of manganese in young men consuming diets containing five levels of dietary manganese. *J Nutr* **118**: 764–773.
46) Nishimuta M, Inoue N, Kodama N, Morikuni E, Yoshioka YH, Matsuzaki N, Shimada M, Sato N, Iwamoto T, Ohki K, Takeyama H, Nishimuta H. 2006. Moisture and mineral content of human feces-high fecal moisture is associated with increased sodium and decreased potassium content. *J Nutr Sci Vitaminol* **52**: 121–126.
47) Food and Nutrition Board, Institute of Medicine. 2001. Manganese. *In*: Dietary Reference Intake for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (Institute of Medicine, ed), p 394–419. National Academies Press, Washington DC.
48) Shiraishi K. 1994. Intake of trace elements. *Rinsho Eiyo (Jpn J Clin Nutr)* **84**: 381–389 (in Japanese).
49) Johnson PE, Lykken GI, Korynta ED. 1991. Absorption and biological half-life in humans of intrinsic and extrinsic 54Mn tracers from foods of plant origin. *J Nutr* **121**: 711–717.
50) Schroeder HA, Balassa JJ, Tripton IH. 1966. Essential trace metals in man: Manganese. A studying homeostasis. *J Chron Dis* **19**: 545–571.
51) Fuse Y, Saito N, Tsuchiya T, Shishiba Y, Irie M. 2007. Smaller thyroid gland volume with high urinary iodine excretion in Japanese schoolchildren: Normative reference values in an iodine-sufficient area and comparison with the WHO/ICCIDD reference. *Thyroid* **17**: 145–155.
52) Zimmermann MB, Hess SY, Molinari L, De Benoist B, Delange F, Braverman LE, Fujieda K, Ito Y, Jooste PL, Moosa K, Pearce EN, Pretell EA, Shishiba Y. 2004. New reference values for thyroid volume by ultrasound in iodine-sufficient schoolchildren: a World Health Organization/Nutrition for Health and Development Iodine Deficiency Study Group Report. *Am J Clin Nutr* **79**: 231–237.
53) Nagataki S. 2008. The average of dietary iodine intake due to the ingestion of seaweed is 1.2 mg/day in Japan. *Thyroid* **18**: 667–668.
54) Suzuki M, Tamura T. 1985. Iodine intake of Japanese male university students: Urinary iodine excretion of sedentary and physically active students and sweat iodine excretion during exercise. *J Nutr Sci Vitaminol* **31**: 409–415.
55) Katamine S, Mamiya Y, Sekimoto K, Hoshino N, Totsuka K, Naruse U, Watabe A, Sugiyama R, Suzuki M. 1986. Iodine content of various meals currently consumed by urban Japanese. *J Nutr Sci Vitaminol* **32**: 487–495.
56) Food and Nutrition Board, Institute of Medicine. 2001. Iodine. *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 258–289. National Academies Press, Washington DC.
57) Fisher DA, Oddie TH. 1969. Thyroidal radioiodine clearance and thyroid iodine accumulation: contrast between random daily variation and population data. *J Clin Endocrinol Metab* **29**: 111–115.
58) Fisher DA, Oddie TH. 1969. Thyroid iodine content and turnover in euthyroid subjects: validity of estimation of thyroid iodine accumulation from short-term clearance studies. *J Clin Endocrinol Metab* **29**: 721–727.
59) Muramatsu Y, Sumiya M, Ohmomo Y. 1983. Stable iodine contents in human milk related to dietary algae consumption. *Hoken Butsuri* **18**: 113–117.
60) Nishiyama S, Mikeda T, Okada T, Nakamura K, Kotani T, Hishinuma A. 2004. Transient hypothyroidism or persistent hyperthyrotropinemia in neonates born to mothers with excessive iodine intake. *Thyroid* **14**: 1077–1083.
61) Delange F. 1989. Iodine nutrition and congenital hypothyroidism. *In*: Research in Congenital Hypothyroidism (Delange F, Fisher DA, Glinoer D, eds), p 173–185. Plenum press, New York.
62) Konno N, Makita H, Yuri K, Iizuka N, Kawasaki K. 1994. Association between dietary iodine intake and prevalence of subclinical hypothyroidism in the coastal regions of Japan. *J Clin Endocrinol Metab* **78**: 393–397.
63) Konno N, Iizuka N, Kawasaki K, Taguchi H, Miura K, Taguchi S, Murakami K, Hagiwara K, Noda Y, Ukawa S. 1994. Screening for thyroid dysfunction in adults residing in Hokkaido Japan, in relation to urinary iodine concentration and thyroid autoantibodies. *Hokkaido Igaku Zasshi* **69**: 614–626 (in Japanese).
64) Zimmermann MB, Ito Y, Hess SY, Fujieda K, Molinari L. 2005. High thyroid volume in children with excess dietary iodine intake. *Am J Clin Nutr* **81**: 840–844.
65) Chen X, Yang G, Chen J, Chen X, Wen Z, Ge K. 1980. Studies on the relations of selenium and Keshan disease. *Biol Trace Elem Res* **2**: 91–107.
66) van Rij AM, Thomson CD, McKenzie JM, Robinson MF.

1979. Selenium deficiency in total parenteral nutrition. *Am J Clin Nutr* **32**: 2076–2085.

67) Lockitch G, Taylor GP, Wong LT, Davidson AG, Dison PJ, Riddell D, Massing B. 1990. Cardiomyopathy associated with nonendemic selenium deficiency in a Caucasian adolescent. *Am J Clin Nutr* **52**: 572–577.

68) Food and Nutrition Board, Institute of Medicine. 2000. Selenium. *In*: Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (Institute of Medicine, ed), p 284–324. National Academies Press, Washington DC.

69) WHO/FAO/IAEA. 1996. Selenium. *In*: Trace Elements in Human Nutrition and Health, p 105–122. WHO, Geneva.

70) Yang GQ, Ge KY, Chen JS, Chen XS. 1988. Selenium-related endemic diseases and the daily selenium requirement of humans. *World Rev Nutr Diet* **55**: 98–152.

71) Schroeder HA, Frost DV, Balassa JJ. 1970. Essential trace metals in man: selenium. *J Chronic Dis* **23**: 227–243.

72) Himeno S. 2010. Selenium. *Nippon Rinsho* **68** (Suppl 1): 329–332 (in Japanese).

73) Sunde RA. 2006. Selenium. *In*: Present Knowledge in Nutrition (Bowman BA, Russell RM, eds), 9th ed, p 480–497. ILSI Press, Washington DC.

74) Yang GQ, Zhou RH. 1994. Further observations on the human maximum safe dietary selenium intake in a seleniferous area of China. *J Trace Elem Electrolytes Health Dis* **8**: 159–165.

75) Stranges S, Marshall JR, Natarajan R, Donahue RP, Trevisan M, Combs GF, Cappuccio FP, Ceriello A, Reid ME. 2007. Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. *Ann Intern Med* **147**: 217–223.

76) Yoshida M. 1992. Selenium intake and blood selenium level in Japanese. *Nippon Eiyô Shokuryô Gakkaishi* (*J Jpn Soc Nutr Food Sci*) **45**: 485–494 (in Japanese).

77) Jeejeebhoy KN, Chu RC, Marliss EB, Greenberg GR, Bruce-Robertson A. 1977. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. *Am J Clin Nutr* **30**: 531–538.

78) Food and Nutrition Board, Institute of Medicine. 2001. Chromium. *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 197–223. National Academies Press, Washington DC.

79) Bunker VW, Lawson MS, Delves HT, Clayton BE. 1984. The uptake and excretion of chromium by the elderly. *Am J Clin Nutr* **39**: 797–802.

80) Yoshida M, Takada A, Hirose J, Endo M, Fukuwatari T, Shibata K. 2008. Molybdenum and chromium concentrations in breast milk from Japanese women. *Biosci Biotechnol Biochem* **72**: 2247–2250.

81) Abumrad NN, Schneider AJ, Steel D, Rogers LS. 1981. Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. *Am J Clin Nutr* **34**: 2551–2559.

82) Turnlund JR, Keyes WR, Peiffer GL. 1995. Molybdenum absorption, excretion, and retention studied with stable isotopes in young men at five intakes of dietary molybdenum. *Am J Clin Nutr* **62**: 790–796.

83) Food and Nutrition Board, Institute of Medicine. 2001. Molybdenum. *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 420–441. National Academies Press, Washington DC.

84) Yoshida M, Itô C, Hattori H, Tsuchita H, Yonekubo A, Nishimuta M. 2004. Molybdenum contents in human milk and formula milk, and estimation of molybdenum intake of infants in Japan. *Trace Nutr Res* **21**: 59–64 (in Japanese).

85) Yoshida M, Hattori H, Ota S, Yoshihara K, Kodama N, Yoshitake Y, Nishimuta M. 2006. Molybdenum balance in healthy young Japanese women. *J Trace Elem Med Biol* **20**: 245–252.

86) Fungwe TV, Buddingh F, Demick DS, Lox CD, Yang MT, Yang SP. 1990. The role of dietary molybdenum on estrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats. *Nutr Res* **10**: 515–524.

J Nutr Sci Vitaminol, **59**, S103–S109, 2013

Dietary Reference Intakes for Japanese 2010: Lifestage

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(Received October 26, 2012)

Summary The Dietary Reference Intakes for Japanese 2010 (DRIs-J 2010) included a new chapter for lifestage. In this chapter, important characteristics of the nutritional status and the special considerations in applying for DRIs in each lifestage—infants and children, pregnant and lactating women, and the elderly—were described. In infants, the references of nutrient requirement are mostly presented by adequate intake (AI) because of the impossibility of human experiments to determine the estimated average requirement (EAR). The quality and quantity of breast milk is assumed to be nutritionally desirable for every infant. Therefore, AI was determined on the basis value obtained by nutritional concentration and average amount of breast milk consumed by healthy infants. In addition, the anthropometric references for 4 periods based on the 50th percentiles in growth curves were newly demonstrated. The nutrient requirement increased in the pregnant and lactating stage. Increments were estimated based on the fetal growth during whole pregnancy period in pregnant women and on the daily milk production of 780 mL/d in lactating women. In the elderly stage, the scarcity of nutritional studies regarding the Japanese elderly makes it difficult to determine the appropriate DRI values for the elderly. Furthermore, the changes in nutritional status and physical function with aging have been influenced by not only the chronological age but also various other factors, which complicates the establishment of DRIs for the elderly. In light of these facts, the promotion of further and more comprehensive studies of the elderly is desirable.

Key Words infants and children, pregnant and lactating women, elderly, lifestage

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Table 1. Reference values for body size in infants for 4 periods.

Age	Boys		Girls	
	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)
0–2 (1.5) mo	56.2	4.9	54.8	4.6
3–5 (4.5) mo	65.3	7.4	63.7	6.8
6–8 (7.5) mo	69.7	8.5	68.1	7.8
9–11 (10.5) mo	73.2	9.1	71.6	8.5

Table 2. Reference values for body size in infants for 2 periods.

Age	Boys		Girls	
	Height (cm)	Weight (kg)	Height (cm)	Weight (kg)
0–5 (3) mo	61.5	6.4	60.0	5.9
6–11 (9) mo	71.5	8.8	69.9	8.2

Infants and Children

Background

During the early stages of life, special considerations should be taken regarding the nutritional conditions in utero, nutritional intake from breast milk, and nutritional status in all growing stages. The possibility that nutrition in utero and in infants may influence the subsequent health status in adulthood has stressed the importance of maintaining good dietary habits throughout life (*1*).

Infants

There are 2 important assumptions in this stage. Human experiments to determine the estimated average requirement (EAR) are not possible in infants. Further, it has been shown that the quality and quantity of breast milk consumed in healthy infants is nutritionally desirable for them. Therefore, in the Dietary Reference Intakes (DRIs) for infants, adequate intake (AI) was determined on the basis of values obtained by calculating the product of concentration of nutrients and average amount of breast milk consumed by healthy infants.

For infants older than 6 mo, the dietary intake data both from breast milk and from weaning foods were reviewed for a period of 6–8 and 9–11 mo to determine the AI of selected nutrients. As the intake data for these periods were limited, AI of other nutrients was determined by extrapolating the values for 0–5 mo and 1–2 y.

The anthropometric references (Table 1) for 4 periods were based on the 50th percentile in growth curves (1.5, 4.5, 7.5, and 10.5 mo, respectively) as shown in the infant–child growth survey (Ministry of Health, Labour and Welfare, 2000). The reference values for 2 periods are shown in Table 2.

The average amount of breast milk intake in the period before weaning and beginning solid food intake (15 d–5 mo after birth) was considered to be 780 mL/d for Japanese infants according to published reports (*2*, *3*), which was the same value adopted in previous DRIs (2005 version). The average amount of breast milk intake after weaning and during food intake at 6–8 and 9–11 mo was considered to be 600 and 450 mL/d, respectively. In the case that these 2 periods (6–8 and 9–11 mo) are combined to a single period (6–11 mo), the breast milk requirement will be 525 mL/d as the average value.

The data on nutrient concentration in breast milk were adopted from published reports (*4*–*6*) that were thought to be the most appropriate references (Table 3; left). Nutrient intake data for weaning foods adopted from published reports are shown as references for determining the AI (Table 3; right).

Children

In cases where sufficient information was not available to determine the DRIs for children, they were extrapolated from the values for adults (See also "Dietary Reference Intakes for Japanese 2010: Basic Theories for the Development"). Especially for the tolerable upper intake level (UL), due to the scarcity of information, the values for many nutrients could not be determined. It should never be taken as granted that large amounts of intake will not lead to any health impairments.

Special considerations

To utilize the DRIs for nutritional assessment and planning for infants and children, continuous growth monitoring with a growth chart is important in addition to the judgment of shortage/adequacy of nutrient intakes based on the values shown in the DRIs. In spite of the lack of values for UL in this period, choices and amount of the intake of Food with Nutrient Function Claims or other foods fortified with specific nutrients should be more cautiously considered in children than in adults.

Pregnant and Lactating Women

Background

The dietary habits of pregnant and lactating women are important for meeting the nutritional needs of both the women and their children, especially in the early stages of the growth of the child. Recently, nutrition in utero has been considered to affect subsequent health conditions in adulthood. Nutritional management is, therefore, essential and with special consideration to the nutritional status before pregnancy and appropriate range of body weight gain during pregnancy.

Pregnant women

The age-categorized DRI values were increased for pregnant women to consider the fetal growth. These increments were converted to daily values assuming that the pregnancy period lasts for 280 d. The whole pregnancy period was divided into early (under 16 wk), mid (16–27 wk), and late (28 wk and above) gestation (*7*).

Energy and protein intake increments were estimated on the basis of healthy pregnant women who had nor-

Table 3. Nutrient concentration in breast milk and nutrient intake data for complementary foods.

Nutrients			Concentration in breast milk			Intake data for weaning foods	
			0–5 mo	6–8 mo	9–11 mo	6–8 mo	9–11 mo
Protein (g/d)			12.6 g/L	10.6 g/L	9.2 g/L	6.1 g/d	17.9 g/d
Fat		Total fat	35.6 g/L[1]	—	—	—	—
		(% energy)	48.5%	—	—	—	—
		n-6 fatty acids	5.16 g/L	—	—	—	—
		n-3 fatty acids	1.16 g/L	—	—	—	—
Carbohydrates		Carbohydrates	—	—	—	—	—
		Dietary fibers	—	—	—	—	—
Vitamins	Fat-soluble	Vitamin A	411 μgRE/L	—	—	—	—
		Vitamin D	3.05 μg/L	—	—	—	—
		Vitamin E	3.5–4.0 mg/L	—	—	—	—
		Vitamin K	5.17 μg/L	—	—	—	—
	Water-soluble	Vitamin B_1	0.13 mg/L	—	—	—	—
		Vitamin B_2	0.40 mg/L	—	—	—	—
		Niacin	2.0 mg/L	—	—	—	—
		Vitamin B_6	0.25 mg/L	—	—	—	—
		Vitamin B_{12}	0.45 μg/L	—	—	—	—
		Folic acid	54 μg/L	—	—	—	—
		Pantothenic acid	5.0 mg/L	—	—	—	—
		Biotin	5 μg/L	—	—	—	—
		Vitamin C	50 mg/L	—	—	—	—
Minerals	Macro	Sodium	135 mg/L	135 mg/L		487 mg/d	
		Potassium	470 mg/L	470 mg/L		492 mg/d	
		Calcium	250 mg/L	250 mg/L		128 mg/d	
		Magnesium	27 mg/L	27 mg/L		46 mg/d	
		Phosphorus	150 mg/L	150 mg/L		183 mg/d	
	Micro	Iron	0.426 mg/L	—	—	—	—
		Zinc	2 mg/d[2]	—	—	—	—
		Copper	0.35 mg/L	0.16 mg/L		0.20 mg/d	
		Manganese	11 μg/L	11 μg/L		0.44 mg/d	
		Iodine	133 μg/L	—	—	—	—
		Selenium	17 μg/L	—	—	—	—
		Chromium	1.00 μg/L	—	—	—	—
		Molybdenum	3.0 μg/L	—	—	—	—

[1] Calculated by the weight concentration (3.5 g/100 g) and the specific gravity (1.017) of breast milk.
[2] Daily intake from breast milk.

mal sizes before pregnancy, adequate physical activity, and could deliver normal-sized infants at term. Japanese term-born infants have an average birth weight of 3 kg and the corresponding maternal weight gain is estimated to be approximately 11 kg (*8*).

Lactating women

Increments were estimated based on daily milk production of 780 mL/d. Nutrients that are affected by maternal dietary intake or body stores are listed in Table 4.

Special considerations

DRIs for pregnant and lactating women were derived assuming that these women were neither underweight nor obese before pregnancy. For underweight or obese women, special considerations should be taken based on their prevailing health conditions.

Elderly

Background

Japan is facing the unprecedented prospect of a super-aging society. According to a 2008 estimate, the number of individuals aged 70 y and above, the population defined as elderly in the Dietary Reference Intakes for Japanese (DRIs-J), exceeded 20 million. It is predicted that the percentage of the elderly will only increase in coming years, reaching 19.3% for 70 y and above by

Table 4. Factors affecting the nutrient content in breast milk.

Factors	Nutrients
Maternal dietary intake	Fats[1] (*9*, *10*), vitamins A (*11*), C, K (*12*), E (*13*), B_1 (*14*, *15*), B_2 (*14*, *15*), B_6 (*14*, *15*), niacin (*14*, *15*), biotin (*14*, *15*), pantothenic acid (*14*, *15*), manganese (*14*, *15*), selenium (*16*), iodine (*17*)
Maternal body storage	fats (*9*, *10*), vitamin D (*18*), folate (*14*, *15*)
Neither maternal dietary intake nor body storage	protein (*14*, *15*), vitamin B_{12} (*14*, *15*), magnesium (*14*, *15*), calcium (*14*, *15*), phosphorus (*14*, *15*), chromium (*19*), iron (*20*), copper (*20*), zinc (*20*), sodium (*14*, *15*), potassium (*14*, *15*)
Unknown	molybdenum

[1] Fat composition was affected by maternal diet.

2015 (*21*). The review is to present the status of the elderly concerning the nutritional requirements based on the currently available scientific evidence.

Basic concept

Subjects. The typical subjects of the DRIs are "healthy individuals and groups." However, in the case of the elderly, significant changes in physical functioning as a result of aging are common, and in most cases a decline in nutritional intake, absorption, elimination and physical activity level (PAL) are observed.

Moreover, susceptibility to disease is also significantly higher in the elderly. For example, 16% of individuals aged 65 y and above are certified as requiring long-term care, with the number of such health-care users currently 3.5 million nationally (*22*).

In light of these facts, we conducted a review of studies which included the elderly who are able to lead a quasi self-supporting life, i.e., those who have diseases and/or disorders associated with changes in physical functioning as a result of aging, and those who require minor support and/or have minor ailments as their target subjects.

Ages of subjects and definition of aging. Unlike other criteria for age classification of government reports in the Ministry of Health, Labour and Welfare (MHLW) of Japan, those aged 70 y and above are categorized as elderly in the DRIs-J, which reflect differences in basal metabolic rate, etc.

Another possible approach to classifying the elderly would be to regard regressive change in bodily functioning resulting from aging, and not chronological age, as the primary index of aging and senescence. However, no such index has yet been provided for characterizing aging and senescence accurately and objectively.

The degree of functional decline due to aging varies among the elderly, and it has been reported that total mortality was strongly correlated with the degree of functional decline rather than chronological age. For this reason, the appropriate nutritional intake of the elderly should to take into account their current physical and mental condition more than their chronological age.

Changes in digestion, absorption, and metabolism with aging

It is recognized that the elderly are prone to nutritional disorders owing to appetite decline, various diseases and/or defects, defective body functioning, the use of medication, and so on. The elderly experience decreases in gastric-acid secretion due to atrophic gastritis accompanied by bacterial over-proliferation in the small intestine, resulting in a decrease in nutrient absorption from the small intestine. It has recently been suggested that atrophic gastritis and decreased gastric-acid secretion result from *Helicobacter pylori* infection, whose incidence typically increases with advancing age. Nevertheless, the human small intestine is not significantly affected, at least morphologically, by aging (*23*), which suggests that the absorption of nutrients is not greatly affected by changes in the function and morphology of the small intestine. Therefore, there is currently no evidence that aging-related disorders in the absorption of nutrients from the intestinal tract are the main cause of undernutrition in the elderly.

Nutritional intake status of the elderly

Very little data are available concerning age-specific nutritional intake status in elderly community residents. For this reason, data collected by both the NHNS and the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA), a survey of the status of nutritional intake conducted by the National Institute for Longevity Sciences, were examined to clarify the characteristics of the nutritional intake status of the elderly (*24*).

The results indicate that the intake level of the energy and macronutrients—proteins and fats—tends to decrease with age in males (energy 2,139±542, 2,178±578, 2,073±559, 1,898±488, 1,793±523 kcal/d; protein 81.2±23.9, 78.2±23.8, 75.8±23.7, 72.1±20.0, 68.0±25.2 g/d; fat 54.1±22.1, 50.4±23.0, 48.7±21.5, 43.0±19.4, 43.7±22.0 g/d by the NHNS 2006 and energy 2,305±408, 2,226±365, 2,144±375, 2,076±369, 1,927±292 kcal/d; protein 86.8±18.0, 85.3±16.9, 82.2±14.6, 81.2±15.7, 74.0±14.0 g/d; fat 59.2±16.9, 55.7±13.7, 52.9±14.8, 50.8±13.1, 48.9±12.8 g/d in the fourth wave of the NILS-LSA (means±SD), in the elderly aged

60–64, 65–69, 70–74, 75–79, and 80 y and above, respectively). However no significant age-related differences are seen in the intake of other nutrients in either males or females. While these findings could be used to argue against the opinion that the DRIs for the elderly should be further subdivided by age, those making such an argument should carefully consider that the values reflect only intakes and not requirements.

Energy and nutrients relevant to the elderly

Elderly-specific DRIs-J has been obtained only for energy, proteins, calcium, and iron. Energy and each nutrient will be described in further detail below:

Energy. Using the doubly labeled water (DLW) method, the average gross energy expenditure of healthy elderly males and females was found to be 2,141 kcal/d and 1,670 kcal/d, respectively, and the average PAL to be 1.73 and 1.65, respectively (*25*). The reference basal metabolic rate (BMR) of males and females aged 70 y and above has been found to be the same as that of males and females aged 50 to 69 y: 21.5 and 20.7 kcal/(kg body weight·d), respectively. However, as very few reports have examined BMR in the elderly, the reference BMR for the elderly may be revised in light of future evidence.

Regarding body composition, although it has long been thought that fat-free mass declines rapidly in the elderly, particularly in women as a result of menopause, one study revealed that the amount of fat-free mass did not significantly differ before and after menopause (*26*). Since basal metabolic rate is more strongly correlated with fat-free mass than body weight, evaluation of body composition is important in determining a more suitable basal metabolic standard for the elderly.

With respect to PAL, examinations of relevant reports focusing on individuals aged 70 to 80 y identified 1.70 as the reference value for both males and females. The institutionalized elderly tended to have a lower PAL compared to the independent, and the BMR of residents of long-term care facilities in Japan was extremely low, even that of healthy residents (*27*). These findings indicate that elderly should receive an appropriate energy intake based on estimation of their PAL, taking into account not merely individual body size and overall health but also other parameters, such as living conditions.

Based on the findings of previous studies, the estimated energy requirement (EER) for the elderly in terms of PAL 1.70 was determined to be 2,200 kcal/d for male and 1,700 kcal/d for females, respectively.

Protein. Protein requirements for the elderly were calculated using the nitrogen balance method. Several reviews of studies on nitrogen balance suggest that despite decreases in skeletal muscle mass with age, the protein requirements of the elderly are not lower than those of younger individuals per kg of fat-free mass, while some reports suggest that their protein requirement levels should be set higher to maintain muscle mass and strength for the elderly. No definitive conclusions have yet been reached. Currently, the EAR and recommended dietary allowance (RDA) for protein are the principal values applied to the maintenance of nitrogen equilibrium, but it is unknown whether the protein intake above the EAR or RDA is effective in preventing the decline in fat-free mass caused by aging. A decline in PAL, meanwhile, leads to a decline in the protein metabolism of skeletal muscle, thereby suggesting the need for a high protein requirement (*28*), which is also suggested by a decline in energy intake (*29*). Thus, for the elderly and other subjects whose PAL or energy intake decreases, protein requirements should be determined independently of those for healthy individuals.

n-3 fatty acids. The intake of *n*-3 fatty acids reduces the risk of age-related macular degeneration, a serious disease resulting in loss of eyesight (*30*).

Vitamin B. A deficiency in any one of three vitamins—vitamin B_6, vitamin B_{12}, or folic acid—leads to an elevation in plasma homocysteine, which is also elevated with aging. It has been reported that elevated homocysteine level can be a risk factor for cardiovascular diseases (*31*) and dementia (*32*). Although many intervention studies of vitamin B_6, vitamin B_{12}, and folic acid have recently been conducted with the aim of reducing the homocysteine level, no definitive conclusions have emerged regarding the effect of supplementation of these vitamins on diseases in elderly individuals.

Sodium and potassium. Sodium and potassium are well known as nutrients associated with blood pressure regulation and several lifestyle-related diseases. In Japan, the average intake of sodium in the form of salt exceeds the dietary goal for preventing lifestyle-related diseases (DGs) in every age group. Since there is a tendency among the elderly toward even higher intake, they are more greatly encouraged than other age groups to reduce their salt intake for the prevention of lifestyle-related diseases. However, as sodium is strongly involved in the sense of taste, which declines in elderly individuals (*33*), it is important to ensure that adherence to a low-sodium diet does not increase the risk of under nutrition. With respect to potassium, although individuals aged 50 y and above (middle-aged and elderly individuals) have higher potassium intakes than young adults, the 2005 and 2006 NHNS found that the average intake of individuals aged 70 y and above was below the DGs.

Calcium and vitamin D. In a Japanese cohort study, calcium deficiency in elderly individuals was found to be associated with increased risk of not only osteoporosis but also cerebral apoplexy and colorectal cancer. In the 2005 and 2006 NHNS, the average calcium intake for individuals aged 70 y and above was found to be below 600 mg, which is the EAR for males and the RDA for females. In an epidemiological study conducted in Japan, a significant increase in the number of fractures was observed in females with a calcium intake of less than 350 mg/d (*34*). On the other hand, a randomized controlled trial (RCT) of elderly females in New Zealand revealed an increased prevalence of cardiovascular disease with calcium supplementation (*35*). While suitable calcium intake is necessary for those with a low intake, careful attention should be paid to the use of such supplements among the elderly.

Vitamin D, which elevates calcium absorption in the intestinal tract, is an important nutrient for the Japanese, especially for those with relatively low calcium intake. Several studies suggest that poor vitamin D nutritional status increases the risk of osteoporosis, diminished physical functioning, and colorectal cancer (*36*), whereas comparatively high intake of vitamin D helps prevent falls in the elderly. Many elderly individuals, however, suffer from latent vitamin D deficiency, especially those with low PAL. In light of these findings and with the aim of preventing lifestyle-related diseases, it is desirable to maintain a superior vitamin D status among the elderly. Since vitamin D is also produced when the skin is exposed to ultraviolet radiation, not only intakes by foods but also moderate exposure to sunlight effective in elevating serum 25-hydroxyvitamin D (25[OH]D) levels. Obtaining moderate sun exposure is relatively easy in the course of daily life, and thus a recommended way of maintaining sufficient vitamin D levels, particularly in the elderly.

Conclusion

As can be observed, DRIs for nearly half of the nutrients listed are exactly the same as those for adults aged below 70 y. In most other nutrients, the reference for the elderly such as per body weight used the same values as that of younger adults; however, the values differ from these for younger adults because of the differences of reference body weight and actual intake for the elderly.

Elderly-specific DRIs-J has been obtained only for energy, proteins, calcium, and iron.

In DRIs-J 2010, we were able to examine or calculate DRIs specific to the elderly for only a few nutrients because of the scarcity of nutritional data regarding the elderly and the Japanese elderly in particular. We also faced the challenge of the lack of a sound scientific basis concerning the association between actual nutritional status and lifestyle-related diseases. It is currently difficult to comprehensively evaluate age-related changes in physical and morphological functions, and the appropriateness of determining DRIs by treating all those aged 70 y and above as one group remains a debatable problem. To address these difficulties and the challenges that await Japan as it increasingly becomes a super-aging society, the promotion of further and more comprehensive studies and surveys of the elderly is desirable.

REFERENCES

1) Darnton-Hill I, Nishida C, James WP. 2004. A life course approach to diet, nutrition and the prevention of chronic diseases. *Public Health Nutr* **7**: 101–121.
2) Suzuki K, Sasaki S, Shinzawa K, Totani M. 2004. Milk intake by breast-fed infants before weaning. *Jpn J Nutr Diet* **62**: 369–372.
3) 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 Breastfeeding Res* **2**: 23–28.
4) Kamao M, Tsugawa N, Suhara Y, Wada A, Mori T, Murata K, Nishino R, Ukita T, Uenishi K, Tanaka K, Okano T. 2007. Quantification of fat-soluble vitamins in human breast milk by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* **859**: 192–200.
5) Sakurai T, Furukawa M, Asoh M, Kanno T, Kojima T, Yonekubo A. 2005. Fat-soluble and water-soluble vitamin contents of breast milk from Japanese women. *J Nutr Sci Vitaminol* **51**: 239–247.
6) Isomura H. 2007. Analysis of the breast milk—Based on the latest Japanese breast milk. *Obstet Gynecol Pract* **56**: 305–313.
7) Japan Society of Obstetrics and Gynecology, ed. 2008. Glossary of Obstetrics and Gynecology, 2nd Ed.
8) Takimoto H, Sugiyama T, Fukuoka H, Kato N, Yoshiike N. 2006. Maternal weight gain ranges for optimal fetal growth in Japanese women. *Int J Gynaecol Obstet* **92**: 272–278.
9) Helland IB, Saugstad OD, Smith L, Saarem K, Solvoll K, Ganes T, Drevon CA. 2001. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. *Pediatrics* **108**: E82.
10) Lauritzen L, Halkjaer LB, Mikkelsen TB, Olsen SF, Michaelsen KF, Loland L, Bisgaard H. 2006. Fatty acid composition of human milk in atopic Danish mothers. *Am J Clin Nutr* **84**: 190–196.
11) Haskell MJ, Brown KH. 1999. Maternal vitamin A nutriture and the vitamin A content of human milk. *J Mammary Gland Biol Neoplasia* **4**: 243–257.
12) Greer FR, Marshall SP, Foley AL, Suttie JW. 1997. Improving the vitamin K status of breastfeeding infants with maternal vitamin K supplements. *Pediatrics* **99**: 88–92.
13) Olafsdottir AS, Wagner KH, Thorsdottir I, Elmadfa I. 2001. Fat-soluble vitamins in the maternal diet, influence of cod liver oil supplementation and impact of the maternal diet on human milk composition. *Ann Nutr Metab* **45**: 265–272.
14) Allen LH. 2005. Multiple micronutrients in pregnancy and lactation: an overview. *Am J Clin Nutr* **81**: 1206S–1212S.
15) Lonnerdal B. 1986. Effects of maternal dietary intake on human milk composition. *J Nutr* **116**: 499–513.
16) Dorea JG. 2002. Selenium and breast-feeding. *Br J Nutr* **88**: 443–461.
17) Dorea JG. 2002. Iodine nutrition and breast feeding. *J Trace Elem Med Biol* **16**: 207–220.
18) Hollis BW, Wagner CL. 2004. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. *Am J Clin Nutr* **80**: 1752S–1758S.
19) Anderson RA, Bryden NA, Patterson KY, Veillon C, Andon MB, Moser-Veillon PB. 1993. Breast milk chromium and its association with chromium intake, chromium excretion, and serum chromium. *Am J Clin Nutr* **57**: 519–523.
20) Domellof M, Lonnerdal B, Dewey KG, Cohen RJ, Hernell O. 2004. Iron, zinc, and copper concentrations in breast milk are independent of maternal mineral status. *Am J Clin Nutr* **79**: 111–115.
21) National Institute of Population and Social Seculity Research. 2007. Population Projection for Japan: 2006–2055 (December 2006). Tokyo.
22) Ministry of Health, Labour and Welfare. 2008. Report on Long-term Care Insurance Service, 2006. Tokyo.
23) Corazza GR, Frazzoni M, Gatto MR, Gasbarrini G. 1986. Ageing and small-bowel mucosa: a morphometric study.

Gerontology **32**: 60–65.
24) National Center for Geriatrics and Gerontology. National Institute for Longevity Sciences—Longitudinal Study of Aging: NILS-LSA, The Fourth Wave. Available at: http://www.ncgg.go.jp/department/ep/monograph4th/monograph4th.htm. Accessed July 7, 2012.
25) 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.
26) Franklin RM, Ploutz-Snyder L, Kanaley JA. 2009. Longitudinal changes in abdominal fat distribution with menopause. *Metab Clin Exp* **58**: 311–315.
27) Yokoseki T. 1993. Basal metabolic rate and physical activity in the ederly. *J Jpn Soc Nutr Food Sci* **46**: 451–458.
28) Millward DJ, Bowtell JL, Pacy P, Rennie MJ. 1994. Physical activity, protein metabolism and protein requirements. *Proc Nutr Soc* **53**: 223–240.
29) 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: Proceedings of an IDECG Workshop (Scrimshaw NS, Schürch B, eds), IDECG, Lausanne.
30) Chong EW, Kreis AJ, Wong TY, Simpson JA, Guymer RH. 2008. Dietary omega-3 fatty acid and fish intake in the primary prevention of age-related macular degeneration: a systematic review and meta-analysis. *Arch Ophthalmol* **126**: 826–833.
31) Cui R, Moriyama Y, Koike KA, Date C, Kikuchi S, Tamakoshi A, Iso H. 2008. Serum total homocysteine concentrations and risk of mortality from stroke and coronary heart disease in Japanese: The JACC study. *Atherosclerosis* **198**: 412–418.
32) Clarke R. 2008. B-vitamins and prevention of dementia. *Proc Nutr Soc* **67**: 75–81.
33) Fukunaga A, Uematsu H, Sugimoto K. 2005. Influences of aging on taste perception and oral somatic sensation. *J Gerontol Ser A Biol Sci Med Sci* **60**: 109–113.
34) Nakamura K, Kurahashi N, Ishihara J, Inoue M, Tsugane S. 2009. Calcium intake and the 10-year incidence of self-reported vertebral fractures in women and men: the Japan Public Health Centre-based Prospective Study. *Br J Nutr* **101**: 285–294.
35) Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, Ames R, Gamble GD, Grey A, Reid IR. 2008. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *BMJ* **336**: 262–266.
36) Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. 2006. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* **84**: 18–28.

J Nutr Sci Vitaminol, 58, 253–262, 2012

Characteristics of Under- and Over-Reporters of Energy Intake among Young Japanese Women

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(Received February 13, 2011)

Summary Evidence on factors associated with misreporting of energy intake is limited, particularly in non-Western populations. We examined the characteristics of under- and over-reporters of energy intake in young Japanese women. Subjects were 3,956 female Japanese dietetic students aged 18–20 y (mean body mass index: 20.9 kg/m^2). Energy intake was assessed using a comprehensive self-administered diet history questionnaire. Estimated energy requirement was calculated based on self-reported information on age, body height and weight, and physical activity with the use of an equation from the US Dietary Reference Intakes. Under-, acceptable, and over-reporters of energy intake were identified based on the ratio of energy intake to estimated energy requirement, according to whether the individual's ratio was below, within, or above the 95% confidence limits of the expected ratio of 1.0 (<0.70, 0.70–1.30, and >1.30, respectively). Risk of being an under- or over-reporter of energy intake compared to an acceptable reporter was analyzed using multiple logistic regression. The percentage of under-, acceptable, and over-reporters of energy intake was 18.4, 73.1, and 8.4%, respectively. Under-reporting was associated with overweight or obesity, perception that one's own weight was too heavy or light, lower dietary consciousness, active lifestyle, living without family, and living in a city (compared with a metropolitan area). Over-reporting was associated with sedentary lifestyle only. This study of lean young Japanese women showed that energy intake misreporting, particularly under-reporting, was common and differential among populations. Particularly, perceived weight status was associated with under-reporting of energy intake, independent of actual weight status.

Key Words energy intake, under-reporting, body weight, young women, Japan

Although accurate assessment of habitual dietary intake is a prerequisite to studies of diet and health, the difficulty of obtaining dietary data that accurately represents what people usually eat is now generally recognized (*1*). Misreporting of dietary intake is a common phenomenon that appears to occur non-randomly (*1–4*) and to be selective for different kinds of foods and nutrients (*5–9*). The resulting potential for differential errors in dietary data complicates the interpretation of studies on diet and health and, at worst, might produce spurious diet-health relationships (*1, 3, 7*). Increasing our understanding of this serious issue therefore requires the identification of different characteristics associated with different kinds of misreporting of dietary intake.

Energy intake is the foundation of the diet, because all other nutrients must be provided within the quantity of food needed to fulfill the energy requirement. Reported energy intake is therefore a surrogate measure of the total quantity of food intake (*1*). In fact, under-reporting of energy intake has long been considered a serious problem in almost all dietary surveys (*1–4, 6–18*). In particular, overweight and obese people tend to under-report energy intake to a greater extent than lean people (*1–4, 6–18*). Moreover, recent studies have shown that, in addition to under-reporting, over-reporting of energy intake also needs to be taken into account, in some populations at least, such as those with low body mass index (BMI) (*3, 10, 12, 14*). Most of these studies have been conducted in Western countries (*1–3, 5–8, 10–16*), however, and research in non-Western countries such as Japan is sparse (*4, 9, 17, 18*). Because the ways people interpret and respond to dietary assessment may differ between Western countries and Japan, mainly due to large differences in dietary habits and body size, the accuracy of reported dietary intake may also differ, hampering the extrapolation of findings in Western countries to Japanese populations.

Here, to better understand the serious problem of dietary misreporting, the objective of this study was to

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examine differences in dietary and non-dietary characteristics between under-, acceptable, and over-reporters of energy intake in a group of young Japanese women. A characteristic of young Japanese women is their relatively low BMI, which is nevertheless accompanied by excessive weight concerns and a strong desire for thinness (*19*, *20*), a combination seldom observed in other countries. In particular, we investigated the hypothesis whether actual and perceived weight statuses were independently associated with energy intake misreporting in this unique population.

MATERIALS AND METHODS

Study population. The present study was based on data from the Freshmen in Dietetic Courses Study II, a cross-sectional, self-administered questionnaire survey among dietetic students (n=4,679) from 54 institutions in 33 of 47 prefectures in Japan. A detailed description of the study design and survey procedure has been published elsewhere (*21–24*). Briefly, a set of two questionnaires on dietary habits and other lifestyle behaviors during the preceding month was distributed to all students at orientation sessions or early lectures for freshman students who entered dietetic courses in April 2005, in almost all institutions within 2 wk after the course began. In accordance with the survey protocol, answered questionnaires were checked at least twice for completeness by trained survey staff (mostly registered dietitians) and, when necessary, forms were reviewed with the subject to ensure the clarity of answers.

In total, 4,394 students (4,168 women and 226 men) completed both questionnaires (response rate: 93.9%). For the present analysis, we selected female participants aged 18–20 y (n=4,060). We then excluded women who were in an institution where the survey was not conducted within 2 wk of entry (n=98) and those with missing information on the variables used (n=8). As some participants were in more than one exclusion category, the final analysis sample consisted of 3,956 women.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethics committee of the National Institute of Health and Nutrition, Japan. Written informed consent was obtained from all subjects; in this survey, the signature of the student on both of the questionnaires was considered to constitute informed consent by both the student and her parent(s)/caregiver(s).

Dietary intake. Dietary habits during the preceding month were assessed using a comprehensive self-administered diet history questionnaire (DHQ) (*4*, *25–28*). Details of the DHQ's structure and method of calculating dietary intake have been published elsewhere (*4*, *25–28*). Briefly, the DHQ is a structured 16-page questionnaire which asks about the consumption frequency and portion size of selected foods commonly consumed in Japan, as well as general dietary behavior and usual cooking methods (*25*, *28*). Estimates of daily intake for foods (150 items in total), energy, and selected nutrients were calculated using an ad hoc computer algorithm for the DHQ (*25*, *28*) based on the Standard Tables of Food Composition in Japan (*29*). Values of nutrient and food intake were energy-adjusted using the density method (i.e., percentage of energy for energy-providing nutrients and amount per 1,000 kcal of energy for other nutrients and foods) (*9*).

Validity of the DHQ with respect to commonly studied nutritional factors has been investigated (*4*, *25–28*). Briefly, Pearson correlation coefficients were 0.48 for energy, 0.37–0.75 for energy-providing nutrients, and 0.38–0.68 for other nutrients between the DHQ and 3-d estimated dietary records in 47 women (*25*); 0.23 for sodium and 0.40 for potassium between the DHQ and 24-h urinary excretion in 69 women (*26*); 0.66 between the DHQ and serum phospholipid concentrations for marine-origin n-3 polyunsaturated fatty acids (sum of eicosapentenoic, docosapentaenoic, and docosahexaenoic acids) in 44 women (*27*); and 0.56 between the DHQ and serum concentrations for carotene in 42 women (*27*). Further, Pearson correlation coefficients between energy intake derived from the DHQ and total energy expenditure measured by doubly labeled water were 0.34 in 67 men and 0.22 in 73 women (*4*).

Non-dietary factors. Body weight and height were self-reported as part of the DHQ. BMI (kg/m^2) was calculated as body weight (kg) divided by the square of body height (m). Weight status was defined according to World Health Organization recommendations as follows (*30*): underweight (BMI: $<$18.5 kg/m^2), normal (BMI: $\geq$18.5 to $<$25 kg/m^2), overweight (BMI: $\geq$25 to $<$30 kg/m^2), and obese (BMI: $\geq$30 kg/m^2).

In a 12-page questionnaire on nondietary lifestyle during the preceding month, subjects reported self-perceived weight status (too heavy, somewhat heavy, just about right, somewhat light, or too light), whether currently trying to lose weight (no or yes), residential status (living with family, living alone, or living with others), and smoking status (never, former, or current). Dietary consciousness was assessed in the lifestyle questionnaire using the following question: 'How often do you think about diet or nutrients to maintain your health?' and classified into five categories (always, often, sometimes, seldom, or never). Residential areas, reported in the lifestyle questionnaire, were grouped into six regions (Hokkaido and Tohoku; Kanto; Hokuriku and Tokai; Kinki; Chugoku and Shikoku; and Kyushu) and into three municipality levels (ward (i.e., metropolitan area); city; and town and village).

Subjects also reported on the lifestyle questionnaire the time they usually got up and went to bed, which was used to calculate sleeping hours, and the frequency and duration of high-intensity activities (e.g., carrying heavy loads; bicycling, moderate effort; jogging; and singles tennis), moderate-intensity activities (e.g., carrying light loads; bicycling, light effort; and doubles tennis), walking, and sedentary activities (e.g., studying; reading; and watching television) during the preceding month. For subjects whose recorded total hours were $<$24 h, unrecorded hours were assumed to be spent on

sedentary activities. For subjects whose recorded total hours were >24 h, the total number of hours spent daily was proportionately decreased to equal 24. Each activity was assigned a metabolic equivalent value from a previously published table (0.9 for sleeping, 1.5 for sedentary activity, 3.3 for walking, 5.0 for moderate-intensity activity, and 7.0 for high-intensity activity) (*31*). The number of hours spent per day on each activity was multiplied by the metabolic equivalent value of that activity, and all metabolic equivalent-hour products were summed to produce a total metabolic equivalent-hour score for the day. These were then divided by 24 h to give a physical activity level (PAL) value, and classified into four categories (sedentary (PAL: <1.4), low active (PAL: ≥1.4 to <1.6), active (PAL: ≥1.6 to <1.9), and very active (PAL: ≥1.9)) according to the US Dietary Reference Intakes (*32*).

Identification of misreporting of energy intake. We calculated each subject's estimated energy requirement (which is equal to total energy expenditure during weight stability) based on self-reported information on age, body height and weight, and physical activity, with the use of the following equation from the US Dietary Reference Intakes (*32*).

Estimated energy requirement (i.e., total energy expenditure during weight stability) [kcal/d]

=387−7.31×age [y]+physical activity coefficient [1.00 for sedentary, 1.14 for low active, 1.27 for active, and 1.45 for very active]×(10.9×body weight [kg]+660.7×body height [m])

This equation was developed for use in lean to obese women (≥19 y) from a meta-analysis of methodologically sound studies using doubly labeled water as the criterion measure of total energy expenditure (n=433, SE fit: 229.1, R^2: 0.79) (*32*). An investigation using two equations for normal weight women and for overweight women (*32*) provided similar results (data not shown), while an investigation among 18-y-old women (n=3,574) using two equations for normal weight girls (9–18 y) and for overweight girls (*32*) provided similar results (data not shown). In this paper, we present the results derived from all 3,956 women aged 18–20 y using the first-mentioned equation, which had a maximum number of subjects and a minimum number of different sources of error.

Subjects were identified as acceptable, under-, or over-reporters of energy intake based on their ratio of reported energy intake to estimated energy requirement, according to whether the individual's ratio was within, below, or above the 95% confidence limits of the expected ratio of 1.0. The 95% confidence limits (± 2 standard deviation (SD) cut-offs) were calculated according to the following equation (*33–35*).

95% confidence limit

$$=\pm 2\times\sqrt{(CV^2_{rEI}/d+CV^2_{pER}+CV^2_{mTEE})}$$

CV_{rEI} is the within-person coefficient of variation in reported energy intake, d is the number of days of dietary assessment, CV_{pER} is the error in predicted energy requirement equation, and CV_{mTEE} is day-to-day variation in total energy expenditure measured by doubly labeled water (*33–35*). The values used were 23 for CV_{rEI} (*36, 37*), 30 for d (i.e., 1 mo), 11.5 for CV_{pER} (*32*), and 8.2 for CV^2_{mTEE} (*38*). The obtained 95% confidence limit was ±29.5 (%). Thus, acceptable reporters were defined as having a ratio of energy intake to estimated energy requirement in the range 0.70–1.30, under-reporters as a ratio <0.70, and over-reporters as a ratio >1.30.

Statistical analyses. All reported p values are 2-tailed, and p values of <0.05 were considered statistically significant. Mean differences in dietary characteristics between under-, acceptable, and over-reporters of energy intake were tested with one-way analysis of variance (ANOVA). When the overall p from ANOVA was <0.05, the post hoc Bonferroni test was performed. The chi-square test was used to test differences in proportions across categories of energy intake reporting.

The risk of being classified as an under-reporter of energy intake compared to an acceptable reporter, or as an over-reporter compared to an acceptable reporter, was estimated using logistic regression. First, crude odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of being classified as an under- or over-reporter were calculated for each category of factors which are possibly associated with energy intake misreporting, namely weight status (reference: normal), self-perceived weight status (reference: just about right), whether currently trying to lose weight (reference: no), dietary consciousness (reference: always), physical activity (reference: sedentary), smoking status (reference: never), residential status (reference: living with family), region (reference: Hokkaido and Tohoku), and municipality level (reference: ward (i.e., metropolitan area)). Multivariate-adjusted ORs and 95% CIs were then calculated by entering all variables simultaneously into the regression model to assess the genuine effect on risk. All statistical analyses were performed using SAS statistical software (version 9.1, 2003, SAS Institute Inc, Cary, NC, USA).

RESULTS

Mean values of physical characteristics were as follows: 18.1 (SD: 0.3) y for age, 1.58 (SD: 0.05) m for height, 52.3 (SD: 7.7) kg for weight, and 20.9 (SD: 2.8) kg/m^2 for BMI. Dietary characteristics across categories of reporting status of energy intake are shown in Table 1. Mean value of the ratio of energy intake to estimated energy requirement was 0.93 (SD: 0.28). The percentage of under-, acceptable, and over-reporters of energy intake was 18.4, 73.1, and 8.4%, respectively. Energy-adjusted intake of most nutrients and foods differed among the categories of energy reporting status. For nutrients, under-reporters had the highest intake of carbohydrate and the lowest intake of protein, fat, cholesterol, potassium, calcium, and vitamin A. Over-reporters had the highest intake of protein, fat, alcohol, potassium, iron, and vitamin A and the lowest intake of carbohydrate. For foods, under-reporters had the highest intake of rice and noodles and the lowest intake of confectioneries, fats and oils, fish and shellfish, meats, and soft drinks. Over-reporters had the highest intake

Table 1. Dietary characteristics across categories of reporting status of energy intake.

	All (n=3,956)		Under-reporters (n=729; 18.4%)		Acceptable reporters (n=2,893; 73.1%)		Over-reporters (n=334; 8.4%)		p (ANOVA)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Ratio of energy intake to estimated energy requirement	0.93	0.28	0.60[a]	0.08	0.94[b]	0.15	1.56[c]	0.32	<0.0001
Energy intake (kcal/d)	1,827	551	1,235[a]	196	1,840[b]	327	3,009[c]	650	<0.0001
Estimated energy requirement (kcal/d)	1,984	194	2,065[a]	222	1,969[b]	184	1,931[c]	164	<0.0001
Nutrient intake									
Protein (% of energy)	13.3	2.1	12.9[a]	2.2	13.4[b]	2.1	13.6[c]	2.5	<0.0001
Fat (% of energy)	29.5	6.0	26.5[a]	5.9	29.8[b]	5.5	33.9[c]	6.6	<0.0001
Carbohydrate (% of energy)	55.7	6.9	59.0[a]	6.8	55.4[b]	6.4	51.3[c]	7.7	<0.0001
Alcohol (% of energy)	0.3	1.6	0.3[a]	1.5	0.3[a]	1.4	0.6[b]	2.8	0.01
Dietary fiber (g/1,000 kcal)	6.5	2.1	6.5	2.4	6.5	2.0	6.6	2.1	0.88
Cholesterol (mg/1,000 kcal)	163.8	64.1	151.9[a]	71.8	165.8[b]	62.0	172.4[b]	61.4	<0.0001
Sodium (mg/1,000 kcal)	2,117	556	2,098	617	2,123	536	2,108	578	0.51
Potassium (mg/1,000 kcal)	1,079	286	1,047[a]	340	1,079[b]	269	1,142[c]	297	<0.0001
Calcium (mg/1,000 kcal)	266.6	99.7	256.2[a]	112.3	268.0[b]	97.0	276.5[b]	91.1	0.003
Iron (mg/1,000 kcal)	3.7	0.9	3.6[a]	1.0	3.7[a]	0.9	3.8[b]	0.9	0.002
Vitamin A (μg retinol equivalents/1,000 kcal)	290.7	248.9	265.2[a]	287.0	292.0[b]	234.2	335.6[c]	275.0	<0.0001
Folate (μg/1,000 kcal)	152.2	55.1	156.9[a]	69.3	151.2[b]	51.3	151.0[a,b]	51.0	0.04
Vitamin C (mg/1,000 kcal)	48.1	22.7	49.0[a,b]	26.6	47.5[a]	21.5	51.6[b]	23.0	0.004
Food intake (g/1,000 kcal)									
Rice	159.2	70.1	185.0[a]	79.4	157.8[b]	65.2	114.5[c]	64.1	<0.0001
Bread	28.3	21.8	29.2[a]	24.6	28.5[a]	21.2	24.8[b]	19.8	0.005
Noodles	36.8	32.7	43.3[a]	43.0	36.0[b]	30.3	29.1[c]	23.4	<0.0001
Confectioneries	38.1	17.6	35.2[a]	17.9	38.0[b]	16.8	44.9[c]	21.0	<0.0001
Fats and oils	13.6	6.7	11.9[a]	6.4	13.7[b]	6.4	16.3[c]	8.1	<0.0001
Fish and shellfish	30.2	17.7	27.5[a]	17.5	30.4[b]	17.0	34.1[c]	22.8	<0.0001
Meats	33.7	16.9	29.2[a]	14.9	34.2[b]	16.6	39.2[c]	21.1	<0.0001
Dairy products	83.9	71.4	79.9	76.5	85.1	71.0	82.5	62.2	0.20
Vegetables	127.4	81.0	126.4	98.9	126.7	75.0	134.8	87.6	0.22
Fruits	50.0	51.9	47.6[a]	53.8	48.8[a]	49.6	65.6[b]	63.9	<0.0001
Soft drinks	33.4	53.1	24.4[a]	40.1	33.7[b]	54.4	50.2[c]	62.4	<0.0001

[a,b,c] Mean values within a row with different superscript letters are significantly different, $p<0.05$ (post hoc Bonferroni test; when the overall p from ANOVA was <0.05 the post hoc Bonferroni test was performed).

of confectioneries, fats and oils, fish and shellfish, meat, fruits, and soft drinks and the lowest intake of rice, bread, and noodles. No differences were observed among the categories of energy reporting status for dietary fiber, sodium, dairy products, or vegetables.

Table 2 shows non-dietary characteristics across categories of reporting status of energy intake. While the proportion of overweight or obese subjects was small (6.2 and 1.3%, respectively), many subjects perceived their own weight as too heavy or somewhat heavy (17.4 and 57.1%, respectively), suggesting excessive weight concerns in spite of actual leanness. Weight status, self-perceived weight status, whether currently trying to lose weight, physical activity, and residential status was associated with energy reporting status. Under-reporters of energy intake had the highest proportion of overweight and obese subjects, subjects who perceived their own weight as too heavy or too light, subjects currently trying to lose weight, subjects with an active lifestyle, and subjects living alone. Over-reporters had the highest proportion of underweight subjects, subjects with a sedentary lifestyle, and subjects living with family.

ORs and 95% CIs for the risk of being an under-reporter compared to an acceptable reporter of energy intake are shown in Table 3. Results for crude and multivariate-adjusted models were generally similar. In multivariate analysis, overweight and obese, perceiving their own weight as too heavy or light, lower dietary consciousness, active lifestyle, living without family, and living in a city were associated with a higher risk of being an under-reporter of energy intake. Currently trying to lose weight was associated with a higher risk of being an under-reporter in the crude model, but the association disappeared after consideration of other factors.

Table 4 shows ORs and 95% CIs for the risk of being an over-reporter compared to an acceptable reporter of energy intake. Results for crude and multivariate-adjusted models were generally similar again. On multi-

Table 2. Non-dietary characteristics across categories of reporting status of energy intake.

	All (n=3,956)		Under-reporters (n=729; 18.4%)		Acceptable reporters (n=2,893; 73.1%)		Over-reporters (n=334; 8.4%)		p[1]
	n	%	n	%	n	%	n	%	
Weight status									<0.0001
Underweight (BMI: <18.5 kg/m^2)	576	14.6	83	11.4	427	14.8	66	19.8	
Normal (BMI: ≥18.5 to <25 kg/m^2)	3,080	77.9	545	74.8	2,287	79.1	248	74.3	
Overweight (BMI: ≥25 to <30 kg/m^2)	247	6.2	77	10.6	151	5.2	19	5.7	
Obese (BMI: ≥30 kg/m^2)	53	1.3	24	3.3	28	1.0	1	0.3	
Self-perceived weight status									<0.0001
Too heavy	690	17.4	200	27.4	430	14.9	60	18.0	
Somewhat heavy	2,260	57.1	386	53.0	1,702	58.8	172	51.5	
Just about right	830	21.0	113	15.5	637	22.0	80	24.0	
Somewhat light	151	3.8	22	3.0	111	3.8	18	5.4	
Too light	25	0.6	8	1.1	13	0.5	4	1.2	
Currently trying to lose weight									0.003
No	2,528	63.9	426	58.4	1,889	65.3	213	63.8	
Yes	1,428	36.1	303	41.6	1,004	34.7	121	36.2	
Dietary consciousness									0.42
Always	775	19.6	136	18.7	578	20.0	61	18.3	
Often	2,162	54.7	381	52.3	1,597	55.2	184	55.1	
Sometimes	571	14.4	113	15.5	410	14.2	48	14.4	
Seldom	390	9.9	84	11.5	269	9.3	37	11.1	
Never	58	1.5	15	2.1	39	1.4	4	1.2	
Physical activity									<0.0001
Sedentary	2,323	58.7	321	44.0	1,769	61.2	233	69.8	
Low active	1,317	33.3	305	41.8	927	32.0	85	25.5	
Active	242	6.1	76	10.4	150	5.2	16	4.8	
Very active	74	1.9	27	3.7	47	1.6	0	0	
Smoking status									0.30
Never	3,827	96.7	698	95.8	2,809	97.1	320	95.8	
Former	68	1.7	15	2.1	46	1.6	7	2.1	
Current	61	1.5	16	2.2	38	1.3	7	2.1	
Residential status									0.0002
Living with family	3,508	88.7	612	84.0	2,592	89.6	304	91.0	
Living alone	365	9.2	96	13.2	247	8.5	22	6.6	
Living with others	83	2.1	21	2.9	54	1.9	8	2.4	
Region									0.44
Hokkaido and Tohoku	388	9.8	69	9.5	293	10.1	26	7.8	
Kanto	1,358	34.3	230	31.6	1,003	34.7	125	37.4	
Hokuriku and Tokai	552	14.0	110	15.1	392	13.6	50	15.0	
Kinki	783	19.8	139	19.1	581	20.1	63	18.9	
Chugoku and Shikoku	427	10.8	93	12.8	302	10.4	32	9.6	
Kyushu	448	11.3	88	12.1	322	11.1	38	11.4	
Municipality level									0.047
Ward (i.e., metropolitan area)	784	19.8	122	16.7	598	20.7	64	19.2	
City	2,570	65.0	505	69.3	1,855	64.1	210	62.9	
Town and village	602	15.2	102	14.0	440	15.2	60	18.0	

[1] Chi-square test.

variate analysis, a higher risk of being an over-reporter of energy intake was associated with sedentary lifestyle only. Underweight was associated with higher risk of being an over-reporter in crude model, but the association disappeared after consideration of other factors.

DISCUSSION

In this study in lean young Japanese women, misreporting, particularly under-reporting, of energy intake was common and differently distributed among populations. Under-reporting was associated with overweight or obesity, perceiving one's own weight as too heavy or

Table 3. Risk of being an under-reporter of energy intake compared to being an acceptable reporter of energy intake.

	n of under-reporters/ acceptable reporters	Crude model[1]			Multivariate-adjusted model[2]		
		OR	95% CI	*p*	OR	95% CI	*p*
Weight status							
Underweight (BMI:<18.5 kg/m^2)	83/427	0.82	0.63, 1.05	0.11	0.91	0.66, 1.25	0.55
Normal (BMI: 18.5 to<25 kg/m^2)	545/2,287	1 (reference)			1 (reference)		
Overweight (BMI: 25 to<30 kg/m^2)	77/151	2.14	1.60, 2.86	<0.0001	1.52	1.10, 2.12	0.01
Obese (BMI: 30 kg/m^2)	24/28	3.60	2.07, 6.25	<0.0001	2.68	1.48, 4.86	0.001
Self-perceived weight status							
Too heavy	200/430	2.62	2.02, 3.40	<0.0001	2.03	1.47, 2.79	<0.0001
Somewhat heavy	386/1,702	1.28	1.02, 1.61	0.04	1.19	0.92, 1.53	0.19
Just about right	113/637	1 (reference)			1 (reference)		
Somewhat light	22/111	1.12	0.68, 1.84	0.66	1.17	0.69, 1.99	0.57
Too light	8/13	3.47	1.41, 8.56	0.007	4.06	1.57, 10.50	0.004
Currently trying to lose weight							
No	426/1,889	1 (reference)			1 (reference)		
Yes	303/1,004	1.34	1.13, 1.58	0.0006	1.11	0.93, 1.34	0.25
Dietary consciousness							
Always	136/578	1 (reference)			1 (reference)		
Often	381/1,597	1.01	0.82, 1.26	0.90	1.14	0.91, 1.44	0.26
Sometimes	113/410	1.17	0.89, 1.55	0.27	1.28	0.95, 1.72	0.11
Seldom	84/269	1.33	0.98, 1.81	0.07	1.54	1.11, 2.14	0.01
Never	15/39	1.64	0.88, 3.05	0.12	2.23	1.16, 4.28	0.02
Physical activity							
Sedentary	321/1,769	1 (reference)			1 (reference)		
Low active	305/927	1.81	1.52, 2.16	<0.0001	1.92	1.60, 2.31	<0.0001
Active	76/150	2.79	2.07, 3.77	<0.0001	3.28	2.40, 4.48	<0.0001
Very active	27/47	3.17	1.94, 5.16	<0.0001	3.90	2.36, 6.47	<0.0001
Smoking status							
Never	698/2,809	1 (reference)			1 (reference)		
Former	15/46	1.31	0.73, 2.36	0.37	1.08	0.58, 2.01	0.81
Current	16/38	1.70	0.94, 3.06	0.08	1.45	0.78, 2.70	0.24
Residential status							
Living with family	612/2,592	1 (reference)			1 (reference)		
Living alone	96/247	1.65	1.28, 2.12	0.0001	1.95	1.50, 2.55	<0.0001
Living with others	21/54	1.65	0.99, 2.75	0.06	1.79	1.05, 3.05	0.03
Region							
Hokkaido and Tohoku	69/293	1 (reference)			1 (reference)		
Kanto	230/1,003	0.97	0.72, 1.31	0.86	0.88	0.64, 1.21	0.43
Hokuriku and Tokai	110/392	1.19	0.85, 1.67	0.31	1.08	0.75, 1.56	0.68
Kinki	139/581	1.02	0.74, 1.40	0.92	0.89	0.64, 1.26	0.52
Chugoku and Shikoku	93/302	1.31	0.92, 1.86	0.13	1.05	0.72, 1.53	0.79
Kyushu	88/322	1.16	0.82, 1.65	0.41	1.15	0.79, 1.68	0.47
Municipality level							
Ward (i.e., metropolitan area)	122/598	0.75	0.60, 0.93	0.01	0.71	0.56, 0.90	0.005
City	505/1,855	1 (reference)			1 (reference)		
Town and village	102/440	0.85	0.67, 1.08	0.18	0.85	0.66, 1.09	0.20

[1] Each of the variables listed was entered into the model separately.
[2] All the variables listed were entered into the model simultaneously.

light, lower dietary consciousness, active lifestyle, living without family, and living in a city (compared with a ward (metropolitan area)); while over-reporting was associated with sedentary lifestyle. The most impressive finding was the association of perceived weight status with energy under-reporting, independent of actual weight status. To our knowledge, this is the first study to examine characteristics associated with under- and over-reporting of energy intake in young Japanese women, with consideration of individual physical activity level.

In this study of young Japanese women, about one-

Table 4. Risk of being an over-reporter of energy intake compared to being an acceptable reporter of energy intake.

	n of over-reporters/ acceptable reporters	Crude model[1]			Multivariate-adjusted model[2]		
		OR	95% CI	*p*	OR	95% CI	*p*
Weight status							
Underweight (BMI: <18.5 kg/m^2)	66/427	1.43	1.07, 1.91	0.02	1.33	0.92, 1.90	0.13
Normal (BMI: ≥18.5 to<25 kg/m^2)	248/2,287	1 (reference)			1 (reference)		
Overweight (BMI: ≥25 to<30 kg/m^2)	19/151	1.16	0.71, 1.90	0.56	0.93	0.54, 1.59	0.79
Obese (BMI: ≥30 kg/m^2)	1/28	0.33	0.05, 2.43	0.28	0.20	0.03, 1.53	0.12
Self-perceived weight status							
Too heavy	60/430	1.11	0.78, 1.59	0.56	1.21	0.79, 1.86	0.38
Somewhat heavy	172/1,702	0.81	0.61, 1.07	0.13	0.85	0.62, 1.17	0.32
Just about right	80/637	1 (reference)			1 (reference)		
Somewhat light	18/111	1.29	0.75, 2.24	0.36	1.17	0.66, 2.09	0.58
Too light	4/13	2.45	0.78, 7.70	0.12	2.22	0.69, 7.18	0.18
Currently trying to lose weight							
No	213/1,889	1 (reference)			1 (reference)		
Yes	121/1,004	1.07	0.84, 1.35	0.58	1.20	0.92, 1.55	0.17
Dietary consciousness							
Always	61/578	1 (reference)			1 (reference)		
Often	184/1,597	1.09	0.81, 1.48	0.57	1.08	0.79, 1.48	0.63
Sometimes	48/410	1.11	0.75, 1.65	0.61	1.13	0.75, 1.70	0.57
Seldom	37/269	1.30	0.85, 2.01	0.23	1.27	0.81, 1.99	0.30
Never	4/39	0.97	0.34, 2.81	0.96	0.84	0.29, 2.47	0.75
Physical activity							
Sedentary	233/1,769	1 (reference)			1 (reference)		
Low active	85/927	0.70	0.54, 0.90	0.007	0.68	0.53, 0.89	0.005
Active	16/150	0.81	0.48, 1.38	0.44	0.78	0.45, 1.33	0.36
Very active	0/47	—	—	—	—	—	—
Smoking status							
Never	320/2,809	1 (reference)			1 (reference)		
Former	7/46	1.34	0.60, 2.98	0.48	1.19	0.53, 2.71	0.67
Current	7/38	1.62	0.72, 3.65	0.25	1.60	0.69, 3.68	0.27
Residential status							
Living with family	304/2,592	1 (reference)			1 (reference)		
Living alone	22/247	0.76	0.48, 1.19	0.23	0.76	0.48, 1.20	0.24
Living with others	8/54	1.26	0.60, 2.68	0.54	1.25	0.58, 2.68	0.57
Region							
Hokkaido and Tohoku	26/293	1 (reference)			1 (reference)		
Kanto	125/1,003	1.40	0.90, 2.18	0.13	1.43	0.91, 2.25	0.12
Hokuriku and Tokai	50/392	1.44	0.87, 2.36	0.15	1.38	0.82, 2.32	0.23
Kinki	63/581	1.22	0.76, 1.97	0.41	1.24	0.76, 2.02	0.40
Chugoku and Shikoku	32/302	1.19	0.69, 2.05	0.52	1.23	0.70, 2.15	0.48
Kyushu	38/322	1.33	0.79, 2.24	0.29	1.31	0.76, 2.25	0.34
Municipality level							
Ward (i.e., metropolitan area)	64/598	0.95	0.70, 1.27	0.71	1.04	0.76, 1.42	0.83
City	210/1,855	1 (reference)			1 (reference)		
Town and village	60/440	1.21	0.89, 1.63	0.23	1.19	0.87, 1.63	0.27

[1] Each of the variables listed was entered into the model separately.
[2] All the variables listed were entered into the model simultaneously.

fourth of the participants were classified as either under- or over-reporters of energy intake (18.4 and 8.4%, respectively). In Western countries, the percentage of under-reporters ranged from 3 to 54% and that of over-reporters from 0.1 to 22% (*2, 3, 6, 7, 10–16*). In a Japanese study using total energy expenditure measured by doubly labeled water (n=140), 44% of subjects were defined as under-reporters and 20% as over-reporters (*4*). Other studies in Japan using the ratio of reported energy intake to estimated basal metabolic rate without consideration of individual physical activity reported that the prevalence of under-reporters was 20–37% while that of over-reporters was 2–10% (*17, 18*). Although comparisons of the prevalence of misre-

porting of energy intake between studies are hampered by differences in the criteria used to classify under- and over-reporting, dietary assessment instruments, and population characteristics, these findings suggest that not only under- but also over-reporting of energy intake is likely in many dietary surveys in both Western and Japanese populations.

In this lean Japanese population, we found that overweight and obese subjects were more likely to underreport energy intake. This finding is consistent with numerous previous findings in Western countries (*1–3, 6, 7, 10–16*) and Japan (*4, 17, 18*). Further, subjects who perceived their own weight as too heavy were predominant, and were more likely to under-report energy intake, independent of their actual weight status. Moreover, under-reporting was also independently associated with perceiving one's weight as too light. This may be due to the excessive weight concerns and strong desire for thinness commonly observed in young Japanese women, irrespective of actual weight status (*19, 20*). A similar independent influence of both actual weight status and perceived weight consciousness on underreporting has been observed in other obese populations (*10, 14*).

In this study, higher physical activity was associated with under-reporting of energy intake. This appears reasonable, given that active subjects with greater energy requirements can fall into the category of underreporting (*39*). A similar association was observed in Japanese adult men and women (*4*). Although several studies have suggested an association between smoking status and energy misreporting (*1, 3, 7, 14, 16*), we found no such association, possibly due to the small percentage of former and current smokers in the present study. We found some influence of variables related to residence (residential status and municipality level) on energy under-reporting, which is in accordance with several previous studies (*3, 14*). In contrast to a previous study (*14*), lower dietary consciousness was associated with energy under-reporting, which may reflect carelessness or poor memory of dietary habits, or factors potentially associated with dietary reporting such as knowledge of food and diet and enthusiasm in dietary assessment (*18*).

While previous studies have suggested several lifestyle factors as a risk factor of energy over-reporting, including low BMI (*3, 10, 12, 14*), none of these factors, including weight status, was associated with the risk of an being over-reporter in this study of relatively lean young Japanese women (except for sedentary lifestyle). On this basis, over-reporting may be a random rather than a systematic phenomenon compared with underreporting, in the present population at least.

Consistent with previous Western studies (*1, 3, 7, 10, 12–14, 16*), energy-adjusted nutrient and food intakes differed among under-, acceptable, and over-reporters of energy intake, although nutrient and food intake in Japanese subjects appears to provide no clue as to whether the diet of under- and over-reporters is healthier or unhealthier than that of acceptable reporters (*9, 17*). This supports the hypothesis that the under- and overreporting of foods is selective and that this selective misreporting affects the energy-adjusted nutrient and food intake in a biased way (*5–9*), which in turn affects the diet-disease relationships thereby obtained (*1, 3, 7*).

Several limitations of the present study deserve mention. First, the participants selected were female dietetic students, not a random sample of Japanese people. To minimize the influence of nutritional education, the present survey was conducted in most institutions within 2 wk after the course began. Nevertheless, the participants may have had healthier dietary habits and lifestyles than the general population, although with regard to the reported intake of energy, fat, and carbohydrate and BMI at least, mean and SD values in the present study were reasonably comparable to those of a representative sample of Japanese women aged 15–19 y (1,852 (SD: 480) kcal/d, 29.3% (SD: 6.8%) of energy, 55.5% (SD: 7.8%) of energy, and 20.7 (SD: 3.0) kg/m^2, respectively) (*40*). Our results might not therefore be extrapolatable to the general Japanese population.

At present, the only way to obtain unbiased information on energy requirements in free-living settings is to use doubly labeled water as a biomarker (*1*). This technique is expensive and impractical for application to large-scale epidemiologic studies, and alternative procedures are accordingly used (*3, 7–18*). In the present study, we calculated estimated energy requirements based on self-reported information on age, body height and weight, and physical activity with the use of an equation from the US Dietary Reference Intakes (*32*). Although the equation was developed based on a large number of highly accurate measurements of total energy expenditure by the doubly labeled water method, these were predominantly conducted in Caucasians (*32*), and might therefore be inappropriate for the present Japanese population. Moreover, this calculation used self-reported rather than measured body weight and height, although previous studies have generally shown that while weights are on average underestimated and heights are on average overestimated, the correlations between self-reported and measured values are markedly high (*41, 42*). Additionally, we are unable to determine whether the associations found between misreporting of energy intake and several characteristics are true, or were artifacts caused by the procedure used to identify misreporters or to calculate energy requirements.

Energy intake was assessed using a self-administered dietary assessment questionnaire (i.e., DHQ). Actual dietary habits were not observed and, as is often the case in such dietary questionnaires (*6, 43–46*), the validity of the DHQ in terms of energy intake appears somewhat insufficient against total energy expenditure as measured by doubly labeled water (*4*). Thus, the present findings might be specific to this dietary assessment questionnaire and should be interpreted in this context, albeit there is some evidence that people tend to report dietary intake similarly across dietary assessment methods (*1*).

All the variables used in this study were based on

self-reporting, which might have been biased and hence influenced the results. For example, BMI calculated based on self-reported measures are generally underestimated, although the correlation between self-reported and measured BMI is markedly high (*41*, *42*). It is thus likely that the percentages of overweight and obese subjects based on self-reported data in this study are underestimated, which might have influenced the results by attenuating or strengthening the association.

In conclusion, this study in lean young Japanese women showed that misreporting, particularly under-reporting, of energy intake was common and differently distributed among populations. Under-reporting was associated with overweight or obesity, perception that one's weight was too heavy or light, lower dietary consciousness, active lifestyle, living without family, and living in a city (compared with a ward (metropolitan area)); while over-reporting was associated with a sedentary lifestyle. The most impressive finding was the association of perceived weight status with energy under-reporting, independent of actual weight status. These results suggest that dietary data in young Japanese women should be treated and interpreted with marked caution. Further studies are needed to examine whether the associations observed in the present study are commonly observed across different dietary assessment methods and in other populations.

Acknowledgments

This study was supported by grants from the Ministry of Health, Labour, and Welfare of Japan. None of the authors had any personal or financial conflict of interest to declare.

REFERENCES

1) Livingstone MBE, Black AE. 2003. Markers of the validity of reported energy intake. *J Nutr* **133**: 895S–920S.
2) Tooze JA, Subar AF, Thompson FE, Troiano R, Schatzkin A, Kipnis V. 2004. Psychosocial predictors of energy underreporting in a large doubly labeled water study. *Am J Clin Nutr* **79**: 795–804.
3) Mattisson I, Wirfalt E, Aronsson CA, Wallstrom P, Sonestedt E, Gullberg B, Berglund G. 2005. Misreporting of energy: prevalence, characteristics of misreporters and influence on observed risk estimates in the Malmo Diet and Cancer cohort. *Br J Nutr* **94**: 832–842.
4) Okubo H, Sasaki S, Rafamantanantsoa HH, Ishikawa-Takata K, Okazaki K, Tabata I. 2008. Validation of self-reported energy intake by a self-administered diet history questionnaire using the doubly labeled water method in 140 Japanese adults. *Eur J Clin Nutr* **62**: 1343–1350.
5) Poppitt SD, Swann D, Black AE, Prentice AM. 1998. Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *Int J Obes Relat Metab Disord* **22**: 303–311.
6) Subar AF, Kipnis V, Troiano RP, Midthune D, Schoeller DA, Bingham S, Sharbaugh CO, Trabulsi J, Runswick S, Ballard-Barbash R, Sunshine J, Schatzkin A. 2003. Using intake biomarkers to evaluate the extent of dietary misreporting in a large sample of adults: the OPEN study. *Am J Epidemiol* **158**: 1–13.
7) Rosell MS, Hellenius MLB, De Faire UH, Johansson GK. 2003. Associations between diet and the metabolic syndrome vary with the validity of dietary intake data. *Am J Clin Nutr* **78**: 84–90.
8) Heitmann BL, Lissner L. 1995. Dietary underreporting by obese individuals: is it specific or non-specific? *BMJ* **311**: 986–989.
9) 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.
10) Lutomski JE, van den Broeck J, Harrington J, Shiely F, Perry IJ. 2011. Sociodemographic, lifestyle, mental health and dietary factors associated with direction of misreporting of energy intake. *Public Health Nutr* **14**: 532–541.
11) Nielsen BM, Nielsen MM, Toubro S, Pedersen O, Astrup A, Sorensen TI, Jess T, Heitmann BL. 2009. Past and current body size affect validity of reported energy intake among middle-aged Danish men. *J Nutr* **139**: 2337–2343.
12) Klingberg S, Hallenberg E, Lorentzon M, Mellstrom D, Ohlsson C, Hulthen L. 2008. Characteristics of under- and over-reporters of energy intake among 18–20-year-old males: the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study. *Public Health Nutr* **11**: 1117–1123.
13) Bedard D, Shatenstein B, Nadon S. 2004. Underreporting of energy intake from a self-administered food-frequency questionnaire completed by adults in Montreal. *Public Health Nutr* **7**: 675–681.
14) Johansson L, Solvoll K, Bjorneboe GEA, Drevon CA. 1998. Under- and overreporting of energy intake related to weight status and lifestyle in a nationwide sample. *Am J Clin Nutr* **68**: 266–274.
15) Ferrari P, Slimani N, Ciampi A, Trichopoulou A, Naska A, Lauria C, Veglia F, Bueno-de-Mesquita HB, Ocke MC, Brustad M, Braaten T, Tormo JM, Amiano P, Mattisson I, Johansson G, Welch A, Davey G, Overvad K, Tjonneland A, Clavel-Chapelon F, Thiebaut A, Linseisen J, Boeing H, Hemon B, Riboli E. 2002. Evaluation of under- and over-reporting of energy intake in the 24-hour diet recalls in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* **5**: 1329–1345.
16) Johansson G, Wikman A, Ahren AM, Hallmans G, Johansson I. 2001. Underreporting of energy intake in repeated 24-hour recalls related to gender, age, weight status, day of interview, educational level, reported food intake, smoking habits. *Public Health Nutr* **4**: 919–927.
17) Okubo H, Sasaki S. 2004. Underreporting of energy intake among Japanese women aged 18–20 years and its association with reported nutrient and food group intakes. *Public Health Nutr* **7**: 911–917.
18) 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 to relative accuracy of energy intake among Japanese adults. *Public Health Nutr* **9**: 651–657.
19) Hayashi F, Takimoto H, Yoshita K, Yoshiike N. 2006. Perceived body size and desire for thinness of young Japanese women: a population-based survey. *Br J Nutr* **96**: 1154–1162.
20) Kagawa M, Kuroiwa C, Uenishi K, Mori M, Dhaliwal S, Hills AP, Binns CW. 2007. A comparison of body perceptions in relation to measured body composition in young

Japanese males and females. *Body Image* **4**: 372–380.
21) Murakami K, Sasaki S, Okubo H, Takahashi Y, Hosoi Y, Itabashi M. 2007. Association between dietary fiber, water and magnesium intake and functional constipation among young Japanese women. *Eur J Clin Nutr* **61**: 616–622.
22) Murakami K, Sasaki S, Okubo H, Takahashi Y, Hosoi Y, Itabashi M. 2007. Dietary fiber intake, dietary glycemic index and load, and body mass index: a cross-sectional study of 3931 Japanese women aged 18–20 years. *Eur J Clin Nutr* **61**: 986–995.
23) Murakami K, Sasaki S, Okubo H, Takahashi Y, Hosoi Y, Itabashi M. 2007. Monetary costs of dietary energy reported by young Japanese women: association with food and nutrient intake and body mass index. *Public Health Nutr* **10**: 1430–1439.
24) Murakami K, Sasaki S, Okubo H, Takahashi Y. 2009. Neighborhood socioeconomic status in relation to dietary intake and body mass index in female Japanese dietetic students. *Nutrition* **25**: 745–752.
25) Sasaki S, Yanagibori R, Amano K. 1998. Self-administered diet history questionnaire developed for health education: a relative validation of the test-version by comparison with 3-day diet record in women. *J Epidemiol* **8**: 203–215.
26) Sasaki S, Yanagibori R, Amano K. 1998. Validity of a self-administered diet history questionnaire for assessment of sodium and potassium: comparison with single 24-hour urinary excretion. *Jpn Circ J* **62**: 431–435.
27) Sasaki S, Ushio F, Amano K, Morihara M, Todoriki T, Uehara Y, Toyooka T. 2000. Serum biomarker-based validation of a self-administered diet history questionnaire for Japanese subjects. *J Nutr Sci Vitaminol* **46**: 285–296.
28) Murakami K, Sasaki S, Takahashi Y, Okubo H, Hirota N, Notsu A, Fukui M, Date C. 2008. Reproducibility and relative validity of dietary glycaemic index and load assessed with a self-administered diet-history questionnaire in Japanese adults. *Br J Nutr* **99**: 639–648.
29) Science and Technology Agency. 2005. Standard Tables of Food Composition in Japan, 5th revised and enlarged ed. Printing Bureau of the Ministry of Finance, Tokyo (in Japanese).
30) World Health Organization. 1998. Obesity: Preventing and Managing the Global Epidemic. International Obesity Task Force. World Health Organization, Geneva.
31) Ainsworth BE, Haskell WL, Leon AS, Jacobs DR Jr, Montoye HJ, Sallis JF, Paffenbarger RS Jr. 1993. Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* **25**: 71–80.
32) Institute of Medicine. 2002. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids, Part I. National Academy Sciences, Washington, DC.
33) McCrory MA, Hajduk CL, Roberts SB. 2002. Procedures for screening out inaccurate reports of dietary energy intake. *Public Health Nutr* **5**: 873–882.
34) Huang TT, Roberts SB, Howarth NC, McCrory MA. 2005. Effect of screening out implausible energy intake reports on relationships between diet and BMI. *Obes Res* **13**: 1205–1217.
35) Huang TT, Howarth NC, Lin BH, Roberts SB, McCrory MA. 2004. Energy intake and meal portions: associations with BMI percentile in U.S. children. *Obes Res* **12**: 1875–1885.
36) Black AE. 2000. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord* **24**: 1119–1130.
37) Black AE. 2000. The sensitivity and specificity of the Goldberg cut-off for EI:BMR for identifying diet reports of poor validity. *Eur J Clin Nutr* **54**: 395–404.
38) Black AE, Cole TJ. 2000. Within- and between-subject variation in energy expenditure measured by the doubly-labelled water technique: implications for validating reported dietary energy intake. *Eur J Clin Nutr* **54**: 386–394.
39) Barnard JA, Tapsell LC, Davies PSW, Brenninger VL, Storlien LH. 2002. Relationship of high energy expenditure and variation in dietary intake with reporting accuracy on 7 day food records and diet histories in a group of healthy adult volunteers. *Eur J Clin Nutr* **56**: 358–367.
40) Ministry of Health, Labour and Welfare of Japan. 2009. The National Health and Nutrition Survey in Japan, 2006. Daiichi Shuppan Publishing, Tokyo (in Japanese).
41) Gorber SC, Tremblay M, Moher D, Gorber B. 2007. A comparison of direct vs. self-report measures for assessing height, weight and body mass index: a systematic review. *Obes Rev* **8**: 307–326.
42) Wada K, Tamakoshi K, Tsunekawa T, Otsuka R, Zhang H, Murata C, Nagasawa N, Matsushita K, Sugiura K, Yatsuya H, Toyoshima H. 2005. Validity of self-reported height and weight in a Japanese workplace population. *Int J Obes* **29**: 1093–1099.
43) Bathalon GP, Tucker KL, Hays NP, Vinken AG, Greenberg AS, McCrory MA, Roberts SB. 2000. Psychological measures of eating behavior and the accuracy of 3 common dietary assessment methods in healthy postmenopausal women. *Am J Clin Nutr* **71**: 739–745.
44) Kroke A, Klipstein-Grobusch K, Voss S, Moseneder J, Thielecke F, Noack R, Boeing H. 1999. Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. *Am J Clin Nutr* **70**: 439–447.
45) Sawaya AL, Tucker K, Tsay R, Willett W, Saltzman E, Dallal GE, Roberts SB. 1996. Evaluation of four methods for determining energy intake in young and older women: comparison with doubly labeled water measurements of total energy expenditure. *Am J Clin Nutr* **63**: 491–499.
46) Andersen LF, Tomten H, Haggarty P, Lovo A, Hustvedt BE. 2003. Validation of energy intake estimated from a food frequency questionnaire: a doubly labelled water study. *Eur J Clin Nutr* **57**: 279–284.

Towards a better National Health and Nutrition Survey in Japan

In his Comment (Oct 1, p 1205),[1] Satoshi Sasaki doubts the value of the National Health and Nutrition Survey in Japan[2] (hereafter, the Survey), mentioning that "as long as the Survey continues to be done and reported in the current manner, it will not fulfil its potential as a valuable resource for health." He raises three points. First, the use of data from the Survey is limited; second, there are problems with methods and quality control; and third, access to Survey information is limited. We would like to address the first point, and offer proposals as to the other two points, in light of his comments.

Since 1948, the Survey has been carried out annually by the Ministry of Health, Labour and Welfare, together with the National Institute of Health and Nutrition and in collaboration with local or registered dietitians and randomly selected Japanese people (currently about 9000 individuals of 4000 households). The Survey is, a priori, meant to obtain a set of national statistics to get an overview of the present status of health and nutrition in Japan, and of long-term trends for launching governmental policy and initiatives. It is also concurrently serving to provide a wide range of basic information for setting dietary reference intakes for Japanese people;[3] an exercise and physical activity reference for health promotion;[4] regulatory measures for food additives and contamination with insecticides or pesticides, organic mercury, and radioactive substances; and reference values for consumption of energy and nutrients for the victims of the Great Eastern Japan Earthquake of March 11, 2011.

Sasaki's first comments do not seem reasonable because the Survey is a set of cross-sectional observations that show the status quo of health and nutrition in Japan as a whole, and has its own limits in showing how the traditional Japanese diet has contributed towards achieving the world's highest longevity. Furthermore, the Survey cannot be counted on to have a role in analytical epidemiological approaches, including case-control studies, cohort studies, or randomised controlled trials, to investigate the associations between individual health and disease and physical activity and nutrition, and the interactions between environmental factors and host genetic factors.

Second as Sasaki points out, the participation rate is rather low at about 60%, suggesting that there is non-response bias. Descriptions remain somewhat unclear about presently adopted semi-weighed food records, assessment of individual intake from household data, standardisation of consumption data, validity, and reproducibility. Thus, there might be issues of generalisability. We at the National Institute of Health and Nutrition have committed ourselves to managing data quality control and standardisation of the Survey methods, but we should keep on exerting every effort to improve the Survey. Since information on energy and nutrients is scarcely given for cooked dishes and prepared food, in particular, in the Standard Tables of Food Composition in Japan, the quality and quantity of table data should be improved with all due speed.

A research group under the auspices of the Ministry of Health, Labour and Welfare suggested transfer of the Survey method from semi-weighed food records to 1-day (or multiple-day) 24-h dietary recall (with or without photos),[5] which is currently adopted worldwide, making international comparisons possible. This approach allows us to estimate individual consumption of energy, food, and nutrients; clarify the causative factors for health promotion and prevention of diseases; and elucidate the factors associated with life expectancy.

The third point relates to governmental statistics: that is, secondary (post-tabulated) data are provided to researchers. Thus, to obtain the Survey primary data, researchers must go through formalities and secure approval from the Ministry. Round table discussion on tabulation items to meet the current needs, open access to the Survey information, and provision of the primary data (or setting-up a data archive) should be made. The National Institute of Health and Nutrition proposes to launch a cohort study based on the Survey individual data to verify the associations between health and disease, physical activity and sports, and consumption of food and nutrients along with information on smoking, alcohol drinking, anthropometric measurements, and blood biomarkers.

Sasaki's comments serve to alert the Ministry and the Institute to modify the framework of the Survey, including replacement of the Survey methods, and to guarantee quality control, standardisation, and access to the Survey data.

We declare that we have no conflicts of interest.

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1 Sasaki S. The value of the National Health and Nutrition Survey in Japan. *Lancet* 2011; **378:** 1205–06.
2 Ministry of Health, Labour and Welfare, Japan. The National Health and Nutrition Survey in Japan 2008. Tokyo: Office for Life-style Related Diseases Control, General Affairs Division, Health Service Bureau, Ministry of Health, Labour and Welfare, 2011 (in Japanese).
3 Ministry of Health, Labour and Welfare, Japan. Dietary reference intakes for Japanese 2010. Report from the Scientific Committee of Dietary Reference Intakes for Japanese. Tokyo: Office for Life-style Related Diseases Control, General Affairs Division, Health Service Bureau, Ministry of Health, Labour and Welfare, 2011 (in Japanese).
4 Ministry of Health, Labour and Welfare, Japan. Exercise and physical activity reference for health promotion 2006: physical activity, exercise, and physical fitness. Tokyo: Office for Life-style Related Diseases Control, General Affairs Division, Health Service Bureau, Ministry of Health, Labour and Welfare, 2006 (in Japanese).
5 Willett W. Nutritional epidemiology, 2nd edn. New York: Oxford University Press, 1998.

Biosci. Biotechnol. Biochem., **76** (1), 186–188, 2012

Note

The Urinary Excretory Ratio of Nicotinamide Catabolites Was Associated with the Conversion Ratio of Tryptophan to Nicotinamide in Growing Rats Fed a Niacin-Free 20% Casein Diet

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Received August 1, 2011; Accepted October 14, 2011; Online Publication, January 7, 2012
[doi:10.1271/bbb.110564]

Weaning rats were fed a niacin-free 20% casein diet. Twenty-four-h-urine samples were collected, and nicotinamide and its catabolites were measured. A correlation was found between the urinary excretory ratio of nicotinamide catabolites (N^1-methyl-2-pyridone-5-carboxamide + N^1-methyl-4-pyridone-3-carboxamide)/N^1-methylnicotinamide and the tryptophan-nicotinamide conversion ratio during growing period of the rats. This indicates the possibility that the conversion ratio can be deduced from the excretory ratio.

Key words: N^1-methylnicotinamide; N^1-methyl-2-pyridone-5-carboxamide; N^1-methyl-4-pyridone-3-carboxamide; tryptophan-nicotinamide conversion ratio

The vitamin Nam is biosynthesized from the essential amino acid Trp in mammalian liver, including the human liver.[1,2] The metabolism of nicotinic acid, Nam, and Trp in mammals is given in reference 3. It is said that the pathway Trp to Nam plays a critical role in preventing Nam deficiency pellagra in humans, because protein malnutrition frequently causes pellagra.[4] In order to calculate the conversion ratio of Trp to Nam, animals and humans must eat a special diet that configures a preformed niacin-free refined diet for several days.[5] This means that calculating the conversion ratio is very difficult.

Shibata[6] had found that the conversion ratio of Trp to Nam is affected by age, and the excretory ratio of (2-Py + 4-Py)/MNA is too, but the conversion ratio could not be calculated in the experiment[6] because the diet of rats contained a pre-formed niacin (niacin is a generic name for Nam and nicotinic acid).

We thought of the possibility that the excretory ratio of (2-Py + 4-Py)/MNA can be used as a surrogate biomarker of the conversion ratio of Trp to Nam during the growing period of rats. As a first step, we investigated the relationship between the excretory ratio and the conversion using 24-h urine samples. The urinary excretory ratio of Nam catabolites was associated with the conversion ratio of Trp to Nam in growing rats fed a niacin-free 20% casein diet. We report these results in detail here.

The care and treatment of the experimental animals confirmed to The University of Shiga Prefecture Guidelines for the Ethical Treatment of Laboratory Animals. The room temperature was maintained at about 22 °C and about 60% humidity and a 12 h/12 h light/dark cycle (06:00–18:00/18:00–06:00) was imposed.

Male 3-week-old Wistar rats purchased from CLEA Japan (Tokyo) were placed immediately in individual CL-301 metabolism cages purchased from CLEA Japan, and were fed freely with a conventional purified diet consisting of 20% vitamin-free milk casein, 0.2% L-methionine, 46.9% gelatinized cornstarch, 23.4% sucrose, 5% corn oil, 3.5% AIN-93-G mineral mixture,[7] and a 1% AIN-93 vitamin mixture[7] containing chorine bitartrate, but without niacin, for 30 d.

Twenty four-h urine samples were collected from 9:00 to next 9:00 for days 7, 16, 23, and 30 of the experiment in amber bottles containing 1 mL of 1 mol/L HCl, and were stored at −20 °C until needed. The urine contents of Nam, 2-Py, and 4-Py were measured simultaneously by the HPLC method of Shibata *et al.*[8] The urine content of MNA was also measured by this method.[9] The conversion ratio was calculated by comparing the Trp intake during urine collection with the sum of urinary excretion of Nam, MNA, 2-Py, and 4-Py.[10]

Pearson correlation coefficients were calculated to determine the association between the conversion ratio of Trp to Nam and the urinary excretory ratio of (2-Py + 4-Py)/MNA. The calculation was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

The weaning rats had free access to the niacin-free 20% casein diet for 30 d. The changes in food intake and in growth during the experiment were normal. Figure 1 shows the urinary excretion of Nam, MNA, 2-Py, and 4-Py. These compounds increased with age. The conversion ratio of Trp to Nam increased with age, as shown in Fig. 2A, and the excretory ratio of (2-Py + 4-Py)/MNA also increased with age as shown in Fig. 2B.

Figure 3 shows the relation found between the conversion ratio of Trp to Nam and the urinary excretory ratio of Nam catabolites. The Pearson coefficient value

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Abbreviations: Trp, tryptophan; Nam, nicotinamide; MNA, N^1-methylnicotinamide; 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-3-carboxamide

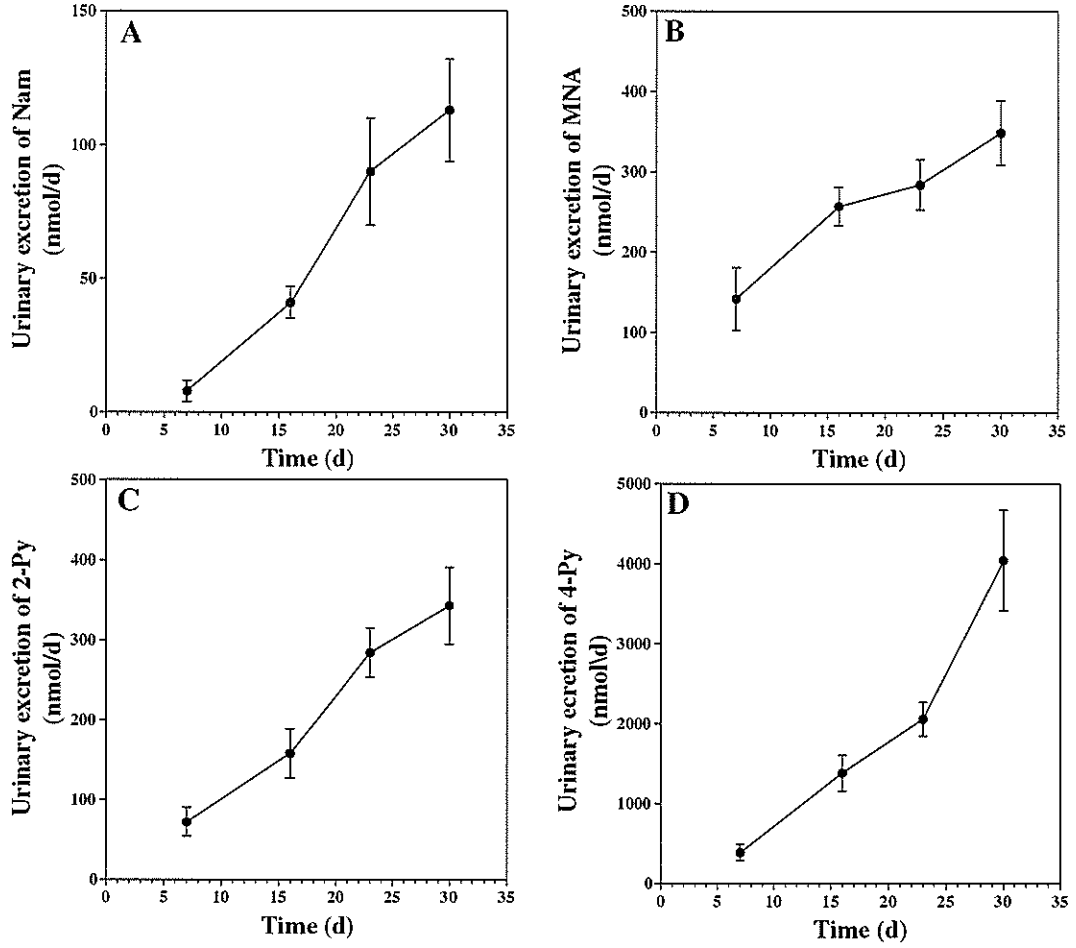

Fig. 1. Effects of Age on the Urinary Excretion of Nam (A), MNA (B), 2-Py (C), and 4-Py (D). Symbols mean represent ± SEM for six rats.

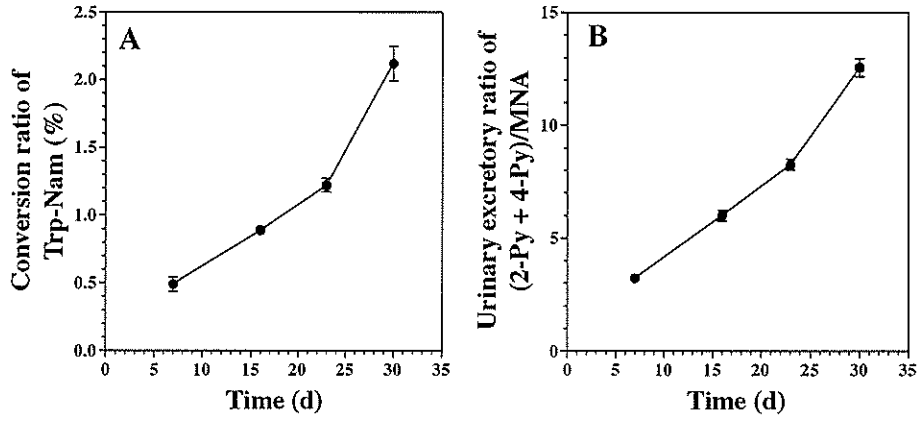

Fig. 2. Effects of Age on the Conversion Ratio of Trp to Nam (A) and the Urinary Excretory Ratio of (2-Py + 4-Py)/MNA (B). Symbols mean represent ± SEM for six rats.

was 0.90, and p was 0.03. This correlation is significant. A very strong correlation was found between the urinary excretory ratio of Nam catabolites (2-Py + 4-Py)/MNA in the 24-h urine samples and the Trp-Nam conversion during the growing period of the rats.

Pellagra results from a diet deficient in Nam and/or Trp. This disease is considered a public health problem in many maize-consuming African and Asian countries, especially populations facing to emergency and conflict.[11-15]

Krehl *et al.*[16] found that Trp could completely counteract the growth retardation caused by corn grits diet in rats. The conversion ratio of Trp to Nam is not constant: It is affected by age,[5] various nutritional factors,[10,17-25] hormones,[26-28] and chemicals.[29-31] Therefore, it is important in preventing a pellagra outbreak to know the conversion ratio of Trp to Nam under the conditions, but it is not possible to know this in case of emergency and conflict.

As for the biomarkers of pellagra, it is known that the blood NAD level does not reflect Nam nutritional status in pellagra patients,[32] and that the Nam itself does not appear in the urine even in healthy people.[3] On the contrary, urinary excretion of Nam catabolites such as

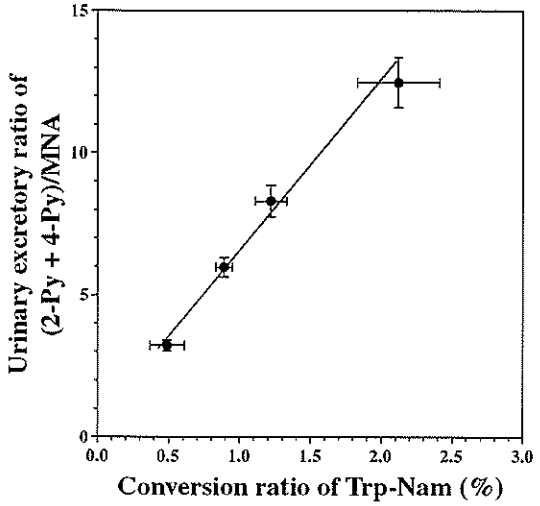

Fig. 3. Relation between the Conversion Ratio of Trp to Nam and the Urinary Excretory Ratio of Nam Catabolites.

Symbols mean represent ± SEM for six rats. The Pearson coefficient value was 0.90, and p was 0.03. The correlation is significant.

MNA, 2-Py, and 4-Py, and the excretory ratio of (2-Py + 4-Py)/MNA in spot urine samples, are generally used as a laboratory test.[14]

Shibata and co-workers[10,17–22,33] found that the urinary excretory ratio of (2-Py + 4-Py)/MNA primarily reflected protein nutritional status, not Nam nutritional status, because the excretory ratio was decreased by the administration of an extremely large amount of Nam[34] and MNA[35] in rats. In addition, Shibata *et al.*[36] reported that the administration of 150 mg/d of Nam did not affect the excretory ratio in humans. Thus, increases in the excretory ratio do not bring improved Nam nutritional status. Shibata[33] proposed that the Nam catabolite excretory ratio reflects protein nutritional status.

Collection of a 24-h urine sample and feeding of a niacin-free refined diet are very hard to achieve in emergency and conflict situations. (2-Py + 4-Py)/MNA can be measured by using a spot urine sample instead of a 24-h urine sample. Therefore, it appears to be possible that the conversion ratio of Trp to Nam can be deduced by a spot urine sample instead of using a 24-h urine sample.

It is necessary to examine whether the same result obtains when weaning rats are fed a diet containing other proteins or different concentrations of dietary proteins. In addition, it is also necessary to examine diurnal variations in the urinary excretory ratio of (2-Py + 4-Py)/MNA, even though the collection of spot urine samples from rats is difficult.

In the future, we plan to study the relation between the conversion ratio of Trp to Nam in 24-h urine samples and the urinary excretory ratio of (2-Py + 4-Py)/MNA in spot urine samples the growing period of humans.

Acknowledgments

This investigation was part of the project "Studies on the Dietary Reference Intakes for Japanese" (principal investigator, Katsumi Shibata), which was supported by a Research Grant for Comprehensive Research on Cardiovascular and Life-Style Related Diseases from the Ministry of Health, Labour and Welfare of Japan.

References

1) Nishizuka Y and Hayaishi O, *J. Biol. Chem.*, **238**, 3369–3377 (1963).
2) Goldsmith GA, Sarett HP, Register UD, and Gibbens J, *J. Clin. Invest.*, **31**, 533–542 (1952).
3) Shibata K and Matsuo H, *Am. J. Clin. Nutr.*, **50**, 114–119 (1989).
4) Goldberger J, Wheeler GA, and Sydenstricker VP, *Public Health Rep.*, **35**, 648–713 (1920).
5) Fukuwatari T, Ohta M, Sasaki R, and Shibata K, *J. Nutr. Sci. Vitaminol.*, **50**, 385–391 (2004).
6) Shibata K, *Agric. Biol. Chem.*, **54**, 2953–2959 (1990).
7) Reeves PG, *J. Nutr.*, **127**, 838S–841S (1997).
8) Shibata K, Kawada T, and Iwai K, *J. Chromatogr.*, **424**, 23–28 (1988).
9) Shibata K, *Vitamins*, **61**, 599–604 (1987).
10) Shibata K and Matsuo H, *J. Nutr.*, **119**, 896–901 (1989).
11) Moren A, Lemoult D, and Brodel A, *Lancet*, **335**, 1403–1404 (1990).
12) Malfait P, Moren A, Dillon JC, Brodel A, Begkoyian G, Etchegorry MG, Malenga G, and Hakewill P, *Int. J. Epidemiol.*, **22**, 504–511 (1993).
13) Baquet S, Wullaume F, Egmond KV, and Ibañez F, *Lancet*, **355**, 1829–1830 (2000).
14) World Health Organization, Pellagra and its prevention and control in major emergencies, Geneva (2002).
15) Seal AJ, Greeke PI, Dibari F, Cheung E, Kyroussis E, Semedo P, and van den Briel T, *Am. J. Clin. Nutr.*, **85**, 218–224 (2007).
16) Krehl WA, Teply LJ, Sarma PS, and Elvehjem CA, *Science*, **101**, 489–490 (1945).
17) Shibata K, Nomamoto R, and Iwai K, *Agric. Biol. Chem.*, **53**, 1765–1769 (1988).
18) Shibata K and Onodera M, *Agric. Biol. Chem.*, **55**, 1291–1298 (1991).
19) Shibata K, Murotani M, and Onodera M, *Biosci. Biotechnol. Biochem.*, **56**, 665–669 (1992).
20) Shibata K, *Biosci. Biotechnol. Biochem.*, **58**, 442–443 (1994).
21) Shibata K, Taniguchi I, and Onodera M, *Biosci. Biotechnol. Biochem.*, **58**, 970–971 (1994).
22) Shibata K, *Biosci. Biotechnol. Biochem.*, **59**, 715–716 (1995).
23) Shibata K, Mushiage M, Kondo T, Hayakawa T, and Tsuge H, *Biosci. Biotechnol. Biochem.*, **59**, 2060–2063 (1995).
24) Shibata K, Shimada H, and Kondo T, *Biosci. Biotechnol. Biochem.*, **60**, 1660–1666 (1996).
25) Shibata K, Kondo T, and Yonezima M, *J. Nutr. Sci. Vitaminol.*, **43**, 479–483 (1997).
26) Shibata K and Kondo T, *Biosci. Biotechnol. Biochem.*, **57**, 1890–1893 (1993).
27) Shibata K and Toda S, *Biosci. Biotechnol. Biochem.*, **58**, 1757–1762 (1994).
28) Shibata K, *Biosci. Biotechnol. Biochem.*, **59**, 2127–2129 (1995).
29) Shibata K, Kondo T, Marugami M, and Umezawa C, *Biosci. Biotechnol. Biochem.*, **60**, 1455–1459 (1996).
30) Shibata K, Ishikawa A, and Kondo T, *Biosci. Biotechnol. Biochem.*, **61**, 1679–1683 (1997).
31) Shibata K, Fukuwatari T, Enomoto A, and Sugimoto E, *J. Nutr. Sci. Vitaminol.*, **47**, 263–266 (2001).
32) Greeke PI, Dibari F, Cheung E, van den Briel T, Kyroussis E, and Seal AJ, *J. Nutr.*, **137**, 2013–2017 (2007).
33) Shibata K, *Vitamins*, **64**, 1–18 (1990).
34) Shibata K and Matsuo H, *Agric. Biol. Chem.*, **53**, 2031–2036 (1989).
35) Shibata K and Matsuo H, *Agric. Biol. Chem.*, **53**, 2393–2397 (1989).
36) Shibata K, Onodera M, Shimada S, and Yasuda K, *Vitamins*, **66**, 309–314 (1992).

British Journal of Nutrition (2012), **108**, 1034–1041
doi:10.1017/S0007114511006192

Effects of ethanol consumption on the B-group vitamin contents of liver, blood and urine in rats

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(Submitted 11 July 2011 – Final revision received 14 October 2011 – Accepted 17 October 2011 – First published online 15 December 2011)

Abstract

Several studies have shown that blood vitamin levels are lower in alcoholic patients than in control subjects. Acute ethanol exposure enhances the release of vitamins from liver cells *in vitro*. The aim of the present study is to confirm the effects of ethanol consumption on vitamin contents *in vivo*. We compared the contents of B-group vitamins in the liver, blood and urine between ethanol-fed and control rats fed a diet containing a sufficient- and low-vitamin mixture. The experimental rats were fed a 15 % ethanol solution freely for 28 d, and then 24 h urine samples were collected, after which the animals were killed. The B-group vitamin contents in the liver, blood and urine were measured. No differences in liver, blood and urine contents were observed between the control and ethanol-fed rats fed a diet containing a sufficient-vitamin mixture. On the contrary, in rats fed a diet containing a low-vitamin mixture, consumption of ethanol caused a decrease in the contents of vitamins B_1, B_2 and pantothenic acid in the liver; however, the contents of the other vitamins did not decrease. In the blood, the contents of vitamins B_1, B_2, B_6 and pantothenic acid were lower in the ethanol-fed rats than in the controls. Urinary excretion of the B-group vitamins, except for niacin, was lower in the ethanol-fed rats. These results show that ethanol consumption affects the absorption, distribution and excretion of each of the vitamins in rats fed a diet containing a low-vitamin mixture.

Key words: Vitamins: Urine: Blood: Liver: Ethanol

Numerous studies have shown that vitamin status of alcoholic patients differs from non-drinking subjects[1–7], and the majority have shown that blood vitamin levels are lower in alcoholic patients than in controls[8–10]. In addition, several reports have suggested that chronic alcohol feeding may lead to a significant inhibition of carrier-mediated thiamin[11,12] and folate[13–19] uptake in the intestine and kidney. This phenomenon is observed only in alcoholic patients who drink ethanol chronically. On the contrary, a reduction in circulating levels of B-complex vitamins often occurred without clinical evidence of hypovitaminosis[20]. Sorrell *et al.*[21] reported that the *in vitro* perfusion of rat liver with ethanol caused the release of all B-vitamins except biotin from the liver stores. Israel & Smith[22] reported that acute ethanol feeding to rats inhibited the conversion of pantothenic acid to CoA. These studies in animal models suggested that acute ethanol intake results in an increased hepatic release of vitamins and an impaired utilisation, which means increased levels of free forms of vitamins in the liver which can in turn permeate the cell membranes[21,22]. This might lead to increases in blood vitamin contents and in urinary excretion. Although there are many reports concerning the effects of ethanol on the absorption and metabolism of vitamins, the conclusion concerning the controversy remains elusive. The reason might be that there is no study regarding the simultaneous measurement of vitamin contents of liver (as a biomarker of the storage amount of vitamins), blood (as a biomarker of the circulation amount of vitamins) and urine (as a biomarker of the reabsorption ability of kidney and an extra amount of vitamins).

In the present study, we examined the effects of ethanol consumption on the contents of B-group vitamins of the liver, blood and urine in rats fed two kinds of diets containing either a sufficient- or a low-vitamin mixture.

Materials and methods

Chemicals

Vitamin-free milk casein, sucrose and L-methionine were purchased from Wako Pure Chemical Industries. Maize oil was purchased from Ajinomoto. Gelatinised maize starch, a mineral mixture (AIN-93G mineral mixture)[23] and a vitamin mixture (nicotinic acid-free AIN-93 vitamin mixture containing

Abbreviations: 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-3-carboxamide.

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25 % choline bitartrate)[23] were obtained from Oriental Yeast Company, Limited.

Thiamin hydrochloride ($C_{12}H_{17}ClN_4OS$-HCl; molecular weight 337·27), riboflavin ($C_{17}H_{20}N_4O_6$; 376·37), pyridoxine hydrochloride ($C_8H_{11}NO_3$-HCl; 205·63), cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$; 1355·40), nicotinamide ($C_6H_6N_2O$; 122·13), calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca; 476·54), folic acid ($C_{19}H_{19}N_7O_6$; 441·40) and D(+)-biotin ($C_{10}H_{16}N_2O_3S$; 244·31) were purchased from Wako Pure Chemical Industries. 4-Pyridoxic acid ($C_8H_9NO_4 = 183·16$) was made by ICN Pharmaceuticals and obtained through Wako Pure Chemical Industries.

N^1-Methylnicotinamide chloride ($C_7H_9N_2O$-HCl; 159·61) was purchased from Tokyo Kasei Kogyo. N^1-Methyl-2-pyridone-5-carboxamide (2-Py, $C_7H_8N_2O_2$ 152·15) and N^1-methyl-4-pyridone-3-carboxamide (4-Py, $C_7H_8N_2O_2$ 152·15) were synthesised by the methods of Pullman & Colowick[24] and Shibata *et al.*[25], respectively. All other chemicals used were of highest purity available from commercial sources.

Animals and treatment

The care and treatment of the experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals. The animals were maintained under controlled temperature (22°C), 60 % humidity and light conditions (12 h light–12 h dark cycle).

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a sufficient-vitamin mixture (Expt 1)

Male Wistar rats (3 weeks old) obtained from CLEA Japan were fed freely with a conventional purified diet, consisting of 20 % vitamin-free milk casein, 0·2 % L-methionine, 46·9 % gelatinised maize starch, 23·4 % sucrose, 5 % maize oil, 3·5 % AIN-93-G mineral mixture[14] and 1 % AIN-93 vitamin mixture[14] containing chorine bitartrate, but without nicotinic acid, to acclimatise for 7 d. Nicotinic acid had not been added to this diet because it is supplied enough from tryptophan in casein[26], and a dietary fibre-free diet was used because it is a tradition not to use dietary fibre in our laboratory which is not essential for normal growth[27].

The rats were divided into two groups (n 5 each). Group 1 was fed with a diet containing the 1 % vitamin mixture (a sufficient-vitamin diet) and allowed to drink water for 28 d. Group 2 was fed with a diet containing the 1 % vitamin mixture (a sufficient-vitamin diet) and forced to drink a 15 % ethanol solution instead of water for 28 d. The 24 h urine samples were collected in amber bottles containing 1 ml of 1 M-HCl at 09.00–09.00 hours of the last day and were stored at −25°C until required. The rats were killed at about 09.00 hours; blood was collected and tissues were taken to measure the weights and the contents of B-group vitamins in the liver, blood and urine. Liver samples were preserved at −25°C until required.

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a low-vitamin mixture (Expt 2)

A preliminary experiment revealed that the body-weight gain of young rats was the same when fed a diet containing the 1 % AIN-93 vitamin mixture and the 0·3 % AIN-93 vitamin mixture, whereas the body-weight gain was lower in rats fed a diet containing the 0·2 % AIN-93 vitamin mixture than in those fed a diet containing the 1 or 0·3 % diets. Thus, we determined tentatively whether the diet containing the 0·3 % AIN-93 vitamin mixture could supply a minimum amount of vitamins for the growing rats.

Male Wistar rats (3 weeks old) obtained from CLEA Japan were fed freely with the conventional purified diet (mentioned above) to acclimatise for 7 d. The rats were then divided into two groups (n 5 each). Group 1 was fed a diet containing the 0·3 % vitamin mixture and allowed to drink water for 28 d. Group 2 was fed a diet containing the 0·3 % vitamin mixture and forced to drink a 15 % ethanol solution instead of water for 28 d. The 24 h urine samples and tissues were collected. Levels of alanine aminotransferase, aspartate aminotransferase and γ-glutamyltranspeptidase were measured at Mitsubishi Chemical Medience (Tokyo, Japan).

Measurement of B-group vitamins in urine and blood

Preparation and measurement of the extracts of the B-group vitamins from the urine and blood are described as follows[28].

Vitamin B_1

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to ten volumes of 5 % ice-cold TCA and homogenised with a Digital Homogenizer Hom (Iuchi). The acidified homogenate was centrifuged at 10 000 ***g*** for 10 min at 4°C, and the supernatant was retained and used for the measurement of vitamin B_1[29].

Vitamin B_2

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to ten volumes of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0) and homogenised with a Teflon/glass homogeniser (Nikko Hansen). To 0·1 ml of the homogenate, 0·44 ml of water and 0·26 ml of 0·5 M-H_2SO_4 were added and then kept at 80°C for 15 min. After cooling, 0·2 ml of 10 % TCA were added and centrifuged at 10 000 ***g*** for 3 min at 4°C. From the supernatant obtained, 0·2 ml was withdrawn and added to 0·2 ml of 1 M-NaOH. The alkalised mixture was irradiated with a fluorescent lamp for 30 min and then 0·02 ml of glacial acetic acid were added to the mixture. The neutralised mixture was passed though a 0·45 μm microfilter and the filtrate was directly injected into the HPLC system for measuring lumiflavin[30].

Vitamin B_6

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to 90 ml of 55 mM-HCl and homogenised with a Waring blender. The homogenate was autoclaved at 121°C for 3 h. After cooling, the mixture was adjusted to pH 5·0 with 1 M-NaOH and then made up to 100 ml with water. The solution was filtered with qualitative filter no. 2 (ADVANTEC MFS, Inc.). The filtrate was used for measuring vitamin B_6 as described previously[31].

Vitamin B_{12}

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to 2·5 ml of 0·57 M-acetic acid–sodium acetate buffer (pH 4·5) plus 5 ml of water and 0·1 ml of 0·05 % potassium cyanide (KCN). The suspension was homogenised with a Teflon/glass homogeniser. The homogenate was then put into a boiling water-bath for 5 min. After cooling, 0·15 ml of 10 % metaphosphoric acid were added and made up to 10 ml with water. The solution was filtered with qualitative filter no. 2 (ADVANTEC MFS, Inc.). The filtrate was used for measuring vitamin B_{12} as described previously[32].

Nicotinamide

Frozen liver samples, about 0·6 g, were thawed, minced, and then added to five volumes of 0·1 g/ml isonicotinamide. The suspension was homogenised with a Teflon/glass homogeniser. The homogenate (1 ml) was withdrawn and added to 4 ml of water, and then autoclaved at 121°C for 10 min. After cooling, the mixture was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was retained and the precipitated materials were extracted again with 5 ml of water, and the supernatant was retained. Both the retained supernatants were combined, and the extract was used for measuring nicotinamide as described previously[25].

Pantothenic acid

Frozen liver samples, about 0·2 g, were thawed, minced, and then added to ten volumes of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0). The suspension was homogenised with a Teflon/glass homogeniser. The homogenate was incubated at 37°C overnight to convert free pantothenic acid from the bound type of pantothenate compounds. The reaction was stopped by putting it into a boiling water-bath for 5 min. After cooling, the mixture was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was retained and the precipitated materials were extracted again with 2 ml of water, and the supernatant was retained. Both the retained supernatants were combined, and the extract was used for measuring pantothenic acid as described previously[33].

Folate

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to ten volumes of 0·1 M-KH_2PO_4–K_2HPO_4 buffer (pH 6·1). The suspension was homogenised with a Teflon/glass homogeniser. The homogenate was autoclaved at 121°C for 5 min. After cooling, 2·5 ml of pronase (5 mg/ml; Pronase MS; Kaken Pharmaceutical Company, Limited) were added and then incubated at 37°C for 3 h. The reaction was stopped by putting it into a boiling water-bath for 10 min. After cooling, 0·5 ml of conjugase (extract from porcine kidney acetone powder, Type II; Sigma-Aldrich) were added and incubated at 37°C overnight. The reaction was stopped by putting it into a boiling water-bath for 10 min. After cooling, the mixture was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was retained, and the precipitated materials were extracted again with 3 ml of water, and the supernatant was retained. Both the retained supernatants were combined, and the extract was used for measuring folate as described previously[34]. The conjugase solution was made as follows: 60 ml of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0) were added to 20 g porcine kidney acetone powder and stirred for 30 min at 4°C. The suspension was centrifuged at 10 000 ***g*** for 10 min at 4°C. The supernatant was dialysed against a large amount of 50 mM-KH_2PO_4–K_2HPO_4 buffer (pH 7·0) to remove endogenous folate of the kidney acetone powder. The dialysed conjugase solution was used.

Biotin

Frozen liver samples, about 0·5 g, were thawed, minced, and then added to two volumes of 2·25 M-H_2SO_4 and then homogenised with a Waring blender. The suspension was hydrolysed by autoclaving for 1 h at 121°C. After cooling, the suspension was centrifuged at 10 000 ***g*** for 10 min at 4°C, and the supernatant was used for measuring biotin[35].

Analyses

The measurements of the B-group vitamins except for vitamin B_6 were described previously[19]. The urinary excretion of 4-pyridoxic acid, a catabolite of vitamin B_6, was measured according to the method of Gregory & Kirk[36].

Statistical analysis

Mean values between the treatment groups were compared using the Mann–Whitney *U* two-tailed *t* test. $P<0·05$ was considered to be statistically significant. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software).

Results

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a sufficient-vitamin mixture (Expt 1)

There were no differences in body-weight gain and liver weights between the groups. No differences in the levels of vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, nicotinamide, pantothenic acid, folate and biotin were observed in the liver

and blood. Although the 24 h urinary excretion of some of the vitamins was slightly lower in the ethanol-treated group than in the control, the differences were not significant (data not shown). Thus, ethanol consumption did not affect the B-group vitamin contents in the liver, blood and urine when the rats were fed a diet containing sufficient amounts of the vitamins.

Effects of ethanol feeding on the B-group vitamin contents of liver, blood and urine in rats fed a diet containing a low-vitamin mixture (Expt 2)

As shown in Table 1, body-weight gain, food intake and liver weights were lower in the ethanol-fed group than in the controls. The overall food intake was lower in the ethanol-fed group than in the controls, but energy intake was almost the same because of ethanol intake.

The effects of ethanol consumption on the activities of alanine aminotransferase, aspartate aminotransferase and γ-glutamyltranspeptidase in plasma are shown in Table 2. No significant effects of ethanol consumption were observed for these indices of liver function.

The effects of ethanol consumption on the B-group vitamin contents of the liver are shown in Table 3. The contents of the vitamins in liver are measured as storage amounts of the vitamins, thus are expressed as mol/liver. The contents of vitamin B_1, vitamin B_2 and pantothenic acid were lower in the ethanol-fed group than in the controls, whereas the contents of vitamin B_6, vitamin B_{12}, nicotinamide, folate and biotin were not significantly different.

The effects of ethanol consumption on the B-group vitamin contents of the blood are shown in Table 4. The contents of vitamin B_1, vitamin B_2, vitamin B_6 and pantothenic acid were lower in the ethanol-fed group than in the controls, whereas the contents of vitamin B_{12}, nicotinamide, folate and biotin were not significantly different.

The effects of ethanol consumption on the 24 h urinary excretion of the B-group vitamins are shown in Table 5. The excretion of vitamin B_1, vitamin B_2, 4-pyridoxic acid (a catabolite of vitamin B_6), vitamin B_{12}, pantothenic acid, folate and biotin was lower in the ethanol-fed group than in the controls, whereas the contents of nicotinamide (sum of the contents of nicotinamide and its catabolites such as N^1-methylnicotinamide, 2-Py and 4-Py) were not significantly different.

Food intake was different in the two groups, so that urinary excretion ratios of the vitamins were calculated. As shown in Table 5, the excretion ratios of all vitamins except for vitamin B_{12} were lower in the ethanol-fed group.

Table 1. Effects of ethanol consumption on rat body-weight gain, food intake, ethanol intake, water intake, energy intake, food efficiency ratio and liver weight (Expt 2)

(Mean values with their standard errors for five rats per group)

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Initial body weight (g)	36	1	36	1
Final body weight (g)	204	7	164*	8
Body-weight gain (g/28 d)	168	7	128*	3
Food intake (g/28 d)	363	14	258*	6
Ethanol intake† (g/28 d)	–		45	3
Water intake (ml/28 d)	396	26	–	
Energy intake‡ (kcal/28 d)	1488	58	1396	56
Energy intake‡ (kJ/28 d)	6230	242	5845	234
Food efficiency ratio§	0·46	0·01	0·50	0·00
Energy efficiency ratio‖	0·113	0·020	0·092	0·006
Liver weight (g)	9·70	0·55	8·47	0·36

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney U two-tailed t test).
† The value is expressed in g of pure ethanol and not as the volume of 15 % ethanol.
‡ Energy of 1 g ethanol was calculated as 29·3 kJ (7 kcal)/g.
§ (Body-weight gain/food intake) × 100.
‖ (Body-weight gain/energy intake) × 100.

Table 2. Effects of ethanol consumption on the activities of alanine aminotransferase, aspartate aminotransferase and γ-glutamyltranspeptidase in plasma

(Mean values with their standard errors for five rats per group)

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Alanine aminotransferase (IU/l)	22·4	1·9	24·8	2·0
Aspartate aminotransferase (IU/l)	157	11	136	10
γ-Glutamyltranspeptidase (IU/l)	3·2	0·9	3·2	0·9

Discussion

An ordinary diet for rats generally contains sufficient amounts of nutrients including vitamins[23]. Under well-nourished conditions, rats are generally little affected by factors such as ethanol consumption. In fact, the present study proves that ethanol consumption did not affect the body-weight gain or the vitamin contents in the liver and blood when rats were fed a diet containing sufficient amounts of vitamins. On the other hand, when rats were fed a diet low in vitamins, body-weight gain was lower in the ethanol-fed group than in the control group and some vitamin contents of the liver and blood, and urinary excretion were decreased. These results show that chronic ethanol consumption affects

Table 3. Effect of ethanol consumption on liver B-group vitamin contents (Expt 2)

(Mean values with their standard errors for five rats per group)

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Vitamin B_1 (nmol/liver)	127	6	100*	4
Vitamin B_2 (nmol/liver)	686	62	422*	16
Vitamin B_6 (nmol/liver)	229	16	281	23
Vitamin B_{12} (nmol/liver)	0·39	0·03	0·38	0·02
Niacin (μmol/liver)	18·2	1·8	16·6	1·3
Pantothenic acid (μmol/liver)	3·16	0·19	2·42*	0·18
Folate (nmol/liver)	70·0	9·7	73·6	9·3
Biotin (nmol/liver)	9·31	1·10	9·65	0·46

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney U two-tailed t test).

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Table 4. Effect of ethanol consumption on blood B-group vitamin contents (Expt 2)

(Mean values with their standard errors for five rats per group)

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Vitamin B_1 (pmol/ml)	159	4	139*	6
Vitamin B_2 (pmol/ml)	177	5	142*	4
Vitamin B_6 (nmol/ml)	0·49	0·04	0·34*	0·02
Vitamin B_{12} (pmol/ml)	1·55	0·03	1·41	0·01
Niacin (nmol/ml)	127	6	117	2
Pantothenic acid (nmol/ml)	1·13	0·04	0·89*	0·04
Folate (pmol/ml)	149	4	138	10
Biotin (pmol/ml)	30·4	3·4	25·9	1·0

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney *U* two-tailed *t* test).

absorption, distribution and excretion of vitamins, as reported previously[1–19]. The present findings are not consistent with the *in vitro* perfusion of rat liver with ethanol, which caused the release of all B-vitamins except biotin from the liver stores[23]. This phenomenon was not observed in the present whole-body experiment, because the vitamin contents of the blood were not increased by ethanol consumption. In the present *in vivo* experiment, any vitamins released from the liver were quickly absorbed by non-hepatic tissues. In humans, the typical dietary vitamin intakes are generally around the minimum requirements. Thus, the nutritional status of rats fed a diet low in vitamins was similar to that of humans. Ethanol consumption was 45 g over 28 d, so that daily average ethanol consumption was about 1·6 g/d, which corresponds to an energy intake of 46·9 kJ (11·2 kcal)/d. The energy intake in the ethanol-fed group, including ethanol energy, was 5845 kJ (1396 kcal) over 28 d (about 209 kJ (50 kcal)/d). Thus, ethanol accounted for 20 % of dietary energy. Under these conditions, liver functions in rats were not injured. If humans were to consume 10 467 kJ (2500 kcal)/d, the equivalent ethanol consumption would be about 70 g/d, which corresponds to 1 litre of typical beer.

Vitamin depletion, common in malnourished alcoholic patients[10], can occur despite vitamin supplementation. Vitamin malabsorption[37], exacerbated by malnutrition, contributes to this depletion[38]. Also, in alcoholic patients, the impaired ability of the liver to bind and store vitamins might contribute to this depletion. This may probably be due to the hepatotoxicity of ethanol, which impairs not only the vitamin-binding capacity but also the vitamin storage of the liver. In the present study, a diet containing 20 % casein supplemented with methionine was used, which is an excellent protein source from a nutritional standpoint. This suggest the reasons why ethanol consumption did not cause any severe damage, such as an extremely low food intake and body-weight gain and roughness of fur for the rats, even when they were fed a low-vitamin diet.

Sorrell *et al.*[21] reported that the *in vitro* perfusion of rat liver with ethanol caused the release of all vitamins from the liver stores, especially thiamin. It is generally considered that this phenomenon causes increased urinary excretion of vitamins, but in the present *in vivo* experiments, ethanol consumption did not cause increased urinary excretion, but rather decreased it. This discrepancy between the expected and the actual findings may be attributed to the difference between the *in vitro* and *in vivo* experiments. Moreover, there are differences in short-term and long-term adjustment mechanisms for ethanol toxicity. The protein nutritional status was high in the present study because the diet used 20 % casein supplemented with methionine. Protein plays a pivotal role in vitamin absorption and storage in hepatocytes. Protein malnutrition causes malabsorption, reduced storage and impaired utilisation of vitamins. Thus, an adequate intake of vitamins, and also protein, is essential for preventing ethanol toxicity.

In the present study on the low-vitamin diet, vitamin B_1, vitamin B_2 and pantothenic acid contents in the liver and blood were lower in the ethanol-fed group than in the controls, even when rats were fed a high-protein diet. Furthermore, the total urinary excretion and excretion ratios of all three vitamins were also lower in the ethanol-fed group. Thus, ethanol consumption reduced the intestinal absorption of these vitamins, as reported by Subramanya *et al.*[12], Hamid *et al.*[13,14,16,17] and Wani & Kaur[19]. Vitamins such as

Table 5. Effect of ethanol consumption on urinary B-group vitamin excretion (upper row) and urinary excretion ratio (lower row) for each of the vitamins (Expt 2)†

(Mean values with their standard errors for five rats per group)

	Control		15 % Ethanol	
	Mean	SEM	Mean	SEM
Vitamin B_1				
nmol/d	3·5	0·1	1·8*	0·1
%	3·4	0·2	2·7*	0·2
Vitamin B_2				
nmol/d	3·6	0·3	0·15*	0·04
%	3·8	0·2	0·24*	0·05
4-PIC‡				
nmol/d	29·4	1·9	7·3*	0·5
%	15·6	0·5	4·5*	0·3
Vitamin B_{12}				
pmol/d	9·1	0·4	6·7*	0·2
%	8·9	0·3	9·1	0·2
Niacin§				
μmol/d	2·00	0·16	1·82	0·24
%	−‖		−‖	
Pantothenic acid				
nmol/d	24·3	2·4	6·3*	0·3
%	6·5	0·5	2·4*	0·2
Folate				
nmol/d	1·85	0·19	0·77*	0·11
%	7·3	0·7	4·4*	0·6
Biotin				
nmol/d	0·21	0·02	0·09*	0·01
%	5·0	0·4	3·0*	0·25

4-PIC, 4-pyridoxic acid.

* Mean values were significantly different from those of the control group ($P<0·05$; Mann–Whitney *U* two-tailed *t* test).

† Percentage urinary excretion ratio was calculated using the following equation: (24 h urinary excretion (mol/d)/intake of the vitamin during urine collection (mol/d)) × 100.

‡ A catabolite of vitamin B_6.

§ Niacin content was calculated as the sum of the nicotinamide content and its catabolites such as N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-3-carboxamide.

‖ Urinary excretion ratio was not calculated as niacin was derived from tryptophan.

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vitamin B_1, vitamin B_2 and pantothenic acid might be directly and/or indirectly involved in the metabolism of ethanol, indicating that the vitamin catabolites increased and were excreted into the urine. Of these three vitamins, only the catabolic fate of vitamin B_1 is relatively well known. It has been reported that the excretion of vitamin B_1 metabolites usually exceeds by far the excretion of intact vitamin B_1 using radioactive tracer experiments[39]. The major metabolites of vitamin B_1 in rat urine are 2-methyl-4-amino-5-pyridinecarboxylic acid[40], 4-methylthiazole-5-acetic acid[41] and thiamine acetic acid[42]. Pearson[39] reported that the sum of the metabolites accounted for about 50 % of the total urinary excretion of vitamin B_1 and its catabolites from radioactive tracer experiments. Although we cannot measure the catabolites of vitamin B_1, these metabolites might increase in the urine of the ethanol-fed rats. It is likely that a similar phenomenon would apply for the fates of vitamin B_2 and pantothenic acid.

The content of vitamin B_6 in the blood was lower in the ethanol-fed group, but the content of vitamin B_6 in the liver was slightly higher in the ethanol-fed group than in the control. The urinary excretion of vitamin B_6, determined from its catabolite 4-pyridoxic acid, was much lower in the ethanol-fed group than in the control. Probably ethanol consumption resulted in an increased storage of vitamin B_6 in the liver.

Other B-group vitamin contents in the liver and blood, such as vitamin B_{12}, nicotinamide, folate and biotin, were not affected by ethanol consumption. The lack of any effect of ethanol consumption on the niacin content in this experiment was probably because nicotinamide was synthesised from tryptophan, which was present in the diet as casein and was supplied adequately[43]. For rats, NAD precursors such as nicotinic acid and nicotinamide are not essential. In fact, the urinary excretion of nicotinamide did not differ between the two groups. Concerning the effect of ethanol consumption on biotin, Sorrell *et al.*[21] reported that the *in vitro* perfusion of rat liver with ethanol did not cause the release of biotin, but caused the release of vitamin B_{12} first. In the present experiment, a similar phenomenon was observed for biotin, but not for vitamin B_{12}. Frank *et al.*[44] reported that the first vitamin released into the circulation during hepatic insult by ethanol is vitamin B_{12}. This disparity between the reported and the present findings might also arise from the difference in protein nutritional status.

There are many reports concerning how ethanol consumption affects folate absorption and metabolism[13–18,45–53]. Some studies have reported that ethanol consumption increased the urinary excretion of folates[46,47,50–53] and caused decreased serum folate levels. Romanoff *et al.*[53] reported that acute ethanol exposure inhibits the apical transport of 5-methyltetrahydrofolate in cultured human proximal tubule cells, and in subchronic ethanol studies, increasing concentrations of ethanol resulted in an up-regulation of folate transporters. Furthermore, Romanoff *et al.*[53] reported that both the folate receptor and reduced folate carrier transporter proteins were up-regulated in rats receiving an ethanol diet. On the contrary, Hamid *et al.*[13,14,16,17] and Wani & Kaur[19] reported that ethanol reduced the intestinal uptake of folate by altering the binding and transport kinetics of the folate transport system and also the expression of folate transporters in the intestine. In addition, Hamid & Kaur[15] reported that ethanol consumption reduces folate re-uptake in the renal absorption system by the decreased expression of transporters. The present data for folate are not consistent with previous reports[13–18,45–53]; the contents of folate in the liver and blood were not affected by ethanol consumption, and the urinary excretion of folate and the excretion ratio were decreased markedly. A study[52] reported that urinary folate excretion increased in ethanol-fed rats consuming folate-containing diets, but not in rats fed folate-deficient diets. In the present study, the urinary excretion of folate did not increase, but decreased. This was because the diet was low in folate. In the present study, the urinary excretion of folate was lower in the ethanol-fed group than in the non-ethanol group, suggesting that ethanol consumption and the feeding of a low-folate diet up-regulated the folate receptor and reduced folate carrier transporter proteins. This up-regulation was probably a compensatory response to counteract the effects of ethanol in inhibiting the reabsorption of folate. Therefore, the effects of ethanol would depend on the dose and duration of treatment.

In summary, these results show that ethanol consumption affects the absorption, distribution and excretion of each of the vitamins in rats fed a diet containing a low-vitamin mixture. On the other hand, when rats were fed a 20 % casein diet containing a sufficient amount of vitamins, ethanol consumption did not affect any factors that we measured.

Acknowledgements

This study was part of the project ‘Studies on the Dietary Reference Intakes for Japanese’ (principal investigator, K. S.), which was supported by a Research Grant for Comprehensive Research on Cardiovascular and Life-Style Related Diseases from the Ministry of Health, Labour and Welfare of Japan. The authors’ responsibilities are as follows: A. M. designed the study, performed the experiments and prepared the manuscript; M. S. and T. F. helped in the study design, performed the experiments and assisted with the data analysis; K. S. contributed to the study design and supervised the study. The authors declare that they have no conflict of interest.

References

1. Bonjour JP (1979) Vitamins and alcoholism. I. Ascorbic acid. *Int J Vitam Nutr Res* **49**, 434–441.
2. Bonjour JP (1980) Vitamins and alcoholism. II. Folate and vitamin B_{12}. *Int J Vitam Nutr Res* **50**, 96–121.
3. Bonjour JP (1980) Vitamins and alcoholism. III. Vitamin B_6. *Int J Vitam Nutr Res* **50**, 215–230.
4. Bonjour JP (1980) Vitamins and alcoholism. IV. Thiamin. *Int J Vitam Nutr Res* **50**, 321–338.
5. Bonjour JP (1980) Vitamins and alcoholism. V. Riboflavin. VI. Niacin. VII. Pantothenic acid. VIII. Biotin. *Int J Vitam Nutr Res* **50**, 425–440.
6. Bonjour JP (1981) Vitamins and alcoholism. IX. Vitamin A. *Int J Vitam Nutr Res* **51**, 166–177.

7. Bonjour JP (1981) Vitamins and alcoholism. X. Vitamin D, XI. Vitamin E, XII. Vitamin K. *Int J Vitam Nutr Res* **51**, 307–318.
8. Leevy CM, Baker H, TenHove W, *et al.* (1965) B-Complex vitamins in liver disease of the alcoholic. *Am J Clin Nutr* **16**, 339–346.
9. Leevy CM, Thompson A & Baker H (1970) Vitamins and liver injury. *Am J Clin Nutr* **23**, 493–499.
10. Baker H, Frank O, Ziffer H, *et al.* (1964) Effect of hepatic disease on liver B-complex vitamin titers. *Am J Clin Nutr* **14**, 1–6.
11. Subramanian VS, Subramanya SB, Tsukamoto H, *et al.* (2010) Effect of chronic alcohol feeding on physiological and molecular parameters of renal thiamin transport. *Am J Physiol Renal Physiol* **299**, F28–F34.
12. Subramanya SB, Subramanian VS & Sain HM (2010) Chronic alcohol consumption and intestinal thiamin absorption: effects on physiological and molecular parameters of the uptake process. *Am J Physiol Gastrointest Liver Physiol* **299**, G23–G31.
13. Hamid A & Kaur J (2007) Long-term alcohol ingestion alters the folate-binding kinetics in intestinal brush border membrane in experimental alcoholism. *Alcohol* **41**, 441–446.
14. Hamid A, Kaur J & Mahmood A (2007) Evaluation of the kinetic properties of the folate transport system in intestinal absorptive epithelium during experimental ethanol ingestion. *Mol Cell Biochem* **304**, 265–271.
15. Hamid A & Kaur J (2007) Decreased expression of transporters reduces folate uptake across renal absorptive surfaces in experimental alcoholism. *J Membr Biol* **220**, 69–77.
16. Hamid A, Wani NA, Rana S, *et al.* (2007) Down-regulation of reduced folate carrier may result in folate malabsorption across intestinal brush border membrane during experimental alcoholism. *FEBS J* **274**, 6317–6328.
17. Hamid A, Kiran M, Rana S, *et al.* (2009) Low folate transport across intestinal basolateral surface is associated with down-regulation of reduced folate carrier in *in vivo* model of folate malabsorption. *IUBMB Life* **61**, 236–243.
18. Hamid A & Kaur J (2009) Role of signaling pathways in the regulation of folate transport in ethanol-fed rats. *J Nutr Biochem* **20**, 291–297.
19. Wani NA & Kaur J (2010) Reduced levels of folate transporters (PCFT and RFC) in membrane lipid rafts result in colonic folate malabsorption in chronic alcoholism. *J Cell Physiol* **226**, 579–587.
20. Leevy CM, George WS, Ziffer H, *et al.* (1960) Pantothenic acid, fatty liver and alcoholism. *J Clin Invest* **39**, 1005.
21. Sorrell MF, Baker H, Barak AJ, *et al.* (1974) Release by ethanol of vitamins into rat liver perfusates. *Am J Clin Nutr* **27**, 743–745.
22. Israel BC & Smith CM (1987) Effects of acute and chronic ethanol ingestion on pantothenate and CoA status of rats. *J Nutr* **117**, 443–451.
23. Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr* **127**, 838S–841S.
24. Pullman ME & Colowick SP (1954) Preparation of 2- and 6-pyridones of N^1-methylnicotinamide. *J Biol Chem* **206**, 121–127.
25. Shibata K, Kawada T & Iwai K (1988) Simultaneous microdetermination of nicotinamide and its major metabolites, N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-3-pyridone-4-carboxamide, by high-performance liquid chromatography. *J Chromatogr* **424**, 23–28.
26. Shibata K, Mushiage M, Kondo T, *et al.* (1995) Effects of vitamin B_6 deficiency on the conversion ratio of tryptophan to niacin. *Biosci Biotechnol Biochem* **59**, 2060–2063.
27. Fukuwatari T, Wada H & Shibata K (2008) Age-related alterations of B-group vitamin contents in urine, blood and liver from rats. *J Nutr Sci Vitaminol* **54**, 357–362.
28. Shibata K, Fukuwatari T, Ohta M, *et al.* (2005) Values of water-soluble vitamins in blood and urine of Japanese young men and women consuming a semi-purified diet based on the Japanese Dietary Reference Intakes. *J Nutr Sci Vitaminol* **51**, 319–328.
29. Fukuwatari T, Suzuura C, Sasaki R, *et al.* (2004) Action site of bisphenol A as metabolic disruptor lies in the tryptophan-nicotinamide conversion pathway. *J Food Hyg Soc Jpn* **45**, 231–238.
30. Ohkawa H, Ohishi N & Yagi K (1982) A simple method for micro-determination of flavin in human serum and whole blood by high-performance liquid chromatography. *Biochem Int* **4**, 187–194.
31. AOAC International (1995) *Official Methods of Analysis*, 16th ed. Arlington, VA: AOAC International.
32. Watanabe F, Abe K, Katsura H, *et al.* (1998) Biological activity of hydroxo-vitamin B_{12} degradation product formed during microwave heating. *J Agric Food Chem* **46**, 5177–5180.
33. Skeggs H & Wright LD (1944) The use of *Lactobacillus arabinosus* in the microbiological determination of pantothenic acid. *J Biol Chem* **156**, 21–26.
34. Aiso K & Tamura T (1998) Trienzyme treatment for food folate analysis. Optimal pH and incubation time for α-amylase and protease treatment. *J Nutr Sci Vitaminol* **44**, 361–370.
35. Fukui T, Iinuma K, Oizumi J, *et al.* (1994) Agar plate method using *Lactobacillus plantarum* for biotin determination in serum and urine. *J Nutr Sci Vitaminol* **40**, 491–498.
36. Gregory JF 3rd & Kirk JR (1979) Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. *Am J Clin Nutr* **32**, 879–883.
37. Thomson AD, Baker H & Leevy CM (1970) Patterns of ^{35}S-thiamine hydrochloride absorption in the malnourished alcoholic patients. *J Lab Clin Med* **76**, 34–45.
38. Leevy CM & Baker H (1968) Vitamins and alcoholism. *Am J Clin Nutr* **21**, 1325–1328.
39. Pearson WN (1967) Blood and urinary vitamin levels as a potential indices of body stores. *Am J Clin Nutr* **20**, 514–525.
40. Neal RA & Pearson WN (1964) Studies of thiamine metabolism in the rat. II. Isolation and identification of 2-methyl-4-amino-5-pyridinecarboxylic acid as a metabolite of thiamine in rat urine. *J Nutr* **83**, 351–357.
41. Suzuoki Z, Tominaga F, Matsuo T, *et al.* (1968) Metabolism of thiamine and thiamine tetrahydrofurfuryl disulfide to 4-methylthiazole-5-acetic acid in conventional and germfree rats under various dosing conditions. *J Nutr* **96**, 433–444.
42. Amos WH & Neal RA (1970) Isolation and identification of 3-(2′-methyl-4′-amino-5′-pyrimidylmethyl)-4-methylthiazole-5-acetic acid (thiamine acetic acid) and 2-methyl-4-amino-5-formylaminomethylpyrimidine as metabolites of thiamine in the rat. *J Biol Chem* **245**, 5643–5648.
43. Shibata K & Onodera M (1992) Changes in the conversion rate of tryptophan-nicotinamide according to dietary fat and protein levels. *Biosci Biotechnol Biochem* **56**, 1104–1108.
44. Frank O, Baker H & Leevy CM (1964) Vitamin-binding capacity of experimentally injured liver. *Nature* **203**, 302–303.
45. Collins TD, Eisenga BH, Bhandari SD, *et al.* (1992) Effects of ethanol on tissue folate incorporation and recovery from folate deficiency in rats. *Alcohol Clin Exp Res* **16**, 757–763.
46. Tamura H & Halsted CH (1983) Folate turnover in chronically alcoholic monkeys. *J Lab Clin Med* **101**, 623–628.

British Journal of Nutrition

47. McMartin KE (1984) Increased urinary folate excretion and decreased plasma folate levels in the rat after acute ethanol treatment. *Alcohol Clin Exp Res* **8**, 172–178.
48. Eichner ER & Hillman RS (1973) Effect of alcohol on serum folate level. *J Clin Invest* **52**, 584–591.
49. Paine CJ, Eichner ER & Dickson V (1973) Concordance of radioassay and microbiological assay in the study of the ethanol-induced fall in serum folate level. *Am J Med Sci* **266**, 134–138.
50. McMartin KE & Collins TD (1983) Role of ethanol metabolism in the alcohol-induced increase in urinary folate excretion in rats. *Biochem Pharmacol* **32**, 2549–2555.
51. McMartin KE, Collins TD & Bairnfather L (1986) Cumulative excess urinary excretion of folate in rats after repeated ethanol treatment. *J Nutr* **116**, 1316–1325.
52. McMartin KE, Collins TD, Eisenga BH, *et al.* (1989) Effects of chronic ethanol and diet treatment on urinary folate excretion and development of folate deficiency in the rat. *J Nutr* **119**, 1490–1497.
53. Romanoff RL, Ross DM & McMartin KE (2007) Acute ethanol exposure inhibits renal folate transport, but repeated exposure upregulates folate transport proteins in rats and human cells. *J Nutr* **137**, 1260–1265.

British Journal of Nutrition

Food and Nutrition Sciences, **2012, 3, 123-128**
doi:10.4236/fns.2012.31018 Published Online January 2012 (http://www.SciRP.org/journal/fns)

Correlation between Mineral Intake and Urinary Excretion in Free-Living Japanese Young Women

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Received October 8th, 2011; revised November 8th, 2011; accepted November 25th, 2011

ABSTRACT

To clarify whether the urinary excretion of calcium, magnesium, phosphorus, iron, zinc, copper, manganese, selenium and molybdenum can be used as an index of their intake, the association between urinary excretion and intake in free-living individuals was examined. A total of 102 healthy free-living female university dietetics students aged 18 - 33 years voluntarily participated in this study, of which 76 students were eligible for this assessment. All food consumed for four consecutive days was recorded accurately by a weighed food record method. A 24-h urine sample was collected on the fourth day, and the urinary levels of sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, manganese, selenium and molybdenum were measured. Significant correlation between urinary excretion and intake was observed in sodium ($r = 0.596$, $p < 0.001$), potassium ($r = 0.583$, $p < 0.001$), calcium ($r = 0.402$, $p < 0.001$), magnesium ($r = 0.365$, $p < 0.01$), phosphorus ($r = 0.509$, $p < 0.001$), selenium ($r = 0.349$, $p < 0.01$) and molybdenum ($r = 0.265$, $p < 0.01$). On the other hand, urinary excretion was very low and completely independent of the intake in iron, zinc, copper and manganese. These results indicate that urinary calcium, magnesium, phosphorus, selenium and molybdenum can be used as an index of their intake, similarly to sodium and potassium.

Keywords: Mineral Intake; Trace Elements; Urinary Excretion; Assessment; Japanese Young Women

1. Introduction

To assess the nutritional status of healthy free-living humans, the weighed food record method has been used widely to record the dietary intake and to calculate nutrient intake [1]. Although this method can provide relatively precise information regarding dietary intake compared with other dietary assessment [2], substantial effort is required for respondents to complete the dietary records and to weigh all food consumed. This often leads to errors in the records, which reveals the limitation of a weighed food record method in terms of accuracy [3]. Alternatively, other methods using quantitative biological information, such as urinary excretion, or concentrations of nutrient or their metabolites in blood, as biomarkers to assess dietary intake or nutritional status have been well studied in recent years.

Many preceding studies have investigated urinary excretion as a biomarker for assessing dietary intake. For example, 24-h urinary nitrogen is established as a marker for protein intake [4], urinary sugars for sugar intake [5,6], and urinary thiamine for thiamine intake [7]. As regards minerals, urinary potassium is established as a marker for potassium intake [8] and urinary iodine for iodine intake [9] as well as urinary sodium for sodium intake [10,11].

In the present study, we measured sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, manganese, selenium and molybdenum in 24-h urine and examined the association between urinary mineral excretion and their intake in free-living individuals. In addition, we examined whether the urinary excretion of calcium, magnesium, phosphorus, iron, zinc, copper, manganese, selenium and molybdenum can be used as an index of their intake, similarly to sodium and potassium.

2. Subjects and Methods

2.1. Subjects

This study was reviewed and approved by the Ethics Committee of The University of Shiga Prefecture. A total of 102 healthy free-living female university dietetics students aged 18 - 33 years voluntarily participated in this

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study. The purpose and protocol of this study was explained to all participants before joining the study, and written informed consent was obtained from each participant, and from parents of participants aged < 20 years. We excluded participants diagnosed with cold or influenza, and those who had taken mineral supplements at least once during the previous month. In addition, we excluded participants whose 24-h urine collection or dietary records were considered as incomplete, with a collection time outside the 22 - 26 h range, urine volume < 250 mL, creatinine excretion in relation to body weight outside the 10.8 - 25.2 mg/kg range [12], or extremely low or high energy intake (<500 or >4000 kcal/d). After screening, 76 participants were found to be eligible. Anthropometric profiles of the 76 participants are shown and compared with those of general Japanese young women in **Table 1**. No difference was observed between subjects and general women.

2.2. Dietary Records

This was a 4-day dietary assessment in which the participants were living freely at college and consuming their normal diet. The first day (Monday) of the experimental period was defined as Day 1, etc. To measure dietary intake during the 4-day period precisely, we used a weighed food record method, which is the highest quality in Japan at this time [13,14]. A digital cooking scale (1 g unit; Tanita Inc., Tokyo, Japan), a set of dietary record forms, a dietary record manual, and a disposable camera were distributed to the participants in advance. Upon entry of the dietary record, the status of food at oral intake was identified as "raw", "cooked", "the presence of skin", "cooking ingredient", or "with or without seasoning", and coded according to the Fifth Revised and Enlarged Edition of the Standard Tables of Food Composition in Japan [15]. The participants took photographs with a disposable camera of the dish before and after eating. Several experienced dietitians used the photographs to complete the data, and asked the participants to resolve any discrepancies or to obtain further information when needed. The food that remained after eating was measured by a digital scale and was deduced from the dietary record. Food, nutrient and energy intake was calculated using the Standard Tables of Food Composition in Japan. For mineral intake, sodium, potassium, calcium, phosphorus, iron, zinc, copper and manganese were assessed. Because selenium and molybdenum are not designated in the Standard Table of Food Composition in Japan, intake of these microminerals was calculated using averaged values of the contents for every food groups described in the literature [16,17].

Table 1. Comparison of anthropometric profiles between subjects and general Japanese young women.

	Subjects (n = 76)	NHNSJ-2008[1] (n = 284)
Age	20.1 ± 2.3	20 - 29
Height (cm)	158.3 ± 5.0	158.3 ± 5.4
Weight (kg)	50.8 ± 5.2	51.9 ± 9.5
Body mass index (kg/m^2)	20.2 ± 1.7	20.7 ± 3.6

Values are the means ±SD. [1]Values for general Japanese young women aged 20 to 29 years described in the National Health and Nutrition Survey of Japan in 2008.

2.3. 24-h Urine Sampling

A single 24-h urine sample was collected on Day 4 to measure urinary mineral excretion. In the morning, participants were asked to discard the first specimen and to record the time on the sheet. The next morning, participants were asked to collect the last specimen at the same time as when the specimen had been discarded the previous morning, and to record the time on the sheet. After the urine sample had been collected, the volume of the sample was measured. The urine samples were stored at –20˚C until analysis.

2.4. Measurement of Urinary Minerals

Urine samples were diluted with 9 or more volumes of 0.1 M HNO_3 and filtrated through a 0.45-μm-membrane filter. Filtrate thus obtained was used for the measurement of minerals. Sodium, potassium, calcium and magnesium were determined by atomic absorption spectrometer (AA-6300; Shimadzu, Kyoto, Japan). Phosphorus, iron, zinc and copper were determined by inductively coupled plasma-atomic emission spectrometer (ULTIMA2; Horiba Ltd., Kyoto, Japan). Manganese, selenium and molybdenum were determined by inductively coupled plasma-mass spectrometer (ICPM-8500; Shimadzu) using rhodium (for manganese and molybdenum) and tellurium (for selenium) as internal standards. In these urinalyses, recovery of each mineral adding urine was 97% to 101%.

2.5. Statistical Analysis

For each subject, means of daily nutrient and energy intake were calculated from the consecutive 4-day dietary records. The mean values of the subjects were calculated based on the resulting individual mean values. Pearson correlation coefficients were calculated to determine the association between urinary and dietary measurements of minerals. These statistical tests were performed using a personal computer (eMac; Apple Computer, Cupertino, CA, USA) with the operating system Mac OS 9.2 and statistical program package StatView-J version 5.0 (Abacus Concept, Berkeley, CA).

3. Results and Discussion

In **Table 2**, the daily energy and nutrient intake of the 76

Table 2. Daily intake of energy, major nutrients and minerals of subjects at experimental period.

	Subjects[1] (n = 76)	NHNSJ-2008[2] (n = 418)
Energy (kcal)	1658 ± 302	1669 ± 475
Protein (g)	57.3 ± 11.9	61.0 ± 21.4
Lipid (g)	52.8 ± 15.5	53.7 ± 22.6
Carbohydrate (g)	232.8 ± 39.8	227.3 ± 66.6
Minerals		
Sodium (mg)	2923 ± 834	3617 ± 1415 [3]
Potassium (mg)	1873 ± 472	1886 ± 710
Calcium (mg)	503 ± 142	406 ± 205 [3]
Magnesium (mg)	194 ± 53	201 ± 70
Phosphorus (mg)	852 ± 193	844 ± 292
Iron (mg)	6.7 ± 1.9	6.7 ± 2.7
Zinc (mg)	6.9 ± 1.5	7.2 ± 2.6
Copper (mg)	0.90 ± 0.21	0.98 ± 0.34
Manganese (mg)	2.8 ± 0.8	-
Selenium (μg)	189 ± 67	-
Molybdenum (μg)	272 ± 77	-

Values are the means ±SD. [1]Daily intake was assessed from the consecutive 4-day dietary records. [2]Values for general Japanese young women aged 18 to 29 years described in the National Health and Nutrition Survey of Japan in 2008. [3]Significant difference was observed between subjects and general Japanese young women at $p < 0.001$ by Student's t-test.

eligible participants is presented and compared with those of general Japanese young women described in the National Health and Nutritional Survey of Japan (NHNSJ) [18]. Similarity was observed between the subjects and general Japanese in the intake of energy and macronutrients. Among minerals, no difference was observed in potassium, magnesium, phosphorus, iron, zinc and copper intake. In addition, manganese and molybdenum intake in the participants was close to the reported values for general Japanese [19,20]. On the other hand, lower sodium intake and higher calcium intake were observed in the subjects than in general young women. In Japan, because excess intake of sodium and low intake of calcium have been major nutritional problems, dietetics students have received education so that sodium intake is reduced and calcium intake is increased; therefore, it is thought that the subjects made efforts to reduce their sodium intake and increase their calcium intake intentionally. Selenium intake in the participants was quite a bit higher than the reported value for general Japanese [16,21]. This indicates that overestimation arose in selenium intake roughly calculated using averaged values of the contents for every food group because no difference was observed between the subjects and general Japanese adolescents in the intake of energy and many nutrients.

Table 3 shows 24-h urinary excretion and the apparent urinary excretion rate of minerals. As regards manganese, since almost all samples showed less than the detection limit (<10 μg/L), it is excluded from the table.

Table 3. Daily urinary mineral excretion in subjects.

	Excretion amounts	Apparent excretion rate
	(mg/d)	(%)
Sodium	2616 ± 1010	90.7 ± 30.8
Potassium	1456 ± 498	79.5 ± 23.0
Calcium	100.5 ± 36.4	20.9 ± 8.2
Magnesium	39.9 ± 16.4	22.4 ± 15.4
Phosphorus	660 ± 223	79.1 ± 23.8
	(μg/d)	
Iron	220 ± 138	3.6 ± 2.5
Zinc	374 ± 125	6.3 ± 2.8
Copper	52.5 ± 37.1	6.3 ± 5.1
Selenium	84.8 ± 26.6	49.7 ± 21.3
Molybdenum	211 ± 93	82.2 ± 44.3

Values are the means ±SD. Apparent excretion rate was calculated as follows: (daily urinary excretion amounts)/(daily intake) × 100.

A high rate of urinary excretion (>70%) was observed for sodium and potassium, which intake has been assessed using urine. In addition, phosphorus and molybdenum also showed a high excretion rate, parallel to sodium and potassium. Because most phosphorus and molybdenum ingested from food are absorbed in the intestine and their main excretion route is urine [20,22], this high excretion rate is valid. Although dietary selenium is also mostly absorbed and its main excretion route is urine [23], the excretion rate was 50%, which was lower than several reported values [24]. This was surely caused by an overestimation of selenium intake; if the excretion rate were 70%, selenium intake would be estimated to be about 120 μg/d, which is almost coincident with the reported value for general Japanese [16,21].

The apparent urinary excretion rate of calcium and magnesium was about 20%, which was coincident with the reported value [22,25]. On the other hand, urinary excretion of iron, zinc and copper was very low, which reflects that urine is not the main excretion route of these minerals [26-28].

Figure 1 shows the correlation between daily intake and 24-h urinary excretion of sodium, potassium, calcium, magnesium and phosphorus. Significant correlation was observed with all of these five minerals. In particular, a strong correlation ($r > 0.5$) was observed for sodium, potassium and phosphorus; therefore, in these three minerals, intake could be estimated from the amount of urinary excretion for every individual with high accuracy. Urinary sodium and potassium are already used as important indices of their intake for individuals [10,11]. In addition, urinary phosphorus could also be used as an index of its intake.

Also, in the case of calcium and magnesium, a significant correlation between urinary excretion and intake was observed. The intestinal absorption rate of calcium and magnesium is 30% to 50% and the main excretion

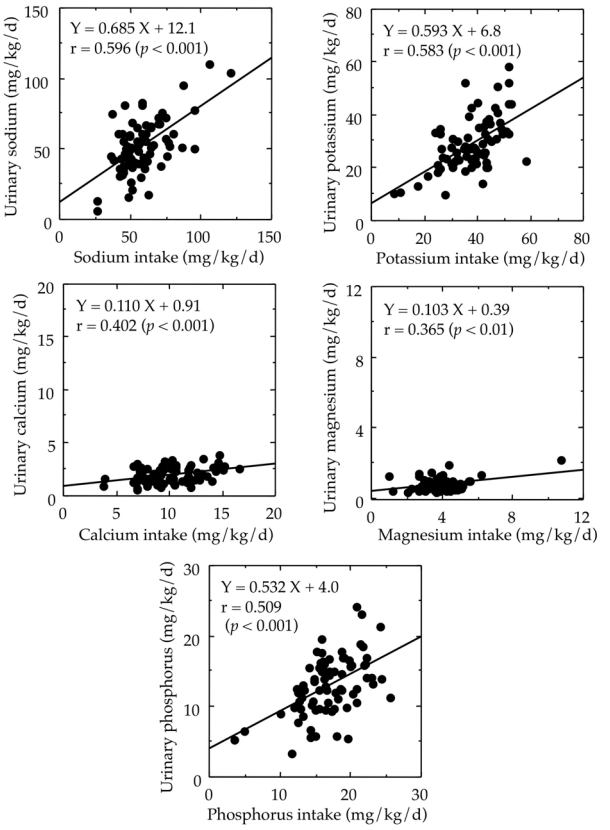

Figure 1. Correlation between daily intake and urinary excretion of sodium, potassium, calcium, magnesium and phosphorus in subjects.

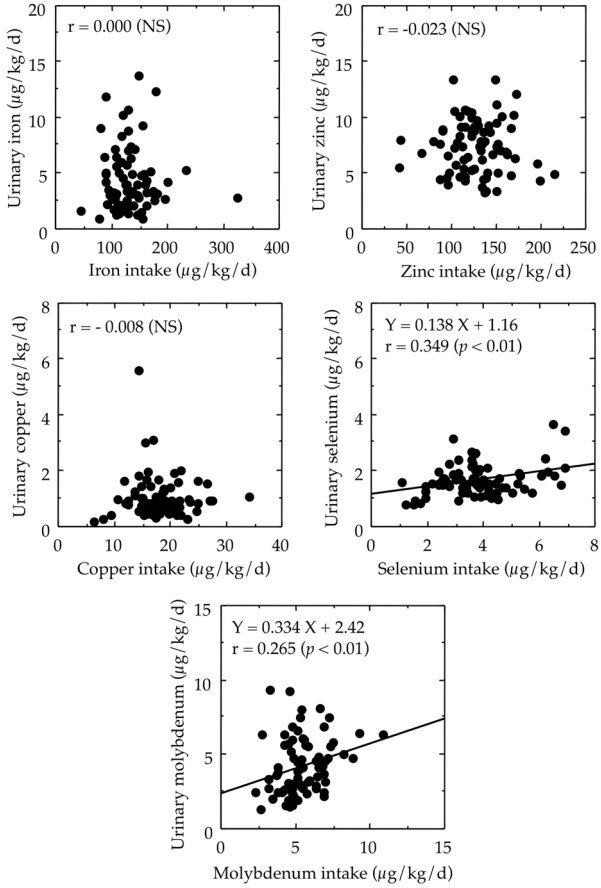

Figure 2. Correlation between intake and urinary excretion of iron, zinc, copper, selenium and molybdenum of subjects.

route is urine [22,25]; therefore, urinary excretion of these minerals reflects absorption amounts. Since intestinal absorption of these minerals changes with various factors [29], it may be difficult to estimate the intake of these minerals from the urinary excretion for every individual. Nevertheless, it will be possible to estimate the intake from urinary excretion at least in a group.

Figure 2 shows correlation between intake and urinary excretion in iron, zinc, copper, selenium and molybdenum. In iron, zinc and copper, the scale is changed between the X- and Y-axis since their excretion rate to urine is very low. In these three minerals, urinary excretion was almost completely independent of the intake. Accordingly, intake of these minerals cannot be estimated from urinary excretion. In addition, because urinary manganese excretion was very low, similarly to iron, zinc and copper, it may be difficult to use urinary manganese as an index of manganese intake. Probably, it is the reason that their urinary excretion is constantly low regardless of the intake, since they are bound to protein in blood. In the case of selenium and molybdenum, a significant correlation was observed; however, in spite of having said that a large part of ingested selenium and molybdenum was excreted into urine, similarly to potassium, sodium and phosphorus [20,23], the correlation coefficients were smaller than those of calcium and magnesium. Probably, these weak correlations were due to rough intake estimation using averaged values of the contents for every food group; therefore, it is considered that a greater correlation coefficient was obtained when intake was estimated using the content of every food, as for other minerals.

In the present study, it was confirmed that excretion amounts in 24-h urine were good indices of daily intake of phosphorus, calcium, magnesium, selenium and molybdenum similarly to sodium and potassium. In minerals, estimation of the intake using 24-h urine is possible when the main excretion route is urine. To estimate the intake of these minerals from the urinary excretion, the precise regression between intake and urinary excretion needs to be established by a balance test in the future.

4. Acknowledgements

This study was supported by a grant for comprehensive research on cardiovascular and lifestyle disease from the Ministry of Health, Labour and Welfare of Japan.

REFERENCES

[1] W. Wilett, "Nutritional Epidemiology," 2nd Edition, Oxford University Press, New York, 1998.

[2] S. A. Bingham, C. Gill, A. Welch, A. Cassidy, S. A. Runswick, S. Oakes, R. Lubin, D. I. Thurnham, T. J. Key, L. Roe, K. T. Khaw and N. E. Day, "Validation of Dietary Assessment Methods in the UK Arm of EPIC Using Weighed Records, and 24-Hour Urinary Nitrogen and Potassium and Serum Vitamin C and Carotenoids as Biomarkers," *International Journal of Epidemiology*, Vol. 26, Suppl. 1, 1997, pp. S137-S151. doi:10.1093/ije/26.suppl_1.S137

[3] M. B. E. Libingstone and A. E. Black, "Markers of the Validity of Reported Energy Intake," *Journal of Nutrition*, Vol. 133, Suppl. 3, 2003, pp. 895S-920S.

[4] S. A. Bingham, "Urine Nitrogen as a Biomarker for the Validation of Dietary Protein Intake," *Journal of Nutrition*, Vol. 133, Suppl. 3, 2003, pp. 921S-924S.

[5] C. Luceri, G. Caderni, M. Lodovici, M. T. Spagnesi, C. Monserrat, L. Lancioni and P. Dolara, "Urinary Excretion of Sucrose and Fructose as a Predictor of Sucrose Intake in Dietary Intervention Studies," *Cancer Epidemiology, Biomarkers and Prevention*, Vol. 5, No. 3, 1996, pp. 167-171.

[6] N. Tasevska, S. A. Runswick, A. A. Welch, A. McTaggart and S. A. Bingham, "Urinary Sugars Biomarker Relates Better to Extrinsic than to Intrinsic Sugars Intake in a Metabolic Study with Volunteers Consuming Their Normal Diet," *European Journal of Clinical Nutrition*, Vol. 63, No. 5, 2009, pp. 653-659. doi:10.1038/ejcn.2008.21

[7] N. Tasevska, S. A. Runswick, A. McTaggart and S. A. Bingham, "Twenty-Four-Hour Urinary Thiamine as a Biomarker for the Assessment of Thiamine Intake," *European Journal of Clinical Nutrition*, Vol. 62, No. 9, 2008, pp. 1139-1147. doi:10.1038/sj.ejcn.1602829

[8] N. Tasevska, S. A. Runswick and S. A. Bingham, "Urinary Potassium Is as Reliable as Urinary Nitrogen for Use as a Recovery Biomarker in Dietary Studies of Free Living Individuals," *Journal of Nutrition*, Vol. 136, No. 5, 2006, pp. 1334-1340.

[9] D. Ristic-Medic, Z. Piskackova, L. Hooper, J. Ruprich, A. Casgrain, K. Ashton, M. Pavlovic and M. Glibetic, "Methods of Assessment of Iodine Status in Humans: A Systematic Review," *American Journal of Clinical Nutrition*, Vol. 89, No. 6, 2009, pp. 2052S-2069S. doi:10.3945/ ajcn.2009. 27230H

[10] L. K. Dahl, "Evidence for an Increased Intake of Sodium in Hypertension Based on Urinary Excretion of Sodium," *Proceedings of Society of Experimental Biology and Medicine*, Vol. 94, No. 1, 1957, pp. 23-26.

[11] Y. Morinaga, T. Tsuchihashi, Y. Ohta and K. Matsumura, "Salt Intake in 3-Year-Old Japanese Children," *Hypertension Research*, Vol. 34, No. 7, 2011, pp. 836-839. doi:10.1038/hr.2011.55

[12] K. Murakami, S. Sasaki, Y. Takahashi, K. Uenishi, T. Watanabe, T. Kohri, M. Yamasaki, R. Watanabe, K. Baba, K. Shibata, T. Takahashi, K. Hayabuchi, K. Ohki and J. Suzuki, "Sensitivity and Specificity of Published Strategies Using Urinary Creatinine to Identify Incomplete 24-h Urine Collection," *Nutrition*, Vol. 24, No. 1, 2008, pp. 16-22. doi:10.1016/j.nut.2007.09.001

[13] T. Imai, S. Sasaki, K. Mori, F. Ando, N. Niino and H. Shimokata, "Nutritional Assessment of 3-Day Dietary Records in National Institute for Longevity Science-Longitudinal Study of Aging (NILS-LSA)," *Journal of Epidemiology*, Vol. 10, Suppl. 1, 2000, pp. S70-S76. doi:10.2188/jea.10.1sup_70

[14] K. Murakami, H. Okubo and S. Sasaki, "Dietary Intake in Relation to Self-Reported Constipation among Japanese Women Aged 18 - 20 Years," *European Journal of Clinical Nutrition*, Vol. 60, No. 5, 2006, pp. 650-657. doi:10.1038/sj.ejcn.1602365

[15] Ministry of Education, Culture, Sport, Science and Technology of Japan, "Standard Tables of Food Composition in Japan 5th Revised and Enlarged Edition (in Japanese)," National Printing Bureau of Japan, Tokyo, 2007.

[16] Y. Miyazaki, H. Koyama, Y. Sasada, H. Sato, M. Nojiri and S. Suzuki, "Dietary Habits and Selenium Intake of Residents in Mountain and Coastal Communities in Japan," *Journal of Nutritional Science and Vitaminology*, Vol. 50, No. 5, 2004, pp. 309-319. doi:10.3177/jnsv.50.309

[17] H. Hattori, A. Ashida, C. Itô and M. Yoshida, "Determination of Molybdenum in Foods and Human Milk, and an Estimate of Average Molybdenum Intake in the Japanese population," *Journal of Nutritional Science and Vitaminology*, Vol. 50, No. 6, 2004, pp. 404-409. doi:10.3177/jnsv.50.404

[18] Ministry of Health, Labour and Welfare of Japan, "The National Health and Nutrition Survey in Japan, 2008 (in Japanese)," Dai-Ichi Shuppan, Tokyo, 2011.

[19] S. Horiguchi, K. Teramoto, T. Kurono and K. Ninomiya, "The Arsenic, Copper, Lead, Manganese and Zinc Contents of Daily Foods and Beverages in Japan and the Estimate of Their Daily Intake," *Osaka City Medical Journal*, Vol. 24, No. 1, 1978, pp. 131-141.

[20] M. Yoshida, H. Hattori, S. Ota, K. Yoshihara, N. Kodama, Y. Yoshitake and M. Nishimuta, "Molybdenum Balance in Healthy Young Japanese Women," *Journal of Trace Elements in Medicine and Biology*, Vol. 20, No. 4, 2006, pp. 245-252. doi:10.1016/j.jtemb.2006.07.004

[21] M. Yoshida and K. Yasumoto, "Selenium Content of Rice Grown at Various Sites in Japan," *Journal of Food Composition and Analysis*, Vol. 1, No. 1, 1987, pp. 71-75. doi:10.1016/0889-1575(87)90013-5

[22] M. Nishimuta, N. Kodama, E. Morikuni, Y. H. Yoshioka, H. Takeyama, H. Yamada, H. Kitajima and K. Suzuki, "Balances of Calcium, Magnesium and Phosphorus in Japanese Young Adults," *Journal of Nutritional Science and Vitaminology*, Vol. 50, No. 1, 2004, pp. 19-25. doi:10.3177/jnsv.50.19

[23] C. A. Swanson, D. C. Reamer, C. Veillon, J. C. King and O. A. Levander, "Quantitative and Qualitative Aspects of Selenium Utilization in Pregnant and Nonpregnant Women: An Application of Stable Isotope Methodology," *American Journal of Clinical Nutrition*, Vol. 38, No. 2,

1983, pp. 169-180.

[24] H. J. Robbrecht and H. A. Deelstra, "Selenium in Human Urine: Concentration Levels and Medical Implications," *Clinica Chimica Acta*, Vol. 136, No. 2-3, 1984, pp. 107-120. doi:10.1016/0009-8981(84)90282-1

[25] N. M. Lewis, M. S. Marcus, A. R. Behling and J. L. Greger, "Calcium Supplements and Milk: Effects on Acid-Base Balance and on Retention of Calcium, Magnesium, and Phosphorus," *American Journal of Clinical Nutrition*, Vol. 49, No. 3, 1989, pp. 527-533.

[26] Food and Nutrition Board Institute of Medicine, "Iron," *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*, National Academy Press, Washington DC, 2001, pp. 290-393.

[27] J. R. Turnlund, "Copper," In: M. E. Shils, J. A. Olson, M. Shike and A. C. Ross, Eds., *Modern Nutrition in Health and Disease*, 9th Edition, Wikkiams & Wilkins, Baltimore, 1999, pp. 241-252.

[28] J. C. King and C. L. Keen, "Zinc," In: M. E. Shils, J. A. Olson, M. Shike and A. C. Ross, Eds., *Modern Nutrition in Health and Disease*, 9th Edition, Wikkiams & Wilkins, Baltimore, 1999, pp. 223-239.

[29] T. Hazell, "Minerals in Foods: Dietary Sources, Chemical Forms, Interactions, Bioavailability," *World Review of Nutrition and Dietetics*, Vol. 46, 1985, pp. 1-123.

Osteoporosis Japan

妊娠初期の骨密度とライフスタイル，栄養摂取状態についての検討

－SKY Study（Saitama，Kobe，Yokohama Pregnant Cohort Study）第1報－

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1　目　　的

妊娠・授乳期の骨代謝動態は，胎盤・母乳を介して，カルシウム，ビタミンD，Kなどの栄養素を児へ大量に供給するために大きく変化する[1,2]。近年，世界各国においてビタミンD不足者が高頻度に存在することが問題となっており[3~6]，小児くる病や，新生児頭蓋ろうの発生率増加は，妊婦・授乳婦のビタミンD，カルシウム不足と関連する可能性が高い[7]。現在われわれは，ライフスタイル，栄養摂取状態と，骨量，血清マーカーの推移について妊婦対象のコホート研究を行っている。今回は，妊娠初期の研究結果について報告する。

2　方　　法

対象は2010年11月から2011年2月に当院を受診した，妊娠5週から12週で，本研究内容に同意の得られた妊婦160名である。糖尿病，腎疾患など骨代謝に関連する慢性疾患を有する妊婦，およびステロイド剤，ビタミン剤を服用する妊婦は除外した。

測定項目は，定量的超音波骨密度測定（QUS：GEヘルスケア・ジャパン社A-1000），食物摂取頻度調査（FFQ法：上西らによる），運動量調査，日照時間（UVケアの有無を確認）のほか，骨代謝関連マーカーを測定した。研究プロトコールを**図1**に示す。

なお，本研究は横浜市立大学倫理委員会の認可のもとに行われた。

3　結　　果

全対象の背景は，年齢32±3.7歳，身長159.2±4.9cm，BMI（body mass index）20.3±2.3で，QUSによる骨密度の指標は，stiffness 92±14.2，BUA 115±15.5，SOS 1553±31.30m/sec，標準化SOS 1539±23.70m/sec，T-score 100±15.5%であった。妊娠初期の踵骨骨密度は平均では良好な値を示したが，T-score 80%未満の低骨密度妊婦が6名（5%）存在した。

ライフスタイル調査の結果は，運動回数7±33.9回/月，運動時間9±82.9時間/月，運動力量1±0.6/月，日照時間38±49.8時間/月（UVケアなし14±29.3時間/月）であった。

妊娠初期のFFQ法による食物摂取頻度調査の結果では，2010年版栄養摂取基準に従い総エネルギーは1610±293.0kcal/日で必要量以下，タンパク質65±16.1g/日，脂質61±14.2g/日，糖質204±41.2g/日は目標範囲内，食塩10±1.6g/日は摂取

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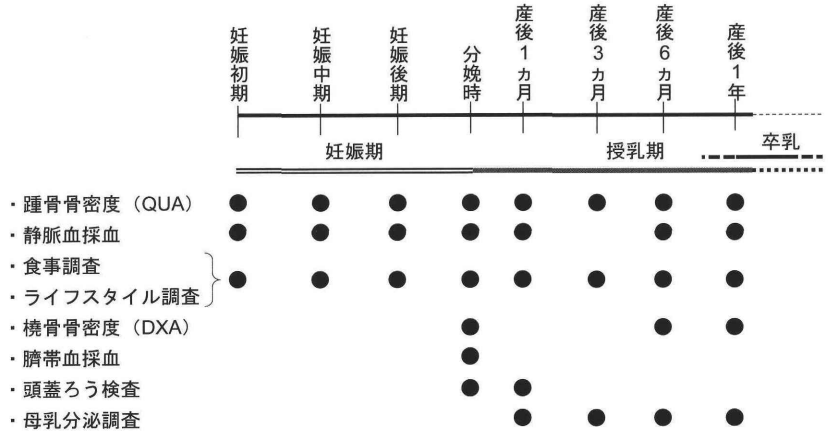

図 1　SKY（Saitama, Kobe, Yokohama Pregnant Cohort）Study のデザイン

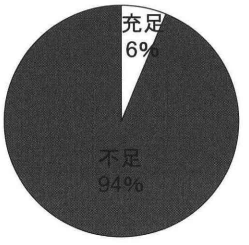

図 2　食物摂取頻度調査におけるカルシウム不足者の割合

表 1　初産・経産での比較

	年齢（歳）	標準化 SOS（m/sec）	総エネルギー（kcal/日）	カルシウム（mg/日）	ビタミン D（μg/日）	ビタミン K（μg/日）	UV なし日照時間（時間/月）
初産婦（52 名）	30.3±3.5	1536±27.2	1629±322	414±136.8	9±2.5	205±12.9	10.9±19.5
経産婦（108 名）	32.8±3.5	1527±23.5	1673±278	421±144.0	9±2.0	204±91.0	15.9±33.8
p	5.6E-0.5	0.04487	0.59307	0.61899	0.38301	0.61894	0.33364

表 2　35 歳以上・35 歳未満での比較

	年齢（歳）	標準化 SOS（m/sec）	総エネルギー（kcal/日）	カルシウム（mg/日）	ビタミン D（μg/日）	ビタミン K（μg/日）	UV なし日照時間（時間/月）
35 歳未満（112 名）	30.3±2.7	1533±27	1602±310	423±146	9±2	207±106	14.1±22.0
35 歳以上（48 名）	36.5±1.6	1523±17	1631±244	408±134	9±2	196±89.4	14.5±45.4
p	2.2E-28	0.0284	0.579	0.551	0.875	0.552	0.939

過多，カルシウム 418±141.4mg/日は目安量以下，鉄 8±4.8mg/日目安量以下，ビタミン A 767±525.6μgRE/日，ビタミン D 9±1.9μg/日，ビタミン K 204±101.7μg/日で推奨量を充足していた。カルシウム摂取は 94%の妊婦において不足していたが（**図 2**），ビタミン D，ビタミン K の摂取量は推奨量に達していた。

対象を初産・経産で比較した結果を**表 1** に，35 歳以上と 35 歳未満で比較した結果を**表 2** に示す。それぞれ栄養摂取やライフスタイルに差はみられなかったが，踵骨骨密度は 35 歳以上妊婦と経産婦で有意に低かった。

4　考察および結語

カルシウム摂取不足は妊娠適齢期女性の多くにみられる傾向にある。2010 年版国民栄養摂取基準では，妊娠・授乳期の付加量はゼロであるが，積極的なカルシウム摂取が推奨される。

本研究の結果，初産・経産婦の栄養摂取に差は認められなかったが，ライフスタイルでは経産婦のほうが日照時間が多い傾向にあり，これは上の子どもと外遊びをするなどの生活パターンの違いによるものと考えられた。

妊娠初期の踵骨骨密度は，35 歳以上，経産婦のほうが有意に低く，年齢因子の影響が考えられた。

文　献

1) Polatti F, et al. Bone mineral changes during and after lactation. Obstet Gynecol 1994;94:52-6.
2) Karlsson MK, et al. Maternity and bone mineral density. Acta Orthop 2005;76:2-13.
3) Kovacs CS. VitaminD in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr 2008;88:520S-528S.
4) Mulligan ML, et al. Implication of vitaminD deficiency in pregnancy and lactation. Am J Obstet Gynecol 2010;202:429.
5) Camargo CA, et al. Vitamin D status of newborns in New Zealand. Br J Nutr 2010;104:1051-7.
6) Ginde AA, et al. Vitamin D insufficiency in pregnant and nonpregnant women of childbearing age in the US. Am J Obstet Gynecol 2010;202:436.
7) Yorifuji J, et al. Craniotabes in normal newborns: the ear- liest sign of subclinical vitamin D deficiency. J Clin Endocrinol Metab 2008;93:1784-8.

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栄養管理報告書を用いた特定給食施設における食事摂取基準の活用に関する調査

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【目的】特定給食施設において適切な栄養管理が行われているかを把握し，必要な指導・助言を行うために，各自治体は特定給食施設に栄養管理報告書の提出を求めている。日本人の食事摂取基準（2010年版）には，給食施設での食事摂取基準の活用の基礎理論としてPDCAサイクルに基づく栄養管理の手順が示されている。本研究では，特定給食施設における食事摂取基準の活用の実態を把握するために，各自治体の栄養管理報告書の書式から基礎理論の手順に基づく栄養管理の実施が把握できるかを調査した。
【方法】栄養管理報告書の書式は2010年3～4月に厚生労働省が収集した。114の自治体（都道府県，保健所を設置する市および特別区）から提出のあった書式のうち，「病院・介護保険社会福祉施設用」の87自治体と「事業所用」86自治体の書式について集計した。集計内容は『対象集団の特性の把握』，『身体状況や食事摂取量の把握』，『食事計画の決定と実施の評価』とした。
【結果】『対象集団の特性の把握』に必要な給食対象集団の特性と人数の両方の記載を求めていない自治体が，「病院・介護保険社会福祉施設用」，「事業所用」ともに2.3%認められた。『身体状況や食事摂取量の把握』に必要な項目として，半数以上の自治体が把握している項目は，「病院・介護保険社会福祉施設用」の身長と体重に関する項目のみであった。『食事計画の決定と実施の評価』に必要な項目として，給与栄養目標量の記載を求めている自治体は「病院・介護保険社会福祉施設用」，「事業所用」ともに約95%であったが，食事摂取量の記載を求めている自治体は約11.5%に過ぎなかった。
【結論】本研究で収集された栄養管理報告書において，給食の食事計画とその評価・計画の見直しにつながる食事摂取量の評価を把握できる項目は限られていた。給食の栄養管理の手順に即した書式の検討が必要である。

栄養学雑誌，Vol.70 No.2 140-151 (2012)

キーワード： 栄養管理報告書，特定給食施設，食事摂取基準，給与栄養目標量，食事摂取量

Ⅰ．緒　言

日本人の食事摂取基準（2010年版）では，活用の基礎理論の中に「給食管理」を目的とした活用理論が示され[1]，さらに「日本人の食事摂取基準」活用検討会報告書（2010年3月）において，給食管理を目的とした食事摂取基準の活用の基本的考え方が示されている[2]。給食管理を目的とした活用では，対象集団の特性の把握を行い，給食を含むすべての食事摂取量のアセスメントを行い，食事計画の決定と実施を行うことと記されている。またそのためのプロセスとして，PDCAサイクルに基づき栄養管理を行う手順が示されている。

一方，栄養管理報告書とは，給食施設で適切な栄養管理が行われているかどうかを把握するために，都道府県，保健所を設置する市および特別区（以下，自治体という）が健康増進法施行細則等に基づき，給食施設の設置者に報告を求めるものである。特定給食施設の指導等に関わる事務は自治事務であることから，栄養管理報告書は自治体ごとに異なる書式が用いられており，報告書の種類（病院，社会福祉施設・介護保険福祉施設，保育所・児童福祉施設，学校，事業所・寄宿舎など）やその記入要領も様々である。

本研究は，自治体が報告を求めている栄養管理報告書の書式から，給食管理を目的とした食事摂取基準の活用の基礎理論に基づき，栄養管理の実施が把握できるかを調査することを目的とした。栄養管理の手順における，『対象集団の特性の把握』，『身体状況や食事摂取量の把握』，および『食事計画の決定と実施の評価』を，各自治体が栄養管理報告書においてどのように確認しているかを実態把握し，その課題や問題点について検討した。

Ⅱ．方　法

1．調査方法

調査には，2010年3月から4月にかけて厚生労働省によって収集された都道府県，保健所を設置する市および特別区の栄養管理報告書の書式を用いた。収集にあたり，特定給食施設における栄養管理のあり方を検討するため

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の資料とすることを示した。114の自治体（都道府県47，政令指定都市18，中核市41，保健所設置市７，特別区１）のうち栄養管理報告書を提出した87の自治体（回収率76%）分を分析対象とした。ただし，東京都23区（特別区）はすべて同じ書式を使用しているため１つの自治体として扱った。

栄養管理報告書の書式の施設別の区分数は，８区分（１自治体），６区分（５自治体），５区分（20自治体），４区分（12自治体），３区分（12自治体），２区分（15自治体），１区分（22自治体）と様々であった。そのうち本研究では，病院および介護保険社会福祉施設をまとめた「病院・介護保険社会福祉施設用」と「事業所用」の２つに分けて集計を行った。「病院・介護保険社会福祉施設用」は，医療および介護を必要としている対象者に３食提供している施設として，「事業所用」は，健康な成人を対象に食事の一部を提供している施設として選択した。施設別区分が１区分しかない場合は，「病院・介護保険社会福祉施設用」と「事業所用」のそれぞれの区分に含めた。事業所用の書式がない自治体があったため，集計に用いたのは「病院・介護保険社会福祉施設用」：87自治体，「事業所用」：86自治体の書式である。

２．調査内容

各自治体が，栄養管理報告書において確認している食事摂取基準の活用に関する記載事項を実態把握するために表１の８項目について集計を行った。具体的には『対象集団の特性の把握』に関する項目として，①給食対象集団の把握内容，『身体状況や食事摂取量の把握』に関する項目として，②給食対象者の身体状況や食事摂取量等の把握の有無，③給食対象者の身体状況等の把握項目，④給食対象者の食事摂取量等の把握項目，『食事計画の決定と実施の評価』に関する項目として，⑤給与栄養目標量・提供量・摂取量の算出の指示および各用語の名称，⑥給与栄養目標量に対する給与栄養量および推定摂取量の確認，⑦給与栄養目標量の記載についての指示，⑧各自治体で報告を求めている栄養素等の項目，である。これらの項目に該当する内容が書式から読み取れる場合は

表１　調査項目

栄養管理の手順	調査項目	確認したい内容	調査の目的
対象集団の特性の把握	①給食対象集団の把握内容	性別・年齢階級・身体活動レベル別給食対象者の人数や給食数	自治体が，施設の人員構成等を把握しているのか，またどのように分類して把握しているのかを実態把握
身体状況や食事摂取量の把握	②給食対象者の身体状況や食事摂取量等の把握の有無	身体状況等の把握の有無	自治体が，施設での身体状況や食事摂取量等の把握の有無を確認しているのかを実態把握
	③給食対象者の身体状況等の把握項目	身体状況等について把握している項目	どのような項目について把握しているのかを確認
	④給食対象者の食事摂取量等の把握項目	食事摂取量等について把握している項目	どのような項目について把握しているのかを確認
食事計画の決定と実施の評価	⑤給与栄養目標量・提供量・摂取量の算出の指示および各用語の名称	給与栄養目標量・提供量・摂取量の算出についての指示	目標量・提供量・摂取量を算出するにあたり，自治体がどのような指示を行っているのかを実態把握
		給与栄養目標量 提供量 摂取量	各用語の整理
	⑥給与栄養目標量に対する給与栄養量および推定摂取量の確認	給与栄養目標量に対する給与栄養量（実施）の内容確認及び評価	施設における食事計画の予定から実施に対する評価について，自治体がどのように指示しているのかを実態把握
		給与栄養目標量に対する推定摂取量の内容確認及び評価	
	⑦給与栄養目標量の記載についての指示	記入要領における給与栄養量の記載についての指示の有無	給与栄養目標量を記載するにあたり，自治体が何らかの指示を行っているのかを実態把握
		記入要領における給与栄養目標量の算出方法についての指示内容	給与栄養目標量を算出するにあたり，自治体がどのような指示を行っているのかを実態把握
	⑧各自治体が報告を求めている栄養素等の項目	栄養素等の項目	各自治体が報告を求めている栄養素等・栄養比率の項目の整理

「該当している書式」として集計した。これらの項目に全く該当しない書式がどのくらいあるのかについても示すこととした。

自治体から提出された書式に示されている内容は，管理栄養士の資格を持ち，給食経営管理論を専門とする研究者（４人，うち１人が保健所および病院勤務経験者）と公衆栄養学を専門とする研究者（２人）が，各項目の該当部分として判定した。判断が難しい事例については，当該の研究者らが内容から判定した。

Ⅲ．結　　果

１．給食対象集団の把握内容

表２に給食対象集団の把握内容について示す。給食対象集団の特性を把握するために必要な性別・年齢階級・身体活動レベル別の人数を同時に把握している自治体は，「病院・介護保険社会福祉施設用」では11自治体（12.6%），「事業所用」では25自治体（29.1%）であり，「事業所用」の方が多かった。性別・年齢階級・身体活動レベル別の人数記入欄がない報告書が，「病院・介護保険社会福祉施設用」では61自治体（70.1%），「事業所用」では50自治体（58.1%）とどちらも全体の自治体の半数以上であった。給食数の把握は「病院・介護保険社会福祉施設用」では85自治体（97.7%），「事業所用」では69自治体（79.3%）であった。給食対象者を把握していない，すなわち性別・年齢階級・身体活動レベル別の人数および給食対象者の人数や給食数のいずれも把握していない自治体は，「病院・介護保険社会福祉施設用」，「事業所用」ともに２自治体（2.3%）であった。

２．給食対象者の身体状況や食事摂取量等の把握の有無

表３に給食対象者における身体状況や食事摂取量等の把握の有無についての結果を示す。給食対象者の身体状況や食事摂取量等の把握をしている自治体は，「病院・介護保険社会福祉施設用」で64自治体（73.6%），「事業所用」で56自治体（65.1%）であった。身体状況や食事摂取量等の把握に関する項目がない自治体は，「病院・介護保険社会福祉施設用」で23自治体（26.4%），「事業所用」で30自治体（34.9%）であった。

表２　給食対象集団の把握内容

			病院・介護保険社会福祉施設用（$n=87$）		事業所用（$n=86$）	
			自治体数	（%）	自治体数	（%）
把握したい項目に該当している書式*	性別・年齢階級・身体活動レベル別の人数	年齢階級別人数	1	1.1	0	0.0
		性別・年齢階級別人数	20	22.9	9	10.5
		性別・年齢階級・身体活動レベル別人数	11	12.6	25	29.1
		区分別の人数記載欄なし	61	70.1	50	58.1
	給食対象者の人数や給食数	給食対象者の合計人数	77	88.5	23	26.4
		給食対象者の朝・昼・夕別人数	1	1.1	7	8.0
		給食数（朝・昼・夕別）	85	97.7	69	79.3
把握したい項目に該当していない書式	給食対象者の把握なし	性別・年齢階級・身体活動レベル別人数および給食対象者人数・給食数とも全て記入欄なし	2	2.3	2	2.3

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。

表３　給食対象者における身体状況や食事摂取量等の把握の有無

		病院・介護保険社会福祉施設用（$n=87$）		事業所用（$n=86$）	
		自治体数	（%）	自治体数	（%）
把握したい項目に該当している書式*	身体状況や食事摂取量等の把握あり	64	73.6	56	65.1
	性別・年齢階級・身体活動レベル別の人数の記入はないが，身体状況や食事摂取量等の把握の有無はあるもの	44	50.6	35	40.7
把握したい項目に該当していない書式	身体状況や食事摂取量等の把握なし	23	26.4	30	34.9

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。

3．給食対象者の身体状況等の把握項目

表4に給食対象者の身体状況等の把握に関する項目を示す。性別，年齢，身体特性（身長・体重），身体活動レベルに関する項目として，性別と年齢の項目をあげていたのは，「病院・介護保険社会福祉施設用」では28自治体（32.2%）と3割程度，「事業所用」では性別で17自治体（19.8%），年齢で14自治体（16.3%）であった。身長と体重の項目をあげていたのは「病院・介護保険社会福祉施設用」では46自治体（52.9%）と半数以上であり，「事業所用」では41自治体（47.7%）と半数に近かった。皮下脂肪厚または体脂肪量等の項目をあげていたのは，「病院・介護保険社会福祉施設用」では9自治体（10.3%）であったが，「事業所用」では0自治体（0%）であった。身体活動レベルについては，「病院・介護保険社会福祉施設用」では27自治体（31.0%），「事業所用」では21自治体（24.4%）であった。身体状況・運動・生活習慣の把握に関する項目は，いずれも「病院・介護保険社会福祉施設用」の方が「事業所用」よりも把握している自治体が多かった。

個別の状況把握に関する項目としては，生化学的検査値の項目では「病院・介護保険社会福祉施設用」では32自治体（36.8%）であったが，「事業所用」では8自治体（9.3%）と少なかった。疾病状況の項目は「病院・介護保険社会福祉施設用」では27自治体（31.0%）であったが，「事業所用」では11自治体（12.8%）であった。

肥満・やせの割合や有所見者の割合に関する項目として，Body mass index（BMI）（kg/m^2）別（肥満とやせ）人数・割合および，糖尿病・高血圧・高脂血症（脂質異

表4　給食対象者の身体状況等の把握項目

		病院・介護保険社会福祉施設用（n=87）		事業所用（n=86）	
		自治体数	（%）	自治体数	（%）
把握したい項目に該当している書式*	性別，年齢，身体特性（身長・体重），身体活動レベルに関する項目				
	性別	28	32.2	17	19.8
	年齢	28	32.2	14	16.3
	身長	46	52.9	41	47.7
	体重	46	52.9	41	47.7
	BMI	38	43.7	31	36.0
	皮下脂肪厚または体脂肪量等	9	10.3	0	0.0
	腹囲の把握	0	0.0	1	1.2
	身体活動レベルの把握	27	31.0	21	24.4
	身体状況	12	13.8	5	5.8
	運動	16	18.4	3	3.5
	生活習慣の把握	24	27.6	7	8.1
	個別の状況把握に関する項目				
	生化学的検査値の把握	32	36.8	8	9.3
	疾病状況	27	31.0	11	12.8
	栄養状態	8	9.2	6	7.0
	摂食・嚥下機能	5	5.7	0	0.0
	褥瘡	8	9.2	0	0.0
	体重減少率	5	5.7	0	0.0
	個別の栄養管理計画	5	5.7	2	2.3
	献立への配慮の有無	4	4.6	6	7.0
	食物アレルギー	1	1.1	1	1.2
	喫煙	16	18.4	3	3.5
	肥満・やせの割合や有所見者の割合に関する項目				
	BMI別（肥満とやせ）人数・割合	16	18.4	21	24.4
	糖尿病・高血圧・高脂血症（脂質異常症）等の人数・割合	14	16.1	22	25.6
	その他の項目				
	行っているアセスメントを記入する	15	17.2	5	5.8
	アセスメントを定期的に行っているかどうか	6	6.9	5	5.8

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。

常症）等の人数・割合があげられた。BMI別（肥満とやせ）人数・割合を把握している自治体は，「病院・介護保険社会福祉施設用」（16自治体：18.4%）に比べて，「事業所用」（21自治体：24.4%）の方が多かった。また，糖尿病・高血圧・高脂血症（脂質異常症）等の生活習慣病の項目を把握している自治体は，「病院・介護保険社会福祉施設用」（14自治体：16.1%）に比べて，「事業所用」（22自治体：25.6%）の方が多かった。

4．給食対象者の食事摂取量等の把握項目

表5に給食対象者の食事摂取量等の把握に関する項目を示す。喫食状況調査をあげている自治体は，「病院・介護保険社会福祉施設用」では21自治体（24.1%）であったが，「事業所用」では4自治体（4.7%）と少なかった。摂取量の把握を求める方法として，全体の残菜から給食の摂取量を把握する残菜調査や，個人の摂取した割合を目測で調査して摂取量を把握する摂取量調査がある。残菜調査をあげている自治体は「病院・介護保険社会福祉施設用」では38自治体（43.7%），「事業所用」では28自治体（32.6%）であった。摂取量調査は「病院・介護保険社会福祉施設用」では36自治体（41.4%），「事業所用」では31自治体（36.0%）であった。摂取量調査の頻度を求めている自治体は，「病院・介護保険社会福祉施設用」では41自治体（47.1%），「事業所用」では26自治体（30.2%）であった。給食以外の食事について把握していたのは「病院・介護保険社会福祉施設用」では17自治体（19.5%）であったが，「事業所用」では0自治体（0%）であった。食習慣（食事内容）の把握・間食の把握を挙げている自治体は，「病院・介護保険社会福祉施設用」，「事業所用」ともに1～3自治体と少なかった。嗜好調査や飲酒の把握は「病院・介護保険社会福祉施設用」の方が「事業所用」よりも把握している自治体が多かった。

5．自治体が算出を求める給与栄養目標量・提供量・摂取量の指標と使用している名称

表6に自治体が算出を求めている給与栄養目標量・提供量・摂取量の指標と使用している名称についての結果を示す。給与栄養目標量の記載は「病院・介護保険社会福祉施設用」では83自治体（95.4%），「事業所用」では81自治体（94.2%）が報告を求めていた。給与栄養目標量として使用している名称は，“給与栄養目標量”の名称が「病院・介護保険社会福祉施設用」で50自治体（57.5%），「事業所用」で50自治体（58.1%）と最も多かった。給与栄養目標量に関する記載を求めていない自治体は，「病院・介護保険社会福祉施設用」では4自治体（4.6%），「事業所用」では5自治体（5.8%）であった。

提供量の記載を求めている自治体は「病院・介護保険社会福祉施設用」では77自治体（88.5%），「事業所用」では75自治体（87.2%）であった。提供量で用いられている名称は，“給与栄養量”の名称が「病院・介護保険社会福祉施設用」で29自治体（33.3%），「事業所用」で30自治体（34.9%）と最も多かった。次いで“実施給与栄養量”の名称が「病院・介護保険社会福祉施設用」で15自治体（17.2%），「事業所用」で14自治体（16.3%）であった。提供量に関する記載を求めていない自治体は，「病院・介護保険社会福祉施設用」では10自治体（11.5%），「事業所用」では11自治体（12.8%）であった。

摂取量の記載を求めている自治体は「病院・介護保険社会福祉施設用」では10自治体（11.5%），「事業所用」では10自治体（11.6%）と少なかった。名称としては“推定摂取量”が「病院・介護保険社会福祉施設用」で6自治体（6.9%），「事業所用」で6自治体（7.0%）と最

表5　給食対象者の食事摂取量等の把握項目

		病院・介護保険社会福祉施設用（$n=87$）		事業所用（$n=86$）	
		自治体数	（%）	自治体数	（%）
把握したい項目に該当している書式*	喫食状況調査	21	24.1	4	4.7
	摂取量の把握方法（残菜調査）	38	43.7	28	32.6
	摂取量の把握方法（摂取量調査）	36	41.4	31	36.0
	摂取量調査の頻度	41	47.1	26	30.2
	給食以外の食事の把握	17	19.5	0	0.0
	食習慣（食事内容）の把握	3	3.4	2	2.3
	間食の把握	2	2.3	1	1.2
	嗜好調査	16	18.4	4	4.7
	飲酒	16	18.4	3	3.5

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。いずれの項目も「給食対象者の把握」に関する項目で確認している。

表6　給与栄養目標量・提供量・摂取量の算出の指示と使用している名称

	自治体が算出を求めている指標	使用している名称	病院・介護保険社会福祉施設用（$n=87$）		事業所用（$n=86$）	
			自治体数	（%）	自治体数	（%）
把握したい項目に該当している書式*	給与栄養目標量		83	95.4	81	94.2
		給与栄養目標量	50	57.5	50	58.1
		目標栄養量	7	8.0	7	8.1
		栄養目標量	5	5.7	5	5.8
		目標量	5	5.7	3	3.5
		給与目標量	3	3.4	3	3.5
		給与栄養基準量	2	2.3	2	2.3
		基準量	2	2.3	2	2.3
		荷重平均栄養所要量	2	2.3	2	2.3
		目標とする栄養量・目標給与栄養量	1	1.1	1	1.2
		目標	1	1.1	1	1.2
		1人1日目標量	1	1.1	1	1.2
		給与栄養量	1	1.1	1	1.2
		基本栄養量	1	1.1	1	1.2
		基本の栄養量	0	0.0	1	1.2
		栄養所要量	1	1.1	1	1.2
		1人1日基本の栄養量	1	1.1	0	0.0
	提供量		77	88.5	75	87.2
		給与栄養量	29	33.3	30	34.9
		実施給与栄養量	15	17.2	14	16.3
		提供栄養量	7	8.0	7	8.1
		給与量	6	6.9	6	7.0
		給与栄養量（実際）	3	3.4	3	3.5
		平均給与栄養量	2	2.3	2	2.3
		予定給与栄養量	2	2.3	2	2.3
		1ヵ月平均給与栄養量	1	1.1	1	1.2
		1日1人あたりの給与栄養量	1	1.1	1	1.2
		1人1日当り平均栄養量	1	1.1	1	1.2
		栄養量	1	1.1	1	1.2
		給与栄養量（予定・実際）	1	1.1	1	1.2
		実施給与栄養量	1	1.1	1	1.2
		実施給与栄養量（平均）	1	1.1	1	1.2
		1人1日当たり平均栄養量	1	1.1	1	1.2
		1人1日給与量	1	1.1	1	1.2
		一人あたり給与栄養量	1	1.1	1	1.2
		平均値	1	1.1	1	1.2
		1人1日給与栄養量	1	1.1	0	0.0
		1人1日当たり給与栄養量	1	1.1	0	0.0
	摂取量		10	11.5	10	11.6
		推定摂取量	6	6.9	6	7.0
		摂取栄養量	3	3.4	3	3.5
		実施給与栄養量	1	1.1	1	1.2
把握したい項目に該当していない書式	給与栄養目標量に関する項目なし		4	4.6	5	5.8
	提供量に関する項目なし		10	11.5	11	12.8
	摂取量に関する項目なし		77	88.5	76	88.4

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。

も多く使われていた。また，実施給与栄養量を摂取量としているところも認められた（「病院・介護保険社会福祉施設用」で1自治体：1.1%，「事業所用」で1自治体：1.2%）。摂取量に関する記載を求めていない自治体は，「病院・介護保険社会福祉施設用」では77自治体（88.5%），「事業所用」では76自治体（88.4%）であった。

6．給与栄養目標量に対する給与栄養量および推定摂取量の確認

表7に給与栄養目標量に対する給与栄養量および推定摂取量の確認についての結果を示す。給与栄養目標量に対して給与栄養量（実施）の内容確認および評価の項目を求めている自治体は，「病院・介護保険社会福祉施設用」では14自治体（16.1%），「事業所用」では15自治体（17.4%）であった。また給与栄養目標量に対する推定摂取量の内容確認および評価の項目を求めているのは，「病院・介護保険社会福祉施設用」，「事業所用」ともに2自治体（2.3%）のみであった。給与栄養目標量に対する給与栄養量（実施）／推定摂取量の内容確認および評価に関

表7　給与栄養目標量に対する給与栄養量および推定摂取量の確認

		病院・介護保険社会福祉施設用（$n=87$）		事業所用（$n=86$）	
		自治体数	(%)	自治体数	(%)
把握したい項目に該当している書式*	給与栄養目標量に対する給与栄養量（実施）の内容確認および評価	14	16.1	15	17.4
	給与栄養目標量に対する推定摂取量の内容確認および評価	2	2.3	2	2.3
把握したい項目に該当していない書式	給与栄養目標量に対する給与栄養量（実施）／推定摂取量の内容確認および評価に関する項目なし	73	83.9	71	82.6

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。

表8　給与栄養目標量の記載についての指示

		病院・介護保険社会福祉施設用（$n=87$）		事業所用（$n=86$）	
		自治体数	(%)	自治体数	(%)
把握したい項目に該当している書式*	記入要領に給与栄養目標量の記載について何らかの指示がある	53	60.9	52	60.5
	記入要領に具体的な算出方法の指示があるもの	12	13.8	11	12.8
	・喫食者の性別・年齢階級・身体活動レベル別人員構成に基づいて算出	5	5.7	4	4.7
	・日本人の食事摂取基準（2005年版）から求める	2	2.3	2	2.3
	・食事摂取基準を基に利用者の状況把握（アセスメント）を行ったうえで算出	2	2.3	2	2.3
	・給与栄養目標量・予定給与栄養量の算出方法を記入（該当するものに○）	2	2.3	2	2.3
	・利用者の身体状況等に基づき給与栄養目標量を算出	1	1.1	1	1.2
	記入要領に算出方法は明記していないが，栄養管理報告書から読み取れるもの	11	12.6	7	8.1
	・給与栄養目標量の算出方法を記入させる	5	5.7	2	2.3
	・施設の食事摂取基準（給与栄養目標量）の設定者・設定年月日・設定頻度を記入させる	4	4.6	3	3.5
	・給与栄養目標量の設定に使用する項目・見直しの頻度等項目がある	1	1.1	1	1.2
	・施設の食事摂取基準の内容が分かる資料（1人1日当たり基本の栄養量・食品構成及び給与栄養量等）を添付させる	1	1.1	1	1.2
把握したい項目に該当していない書式	記入要領に給与栄養目標量の記載に関する指示なし	34	39.1	34	39.5

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。

する項目がない自治体は，「病院・介護保険社会福祉施設用」では73自治体（83.9%），「事業所用」では71自治体（82.6%）であった。

7．給与栄養目標量の記載についての指示

表8に給与栄養目標量の記載に関する指示についての結果を示す。給与栄養目標量の記載にあたり自治体が何らかの指示をしているのは，「病院・介護保険社会福祉施設用」では53自治体（60.9%），「事業所用」では52自治体（60.5%）と，ともに60%以上の自治体が記入要領に記載に関する指示があった。しかし，記入要領に具体的な給与栄養目標量の算出方法について記述があるのは，「病院・介護保険社会福祉施設用」で12自治体（13.8%），「事業所用」で11自治体（12.8%）であった。

記入要領に給与栄養目標量の記載について具体的な算出方法の指示がある栄養管理報告書の指示内容の中では，喫食者の性別・年齢階級・身体活動レベル別人員構成に基づいて算出する手順を示す自治体が最も多く，「病院・介護保険社会福祉施設用」では5自治体（5.7%），「事業所用」で4自治体（4.7%）であった。日本人の食事摂取基準（2005年版）から求めると指示している自治体が，「病院・介護保険社会福祉施設用」，「事業所用」ともに2自治体（2.3%）であり，日本人の食事摂取基準（2010年版）による改定がまだなされていない自治体も見受けられた。記入要領に給与栄養目標量の算出方法が明記され

表9　各自治体が報告を求めている栄養素等の項目

		病院・介護保険社会福祉施設用（$n=87$）		事業所用（$n=86$）	
		自治体数	（%）	自治体数	（%）
把握したい項目に該当している書式*	エネルギー	83	95.4	81	94.2
	たんぱく質	83	95.4	81	94.2
	脂質	78	89.7	77	89.5
	炭水化物	24	27.6	24	27.9
	食物繊維	63	72.4	60	69.8
	ビタミンA	79	90.8	77	89.5
	ビタミンB_1	79	90.8	77	89.5
	ビタミンB_2	79	90.8	77	89.5
	ビタミンC	79	90.8	76	88.4
	カルシウム	79	90.8	77	89.5
	鉄	79	90.8	77	89.5
	食塩相当量	67	77.0	64	74.4
	ナトリウム	17	19.5	12	14.0
	カリウム	4	4.6	5	5.8
	ビタミンD	3	3.4	3	3.5
	ビタミンE	3	3.4	3	3.5
	ビタミンK	3	3.4	3	3.5
	ビタミンB_6	3	3.4	3	3.5
	ビタミンB_{12}	3	3.4	3	3.5
	葉酸	3	3.4	3	3.5
	亜鉛	1	1.1	0	0.0
	たんぱく質エネルギー比率（%）	41	47.1	34	39.5
	脂肪エネルギー比率（%）	78	89.7	61	70.9
	炭水化物エネルギー比率（%）	48	55.2	41	47.7
	穀類エネルギー（kcal）	1	1.1	1	1.2
	穀類エネルギー比率（%）	22	25.3	17	19.8
	動物性たんぱく質（g）	2	2.3	3	3.5
	動物性たんぱく質比率（%）	25	28.7	15	17.4
	動物性脂質比率（%）	3	3.4	2	2.3
	脂肪酸構成比率（%）	5	5.7	3	3.5
把握したい項目に該当していない書式	栄養素等の項目の記入欄なし	4	4.6	5	5.8

* それぞれの項目に該当する内容が書式から読み取れる場合は「該当している書式」として集計した。

ているわけではないが，給与栄養量の記述内容に関する項目が栄養管理報告書から読み取れる内容として，給与栄養目標量の算出方法を記入させる（「病院・介護保険社会福祉施設用」では5自治体（5.7%），「事業所用」で2自治体（2.3%））などがあげられた。記入要領に給与栄養目標量の記載に関する指示がない自治体は，「病院・介護保険社会福祉施設用」では34自治体（39.1%），「事業所用」で34自治体（39.5%）であった。

8．各自治体が報告を求めている栄養素等

表9に各自治体が報告を求めている栄養素等の項目を示す。エネルギー，たんぱく質，脂質，食物繊維，カルシウム，鉄，ビタミンA，ビタミンB_1，ビタミンB_2，ビタミンC，食塩相当量の項目は，「病院・介護保険社会福祉施設用」，「事業所用」ともに70%以上の自治体で報告を求めていた。カリウム，ビタミンD，ビタミンE，ビタミンK，ビタミンB_6，ビタミンB_{12}，葉酸，亜鉛についての報告は共に6%未満と少なかった。エネルギー比率では，たんぱく質エネルギー比率が「病院・介護保険社会福祉施設用」で41自治体（47.1%），「事業所用」で34自治体（39.5%），脂肪エネルギー比率は「病院・介護保険社会福祉施設用」で78自治体（89.7%），「事業所用」で61自治体（70.9%）であり，70%を超えていた。炭水化物エネルギー比率は「病院・介護保険社会福祉施設用」で48自治体（55.2%），「事業所用」で41自治体（47.7%）であり，約50%が報告を求めていた。穀類エネルギー比率は「病院・介護保険社会福祉施設用」で22自治体（25.3%），「事業所用」で17自治体（19.8%），動物性たんぱく質比率は「病院・介護保険社会福祉施設用」で25自治体（28.7%），「事業所用」で15自治体（17.4%）と20%前後であった。栄養素等の記入欄のない自治体（報告を求めていない自治体）は，「病院・介護保険社会福祉施設用」で4自治体（4.6%），「事業所用」で5自治体（5.8%）であった。

Ⅳ．考　察

本研究では，特定給食施設における日本人の食事摂取基準の活用の実態を把握することを目的に，自治体が報告を求めている栄養管理報告書の書式から，栄養管理の手順における，『対象集団の特性の把握』，『身体状況や食事摂取量の把握』，および『食事計画の決定と実施の評価』を各自治体が栄養管理報告書において，どのように確認しているかを調査した。

自治体が特定給食施設に栄養管理報告書を求める目的の一つは，健康増進法に照らして，設置者が適切な栄養管理を実施しているかを把握し，必要な指導・助言を行うことにある。健康増進法施行規則に示された「栄養管理の基準」[3]にそった給食運営が実施されているか，が適切な栄養管理の実施の評価の基準となる。健康増進法施行規則第9条「栄養管理の基準」の第1項には「利用者の身体の状況，栄養状態，生活習慣等を定期的に把握し，これらに基づき，適当な熱量および栄養素の量を満たす食事の提供およびその品質管理を行うとともに，これらの評価を行うよう努めること」とある[3]。これはPDCAサイクルに基づく栄養管理の手順を示しており，食事摂取基準の給食管理における活用の基本的な考え方と一致している。それゆえ，栄養管理報告書の書式から食事摂取基準の活用の状況を把握することを試みた。

特定給食施設で適切な栄養管理を行うために，また，食事摂取基準を活用するためには，『対象集団の特性の把握』が不可欠である。しかし，本研究の調査では，対象集団の特性を把握できる項目を設定していない自治体が，「病院・介護保険社会福祉施設用」および「事業所用」ともに2自治体（2.3%）あった。また，給食対象集団の人数や給食数の規模を把握していても，性別・年齢階級・身体活動レベル別の人数を同時に把握している自治体は，「病院・介護保険社会福祉施設用」で11自治体（12.6%），「事業所用」で25自治体（29.1%）と少ないことが認められた（表2）。

給食の食事計画を行うための食事摂取基準の活用には『身体状況や食事摂取量の把握』を行い，アセスメントすることが不可欠となる。病院の栄養管理は個別対応を基本とするが，宮下らは一般治療食患者の事前アセスメントとして必ず把握しておかなければならない内容として，①主たる疾病名，性別，年齢，②身長，体重，体格指数（BMIなど），③身体活動レベル，日常の生活習慣，食習慣（欠食，間食，外食，サプリメント等使用状況，服薬状況）があげられ，把握しておくことが望ましい内容として，①腹囲，上腕三頭筋部皮下脂肪厚，上腕囲，上腕筋囲，体脂肪率，体重歴，②臨床検査値，③食環境，生活環境，習慣的な栄養素等摂取量，食についての態度・知識・スキル，があげられるとしている[4]。一方，石田らは事業所給食で必ず把握しておかなければならない内容として，①対象者数と給食数，②対象集団の性別・年齢階級別の人員構成，③身体活動レベルの把握につながる情報として主な業務内容，があげられ，把握しておくことが望ましい内容として，①対象集団の身体状況（BMI 25以上，18.5未満の割合，高血圧・脂質異常・高血糖等の割合），②事業所の健康管理の課題，③販売状況（よく売れる料理・定食や人気のある料理・定食），④残菜状

況，などがあげられるとしている[4]。

本研究の結果から「病院・介護保険社会福祉施設用」では，必ず把握しておきたい内容に該当する項目において“身体活動レベルの把握”をあげている自治体は27自治体（31.0%），“生活習慣の把握”は24自治体（27.6%），“給食以外の食事の把握”は17自治体（19.5%），“食習慣の把握”は3自治体（3.4%），“間食の把握”は2自治体（2.3%）であり（表4，表5），サプリメントや服薬状況に関する項目はあげられていなかった。さらに，把握しておくことが望ましい内容に該当する項目において，腹囲，上腕囲，上腕筋囲，体重歴，食についての態度・知識・スキル等の項目はあげられていなかった。また，「事業所用」では，必ず把握しておきたい内容に該当する項目において，“身体活動レベルの把握”をあげている自治体は21自治体（24.4%）であった。さらに，把握しておくことが望ましい内容に該当する項目において，“BMI別（肥満とやせ）人数・割合”は21自治体（24.4%），“糖尿病・高血圧・高脂血症（脂質異常症）等の人数・割合”は22自治体（25.6%），“摂取量の把握方法（残菜調査）”は28自治体（32.6%）があげられた（表4，表5）。把握しておくことが望ましい内容に該当する項目において，事業所の健康管理の課題や，販売状況（よく売れる料理・定食や人気のある料理・定食）に関する項目はあげられていなかった。

給食の『食事計画の決定と実施の評価』を行うための食事摂取基準の活用には，食事摂取量のアセスメントが不可欠である。それゆえ，給食の摂取量および給食以外の摂取量の把握が必要とされる。給与栄養目標量の算出を求めている自治体は，「病院・介護保険社会福祉施設用」で83自治体（95.4%），「事業所用」で81自治体（94.2%）であり，提供量は「病院・介護保険社会福祉施設用」で77自治体（88.5%）と75自治体（87.2%）と80%を超えている。しかし，摂取量になると「病院・介護保険社会福祉施設用」で10自治体（11.5%），「事業所用」で10自治体（11.6%）と少ない結果であった（表6）。また，摂取量の評価方法や調査頻度の把握は，“給食対象者の把握”に関する項目で必要としているものの（表5），摂取量の算出を指示していない自治体が多いことがうかがえた。さらに，給与栄養目標量に対する給与栄養量の評価の確認をしている自治体は「病院・介護保険社会福祉施設用」で14自治体（16.1%），「事業所用」で15自治体（17.1%）であり，給与栄養目標量に対する推定摂取量の確認をしている自治体は，「病院・介護保険社会福祉施設用」で2自治体（2.3%），「事業所用」で2自治体（2.3%）であり（表7），食事計画の評価や見直しに必要な項目は限られていた。

さらに，給与栄養目標量の記載方法について何らかの指示をしている自治体は，「病院・介護保険社会福祉施設用」で53自治体（60.9%），「事業所用」で52自治体（60.5%）であるものの，具体的な算出方法を指示している自治体は，「病院・介護保険社会福祉施設用」で12自治体（13.8%），「事業所用」で11自治体（12.8%）とわずかであった（表8）。給食対象者のアセスメント項目も多様であり，また，対象者のアセスメントに関する項目がない場合もあるため，給食の給与栄養目標量の報告を求めても，その給食の適否を評価することは難しいと思われる。

日本人の食事摂取基準の給食管理における活用理論[1]では，集団の摂取量を把握し，エネルギー量に関しては，体重やBMIを指標として過不足の状態を評価，栄養素に関しては推定平均必要量（EAR）未満のものの割合を減らすことを目標に食事計画を行っていくことが示されている。本研究の結果から，エネルギー量に関しては体重やBMIがどの程度の施設で把握されているか，栄養管理報告書を通じて把握できることが確認できた。しかし，栄養素に関しては，給食以外の食事を含む摂取量の分布が把握できるような項目は栄養管理報告書からは確認できなかった。また，栄養管理報告書の提出は施設設置者に求められているが，栄養の専門職でない施設設置者に給与栄養目標量，提供量，摂取量の報告を求めても理解しにくいものと考えられる。また，栄養管理報告書に給与栄養目標量，提供量，摂取量の記載を求めても，その適否を評価することは困難であると考えられた。さらに，給食運営業務を委託している施設が多い中で，委託側，受託側の双方がこれらの数値を把握，評価できる状況になっているか否かが報告書の書式からは読み取れなかった。同時に，これらの数値を栄養管理報告書に求めた場合に，自治体がその内容の適正さを評価できる項目が整っていないと考えられた。

今後の課題として，給食利用者の栄養管理に資するためには，自治体は給食施設の指導・助言業務におけるPDCAサイクルの中で，栄養管理報告書をどのように用いているかを明確にする必要があると考えられる。すなわち自治体は，これまでの栄養管理報告書から明らかになったことを明確にし，それらを基にどのような指導・助言計画を立てたのか，その指導・助言の結果としてどのように施設の栄養管理の水準が変化してきたのかを評価し，公表していくことで，給食施設に栄養管理報告書の意義を伝えていく必要がある。自治体には，栄養管理報告書の書式が適正な栄養管理の実施を評価できるもの

になっているかを見直すことが求められる。また，全国で約4万7千施設ある特定給食施設の栄養管理が適切に実施されているかどうかは，国民の健康の維持・増進の観点から重要であり，国においては，自治体毎の特定給食施設の栄養管理状況の評価を踏まえ，健康・栄養施策の一つとして評価し，必要に応じて健康増進を目的とした適切な栄養管理の基準の内容や指導のあり方などについて改善を行う必要がある。さらに研究者としては，食事摂取基準の理論を現場に活用する方法として，適切な栄養管理のために特に食事摂取量の把握や評価をどのように行っていくかを示していく必要があると考えられる。

本研究の限界は次に示すとおりである。本研究の結果は収集された自治体の書式から得られた情報のみであるため未提出の自治体の状況は不明であること，また栄養管理報告書で把握できる内容には限りがあるため，本研究の結果で栄養管理の質の良否を判断できるものではないことである。

V．結　論

特定給食施設において日本人の食事摂取基準を適用し，給食管理における活用の基礎理論に示されたPDCAサイクルの手順に基づいて栄養管理を実施している状況を，自治体が給食施設に提出を求めている栄養管理報告書の書式から検討した。「病院・介護保険社会福祉施設用」と「事業所用」と給食の目的や対象者特性の異なる2つの書式に絞って集計した。

その結果，報告書の書式において，『対象集団の特性の把握』に必要な給食対象集団の特性（性別・年齢階級・身体活動レベル別の人数）と給食対象者人数の両方の記載を求めてない自治体が「病院・介護保険社会福祉施設用」，「事業所用」ともに2.3%認められた。『身体状況や食事摂取量の把握』において，半数以上の自治体で把握している項目は，「病院・介護保険社会福祉施設用」の身長と体重に関する項目のみであった。『食事計画の決定と実施の評価』において，給与栄養目標量の記載を求めている自治体は「病院・介護保険社会福祉施設用」，「事業所用」ともに約95%認められたが，食事摂取量の記載を求めている自治体は約11.5%に過ぎず，給食の食事計画とその評価や計画の見直しにつながる食事摂取量の評価に関する把握が行える項目は限られていた。栄養管理報告書を通して設置者および自治体が栄養管理の質を評価できるように，給食の栄養管理の手順に即した書式の検討が必要である。

謝　辞

本研究は平成22年度厚生労働科学研究費補助金（循環器疾患・糖尿病等生活習慣病対策総合研究事業）日本人の食事摂取基準の改定と活用に資する総合的研究（研究代表者　徳留信寛）の「給食施設における食事摂取基準の活用および栄養管理報告書の活用に関する研究」（研究分担者　石田裕美）の一環として行った。

栄養管理報告書の提出にご協力いただきました自治体の皆様に感謝申し上げます。

利益相反

本研究には利益相反に相当する事項はない。

文　献

1）厚生労働省「日本人の食事摂取基準」策定検討会：日本人の食事摂取基準（2010年版），pp. 17–42（2009）第一出版，東京
2）厚生労働省：「日本人の食事摂取基準」活用検討会報告書，http://www.mhlw.go.jp/shingi/2010/03/s0331-9.html，（2010年11月1日）
3）健康増進法施行規則：第9条「栄養管理の基準」，平成15年4月30日厚生労働省令第86号，最終改正平成21年8月28日厚生労働省令第138号
4）由田克士，石田裕美編：PDCAサイクルと食事摂取基準による栄養管理・給食管理，pp. 105–111（2011）建帛社，東京

（受付：平成23年11月11日，受理：平成24年2月16日）

Investigating the Application of Dietary Reference Intakes for Nutrition Management in Specific Food Service Facilities Using the Nutrition Management Report

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ABSTRACT

Objective: In order to evaluate the appropriate application of "the dietary reference intakes for Japanese, 2010" (DRIs-J-2010) in specific food service facilities, local governments in Japan asked these facilities to submit a nutrition management report. In the DRIs-J-2010, nutrition management based on the Plan-Do-Check-Action cycle (PDCA) is suggested as a fundamental theory for application of the DRIs-J-2010 by service facilities. In order to confirm the current state of the application of the DRIs-J-2010 in food service facilities, we investigated whether the present practice of nutrition management based on the fundamental theory was encompassed by the file formats of the nutrition management report.

Methods: The Ministry of Health, Labor and Welfare asked all 114 local Japanese governments (prefectures as well as cities and special wards with public health centers) to submit the nutrition management file formats in March–April 2010. The "hospital and facility" file format was submitted by 87 local governments and the "office" file format was submitted by 86 local governments. We collected survey items related to "assessing the characteristics of target groups," "assessing the physiological aspects and dietary intakes of target groups," and "meal planning and evaluation of the implementation of the plan" from the submitted file formats.

Results: Neither the number of people in the food service target group nor their characteristics (sex, age, physical activity level)—items necessary for "assessing the characteristics of target groups"—were confirmed by 2.3% of local governments submitting either the "hospital and facility" or "office" file format. With regards to the necessary survey items concerning "assessing the physiological aspects and dietary intakes of target groups," more than half of the local governments submitting the "hospital and facility" file format confirmed only height and weight. With regards to the necessary survey items concerning "meal planning and evaluation of the implementation of the plan," approximately 95% of the local governments submitting either the "hospital and facility" or "office" file formats confirmed the food service target energy and nutrients, while approximately 11.5% of the local governments submitting either the "hospital and facility" or "office" file format confirmed the dietary intake.

Conclusion: In the submitted nutrition management file formats, limited survey items were available to evaluate the meal planning and the dietary intakes of the target groups. A file format for these nutrition management reports that is in line with the procedures for nutrition management carried out by food service facilities is required.

Jpn. J. Nutr. Diet., **70** (2) 140～151 (2012)

Key words: nutrition management report, specific food service facilities, dietary reference intakes for Japanese, food service standard of energy and nutrients, dietary intake

日衛誌 (Jpn. J. Hyg.), 67, 485–491 (2012)

総　説

シリーズ　リフレッシュが必要な微量元素に関する常識

クロムはヒトの栄養にとって必須の微量元素だろうか？

吉　田　宗　弘

関西大学化学生命工学部

Is Chromium an Essential Trace Element in Human Nutrition?

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Abstract　**It has been recognized that chromium is an essential trace element associated with carbohydrate metabolism, and chromium deficiency causes an impaired glucose tolerance. Recently, however, Vincent *et al.* have reported that chromium is not an essential trace element. In the present report, the author evaluated the nutritional essentiality of chromium by reviewing several previous reports. In almost all previous reports, the chromium concentration in the animal feed used was higher than 0.1 μg/g, and it is difficult to consider that the experimental animals were in a low-chromium state. In addition, the amount of chromium administered to the animals for the improvement of glucose tolerance was at a pharmacological level, and corresponded to a level that far exceeded the human daily chromium intake (20 to 80 μg/day). On the other hand, recent research has clearly shown that feeding with a severely low-chromium diet (0.016 μg/g) does not impair glucose tolerance. The amount of chromium absorbed in humans estimated from chromium intake (20 to 80 μg/day), chromium absorption rate (1%), and urinary chromium excretion (<1 μg/day) is less than 1 μg/day, which is much lower than those of other essential trace elements. In addition, because there is an inconsistency between the chromium concentration in food and chromium intake, chromium intake seems to be dependent on chromium contamination during food processing and cooking. It is concluded that there is a high possibility that chromium is not an essential trace element.**

Key words: chromium（クロム）, essentiality（必須性）, glucose tolerance（耐糖能）, *Torula* yeast（トルラ酵母）, chromodulin（クロモデュリン）, chromium intake（クロム摂取量）

はじめに

栄養学の教科書には,「クロムはヒトを含む高等動物にとって必須の微量元素であり，欠乏した場合には耐糖能が低下する」と記述されている。わが国の食事摂取基準においても，クロムは栄養上必要な微量ミネラルに位置づけられており，成人の摂取に対して推定平均必要量と推奨量が設定されている (1)。糖代謝の維持や糖尿病予防を目的としたクロムサプリメントも販売されており，米国ではカルシウムサプリメントに次ぐ売り上げがあるという (2)。最近では，インスリンの作用を増強するクロム含有オリゴペプチド（クロモデュリン）の存在も報告され (3)，糖代謝におけるクロムの作用について分子レベルでの理解も進んでいる。ところが，昨年，クロモデュリンの命名者である Vincent は,「クロムは必須の栄養素ではない」という論文を発表した (4)。本稿では，栄養学領域におけるクロム研究の推移を概観し，必須性に対する疑義の根拠について述べる。

受付 2012 年 6 月 16 日，受理 2012 年 7 月 20 日
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1. 耐糖因子としてのクロム

第二次世界大戦後，世界の人口が急激に増加し，マル

サスの人口論，すなわち「人口の増加は土地の食糧生産能力よりもはるかに大きく，人口は幾何級数的に増加するが食糧資源は算術級数的にしか増加しないため，やがて深刻な食糧不足が地球規模で発生する」という理論(5)が現実味を帯び始めた。このため，当時の栄養学に課せられた命題は，新たな食糧資源，とくに新タンパク質食糧資源を開拓することにあるとされた。これを受けて多くの栄養学者がsingle cell protein，すなわち酵母やクロレラなどの単細胞生物をタンパク質源として活用するための研究に取り組んだ。他方，工業社会の進展がもたらす環境汚染に対処するため，微生物よる環境浄化が実用化された結果，副産物としての微生物菌体が大量に得られるようになった。このような状況において，主にパルプ廃液の処理に利用されていたトルラ酵母をヒトや家畜のタンパク質源に活用することが検討され，トルラ酵母の乾燥菌体を唯一のタンパク質源とした飼料で実験動物を飼育することが数多く実施された。

米国のSchwarzは，ラットにトルラ酵母をタンパク質源とした飼料を与えると肝臓の壊死が生じることを認めた。彼は，この異常を未知の栄養素の欠乏であると考え，ビール酵母で飼育したラットに異常が出現しないことから，この未知の栄養素をビール酵母から発見しようと試みた。その結果，肝臓壊死の予防には含硫アミノ酸とビタミンEに加えて第3の因子（factor 3）が必要であることを見いだした(6)。そして，factor 3にセレンが含まれることを示し，セレンが高等動物にとって必須の微量元素である可能性が高いと発表した(7)。この発見は，これまで毒性元素とみなされていたセレンを栄養素としてとらえたものであり，栄養学の歴史においてエポックを形成したものといわれている。

Schwarzの共同研究者であったMertzは，セレンの必須性を示す研究の過程で，トルラ酵母で飼育したラットでは肝臓壊死を起こす前に耐糖能低下が生じることを観察し，ビール酵母からの抽出物が耐糖能低下を改善することを認めた(8)。彼らは，肝臓壊死と同様に，微量元素の欠乏が耐糖能低下を起こすと推定して種々の微量元素をラットに投与し，最終的に三価クロム化合物が耐糖能低下を改善することを見いだした(9)。そして，クロム欠乏が耐糖能低下を起こし，ビール酵母抽出物には耐糖能を正常に維持するための耐糖因子（glucose tolerance factor: GTF）というクロムを含む機能性物質が存在すると主張した。セレンの例があったためか，彼らの主張は多くの栄養学者に受け容れられ，クロムもセレンと同様の必須微量元素であるとの認識が広まった。その後，多くの研究者によってGTF単離の試みが様々な食品や動物の臓器を用いて行われ，GTFの構造には数種のアミノ酸とニコチン酸が含まれる可能性が示唆された(10)。しかし，現在にいたるまで，GTFの単離・構造決定はなされていない。

2. クロムによる糖代謝異常の改善とクロモデュリン

2-1. 糖代謝異常の改善

三価クロムに耐糖能改善効果があるというSchwarzとMertzの主張を背景として，糖代謝異常を起こした症例にクロムを投与する試みが開始された。その結果，200～1,000 μg/dayの三価クロム化合物の投与が2型糖尿病の諸症状（血糖値，耐糖能など）を改善することが明らかとなった(11)。とくにクロム非添加高カロリー輸液の長期投与中に発生した糖代謝異常の症例では，クロム出納が負であり，血中および毛髪クロム濃度（それぞれ0.55 ng/mlと154～175 ng/g）が健常者（それぞれ，4.9～9.5 ng/mlと＞500 ng/g）に比較して明らかに低下していた(12)。さらに，糖尿病患者では，クロムの尿中排泄量が増加していることも示された(13)。これらのことから，クロムの摂取不足，もしくはクロム代謝の異常による体内クロムの減少が糖代謝異常を引き起こすことは明らかであり，クロムが糖代謝に関わる必須微量元素であることは疑いようもない事実であると思われるようになった。

2-2. クロム含有オリゴペプチドの発見

クロムの動物体内における挙動を分子レベルで解明する試みも数多く行われた。1980年代にYamamotoとWadaらは，クロムを投与した動物の臓器にクロムの結合した低分子化合物が存在することを示した(14)。ウサギの肝臓から単離されたものは，分子量が約1,500のグリシン，システイン，アスパラギン酸，グルタミン酸によって構成されるオリゴペプチドであり，全アミノ酸残基の半数以上にカルボキシル基が存在し，1分子当たり4分子の3価クロムが結合していた(15)。彼らは，クロムの結合していないアポ体が存在することに着目し，このオリゴペプチドの役割を，クロムを速やかに尿へ排泄してクロム中毒を防ぐことにあると考察した。

2-3. クロモデュリン

先述の高カロリー輸液投与の症例においてインスリン投与のみでは完全な回復が認められなかったことなどから，クロム投与による耐糖能の改善はクロムがインスリンの作用を増強することを意味すると思われた。YamamotoとWadaらは，牛の乳腺から単離されたクロム含有オリゴペプチドは，肝臓などから単離されたものとクロムとのモル比が異なっているが，ラット脂肪細胞においてインスリンに依存したグルコース代謝を増強することを認めた(16)。その後，1990年代後半に，Vincentらは，クロム含有オリゴペプチドがインスリン受容体のチロシンキナーゼ活性と脂肪細胞の膜に結合したホスホチロシンホスファターゼの活性を高めることを認めた(17, 18)。さらに，クロムの結合していないアポ体のオリゴペプチドには活性増強作用のないこと，増強作用はクロム結合数の増加とともに高まって最大作用には4分子の三価クロムの結合が必要であることも判明し，このオ

リゴペプチドの作用にはクロムが必須であることが明らかになった (17)。

Vincentは，これらの結果にもとづき，クロム含有オリゴペプチドは，以下のような機構によってインスリンを介した細胞内シグナル伝達に関わっており，クロモデュリンと命名すべきものと提唱した (3, 19)。すなわち，インスリンが細胞膜のインスリン受容体に結合すると，インスリン受容体の立体構造が変化してチロシンキナーゼ活性が生じ，インスリンを介したシグナル伝達が開始され，最終的にグルコース輸送担体が細胞膜表面に出現して血中グルコースは速やかに細胞内に取り込まれる。このプロセスにおいて，血中クロムは，おそらく血中インスリン濃度の上昇を引き金として細胞内に取り込まれ，貯えられていたアポクロモデュリンに結合する。生じたホロクロモデュリンは，インスリン受容体に結合して立体構造の変化を支え，チロシンキナーゼ活性を維持する。血中グルコース濃度が低下し，血中インスリンレベルが低下すると，インスリン受容体の立体構造はゆるみ，ホロクロモデュリンも細胞内から血中へ移行して最終的に尿に排泄される。なお，血中クロム濃度の維持，および血中から細胞へのクロムの輸送にはトランスフェリンが関わることも示されている (20)。

3. クロムは必須微量元素の条件を満たしているか

クロモデュリン活性の発現にクロムが必須であることから，Vincentが提唱したクロモデュリンの作用機構は必須微量元素としてのクロムの地位を盤石にするものと思われた。ところがVincent自身がクロムの必須性を否定する主張を行っている。ここではクロムを必須微量元素と認めない根拠を述べる。

3-1. 必須微量元素の条件

まず，必須微量元素というためにはどのような基準を満たす必要があるのかを考えてみる。クロムを栄養素の列に加えたMertzはこの基準についてしばしば言及している。彼の定義はしばしば変化しているが，吉野によれば，1980年頃には表1に記す3基準を満たすものが必須微量元素であるとしていた (21)。しかし，分析技術が発達した現在ではほとんどの元素が生体から検出できるので，基準①はあまり意味がない。むしろ②とあわせて，「生体内にその元素を含む機能性成分，もしくはその元素を必要とする反応系が存在する」とするのが適切と判断する。また，基準③については，機能障害の発生と予防・回復に分けるのが議論を進めやすいので2つに分けることにする。以上から，筆者が考える必須微量元素であるための基準も表1に記した。本稿ではこの筆者による基準にもとづき微量元素の必須性を考える。

表1 必須微量元素であるための基準

1980年頃にMertzが示した基準
①生体の常在成分である。
②代謝系に影響を与える能力がある。
③その欠乏によって機能障害を起こすとともに，生理的適量を負荷することによって欠乏症が阻止されるか，または欠乏症から可逆的に回復させる。
筆者が考える基準
①生体内にその元素を含む機能性成分，もしくはその元素を必要とする反応系が存在する。
②その欠乏によって機能障害（欠乏症）を起こす。
③生理的適量の負荷によって機能障害が予防されるか，可逆的に回復する。

3-2. クロム欠乏飼料とクロム投与量

筆者が示した条件に照らして，クロムの必須性を検証してみる。ラットに発生した耐糖能低下は機能障害に含まれ，これがクロム投与によって改善している。クロム投与量が生理的適量であるかの議論があるが，一応，基準③は満たしているとする。また，基準①もクロモデュリンというクロム含有機能性分子の存在によって満たされている。問題は基準②である。クロム欠乏飼料で飼育した動物にクロムを投与して耐糖能が改善したとする報告が数多く存在しているので，一見，満たされているように見える。しかし，これらの報告で用いられた飼料は本当に“クロム欠乏”飼料と呼べるものであっただろうか。Vincentの指摘もこの点にある。

表2は成人のクロム摂取量を推定した報告をまとめたものである (22–29)。クロム摂取量の推定値はおおむね20～80 μg/dayの範囲にある。また，わが国の食事摂取基

表2 クロム摂取量の推定値

国	推定法	クロム摂取量 (μg/day)	発表年	文献
フランス	高齢者献立の分析	40±14	2007	(22)
スペイン	病院一般食の分析	77±17	2008	(23)
ベルギー	病院や軍隊の食事の分析	53±31	1995	(24)
メキシコ	食品分析値からの算定	30±2	2001	(25)
日本	一般家庭献立の分析	47±33	1988	(26)
	菜食者献立の分析	27±8	2011	(27)
	病院一般食の分析	43±20	2011	(28)
アメリカ	一般成人献立の分析	33±3	1985	(29)

準における成人のクロムの推定平均必要量は20～35 μg/dayである(1)。したがって，ヒトでは摂取量が20 μg/dayを下回らなければクロム不足とはいえない。ヒトの1日の食事を凍結乾燥すると400 g程度になるので，ヒトの摂取量 20 μg/day は食事中濃度に換算すると約0.05 μg/gとなる。この食事中濃度はラットの飼料中濃度にほぼ相当するので，低クロム飼料と呼ぶには飼料中クロム濃度0.05 μg/g未満が最低条件である。しかし，Mertzらの実験における欠乏飼料のクロム濃度は記載されているもので0.1 μg/gであり(30)，ヒトの日常的なクロム摂取量の範囲といえる。

クロム投与量についても，ヒトのクロム摂取量が80 μg/day未満であることを念頭におく必要がある。しかし，Mertzらを含めて，ほとんどの研究は，クロム濃度2または5 μg/mLの飲料水をラットに与えている(30–32)。この投与水準は，ヒトに換算すると1,000～3,000 μg/day程度のクロム投与となり，薬理水準といえる。飼料にクロムを添加する場合もヒトの摂取量80 μg/dayが飼料中濃度0.2 μg/gに換算できるので，これを大幅に超える飼料中クロム濃度1～2 μg/gは栄養水準とはいえない。

以上のことは，これまでの研究においてクロム欠乏飼料と称されてきたものの大半がヒトの日常のクロム摂取量の範囲のクロム濃度であり，耐糖能改善を目的として投与されたクロムの量は日常の摂取量の数十倍に相当する高水準だったことを意味している。つまり，過去の実験結果は，薬理水準のクロム投与によって日常的なクロム摂取のラットの耐糖能が“向上”したことを観察したに過ぎないといえる。

なお，SchwarzとMertzの実験では，トルラ酵母を与えたラットの耐糖能の低下を当時の一般的な精製飼料を与えたラットと比較した上で示しており(9)，トルラ酵母投与によって耐糖能低下が生じたことは事実のようである。彼らの用いたトルラ酵母はパルプ廃液を資化したものであると思われるが，このようなトルラ酵母にはリグニン分解物に由来する芳香族化合物が混入しているため，様々なside effectの生じる可能性がある。たとえば筆者らはパルプ廃液資化トルラ酵母を与えたラットにおいて成長抑制と肝臓の薬物代謝系が亢進することを認めている(33)。また，パルプ廃液由来のトルラ酵母は相当な異臭がしており，これをタンパク質源とする飼料をラットに食べさせるには，糖質源として約50%のショ糖が加えて甘味を強くしなければならない(8, 33)。トルラ酵母飼料を投与したラットにおける肝臓壊死や耐糖能低下の発生には，混入していた芳香族化合物や大量に加えられたショ糖が関わっているかもしれない。すなわち，彼らの実験で発生した耐糖能低下の原因をクロム以外に求めることは可能だと思われる。

3-3. Vincentの実験と主張(4)

Vincentは，ラット標準精製飼料であるAIN93Gのミネラル配合からクロムを除き，クロム濃度0.016 μg/gというこれまでにない低クロム飼料を調製した。さらに，飼育用具に金属素材を避けるなど，飼育環境からのクロム汚染を極力除く努力も行った。そして，ラットを4群に分け，1群にはこの低クロム飼料，他の3群には，それぞれ通常のAIN93G飼料（クロム濃度1.135 μg/g)，AIN93Gに0.2 μg/gのクロムを添加した飼料（クロム濃度1.331 μg/g)，AIN93Gに1.0 μg/gのクロムを添加した飼料（クロム濃度2.080 μg/g)を与えて約6か月間飼育した後，耐糖試験を行った。血糖値の変化量を積分したArea under curve（AUC）を比較すると，1.0 μg/gクロム添加群がAIN93G群に比べて有意に低い値となった。また，試験中の血中インスリン濃度のAUCはクロム摂取量に依存して小さくなり，1.0 μg/gクロム投与群が最低値を示した。ただし，いずれの指標においても，低クロム群とAIN93G群との間に有意差は認められなかった。Vincentは，低クロム群とAIN93G群との間に有意差のないことから，耐糖試験において血糖値やインスリン濃度に差が生じるには薬理水準のクロム投与が必要であると述べ，これまでの研究で認められたクロムの効果は栄養素としての作用ではなく薬理作用であると結論している。そして，クロムは必須微量元素ではないと主張している。

AIN93Gのクロム濃度がヒト食事換算では400 μg/day程度のクロム摂取に相当しており，ヒトの摂取範囲に該当する群が設定されていないことにやや不満は感じる。しかし，VincentはAIN93Gがラットの標準飼料であることを重視し，これを栄養的に適切なクロムを摂取する群と位置づけて低クロム飼料投与群と比較したといえる。Vincentと同様に，飼料中濃度0.03 μg/gの低クロム飼料を用いて，飼料中濃度1 μg/gのクロム投与が耐糖能に影響を与えないことを示す研究が存在することから(34)，ラットの耐糖試験においてクロムの効果が生じるには，飼料中濃度1 μg/gでは不十分であり，2 μg/gという高い水準の摂取が必要であることは確かである。先の必須微量元素に関する筆者の基準に照らした場合，低クロム飼料群がAIN93G飼料群に比較して耐糖能低下を起こしていないことから，基準②がクリアできていないことは明白である。つまり，Vincentの主張はきわめて妥当なものといえる。

なお，Mertzは1980年代より後になって，欠乏症発生を必須微量元素の基準からはずし，代わりに「適量を摂取することによって健康の増進に寄与する」を加えることを提唱している(35)。この条件であれば，う歯予防効果を持つフッ素なども必須微量元素の仲間に加わることになり，薬理水準ではあっても耐糖能を“向上”させたクロムも必須微量元素となる。しかし，薬は予防や健康増進に関するものであったとしても栄養素ではない。ゆえにMertzの提唱に同意することはできない。

4. クロムサプリメントの効果

最初にも述べたが，米国では，糖尿病予防などを目的

としたクロムサプリメントの人気が高い。しかし，集団を対象としたクロムサプリメント投与に関するシステマチックレビューは，200～1,000 μg/dayのクロム化合物投与は，2型糖尿病患者の空腹時血糖値とヘモグロビンA1濃度を低下させるが，健常者の糖および脂質代謝に対して有益な効果はいっさいないと述べている (36)。つまり，200～1,000 μg/dayのクロム投与は，起こってしまった糖代謝異常には効果があるが，健常者の糖代謝をさらに向上させる効果はないといえる。ただし，健常者を対象として，クロムサプリメント投与と糖尿病発症率との関連を検討した前向きの疫学研究が見当たらないので，クロムサプリメントに糖尿病予防効果があるかは不明である。なお，このシステマチックレビューでは，クロム源がビール酵母の場合はクロム投与量が10 μg/day未満でも糖尿病患者の血糖値が低下することを示している。これに関して，ビール酵母中のクロムのbioavailabilityが高いとする主張もあるが，ビール酵母にはクロムと無関係なGTFも存在するとも考えられる。

5. クロムパラドックス

食品のクロム含有量，クロム摂取量，吸収率，尿中排泄量，体内量，クロム出納に関するこれまでの報告をつないでいくと辻褄の合わないことがいくつか認められる。

5-1. 食品中含有量と摂取量

これまで日本の食品成分表にはクロム含有量の記載がないため，献立作成時にクロム摂取量が食事摂取基準の数値に見合っているかを確認できなかった。この事態に対処するため，一昨年の秋に刊行された日本食品標準成分表2010（以下，成分表と略記）では，これまで記載のなかったヨウ素，セレン，クロム，モリブデン，およびビオチンの含有量が初めて記載された (37)。数値記載の対象となったのは全体の3分の1に相当する約500食品であるが，日常の食生活において高頻度に出現する食品はほぼ網羅されており，一般的な献立であればクロム摂取量を算定することは可能である。ところが，この成分表を用いて日本人のクロム摂取量を算定すると10 μg/day未満という数値が得られ (38)，表2に示したこれまでの摂取量推定値との間に大きな乖離が認められる。同一献立について成分表からの計算値と実測値を比較しても同様の結果が得られる (28)。乖離の原因は，成分表に記載されている食品のクロム含有量がこれまで報告されてきたものに比較してあまりにも低いことにある。クロム分析値が時代とともに低い値になっていることが有名であるため，数値が低いほど信頼性が高いという思い込みがクロム研究者にあるが，それにしても日本の成分表のクロムの数値は低すぎるのではないかという印象が強い。

成分表からのクロム摂取量算定値は，出納実験にもとづいて設定された食事摂取基準におけるクロム摂取の推定平均必要量を大きく下回っている。このため，単純に摂取量算定値と摂取基準の数値を比較すると，日本の食品はクロム含有量が少なく，日本人はクロム摂取不足であることになってしまう。とくにクロムサプリメントが日本でも販売されていることから，宣伝材料に使われる可能性は高い。必須でない可能性が高い化学物質に対して必要量や摂取の推奨量を定めることの是非も含めて，至急に対応する必要がある。

表3 クロム含有量の高い食品（μg/100 g）

バジル，粉末	47
あおのり，素干し	41
パセリ，乾燥物	38
パプリカ，粉末	33
刻みこんぶ	33
こしょう（黒），粉末	30
ほしひじき	24
ミルクチョコレート	24
カレー粉	21
さんしょう	21
紅茶，葉	18
とうがらし，粉末	17
シナモン，粉末	14
さらしあん	14
黒砂糖	13
かぼちゃ種，味付け	13
こしょう（混合），粉末	12
まこんぶ，素干し	11
カットわかめ	10

日本食品標準成分表2010より抜粋

表3は成分表に記載されたクロム含有量を数値の高い食品から順に抜き出したものである。クロム含有量の高い食品の大半は粉末化した香辛料と加工食品であり，穀物，豆，および生鮮食品の中に100 gあたり10 μgを超えるクロム含有量のものは皆無である。クロムの分析においては周囲からのクロム汚染に細心の注意を払うことが要求される。加工食品のクロム含有量が高いこと，および献立中クロム濃度に関して実測値が成分表からの算定値を大きく上回ることは，献立に含まれるクロムの多くが調理加工中に紛れ込んだ可能性をうかがわせる。つまり，クロム摂取量は汚染に依存して変化しているかもしれないのである。調理加工におけるクロム汚染の実態を検証した研究はないが，このような物質が必須の栄養素であることは考えにくい。

5-2. 吸収量

食事から摂取されたクロムの吸収率は種々の条件によって変動するといわれているが，米国の食事摂取基準ではこれを平均1%と見積もって授乳婦のクロム摂取の目安量を算定している (39)。最近の同位体を用いた動物実験の結果はこの見積もりを支持している (40)。クロム摂取量20～80 μg/dayに吸収率1%を適用すると，食事から体内に吸収されるクロムは1 μg/day未満ということになる。ヨウ素，セレン，モリブデンは，摂取量もしく

は必要量がクロムと同水準であるが，これらは消化管で大半が吸収される。マンガンは吸収率が数％未満といわれるが，1日摂取量がmgのオーダーであるため，吸収量はヨウ素やセレンとほぼ同水準となる。つまり，クロムの吸収量は，これまで知られている必須微量元素に比較して100分の1未満であり，あまりにも少ないといわざるを得ない。この点においてもクロムの必須性には疑問がある。

クロムの主排泄経路は尿であると考えられる (40)。尿クロムの分析値は研究者ごとに差異が大きいが，最近は吸収率1%に見合う尿排泄量 (1 μg/day未満) とする報告が多い (41–43)。一方，クロムの体内量が加齢とともに低下するという報告 (44) があり，連日ではないにしてもクロム出納が負になっている可能性がある。たとえば母乳へのクロム損失は1 μg/dayに近いが (45)，吸収率が1%であるとすると授乳婦では連日100 μg/day程度の摂取がないと出納は負になる。ただし，高齢者を対象として行われた実験では，クロム摂取量が20 ～ 30 μg/dayであっても正の出納値が得られている (46, 47)。

おわりに

クロムの必須性に疑問を投げかける論文は以前から繰り返し発表されていた (48, 49)。Vincentの発表にインパクトがあったのは，彼がクロム含有機能性分子であるクロモデュリン研究の第一人者であったためである。本稿で述べたように，現状ではクロムの必須性を否定する論理が優勢である。しかし，クロムが必須微量元素である可能性はまだ残っている。ただし，Vincent以上のクロム欠乏動物を作成するのは技術的に困難なので，別の方法を考える必要がある。クロモデュリンがクロムを含む機能性分子であることは事実であるから，クロモデュリンが健康維持に必須の生体成分であることを示すことができれば，クロム欠乏による健康障害を実験的に起こさなくても，クロムを必須微量元素の列に加えることができる。クロモデュリンの単離は，クロム投与動物の臓器，あるいは採取後にクロム溶液に浸漬した臓器を材料として行われており，クロモデュリンの大半はアポ体で存在していると考えられる。このアポクロモデュリンの合成に関わる遺伝子をノックアウトし，何が起こるかを調べるのは有効かもしれない。ただし，アポクロモデュリンの役割が別にあって，クロムが結合したクロモデュリンの作用は偶然の産物であるということが判明する可能性もある。

一方，クロムの摂取，吸収，排泄に関するいくつかのパラドックスを解消するには，クロムの正確な定量分析が必須である。食事，血液，尿などを対象としたクロムの分析においては，標準参照試料を用いて測定値の正確性を担保することが必要である。

文　献

(1) 厚生労働省．日本人の食事摂取基準［2010年版］．東京：第一出版，2009:218–275.
(2) Nielsen F. Controversial chromium: does the superstar mineral of the Mountebanks receive appropriate attention from clinicians and nutritionists? Nutrition Today 1997;31:226–233.
(3) Vincent JB. Recent advances in the nutritional biochemistry of trivalent chromium. Proc Nutr Soc 2004;63:41–47.
(4) Di Bona KR, Love S, Rhodes NR, McAdory D, Sinha SH, Kern N, Kent J, Strickland J, Wilson A, Beaird J, Ramage J, Rasco JF, Vincent JB. Chromium is not an essential trace element for mammals: effects of a "low-chromium" diet. J Biol Inorg Chem 2011;16:381–390.
(5) 斉藤悦則訳．マルサス人口論．東京：光文社，2011.
(6) Schwarz K. A hitherto unrecognized factor against dietary necrotic liver degeneration in American yeast (factor 3). Proc Soc Exp Biol Med 1951;78:852–854.
(7) Schwarz K, Foltz CM. Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. J Am Chem Soc 1957;79:3292–3293.
(8) Mertz W, Schwarz K. Impaired intravenous glucose tolerance as an early sign of dietary necrotic liver degeneration. Arch Biochem Biophys 1955;58:504–506.
(9) Schwarz K, Mertz W. Chromium (III) and the glucose tolerance factor. Arch Biochem Biophys 1959;85:292–295.
(10) Steele NC, Althen TG, Frobish LT. Biological activity of glucose tolerance factor in swine. J Anim Sci. 1977;45:1341–1345.
(11) Mertz W. Chromium in human nutrition: a review. J Nutr 1993;123:626–633.
(12) Jeejeebhoy KN, Chu RC, Marliss EB, Greenberg GR, Bruce-Robertson A. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. Am J Clin Nutr 1977;30:531–538.
(13) Morris BW, MacNeil S, Hardisty CA, Heller S, Burgin C, Gray TA. Chromium homeostasis in patients with type II (NIDDM) diabetes. J Trace Elem Med Biol 1999;13:57–61.
(14) Yamamoto A, Wada O, Ono T. A low-molecular-weight, chromium binding substance in mammals. Toxicol Appl Pharmacol 1981;59:515–523.
(15) Yamamoto A, Wada O, Ono T. Isolation of a biologically active low-molecular-mass chromium compound from rabbit liver. Eur J Biochem 1987;165:627–631.
(16) Yamamoto A, Wada O, Suzuki H. Purification and properties of biologically active chromium complex from bovine colostrum. J Nutr 1988;118:39–45.
(17) Davis CM, Vincent JB. Chromium oligopeptide activates insulin receptor tyrosine kinase activity. Biochemistry 1997;36:4382–4385.
(18) Davis CM, Sumrall KH, Vincent JB. A biologically active form of chromium may activate a membrane phosphotyrosine phosphatase (PTP). Biochemistry 1996;35:12963–12969.
(19) Vincent JB. Quest for the molecular mechanism of chromi-

um action and its relationship to diabetes. Nutr Rev 2000; 58:67–72.
(20) Clodfelder BJ, Emamaullee J, Hepburn DD, Chakov NE, Nettles HS, Vincent JB. The trail of chromium(III) in vivo from the blood to the urine: the roles of transferrin and chromodulin. J Biol Inorg Chem 2001;6:608–617.
(21) 吉野芳夫. 無機質の代謝. 細谷憲政編, 栄養学 (2) 代謝と生理. 東京：有斐閣, 1980:141–167.
(22) Roussel AM, Andriollo-Sanchez M, Ferry M, Bryden NA, Anderson RA. Food chromium content, dietary chromium intake and related biological variables in French free-living elderly. Br J Nutr 2007;98:326–331.
(23) Velasco-Reynold C, Navarro-Alarcon M, De La Serrana HL, Perez-Valero V, Lopez-Martinez MC. Determination of daily dietary intake of chromium by duplicate diet sampling: in vitro availability study. Food Addit Contam 2008; 25:604–610.
(24) Van Cauwenbergh R, Hendrix P, Robberecht H, Deelstra HA. Daily dietary chromium intake in Belgium, using duplicate portion sampling. Z Lebensm Unters Forsch 1996; 203:203–206.
(25) Grijalva Haro MI, Ballesteros Vázquez MN, Cabrera Pacheco RM. Chromium content in foods and dietary intake estimation in the Northwest of Mexico. Arch Latinoam Nutr 2001;51:105–110.
(26) 池辺克彦, 田中之雄, 田中涼一. 陰膳方式による15金属元素の一日摂取量について. 食衛誌 1988;29:52–57.
(27) Yoshida M, Ôgi N, Iwashita Y. Estimation of mineral and trace element intake in vegans living in Japan by chemical analysis of duplicate diets. Health 2011;3:672–676.
(28) 吉田宗弘, 児島未希奈, 三由亜耶, 森田明美. 病院および介護施設の食事からの微量ミネラル摂取量の計算値と実測値との比較. 微量栄養素研究 2011;28:27–31.
(29) Anderson RA, Kozlovsky AS. Chromium intake, absorption and excretion of subjects consuming self-selected diets. Am J Clin Nutr 1985;41:1177–1183.
(30) Mertz W, Roginski EE, Schroeder HA. Some aspects of glucose metabolism of chromium-deficient rats raised in a strictly controlled environment. J Nutr 1965;86:107–112.
(31) Striffler JS, Law JS, Polansky MM, Bhathena SJ, Anderson RA. Chromium improves insulin response to glucose in rats. Metabolism 1995;44:1314–1320.
(32) Scroeder HA. Chromium deficiency in rats: A syndrome simulating diabetes mellitus with retarded growth. J Nutr 1966;88:439–445.
(33) 吉田宗弘, 小野聡子, 舘　博, 安藤達彦. セレン欠乏飼料としてのトルラ酵母の再評価. 微量栄養素研究 1995;12:131–135.
(34) Flatt PR, Juntti-Berggren L, Berggren PO, Gould BJ, Swanston-Flatt SK. Effects of dietary inorganic trivalent chromium (Cr3+) on the development of glucose homeostasis in rats. Diabete Metab 1989;15:93–97.
(35) Mertz W. Essential trace metals: new definitions based on new paradigms. Nutr Rev 1993;51:287–295.
(36) Balk EM, Tatsioni A, Lichtenstein AH, Lau J, Pittas AG. Effect of chromium supplementation on glucose metabolism and lipids: a systematic review of randomized controlled trials. Diabetes Care 2007;30:2154–2163.
(37) 文部科学省学術審議会資源調査分科会. 日本食品標準成分表2010. 東京：全国官報販売協同組合, 2010.
(38) 加藤友紀, 大塚　礼, 今井具子, 安藤富士子, 下方浩史. 地域在住中高年者の微量ミネラルおよびビオチンの摂取量. 栄食誌 2012;65:21–28.
(39) Food and Nutrition Board, Institute of Medicine. Chromium. In: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington DC: National Academic Press, 2001:197–223.
(40) Kottwitz K, Laschinsky N, Fischer R, Nielsen P. Absorption, excretion and retention of 51Cr from labeled Cr-(III)-picolinate in rats. Biometals 2009;22:289–295.
(41) Nomiyama H, Yotoriyama M, Nomiyama K. Normal chromium levels in urine and blood of Japanese subjects determined by direct flameless atomic absorption spectrophotometry, and valency of chromium in urine after exposure to hexavalent chromium. Am Ind Hyg Assoc J 1980;41:98–102.
(42) Hajifaraji M, Leeds AR. The effect of high and low glycemic index diets on urinary chromium in healthy individuals: a cross-over study. Arch Iran Med 2008;11:57–64.
(43) Bahijri SM, Alissa EM. Increased insulin resistance is associated with increased urinary excretion of chromium in non-diabetic, normotensive Saudi adults. J Clin Biochem Nutr 2011;49:164–168.
(44) Davies S, Howard JM, Hunnisett A, Howard M. Age-related decreases in chromium levels in 51,665 hair, sweat, and serum sample from 40,872 patients. Implications for the prevention of cardiovascular disease and type II diabetes mellitus. Metabolism 1997;46:469–473.
(45) Yoshida M, Takada A, Hirose J, Endô M, Fukuwatari T, Shibata K. Molybdenum and chromium concentrations in breast milk from Japanese women. Biosci Biotechnol Biochem 2008;72:2247–2250.
(46) Bunker VW, Lawson MS, Delves HT, Clayton BE. The uptake and excretion of chromium by the elderly. Am J Clin Nutr 1984;39:797–802.
(47) Offenbacher EG, Spencer H, Dowling HJ, Pi-Sunyer FX. Metabolic chromium balances in men. Am J Clin Nutr 1986;44:77–82.
(48) Stearns DM. Is chromium a trace essential metal? Biofactors 2000;11:149–162.
(49) Editorial Board of Nutrition Reviews. Is chromium essential for humans? Nutr Rev 1988;46:17–20.