研究成果の刊行に関する一覧表

雑誌

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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

Masato Kasuga
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.

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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-

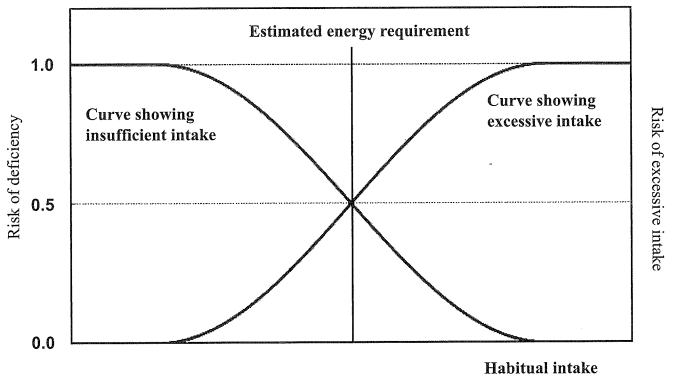

Fig. 1. Theoretical model for understanding estimated energy requirement. Left and right vertical axes show probability of insufficient and excessive intake for individuals, respectively.

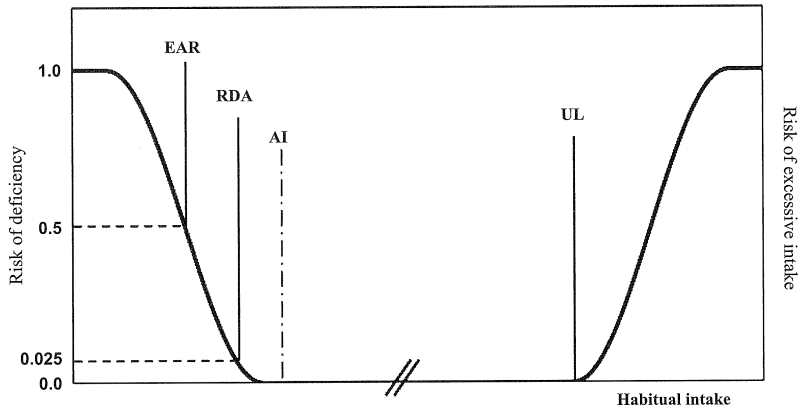

Fig. 2. Theoretical model for understanding EAR, RDA, AI, and UL for nutrients. EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; UL, tolerable upper intake level.

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.

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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.

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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.

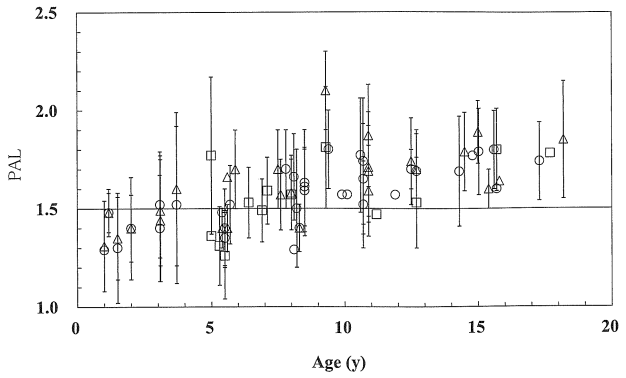

Fig. 1. PAL of children. The data presented for all age groups were taken only from studies that measured basal metabolic rate except for those for children aged 3 to 5 y, for whom data were also taken from studies that estimated basal metabolic rate, and children aged 1 to 2 y, for whom data were also 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.

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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.

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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.

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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.

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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.

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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.

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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.

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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.

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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.

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Review

Excess Intake of Microminerals : Is the Iodine Intake of Japanese People Excessive

微量ミネラルの過剰摂取－日本人のヨウ素摂取は過剰水準か

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Summary

Iodine intake in Japanese people was evaluated and excess intake of other microminerals (essential trace elements) was also described. Since kombu (a kind of kelp) contains iodine at a high level, average intake of this micromineral in Japanese people is estimated to be 1 to 3 mg/d. This intake exceeds not only the recommended dietary allowance (130 μg/d) but also the US tolerable upper limit (UL) (1.1 mg/d). However, adverse effects due to excess iodine intake are not observed Japanese in general. In fact, since detrimental influence is only frequently observed in Japanese after an iodine intake at a level of more than 10 mg/d occurs, a more appropriate UL of iodine for Japanese is estimated to be 3 to 5 mg/d. Among other microminerals, molybdenum intake exceeds the UL in vegans but again no adverse effects have been observed. However, there is a possibility of toxicity through excess intake of iron and zinc in the use of supplements or medicines containing these microminerals.

1. はじめに

必須微量元素（微量ミネラル）は必要量と中毒量の範囲が狭いことが特徴である。このため、わが国の食事摂取基準においては、クロムを除く7種の微量ミネラル（鉄、亜鉛、銅、マンガン、ヨウ素、セレン、モリブデン）に対して、摂取の耐容上限量を策定している。本稿では、これら7種の微量ミネラル中で、日本人の通常の食生活において過剰摂取の可能性があるといわれるヨウ素をとりあげ、摂取の実態とその是非について論じる。また、ヨウ素以外の微量ミネラルに関しては過剰摂取の可能性についてふれる。

2. ヨウ素の生理機能

2-1. 甲状腺ホルモンとヨウ素欠乏[1)]

健常な成人の体内には15～20 mgのヨウ素が存在し、その大半は甲状腺に分布して甲状腺ホルモンの合成に利用される。すなわち、吸収された食事由来のヨウ素はヨウ化物イオン（I^-）の形態で体内を輸送され、甲状腺濾胞細胞の基底膜に存在するタンパク質であるナトリウム/ヨウ化物イオン共輸送体によって能動的に甲状腺内に取り込まれる。取り込まれたヨウ化物イオンは、甲状腺ペルオキシダーゼによって過酸化水素と反応しI_2へと酸化される。生成したI_2はチログロブリンのチロシン残基に結合し、チログロブリン内で甲状腺ホルモンの前駆体であるモノヨードチロシン（MIT）、およびジヨードチロシン（DIT）が生じる。MITとDITは甲状腺ペルオキシダーゼによって重合し、チログロブリンがリソゾーム内のプロテアーゼで加水分解されると、甲状腺ホルモンであるテトラヨードチロニン（チロキシン：T4）、もしくはトリヨードチロニン（T3）が遊離する。図1にヨウ化物イオンからT3とT4が生成するプロセスをまとめた。

甲状腺ホルモンは、成長、発達、生殖などの生命活動の調節を行っている。とくに胎児期における甲状腺ホルモンの役割は重要であり、脳細胞の成長、末梢組織や骨格の成長と成熟を促している。

慢性のヨウ素不足が継続すると甲状腺のヨウ素含量は20 μg以下にまで激減し、甲状腺ホルモンの合成が著しく低下する。成人では、ヨウ素不足によって甲状腺ホルモンの分泌量が減少すると、甲状腺におけるヨウ化物イオンの取り込みを高めるために脳から甲状腺刺激ホルモン（TSH）が分泌される。血清甲状腺ホルモンの低下、およびTSHの上昇した状態を甲状腺機能低下症と呼ぶ。TSHによって刺激を受けた甲状腺はやがて異常

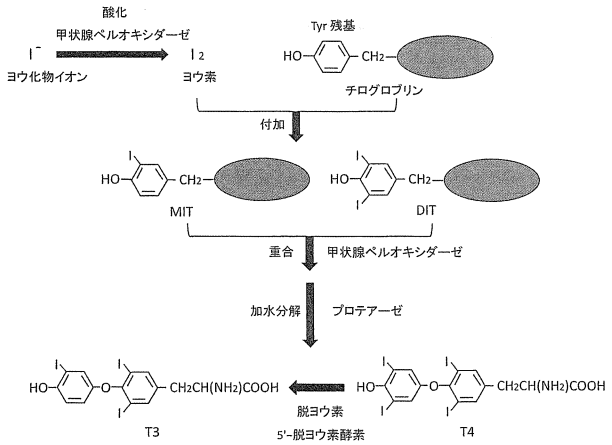

図1. 甲状腺ホルモンの生合成

肥大と過形成を起こし、甲状腺腫（ゴイター）が生じる。

ヨウ素不足の深刻なダメージを受けるのは胎児期、および乳幼児期である。胎児期においては、甲状腺ホルモンが身体の正常な発育に関与するため、ヨウ素不足は脳や末梢組織の致命的な発達不全を起こす。すなわち、妊娠中のヨウ素不足は流産や死産の確率を高めるとともに、出生した子において全般的な精神遅滞、低身長、四肢の麻痺、さらには甲状腺の萎縮と繊維化などを招く。このような乳児おける重篤な甲状腺機能低下症はクレチン症と呼ばれる。とくに甲状腺の器質的変性は致命的であり、粘液水腫型クレチン症という。脳細胞の成長は幼児期も継続するため、乳幼児期のヨウ素不足は知能の発達にも悪影響を及ぼす。

ヨウ素は後述のように海産物、とくに海藻類に多いため、大陸内陸部に居住する民族で摂取不足が目立つ。欧州や米国でもヨウ素不足は深刻であったが、これらの地域では食卓塩にヨウ素を添加することによってヨウ素不足を解消しつつある。それでもアフリカ内陸部を中心に、全世界で10億近い人々がいまだにヨウ素不足といわれており、WHOでは、ヨウ素を鉄、ビタミンAとともに摂取不足を改善すべき三大栄養素としている。

2-2. ヨウ素の必要量

ヨウ素の摂取量が適切な場合、甲状腺へのヨウ素の蓄積と排出は等しく、甲状腺のヨウ素含量はほぼ一定であると考えられる。放射性同位元素を用いた研究において、健常な成人では1日に約95 μgのヨウ素が甲状腺に蓄積することが示されている[2,3]。この蓄積量は甲状腺のヨウ素含量を一定に保つのに必要な量と考えられる。食事中のヨウ素がほぼ100 %吸収されるとすれば、この95 μg/日がヨウ素摂取の必要量と考えられる。この考え方にもとづき、日本人の食事摂取基準2010年版では、成人に対して、ヨウ素の推定平均必要量（ヨウ素欠乏のリスクが50 %である摂取量）を95 μg/日、そして推奨摂取量（ヨウ素欠乏のリスクが2.5 %となる摂取量）を130 μg/日としている[4]。

2-3. ゴイトロゲン

食品成分の中には甲状腺へのヨウ素の取り込みや、甲状腺ペルオキシダーゼの活性を阻害するものがある。ヨウ素摂取不足が起こるような地域では、このような成分はヨウ素不足を加速し、ヨウ素欠乏症を招くことがあるため、ゴイトロゲン（甲状腺腫誘発物質）と呼ばれている。代表的なゴイトロゲンとしては、アブラナ科植物中のイソチオシアネート類、大豆中のイソフラボンの主成分であるゲニスタインなどが知られている[5,6]。

3. 食品中のヨウ素濃度とヨウ素摂取量

3-1. 高ヨウ素の食品

表1に主な食品のヨウ素濃度を日本食品標準成分表2010から

表1. 主な食品のヨウ素濃度（μg/100 g）

食品	ヨウ素濃度	食品	ヨウ素濃度
食パン	1	あおのり、素干し	2,800
精白米	0	焼きのり	2,100
さつまいも	1	長昆布、素干し	210,000
木綿豆腐	5	真昆布、素干し	240,000
春菊	5	昆布、佃煮	11,000
大根	3	昆布だし	8,200
なす	0	干しひじき	47,000
りんご	0	素干しわかめ、水戻し	1,900
しいたけ	0	まいわし	28
牛肉、赤肉	1	しろさけ	5
牛肝臓	4	まだい	6
鯨肉	4	まだら	350
鶏肉、ささみ	0	くろまぐろ	14
卵黄	50	あさり	55
卵白	2	くるまえび	4
普通牛乳	16	こういか	4

日本食品標準成分表2010より抜粋

抜粋した。海藻類、とくに昆布のヨウ素濃度が著しく高いことは明らかである。次に、筆者の研究室において測定した昆布の種類ごとのヨウ素濃度を表2に示した。いずれも1例のみの測定であるため、数値上の差異が種によるものなのか産地によるものなのかは判断できない。しかし、表1に示した成分表記載の数値を大きく超えるヨウ素濃度の昆布が存在することは明らかである。表3に、各種昆布製品のヨウ素濃度について、筆者の研究室での測定結果をまとめた。「削り昆布」(商品名：おぼろ昆布、またはとろろ昆布)、および昆布屑を油で揚げた「昆布チップ」には、素干し昆布とほぼ同水準のヨウ素が含まれていた。これに対して、佃煮昆布や塩昆布のヨウ素濃度は素干し昆布よりも一桁低かった。これは比較的廉価な佃煮昆布の原料に、だしをとった後の昆布が転用されているためである。したがって、佃煮昆布であっても、伝統的な製法、すなわち素干し昆布をそのまま利用して調味料とともに煮詰めるという製法のものは、素干し昆布に近いヨウ素濃度になると推定される。これらの結果は、わずかな量（0.1 g程度）の昆布製品の摂取によって推奨摂取量を上回るヨウ素摂取が達成できることを意味している。

表2. 各種昆布のヨウ素濃度（μg/100 g dry weight）

種類	濃度
真昆布　*Laminaria japonica*	424,000
羅臼昆布　*Laminaria diabolica*	560,000
利尻昆布　*Laminaria ochotensis*	242,000
三石昆布(日高昆布)　*Laminaria angustata*	307,000
籠目昆布　*Kjellmaniella crassifolia*	458,000

表3. 昆布製品中のヨウ素濃度（μg/100 g dry weight）

昆布製品	濃度
塩昆布	28,000
佃煮昆布	36,000
削り昆布	609,000
昆布茶	24,000
昆布飴	20,000
酢昆布	263,000
味付け昆布	516,000
昆布チップ	369,000

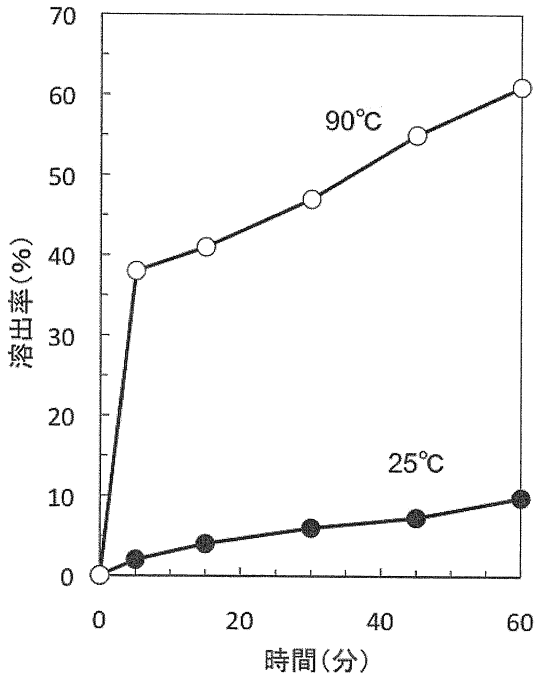

図2. 素干し昆布から出汁へのヨウ素の移行
約4×2.5 cm（約1 g）の素干し真昆布（ヨウ素濃度5.03 mg/g）に100 mlの湯（90 ℃）または水（25 ℃）を加え、静置した。

図2は、素干し昆布1 gに100倍量の水または沸騰水を加えたときのヨウ素の出汁（だし）への移行を調べた結果を示している。浸漬1時間で、水では約10 %、沸騰水では約60 %のヨウ素が出汁へ移行し、出汁中ヨウ素濃度は沸騰水の場合約2〜4 mg/100 gであった。なお、食品成分表では、本実験条件よりも昆布の比率を多くした場合の昆布出汁のヨウ素濃度として8.2 mg/100 gを記載しており、抽出条件による濃度変化は大きいと推定できる。しかし、昆布出汁を数ml摂取するだけで、推奨摂取量を大幅に上回るヨウ素が摂取できることは確かである。

3-2. 日本人のヨウ素摂取

日本人は海産物を多食するため昆布を摂取しなくても十分なヨウ素摂取が達成できると思われている。しかし、筆者らが

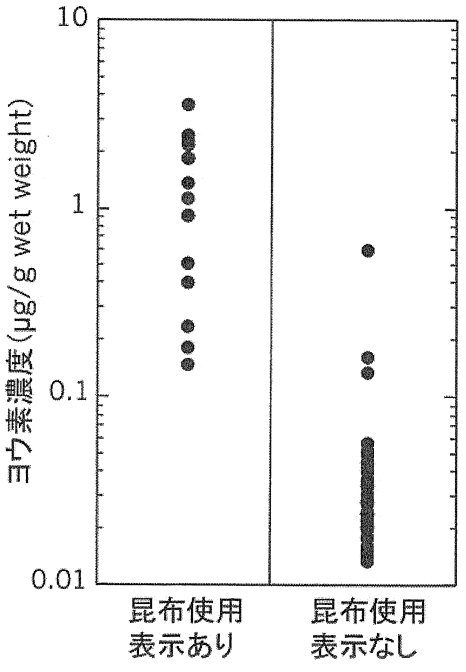

図3. 市販離乳食のヨウ素濃度[7]
母乳は平均的に0.1 μg/gを超えるヨウ素を含んでいるので、昆布使用の表示のないものは3例（いずれもヒジキを使用していた）を除いて母乳よりも低水準のヨウ素濃度ということになる。

市販離乳食について調べた結果では、図3に示すように、昆布未使用のものはヒジキを使用しているものを除くといずれも母乳よりも低水準のヨウ素しか含んでいなかった[7]。このことは、日本人のヨウ素摂取が昆布の摂取状況によって規定されていることを意味している。

日本人のヨウ素摂取量は、これまで陰膳献立の分析[8]、尿中ヨウ素濃度[9,10]、海藻消費量[11]の三方向から推定されており、陰膳献立の分析、および尿中ヨウ素濃度の測定結果では500 μg/日未満の摂取の中に間欠的に2 mg/日以上、場合によっては10 mg/日に近い高ヨウ素摂取が出現すること、海藻消費量の検討では1.2 mg/日という平均摂取量が示されている。また、日本人のヨウ素摂取量に関するレビューにおいては、平均で1から3 mg/日という数値が提示されている[12]。

しかし、後述のヨウ素の過剰障害を考える場合、集団の平均摂取量はあまり意味をもたない。重要なのは、各個人の摂取状況、すなわち、各個人の中で低摂取と高摂取が繰り返されているのか、それとも各個人の中では安定した摂取が保たれているのかである。昆布出汁が日常的に多用されるため、意識していても昆布製品を食生活から除外することは相当に困難であることから、日本人においてヨウ素不足が起こるような連続的低摂取が生じることは考えがたい。したがって、問題となるのは、数mg/日という連続的高摂取が存在するかである。ひとつまみ（約1 g）の削り昆布、コップ一杯（100～150 ml）程度の昆布出汁を連日摂取する日本人はいくらでも存在するように思われるが、日本人を対象としたヨウ素摂取の個人内変動を検討した研究が存在しないため、このことに対しては明確に答えられないのが現状である。

4. ヨウ素中毒

4-1. ウォルフ-チャイコフ効果と脱出現象[13]

ヨウ素を過剰に摂取した場合、ヨウ素不足の場合と同様に甲状腺機能低下が起こり、継続すれば甲状腺腫が出現する。過剰ヨウ素によって甲状腺機能低下が生じることをウォルフ-チャイコフ効果という。この現象は、摂取しすぎたヨウ素が甲状腺にあふれ、甲状腺ホルモンを合成できなくなる（材料がありすぎて作業ができない工場のようなもの）と形容されている。この過剰ヨウ素による甲状腺機能低下は次第に起こりにくくなる。これは一種の慣れといわれており、脱出（エスケープ）現象という。過剰のヨウ素を投与したラットでは甲状腺ペルオキシダーゼと甲状腺膜のナトリウム/ヨウ化物イオン共輸送体のmRNAの発現が低下することが確認されている。つまり、ウォルフ-チャイコフ効果は甲状腺ペルオキシダーゼ活性の低下によりヨウ素の有機化が阻害されることにより生じ、脱出現象は甲状腺へのヨウ素の取り込み能力が低下することによって生じると考えられる。

4-2. 欧米におけるヨウ素過剰障害とヨウ素摂取の耐容上限量

米国では、ヨウ素摂取量が0.3 mg/日程度である人に実験的に1.5 mg/日のヨウ素を連続的に投与すると甲状腺機能低下が生じることが観察されている[14]。この研究は、米国のヨウ素摂取の耐容上限量（成人1.1 mg/日、小児0.2～0.9 mg/日）策定の根拠とされている[15]。中国[16]やアフリカ[17]での疫学研究において、井戸水から連日1.5 mg程度のヨウ素を摂取している人において甲状腺腫の有病率が高いことが判明していることから、この上限量は妥当なものと考えられている。

昆布製品の摂取が耐容上限量を超えるヨウ素摂取量を容易にもたらすため、欧米では甲状腺機能低下の原因を昆布などの海藻類を使用した食品にもとめることがしばしばある。最近でも、オーストラリアのニューサウスウェールズ州において、成人および小児の甲状腺機能低下の原因が昆布出汁入り豆乳の飲用にあるとされ、当該製品がリコールされるという事件が発生した[18]。当該製品のヨウ素濃度が2.5 mg/100 mlであり、成人では約50 ml、小児では約20 mlの摂取で米国の耐容上限量を超えること、および摂取を中止すると甲状腺機能低下から回復したことがその根拠となっている。この昆布出汁入り豆乳のヨウ素濃度は先に述べた昆布出汁に近い水準である。オーストラリアの事例の原因が本当にヨウ素であるとすれば、欧米人は本格的な昆布出汁を20～30 ml摂取し続けると甲状腺機能低下を起こすことになる。欧米から来日した人の中に昆布出汁を使った食品を日常的に摂取しているケースがあるように思うが、日本国内で欧米人が昆布のために甲状腺機能低下を起こしたという事例は聞いたことがない。見過ごされているのであろうか。

4-3. 日本におけるヨウ素過剰障害

日本人は昆布を日常的に調理に用いるため、先に述べたように、平均ヨウ素摂取量（1～3 mg/日）が欧米の耐容上限量（1.1 mg/日）を超えると推定できる。しかし、成人日本人が昆布摂取を原因とする甲状腺機能低下を起こした事例は少ない。つまり、日本人のヨウ素摂取の正確な実態はいまだに明確ではないが、成人に限定すれば、ほとんどの日本人のヨウ素摂取は過剰障害を起こす水準にないと思われる。日本における報告では、乾燥物換算で連日10 g以上の昆布を1年間摂取した事例[19]、昆布チップ1袋を約1ヶ月食べ続けた事例[20]など、明らかに特殊と思われる昆布摂取が行われた場合に甲状腺機能低下や甲状腺腫が認められている。一方、日本の健常人を対象にした実験では、昆布から35～70 mg/日のヨウ素（乾燥昆布15～30 g）を7～10日間摂取した場合に血清TSHの可逆的な上昇が認められている[21]。またヨウ素製剤を用いた実験でも、27 mg/日のヨウ素を28日間摂取することによって、血清TSHの上昇、血清T4のわずかな低下、さらに甲状腺容積の増加が可逆的に生じている[22]。これらのことは、日本人は欧米人に比較してヨウ素過剰障害を起こしにくいが、それでも成人が20 mg/日を超え

るヨウ素を摂取し続ければ確実に過剰障害が起きることを意味している。

一方、疫学調査からは、これより少ないヨウ素摂取でも甲状腺機能低下が生じる可能性を指摘できる。すなわち北海道住民を対象にした調査は、尿中ヨウ素濃度から判断して10 mg/日を上回るヨウ素摂取がある人において甲状腺機能低下の発生率が上昇することを示している[23, 24]。残念なことに、この調査は尿中ヨウ素濃度の測定を1回しか行っておらず、10 mg/日を超える摂取が連日であったかは不明である。しかし、10 mg/日を超えるヨウ素摂取が頻繁に生じる場合には甲状腺機能低下のおそれがあるといえるだろう。

成長期では日本人であってもヨウ素過剰摂取の影響があるとする報告が散見される。たとえば、世界各地の6〜12歳の小児を対象とした疫学研究は、平均で750 μg/日のヨウ素摂取である北海道沿岸地域の小児集団の平均甲状腺容積が他地域よりも有意に大きいことを示している[25]。また、妊娠中に昆布を多食した母親から出生した乳児に高TSH血症が発生したという報告がある[26]。この母親の日常の平均的なヨウ素摂取量が2〜3 mg/日に及んでおり、母乳からの高ヨウ素摂取も加わって高TSH血症が発生したと推定されている。母親のヨウ素摂取量が1.5 mg/日を超えると、母乳のヨウ素濃度が1 μg/gを超えることから[27]、乳児においても1 mg/日を超える高ヨウ素摂取の日があることは確実である。一方、妊娠初期の神戸市在住の女性を対象にした調査では、尿中ヨウ素濃度（中央値、328 μg/L；範囲、25〜78,487 μg/L）は、血中TSHと正、T4およびT3とは負の相関関係を示している。しかし、回帰式は信頼区間がきわめて広く、数%存在する3,000 μg/Lを超える尿中ヨウ素濃度を当てはめても高TSH血症にならない[28]。これまでの研究は尿中ヨウ素の測定を1回しか行っておらず、個人のヨウ素摂取の把握が不十分と思われる。今後は同一人に対して複数回の測定を行い、個人のヨウ素摂取をより正確に把握することが必要であろう。

成人日本人が平均で欧米の耐容上限量を超えるヨウ素摂取であるにもかかわらず過剰障害を起こさない理由はいくつか考えることができる。まず、日本人のヨウ素摂取の形態が、連日の高摂取ではなく、間欠的な高摂取であることがあげられる。しかし、先にも述べたが、削り昆布や昆布出汁の高ヨウ素濃度を考えると、少なくとも欧米の耐容上限量程度のヨウ素を連日摂取する日本人の存在は十分に考えられる。次に、間欠的に生じる高ヨウ素摂取が、ヨウ素に対する慣れ、すなわち脱出現象を成立させていることが考えられる。日本人が欧米人に比べて10倍以上のヨウ素摂取でなければ甲状腺機能低下を起こさないことはその根拠になるだろう。さらに、日本人がゴイトロゲンを含む食品、とくに大豆を多食することも、日本人にヨウ素過剰摂取に起因した甲状腺機能低下が起こりにくい理由に加えることができるかもしれない。しかし、これを立証する調査研究は今のところ見当たらない。

最近、日本人を対象にして海藻類の摂取状況と甲状腺がん発生との関連が検討され、閉経後の女性において、海藻類をほとんど毎日食べる集団は週2日以下しか食べない集団に比較して甲状腺がん、なかでも乳頭がんの発生リスクが有意に上昇することが認められた[29]。海藻類はヨウ素を特異的に多く含む食品であることから、閉経後の高ヨウ素摂取が甲状腺がん発生リスクを高める要因であることを示唆するものといえる。

5. 日本人に対するヨウ素摂取の耐容上限量

現行のヨウ素摂取の耐容上限量2.2 mg/日は、先の北海道での疫学調査[23, 24]に示されていた対象者全員の平均ヨウ素摂取量3.3 mg/日を健康障害非発現量（NOAEL）と考えて、これに不確定因子1.5を加味したものである[4]。昆布は少量の摂取で数mg/日を超えるヨウ素摂取をもたらすが、耐容上限量は連続的摂取に適用されるものであり、間欠的な高摂取を否定するものではない。つまり、数mg/日を超えるヨウ素摂取であっても連日でなければ問題とならない。しかし、献立を作成する側はどの献立でも上限量を超えないようにするのが常である。つまり、現行の2.2 mg/日は、連続摂取を念頭においたものであるが、活用の場ではすべての献立に適用すべきものと受け止められ、献立から昆布を排除することに繋がりかねない。昆布を献立から排除することは和食の否定につながる。また幼少期に昆布を過度に排除することは、味覚発達の点から好ましくなく、さらにヨウ素に対する脱出現象成立を難しくする。したがって、ヨウ素摂取の上限値は、現行よりも高い数値に定めるのが現実的といえる。

論理的には、ヨウ素摂取の上限量は、連日摂取、平均摂取、瞬間最大摂取の3つに分けて示すのが正しいが、それでは理屈倒れであり、活用に適さないものになる。成人において、ヨウ素過剰障害を予防する場合に参考となるのは、10 mg/日を超える摂取が頻繁に出現すると甲状腺機能低下が起こるという報告[23, 24]である。先にも述べたが、摂取基準を活用して献立を作成する場合、常に上限量を超えないような配慮がなされるのであるから、10 mg/日を上限量にすれば、10 mg/日超えの献立は出現しないことになる。ただし、10 mg/日近い摂取量でも大丈夫という印象を与えることは問題があるので、10 mg/日の2〜3分の1に相当する3〜5 mg/日という数値が現実的な上限量になると思える。

上限量を現在の日本人のヨウ素摂取量から設定するという考え方もある。すなわち、一般的な日本人にヨウ素過剰摂取に起因する甲状腺機能障害がほとんど見当たらないことを根拠として、日本人のヨウ素摂取量の上限付近（たとえば、95パーセンタイル値）を耐容上限量とするのである。今のところヨウ素摂取に関する国民全体を対象とした調査報告は存在しない

が、尿中濃度[9, 10, 28]や陰膳献立分析[8]の結果からは3～5 mg/日付近が上限に相当すると考えられる。すなわち、現在のヨウ素摂取を肯定するという立場からも、上限量を3～5 mg/日にすることは妥当といえる。この上限量であれば昆布出汁をある程度利用できるので、献立の幅も広がることになる。

妊婦と授乳婦では2～3 mg/日のヨウ素摂取で乳児に高TSH血症が出現していることから[26]、2～3 mg/日よりも低い上限量を設定せざるを得ないであろう。また、小児に関しても、平均750 μg/日の摂取で甲状腺容積の増大が観察されているという報告[25]を無視できないと思われる。

ただし、離乳食の分析結果が示すように、日本人が間欠的高摂取によってヨウ素摂取を維持している可能性は高いので、間欠的な高濃度摂取が許容されるということを明示する意味で、カドミウムなどの有害微量元素と同様のTWI（Tolerable weekly intake：耐容週間摂取量）を設定するのも一案かもしれない。この場合、成人では20 mg/weekがひとつの目安になると思われる。

なお、最近の研究は、横浜在住の妊娠女性の尿中ヨウ素濃度を測定し、中央値219 μg/L、範囲18～16,300 μg/L、幾何平均値262 μg/L、幾何平均値の95 %信頼区間241～286 μg/Lを報告している[30]。この測定値は先に示した神戸在住の妊娠女性[28]に比較して明らかに低く、都市部であってもヨウ素摂取に地域差のあることを示している。横浜の数値が日本人の多数を代表しているのであれば、現行の上限量である2.2 mg/日を超える摂取の人の割合はわずかということになる。食品標準成分表にヨウ素濃度の数値が記載され、これを補完する各種加工食品のヨウ素濃度の数値も公表されつつある[31, 32]。今後、食事調査にもとづいて、日本人のヨウ素摂取の実態が明らかになることを期待したい。

6. ヨウ素以外の微量ミネラル

6-1. 過剰摂取の可能性

ヨウ素以外の微量ミネラルにおいて、食事からの摂取によって食事摂取基準の耐容上限量を超える摂取が生じる可能性があるのはモリブデンのみである。他の微量ミネラルはサプリメントもしくは医薬品の服用によって、食事とは別に大量に摂取した場合に過剰摂取が生じる可能性がある。

6-2. 菜食主義者におけるモリブデンの大量摂取

表4は、動物性食品をいっさい摂取しないビーガンと称される厳格な菜食主義者のミネラル摂取量を調査した結果をまとめたものである[33]。一般女性に比較して、カリウム、マグネシウム、リン、鉄、銅、マンガン、モリブデンの摂取量が明らかに多い。なかでも、モリブデンの平均摂取量は現行の耐容上限量を明らかに上回っている。日本人におけるモリブデンの主たる供給源が米と大豆製品であり、菜食主義者ではたんぱく質摂取量を確保するために穀物と豆類の摂取が多くなることがその原因である。しかし、対象となったビーガンに健康上の問題はまったく生じていないことから、この耐容上限量を超えるモリブデンの摂取はおそらく問題にならないと判断する。現行の

表4. 日本人女性ビーガン12名の1日当たりミネラル摂取量[33]

	ビーガン女性 (n=12)	30～49歳の一般女性[a] (n=1053)	食事摂取基準2010	
			推定平均必要量	耐容上限量
エネルギー(kcal)	1847±141	1682±469	1750[b]	—
ナトリウム(mg)	3649±1719	3696±1415[c]	590[c]	2950[c,d]
カリウム(mg)	3610±1272[f]	1983±777	2000[e]	—
カルシウム(mg)	361±122	440±224	550	2300
マグネシウム(mg)	494±112[f]	214±80	240	—
リン(mg)	1225±311[f]	854±284	900[e]	3000
鉄(mg)	13.0±2.4[f]	6.9±3.0	9.0	40
亜鉛(mg)	8.3±1.6	7.1±2.4	8.0	35
銅(mg)	1.75±0.37[f]	1.00±0.35	0.6	10
マンガン(mg)	7.5±2.2	—	3.5[e]	11
ヨウ素(μg)	1865±1934	—	95	2200
セレン(μg)	87±34	—	20	230
クロム(μg)	27±8	—	25	—
モリブデン(μg)	540±207	—	20	500

[a] 2008年国民健康・栄養調査成績より引用
[b] 推定エネルギー必要量
[c] 食塩の値から換算
[d] 目標量
[e] 目安量
[f] 一般女性の摂取量との間に危険率0.1 %未満で有意差がある。

耐容上限量は動物実験の結果にもとづいて設定していることから[4]、むしろこのビーガンのモリブデン摂取量にもとづいてモリブデンの耐容上限量をより高い数値に変更できる可能性もあると思われる。

6-3. サプリメントなどによる過剰摂取

日本人は鉄と亜鉛が摂取不足といわれているため、この両ミネラルを含むサプリメント類や強化食品が多数流通している。確かに現在の食生活パターンを維持しつつ、これら両ミネラルの摂取量を増加させるには、サプリメントや強化食品を利用することが有効である。両ミネラルともに過剰摂取では吸収率が低下し、さらに亜鉛ではたとえ吸収量が増加しても臓器中濃度がほぼ一定値に維持される。また、ほとんどのサプリメントは耐容上限量を超える摂取が起こらないように濃度設計されている。したがって、現在のところ、国内においては、これら両ミネラルの過剰摂取に関する報告は見当たらない。しかし、微量ミネラルでは中毒水準と必要水準が近接していることから、今後とも過剰摂取の発生に注意する必要がある。なお、最近、腎不全患者のリン吸収を抑制するリン吸着剤として鉄化合物が開発されている[34]。この場合、グラム単位での投与が行われることから、臓器への鉄沈着などが生じる危険性がある。

サプリメントから付加的にセレンを摂取することが前立腺がんの予防につながる可能性があるとの報告[35]を背景としてセレンサプリメントが市場に出現している。しかし、大規模な疫学介入試験はセレンによる前立腺がん予防効果を否定した[36]。サプリメントからの付加的な200 μg/日のセレン摂取は2型糖尿病発生リスクを高めるという報告も提出されている[37]。したがって、サプリメントからセレンを付加的に摂取することは健康にとって好ましくない影響があると判断できる。

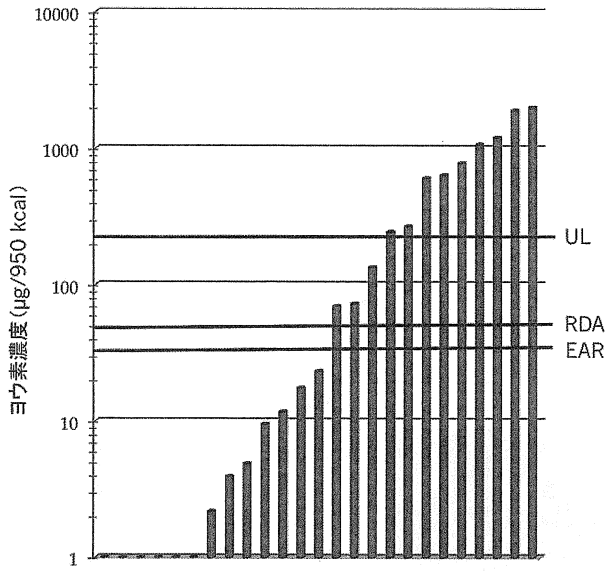

図4. 乳幼児が離乳食から摂取しているヨウ素の推定量[38]
8～16か月児25名が摂取していた離乳食を1日分収集し、ヨウ素濃度を測定した。各離乳食を950 kcal（1歳児の推定エネルギー必要量に相当）摂取した場合のヨウ素摂取量を個別に示した。UL、RDA、EARのラインは、それぞれ食事摂取基準2010年版が定める1～2歳児のヨウ素摂取の耐容上限量、推奨摂取量、推定平均必要量を示している。

7. おわりに

日本人に微量ミネラルの過剰摂取に起因する健康障害はほとんど認められない。つまり、現在の食生活は、機能性食品やサプリメントの誤用さえなければ、微量ミネラルの過剰摂取を起こすものではないといえる。とくにヨウ素に関しては、日本人は昆布を食生活に活かしており、これまで日本人が行ってきた伝統的な昆布の食べ方であればおそらく大きな健康問題は生じないと思われる。つまり、日本人と昆布との間には微妙な間合いが成立しているといえる。したがって、これまでと異なる昆布の食べ方、たとえば、昆布の機能性成分に期待するあまり大量の昆布製品を連日食べるようなことを避ければ十分だといえる。

図4は、乳幼児25名が実際に食べていた離乳食1日分を収集してヨウ素濃度を測定し、ヨウ素摂取量を推定した結果である[38]。摂取基準の推定平均必要量を下回る例が多く、なかにはヨウ素をほとんどまったく摂取していない場合もあったが、逆に、現行の1～2歳児の耐容上限量（250 μg/日）を上回る例も多かった。これらは1日のみの推定であり、同一人において低摂取、あるいは高摂取が連続しているかは明らかでない。しかし、離乳食にはヨウ素含有量が極端に高い献立と低い献立が存在しており、極端に高い献立の出現がなければ摂取不足を引き起こす可能性があることは確かだろう。妊婦や授乳婦に関しては昆布製品の大量消費は控えた方が無難と思われるが、離乳期以降は昆布、とくに昆布出汁を使った食品を適切量摂取することによって、ヨウ素不足を予防し、ヨウ素に対する耐性を獲得することが望ましいといえる。

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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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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

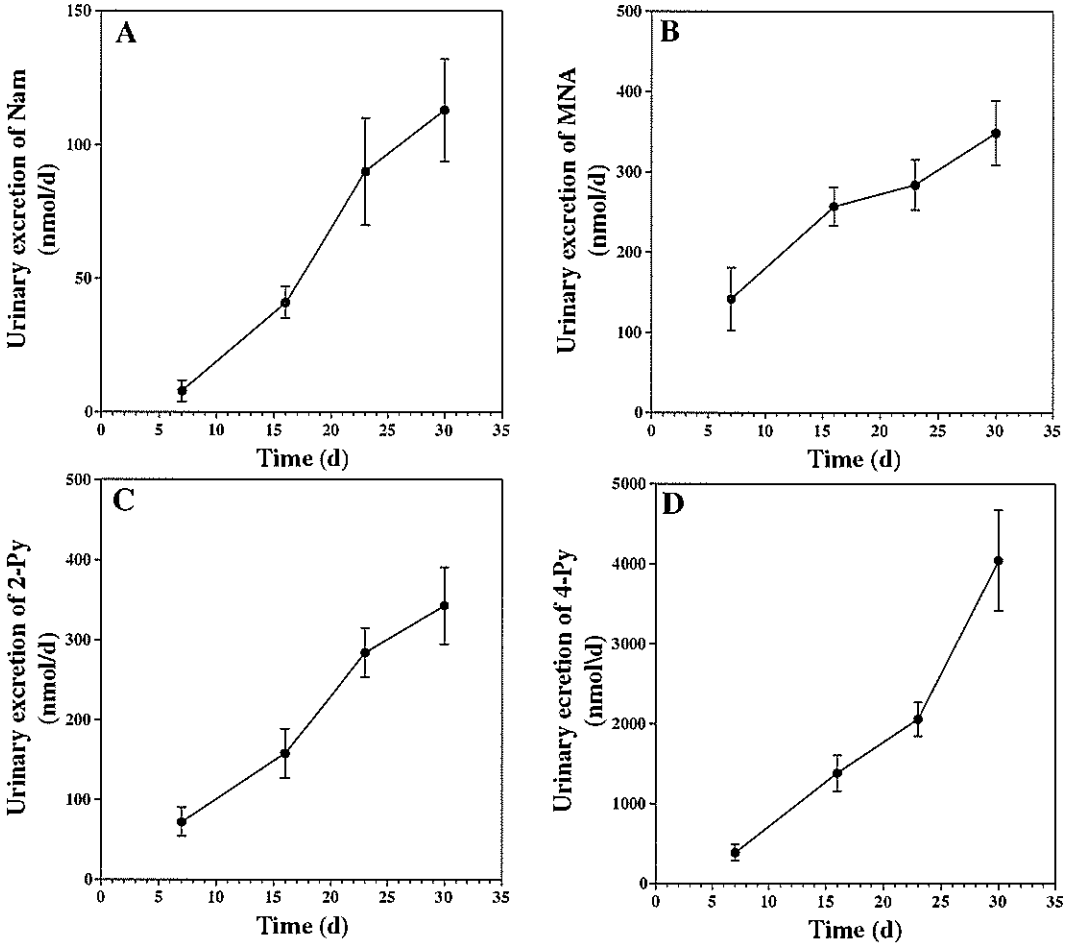

Fig. 1. Effects of Age on the Urinary Excretion of Nam (A), MNA (B), 2-Py (C), and 4-Py (D). Symbols mean represent ± SEM for six rats.

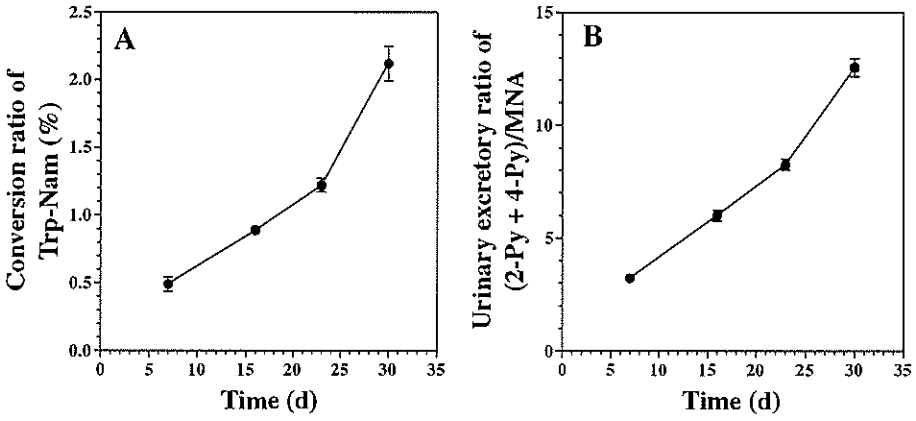

Fig. 2. Effects of Age on the Conversion Ratio of Trp to Nam (A) and the Urinary Excretory Ratio of (2-Py + 4-Py)/MNA (B). Symbols mean represent ± SEM for six rats.

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

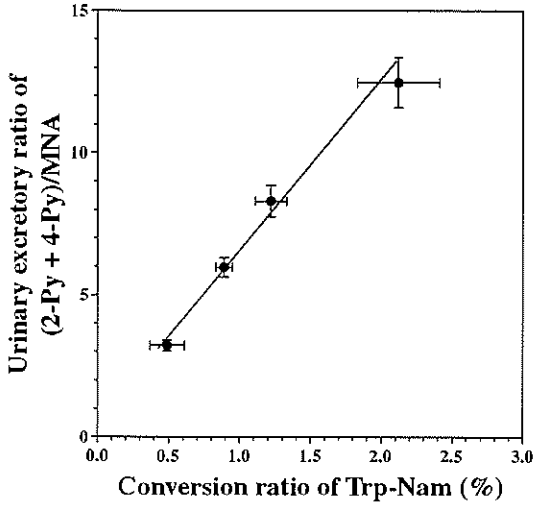

Fig. 3. Relation between the Conversion Ratio of Trp to Nam and the Urinary Excretory Ratio of Nam Catabolites.

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.

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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].

British Journal of Nutrition

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).

British Journal of Nutrition

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.

British Journal of Nutrition

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.

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British Journal of Nutrition

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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

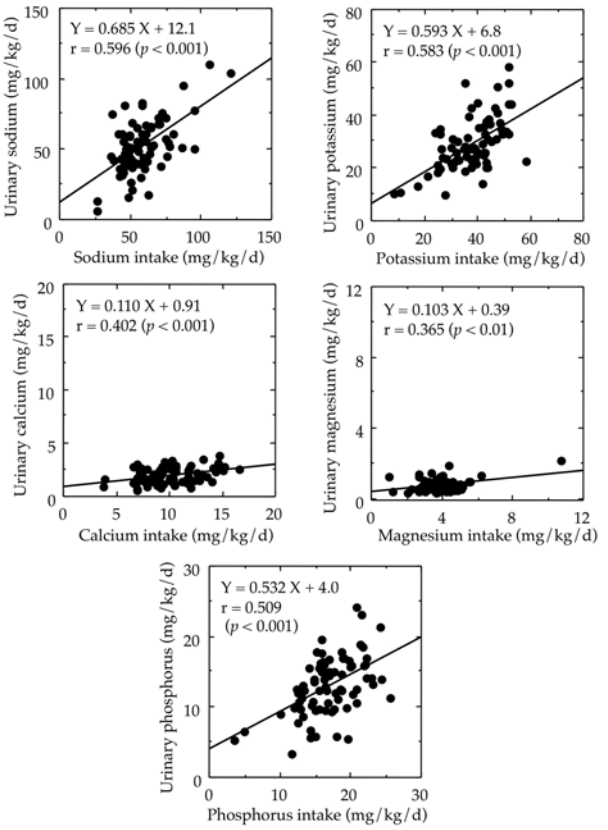

Figure 1. Correlation between daily intake and urinary excretion of sodium, potassium, calcium, magnesium and phosphorus in subjects.

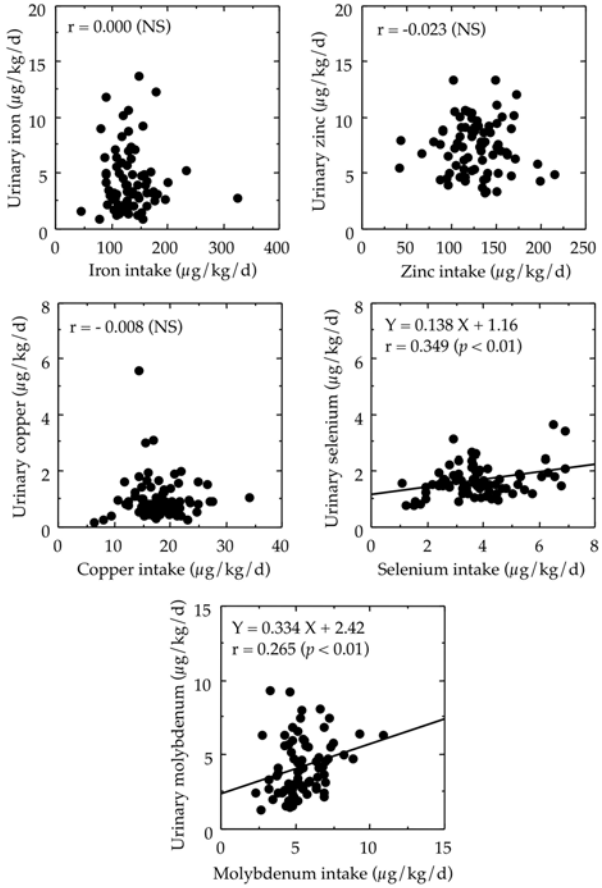

Figure 2. Correlation between intake and urinary excretion of iron, zinc, copper, selenium and molybdenum of subjects.

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.

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British Journal of Nutrition (2012), **107**, 52–60
doi:10.1017/S0007114511002339

Different variations of tissue B-group vitamin concentrations in short- and long-term starved rats

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(Received 3 November 2010 – Revised 24 March 2011 – Accepted 25 March 2011 – First published online 27 June 2011)

Abstract

Prolonged starvation changes energy metabolism; therefore, the metabolic response to starvation is divided into three phases according to changes in glucose, lipid and protein utilisation. B-group vitamins are involved in energy metabolism via metabolism of carbohydrates, fatty acids and amino acids. To determine how changes in energy metabolism alter B-group vitamin concentrations during starvation, we measured the concentration of eight kinds of B-group vitamins daily in rat blood, urine and in nine tissues including cerebrum, heart, lung, stomach, kidney, liver, spleen, testis and skeletal muscle during 8 d of starvation. Vitamin B_1, vitamin B_6, pantothenic acid, folate and biotin concentrations in the blood reduced after 6 or 8 d of starvation, and other vitamins did not change. Urinary excretion was decreased during starvation for all B-group vitamins except pantothenic acid and biotin. Less variation in B-group vitamin concentrations was found in the cerebrum and spleen. Concentrations of vitamin B_1, vitamin B_6, nicotinamide and pantothenic acid increased in the liver. The skeletal muscle and stomach showed reduced concentrations of five vitamins including vitamin B_1, vitamin B_2, vitamin B_6, pantothenic acid and folate. Concentrations of two or three vitamins decreased in the kidney, testis and heart, and these changes showed different patterns in each tissue and for each vitamin. The concentration of pantothenic acid rapidly decreased in the heart, stomach, kidney and testis, whereas concentrations of nicotinamide were stable in all tissues except the liver. Different variations in B-group vitamin concentrations in the tissues of starved rats were found. The present findings will lead to a suitable supplementation of vitamins for the prevention of the re-feeding syndrome.

Key words: **Starvation: Fasting: Energy metabolism**

Starvation produces a series of metabolic changes that lead to a reduction in body weight, alterations in body composition and metabolic gene expression[1,2]. In mammals and birds, three distinct levels of energy depletion have been established[3–10]. The first phase (phase 1) is a rapid period of adaptation marked by an increase in mobilisation of fat stores and a lowering in protein utilisation. During the second phase (phase 2), which is a long period of thrift, most of the energy expenditure is derived from fats, and then fat stores are progressively exhausted, while body proteins are efficiently spared. The third phase (phase 3) is characterised by an increase in protein utilisation. In humans, the negative energy balance resulting from starvation can arise due to disease, eating or psychological disorders, or hunger strikes. Starvation and consequent re-feeding syndrome can lead to electrolyte disorders, especially hypophosphataemia, along with neurological, pulmonary, cardiac, neuromuscular and haematological complications[11]. To avoid the re-feeding syndrome, an additional load of vitamins has been suggested to correct the vitamin deficiencies[11]. However, little is known about B-group vitamin status during starvation.

Several B-group vitamins take part in energy metabolism. For instance, vitamin B_2 functions as FAD and FMN in redox reactions including the electron transport chain and fatty acid oxidation. Nicotinamide is involved in more than 200 reactions, including the metabolism of carbohydrates, amino acids and fatty acids, and also in the electron transport chain. Vitamin B_1 catalyses carbohydrate metabolism including decarboxylation of α-ketoacids and *trans*-ketolation as a cofactor thiamin diphosphate; vitamin B_6 functions as pyridoxal 5′-phosphate in amino acid metabolism including aminotransferases,

Abbreviations: 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-5-carboxamide; 3-HBA, 3-hydroxybutyric acid; MNA, N^1-methylnicotinamide.

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British Journal of Nutrition

decarboxylases, racemases and dehydratases as pyridoxal 5′-phosphate; and pantothenic acid is involved in fatty acid metabolism such as oxidation and synthesis. For these reasons, in the 'Dietary Reference Intakes for Japanese, 2010', dietary requirements for vitamin B_1, vitamin B_2 and niacin are expressed per 4186 kJ (1000 kcal), and the requirement for vitamin B_6 is expressed in terms of protein intake[12].

As mentioned earlier, prolonged starvation sifts the energy source from glucose to fats and then to protein, and B-group vitamins are involved in the metabolism of carbohydrates, fatty acids and amino acids. Thus, in the present study, we investigated how changes in energy metabolism altered B-group vitamin utilisation during starvation. We comprehensively determined eight kinds of B-group vitamin concentrations in rat blood, urine and tissues including the brain, heart, lung, stomach, kidney, liver, spleen, testis and skeletal muscle during 8 d of starvation.

Materials and methods

Diets

The composition of the purified diet is shown in Table 1. Vitamin-free milk casein, L-methionine and sucrose were purchased from Wako Pure Chemical Industries Limited (Osaka, Japan). Maize oil was purchased from Nisshin OilliO Group, Limited (Tokyo, Japan). Gelatinised maize starch, the mineral mixture (AIN-93G) and the vitamin mixture (AIN-93VX) were obtained from Oriental Yeast Company, Limited (Tokyo, Japan).

Animals

Male rats of the Wistar strain, weighing 225–235 g, were obtained from CLEA Japan, Inc. (Tokyo, Japan). The rats were individually housed in a temperature-controlled room (22 ± 2°C and 50–60 % humidity) with a 12 h light–12 h dark cycle and were allowed to acclimate to the environment for 7 d before starting the experiment. Body mass, food consumption and water intake were recorded daily (± 0·1 g). We also collected 24 h urine samples every day.

Experimental procedures

A total of twenty-five rats were randomly divided into five groups. After 1 week of acclimatisation, five rats were killed by decapitation as a control group (CONT, n 5). The other rats were deprived from food for 1 d (S1, n 5), 2 d (S2, n 5), 6 d (S6, n 5) or until they had been in phase 3 for 2 d; that is, they were starved for a total duration of 6–9 d (P3, n 5). The starving phase was determined by calculating the specific daily rate of body mass loss dM/Mdt (g/kg per d) for each animal (dM represents the loss of body mass during $dt = t_1$-t_0 and M is the body mass of the rat at t_0[10,14]. Blood was taken from the tail vein at 09.00 hours every day, and 3-hydroxybutyric acid (3-HBA) concentration in the blood was measured with a 3-HBA Kit (Abbott Japan Company, Limited, Tokyo, Japan) to confirm the metabolic state of each animal because blood 3-HBA reflects fatty acid oxidation.

After the animals were killed, blood samples were collected into EDTA-2K tubes from the carotid artery and were centrifuged at 1700 ***g*** for 10 min at 4°C. Plasma glucose, TAG, urea N, aspartate aminotransferase and alanine aminotransferase were measured with FUJI DRI-CHEM (FUJIFILM Company, Tokyo, Japan).

The cerebrum, heart, lungs, stomach, kidneys, liver, spleen, testes and leg muscles were dissected and weighed (± 0·001 g). The stomach was cleared of its contents. All tissue samples were immediately homogenised in ultra-pure water at 1:10 (w/v) using a Teflon glass homogeniser and stored at −20°C until needed. The present study was conducted according to the guidelines for the care and use of laboratory animals, and was approved by the Ethics Committee of the University of Shiga Prefecture (Shiga, Japan).

Table 1. Composition of the diet

	(g/100 g)
Vitamin-free milk casein	20·0
L-Met	0·2
Gelatinised maize starch	46·9
Sucrose	23·4
Maize oil	5·0
Mineral mixture (AIN-93-G)	3·5
Vitamin mixture (AIN-93VX)*	1·0

* The composition of the vitamin mixture is described by Reeves *et al.*[13].

Analytical methods

Vitamin B_1. Thiamin in urine was measured directly. The vitamin B_1 content in the blood and tissue was determined as the sum of thiamin, thiamin monophosphate and thiamin diphosphate and was expressed as total thiamin. TCA (5 %) was added to whole blood and tissue homogenates, and the blood and homogenates were centrifuged for 5 min at 20 000 ***g***, and the supernatant of the mixture was used for measurement. Vitamin B_1 levels in the urine, blood and tissue were determined by the HPLC post-labelled fluorescence method[15].

Vitamin B_2. Riboflavin in urine was measured directly by HPLC[16]. Riboflavin, FMN and FAD in blood and tissue were converted to lumiflavin by photolysis. Briefly, the supernatant from a TCA-treated blood or tissue sample was added to an equal volume of 1 M-NaOH. The alkalised mixture was irradiated with a fluorescent lamp for 30 min, and acetic acid was added to the mixture. The neutralised mixture was filtered with a 0·45 μm microfilter and the filtrate was directly injected into the HPLC system for the measurement of lumiflavin[17]. The measured lumiflavin was expressed as total vitamin B_2.

Vitamin B_6. 4-Pyridoxic acid, a catabolite of vitamin B_6, in urine was measured directly by HPLC[18]. Serum pyridoxal and pyridoxal 5′-phosphate were determined by the HPLC method[19]. Vitamin B_6 vitamers, including phosphate esters in the tissue, were converted to free vitamin B_6 vitamers such as pyridoxal and pyridoxamine using an autoclave under acidic conditions. Briefly, the homogenate was added

to 0·06 M-HCl at 1:8 (v/v) and autoclaved at 121°C for 3 h, and the mixture was adjusted to pH 5·0 using 1 M-NaOH. These were measured as total vitamin B_6 by the microbioassay method using *Saccharomyces carlsbergensis* strain 4228 ATCC 9080[20].

Vitamin B_{12}. Urine, plasma and tissue homogenates were added to a 0·2 mM-acetate buffer (pH 4·8) with 0·0006 % potassium cyanide. These were put into a boiling water bath for 5 min to be converted to cyanocobalamin, and then 10 % metaphosphoric acid was added to be neutralised. Cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmannii* ATCC 7830[21].

Niacin. Nicotinamide[22] and its catabolites, N^1-methylnicotinamide (MNA)[23], N^1-methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py)[22], in urine were measured directly by HPLC. For measuring the total nicotinamide content in blood and tissues, the whole blood and tissue homogenates were autoclaved at 121°C for 20 min to convert the coenzymes to nicotinamide. The resulting nicotinamide was then determined by the HPLC method[22,24].

Pantothenic acid. Pantothenic acid in urine was determined by HPLC[25]. To digest the bound pantothenic acid including coenzyme A and phosphopantetheine in tissue and plasma to free form, the homogenate or blood was incubated at 37°C for 24 h. Pantothenic acid in the plasma and tissue was determined by the microbioassay method using *Lactobacillus plantarum* ATCC 8014[26].

Folate. Folate in urine and plasma was directly determined by the microbioassay method using *Lactobacillus casei* ATCC 27 773[27]. Folate in tissues was digested to monoglutamate forms by treatment with protease and conjugase. Briefly, 1 M-KH_2PO_4–K_2HPO_4 buffer (pH 6·1) was added to the tissue homogenate at 1:9 (v/v), and the homogenate was autoclaved at 121°C for 5 min. Proteinase MS (Kaken Pharmaceutical Company, Limited, Tokyo, Japan) was added to the homogenate at a final concentration of 2·5 mg/ml and then incubated at 37°C for 3 h. The reaction mixture was added to the conjugase solution (extract from porcine kidney acetone powder, Sigma, Porcine, Type II) at 30:1 (v/v) and incubated at 37°C for 12 h. After centrifugation at 10 000 ***g*** for 10 min, the supernatant was used for determination by the microbioassay.

Biotin. Bound biotin in tissues was converted to the free form using autoclave under acidic conditions. Briefly, 1·5 M-H_2SO_4 was added to the homogenate at 1:1 (v/v), and the homogenate was autoclaved for 1 h at 121°C. The suspension was centrifuged at 10 000 ***g*** for 10 min at 4°C, and the supernatant was used to measure biotin. Biotin in urine and plasma was measured directly. The biotin content in urine, plasma and tissue was determined by the microbioassay method using *L. plantarum* ATCC 8014[28].

Statistical analysis

Values are expressed as means with their standard errors. P3 rats (starved for 6–9 d) were expressed at 8 d on the graph for convenience. To test the significance of the differences in mean values among all groups, one-way ANOVA with Tukey's *post hoc* test was employed. Repeated ANOVA with Bonferroni's *post hoc* test was used to analyse urinary excretion of B-group vitamins in P3 rats, and individual data points were compared with their data at day 0. All differences at $P<0·05$ were considered to be statistically significant. Prism software (version 5; obtained from GraphPad Software, Inc., San Diego, CA, USA) was used for all analyses.

Results

Changes in body mass during starvation

Changes in body mass during starvation are shown in Table 2. Starvation for the first 24 h produced a weight loss of 7 %. From the second day to the last day of starvation, the rats lost 5 % weight for each 24 h (data not shown). The specific daily rate of body mass loss (d*M*/*M*d*t*) *v.* time in starved rats is presented in Fig. 1. The pattern of d*M*/d*Mt* showed a

Table 2. Body mass and organ mass in the control and starved rats (Mean values with their standard errors, *n* 5)

	CONT*		S1		S2		S6		P3†	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial body mass (g)	252·9	3·3	253·1	2·8	246·4	6·2	252·2	3·1	249·0	3·2
Final body mass (g)	252·9^{a}	3·3	235·1^{a,b}	2·3	219·3^{b}	5·6	182·7^{c}	2·5	166·6^{c}	5·4
Organ mass (g, wet wt)										
Cerebrum	1·29	0·02	1·30	0·01	1·28	0·01	1·27	0·02	1·23	0·03
Heart	0·84^{a}	0·04	0·87^{a}	0·03	0·81^{a}	0·04	0·66^{b}	0·02	0·58^{b}	0·02
Lungs	1·28	0·08	1·19	0·10	1·09	0·06	1·09	0·13	0·95	0·06
Stomach	1·16	0·02	1·15	0·04	1·14	0·04	1·20	0·05	1·19	0·08
Kidneys	1·94^{a}	0·05	1·89^{a,b}	0·6	1·69^{b,c}	0·03	1·53^{c,d}	0·02	1·43^{d}	0·04
Spleen	0·75^{a}	0·04	0·67^{a}	0·03	0·50^{a}	0·05	0·30^{b}	0·01	0·25^{c}	0·04
Testes	2·75^{a,b}	0·07	2·66^{b,c}	0·05	2·66^{b,c}	0·04	2·47^{b,c}	0·03	2·45^{c}	0·06
Liver	11·18^{a}	0·23	7·25^{b}	0·22	6·07^{b}	0·19	4·69^{c}	0·12	3·83^{c}	0·42

CONT, non-starved control rats; S1, 1-day starved rats; S2, 2-day starved rats; S6, 6-day starved rats; P3, starved to phase 3 rats.

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different determined by one-way ANOVA with Tukey's multiple comparison tests ($P<0·05$).

* Since the control rats were killed at the beginning of the experiment, the initial body weight was same as the final body weight.

† Phase 3 is determined by the rapid increase in d*M*/d*Mt* (refer to Fig. 1).

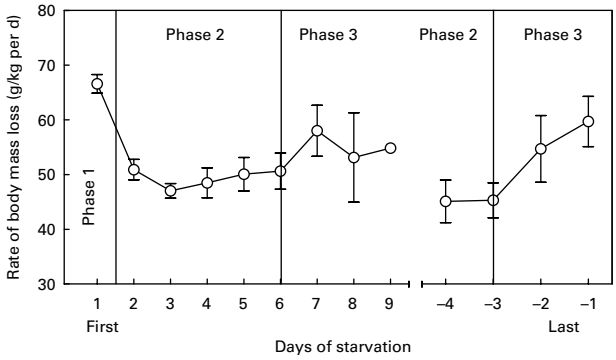

Fig. 1. Rate of body mass loss (d*M*/d*t*) in starved rats. Values are means with their standard errors (*n* 1–20 per d in the left; *n* 5 per d in the right). d*M*/d*t* (d*M* represents the loss of body mass during $dt = t_1 - t_0$ and *M* is the body mass of rat at t_0) was calculated for each animal. Abscissa: left, days counted from the beginning of starvation in all rats; right, counted to the end of starvation in the P3 group.

sharp decrease during the first hours of starvation and a steady rate at days 2–6 of starvation. Since obvious rapid increase was not observed after day 7 of starvation, we showed that the last part of starvation was counted to the end of starvation in the P3 group. The d*M*/d*Mt* in the P3 group clearly showed a rapid increase from 3 d before the end of starvation. These patterns were exactly consistent with previous reports[10,14]. Therefore, we defined the phase at first decrease as phase 1, that of steady rate as phase 2 and the third part of the curve as phase 3 according to previous reports[10,14]. The S6 group showed the steady rate of body mass loss and the low blood 3-HBA concentration, and these were characteristics of both phases 2 and 3. These results showed that the S1 group was representative of phase 1, the S2 was of phase 2, the S6 group was in the marginal range between phases 2 and 3, and the P3 group was in phase 3.

Changes in mass of individual organs during starvation

Table 2 shows the changes in the mass of individual organs during starvation. The cerebrum, lung and stomach mass was not affected by starvation. The liver weight was gradually reduced by starvation, and that in the P3 group was 30% of the control group. From 2 d of starvation, kidney mass decreased. Heart and spleen mass decreased from 6 d. Testes mass decreased in P3 rats. Prolonged starvation reduced the spleen and liver weight the most.

Blood/plasma parameters

Table 3 shows the blood parameters. Blood 3-HBA increased more in S1 and S2 rats than in control rats. In contrast, the urea concentration in plasma was significantly higher in S6 and P3 rats, whereas there was a non-significant increase in the S1 and S2 rats. Plasma glucose level was 60% significantly lower in the S1, S2 and S6 rats than in the control rats. Interestingly, plasma glucose returned to the basal level in the P3 rats. Plasma TAG was dramatically decreased after 1 d of starvation and then continued to decrease gradually throughout the remainder of the starvation period. Plasma aspartate aminotransferase was not affected by starvation. Plasma alanine aminotransferase began to increase after 6 d of starvation.

Effect of starvation on vitamin status

Table 4 shows B-group vitamin content in tissue, blood and urine in the control rats. We determined the B-group vitamin contents in nine tissues including the cerebrum, heart, lung, stomach, kidney, spleen, testis, skeletal muscle and liver, and five tissues were selected as representative variations in Fig. 2.

Cerebrum (Fig. 2(A)) and spleen. With the exception of biotin, all vitamin concentrations were unchanged by starvation. Biotin concentration was initially elevated to 150% in the S1 rats, and then returned to basal level. B-group vitamin concentrations in the spleen showed a similar pattern that starvation did not affect their concentrations except for vitamin B_2. Vitamin B_2 concentration in the testis was elevated to 130% after 6 d of starvation.

Heart (Fig. 2(B)). Vitamin B_1 and folate concentrations significantly decreased to approximately 60% after 6 d of starvation. Pantothenic acid concentration was significantly lower in the S2 and S6 rats than in the control rats. Biotin and vitamin B_6 concentrations significantly increased to 160 and 250% in the S1 and S6 rats, respectively. The other B-group vitamin concentrations were unchanged.

Table 3. Blood parameters in the control and starved rats
(Mean values with their standard errors, *n* 5)

	CONT		S1		S2		S6		P3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3-HBA (mmol/l)*	0·1[a]	0·0	1·8[a]	0·1	2·5[b]	0·2	0·7[a]	0·1	0·3[a]	0·1
Glucose (mmol/l)	6·36[a]	0·28	3·86[b]	0·32	3·84[b]	0·29	3·71[b]	0·37	6·44[a]	0·60
TAG (mmol/l)	3·01[a]	0·45	0·82[b]	0·08	0·81[b]	0·07	0·54[b]	0·10	0·46[b]	0·05
Urea (mmol/l)	7·45[a]	0·27	6·21[a]	0·42	6·21[a]	0·30	8·23[b]	2·36	13·08[b]	0·68
AST (U/l)	263	21	245	10	242	21	240	10	252	8
ALT (U/l)	39·8[a]	3·8	29·4[a]	1·9	31·8[a]	1·8	65·5[b]	10·0	73·3[b]	5·9

CONT, non-starved control rats; S1, 1-day starved rats; S2, 2-day starved rats; S6, 6-day starved rats; P3, starved to phase 3 rats; 3-HBA, 3-hydroxybutyrate; AST aspartate aminotransferase; ALT, alanine aminotransferase.
[a,b] Mean values within a row with unlike superscript letters were significantly different determined by one-way ANOVA with Tukey's multiple comparison tests ($P<0·05$).
* 3-HBA was measured in whole blood, and the others in serum.

British Journal of Nutrition

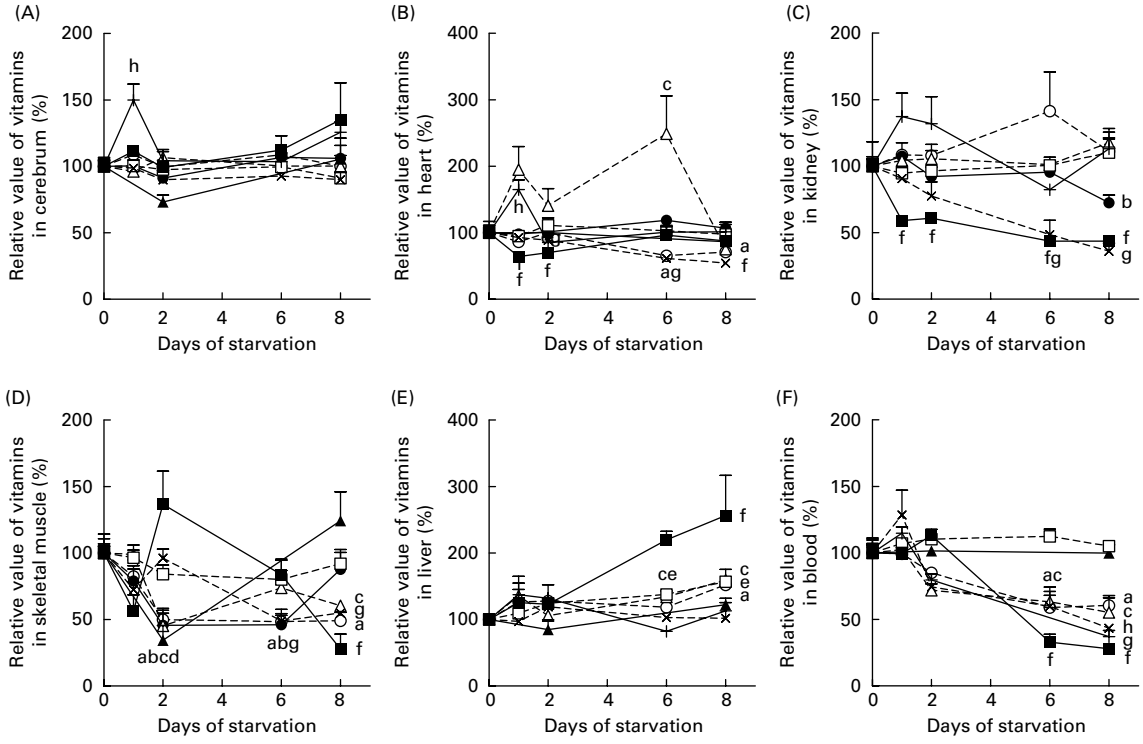

Fig. 2. Relative value of B-group vitamin concentrations in (A) cerebrum, (B) stomach, (C) kidney, (D) skeletal muscle, (E) liver and (F) blood of rats during starvation. * Sum of serum pyridoxal and pyridoxal-5′-phosphate is expressed as serum vitamin B_6. Values are reported as means with their standard errors, n 5 per d. Values of control rats are expressed as 100 %. P3 is expressed at 8 d of starvation. Means with unlike letters were significantly different from day 0 in [a]vitamin B_1 (○), [b]vitamin B_2 (●), [c]vitamin B_6* (Δ), [d]vitamin B_{12} (▲), [e]nicotinamide (□), [f]pantothenic acid (■), [g]folate (×) and [h]biotin (+; $P<0{\cdot}05$).

Kidney (Fig. 2(C)) and testis. Pantothenic acid concentration dramatically decreased to 50 % of control during starvation. Folate concentration significantly decreased to 40 % in the S6 and P3 rats. Vitamin B_2 concentration significantly decreased to 70 % in the P3 rats. The reduction in pantothenic acid concentration from day 1 of starvation was also observed in the stomach and testis, and their maximal reduction was 60 %. In the testis, vitamin B_2 concentration also significantly reduced to 50 % during starvation, and other vitamin concentrations were not changed.

Skeletal muscle (Fig. 2(D)) and stomach. Concentrations of vitamin B_1, vitamin B_2 and vitamin B_6 significantly decreased to 50 % from 2 d of starvation, but only vitamin B_2 concentration returned to control levels in the P3 rats. Vitamin B_{12} concentration significantly decreased to 40 % only in the S2 rats. Pantothenic acid concentration significantly decreased in the P3 rats. Folate concentration significantly decreased to 50 % in the S6 and P3 rats. Pantothenic acid concentration was same as control until 6 d of starvation, and then dramatically decreased to 30 % in the P3 rats. Similar pattern was observed in the stomach that starvation reduced several B-group vitamin concentrations. In brief, pantothenic acid and biotin concentrations reduced to 30 and 60 % from day 1 of starvation, respectively. Vitamin B_6 concentration significantly decreased to 50 % from day 2, and vitamin B_2 did to 40 % from day 6 of starvation.

Liver (Fig. 2(E)). Vitamin B_6 concentration was significantly higher in the S1, S6 and P3 rats, and the relative value in the P3 rats was 160 % of the control animals. Nicotinamide concentration increased in the S2, S6 and P3 rats. Vitamin B_1 and pantothenic acid concentrations were higher in the P3 rats, and their values were 150 and 250 %, respectively. Other B-group vitamins concentrations were unchanged.

Blood (Fig. 2(F)). Whole blood vitamin B_1, serum vitamin B_6 and plasma pantothenic acid concentrations decreased in the S6 and P3 rats. Plasma folate and biotin concentrations decreased in the P3 rats. The relative values of vitamin B_1 and vitamin B_6 in the P3 rats were 60 % of control, those of folate and biotin were 50 %, and those of pantothenic acid were 30 %.

Urinary contents of B-group vitamins (Fig. 3). Vitamin B_1 excretion acutely decreased to 10 % after 1 d of starvation. Urinary excretion of riboflavin, pyridoxal metabolite 4-pyridoxic acid and vitamin B_{12} gradually decreased during 4 d of starvation. Subsequently these values were stable, at approximately 20, 20 and 50 % of each control value. Urinary folate was initially unchanged in the S1 rats and then decreased to 40 % of the baseline value. Urinary pantothenic acid was increased to 170 % in 3rd and 4th days of starvation, and then returned to the control level. Although biotin excretion increased to 460 % during the first 3 d of starvation, it subsequently returned to the basal level.

Urinary contents of nicotinamide and its catabolites (Fig. 4). Nicotinamide excretion increased after 1 d of starvation and then returned to the basal level. 2-Py and 4-Py decreased after an initial increase on day 1. In contrast, MNA excretion

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Table 4. Contents of B-group vitamins in each tissue, blood and urine of control rats
(Mean values with their standard errors, n 4–5)

	Vitamin B_1 (nmol/g tissue)		Vitamin B_2 (nmol/g tissue)		Vitamin B_6 (nmol/g tissue)		Vitamin B_{12} (pmol/g tissue)		Nicotinamide (nmol/g tissue)		Pantothenic acid (nmol/g tissue)		Folate (nmol/g tissue)		Biotin (nmol/g tissue)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cerebrum	9·1	0·4	6·6	0·1	18·3	0·4	35	2	292	12	61	3	0·52	0·02	0·19	0·01
Heart	18·9	1·5	34·4	1·6	14·5	1·6	202	20	578	46	166	9	0·75	0·03	0·23	0·04
Lung	5·7	0·5	8·4	0·6	3·6	0·3	49	4	375	11	59	8	0·78	0·03	0·13	0·02
Stomach	11·7	1·7	11·5	0·3	3·4	0·6	77	12	221	22	51	5	1·14	0·02	0·27	0·01
Kidney	16·8	3·1	57·2	2·7	18·8	1·1	269	19	920	27	250	18	6·47	0·35	1·40	0·12
Spleen	7·0	0·6	6·4	0·4	1·4	0·2	74	8	645	26	33	4	1·17	0·04	0·05	0·01
Testis	25·2	6·4	9·6	0·8	13·9	0·4	61	7	186	7	101	8	0·07	0·00	0·08	0·01
Skeletal muscle	4·3	0·2	7·5	0·5	35·5	1·8	38	5	400	24	22	2	0·30	0·01	0·03	0·00
Liver	32·2	1·8	68·0	3·2	17·1	0·6	144	10	862	34	242	10	14·14	0·69	1·84	0·11
	(pmol/ml WB)		(pmol/ml WB)		(pmol/ml serum)		(pmol/ml plasma)		(nmol/ml WB)		(nmol/ml plasma)		(pmol/ml plasma)		(pmol/ml plasma)	
Blood	285	15	208	12	1·46	0·16	4·97	0·13	121	5	7·02	0·39	43·2	3·6	54·6	3·5
	(nmol/d)		(nmol/d)		(nmol/d)		(pmol/d)		(μmol/d)		(nmol/d)		(nmol/d)		(pmol/d)	
Urine	96	4	65	12	345	4	38	2	3·93	0·17	567	68	6·20	0·68	2·96	0·25

WB, whole blood.

increased during the starvation period. Urinary excretion of the sum of nicotinamide and its catabolites increased 1·4-fold after 1 d of starvation and then decreased by less than half of the food sufficient state.

Discussion

The effects of metabolic changes, which are designated as the changes in the main energy sources such as glucose, lipids and protein, during starvation on the tissue and urine vitamin concentrations are currently poorly understood[11]. Elucidation of the effects will lead to a suitable supplementation for preventing the refeeding syndrome. Therefore, we investigated the effects of short- and long-term starvation on the vitamin concentrations in organs, muscle, blood and urine in rats.

Vitamin concentrations in organs and muscle showed different patterns for each vitamin. For noticeable characteristics, biotin concentration, which means the value in terms of g tissue, was increased in most organs of the S1 rats. A part of the reason is a reduced organ mass at S1. It was unclear why the biotin concentrations in organs remained at the same level regardless of organ mass during starvation. Vitamin B_1 is the vitamin that has the most rapid turnover[30], but the levels in the kidney were maintained. This may point to the necessity of vitamin B_1 in kidneys of starving rats. In terms of the metabolic state, vitamin B_1 was expected to decrease in the early days of starvation, because glucid is the main energy source in this period[3]. However, vitamin B_1 concentrations in tissues and blood were stable in the S1 rats. This is due to the sharp decrease in liver weight and in the urinary excretion of vitamin B_1. Along with the shifts in the main energy source from glucid to fat, vitamin demands appear to change. Next to vitamin B_1, pantothenic acid requirement may be the highest because it is involved in the metabolism of fatty acids[31], and also, biotin requirement may be higher because the gluconeogenesis is more active at the deficient state of glucose[32]. However, the present results were contrary to our expectations. Pantothenic acid concentrations in the heart, stomach, kidneys and testes were decreased in the S1 rats, and a similar phenomenon was observed in biotin concentrations. The urinary excretion of pantothenic acid and biotin was significantly increased by starvation. A similar phenomenon was already reported by Fukuwatari *et al.*[30]. Shibata and co-workers reported[33–38] that the urinary excretion of water-soluble vitamins reflects recent intake of the vitamins over the last few days, and in addition, the decreased urinary excretion of vitamins means the elevated demand for vitamins, whereas the increased urinary excretion of vitamins means the reduced demand for vitamins when their intake of vitamins is almost the same[33]. The increased urinary excretion suggests that the requirement of pantothenic acid and biotin was reduced by starvation, that is, lower concentrations of pantothenic acid and biotin in the body might prefer to live for a long time during starvation. A possible inferable reason for the increase in urinary pantothenic acid and biotin might be a mechanism to prevent the stored fat in the body from overspending or to decrease the amount of acetyl-coenzyme A,

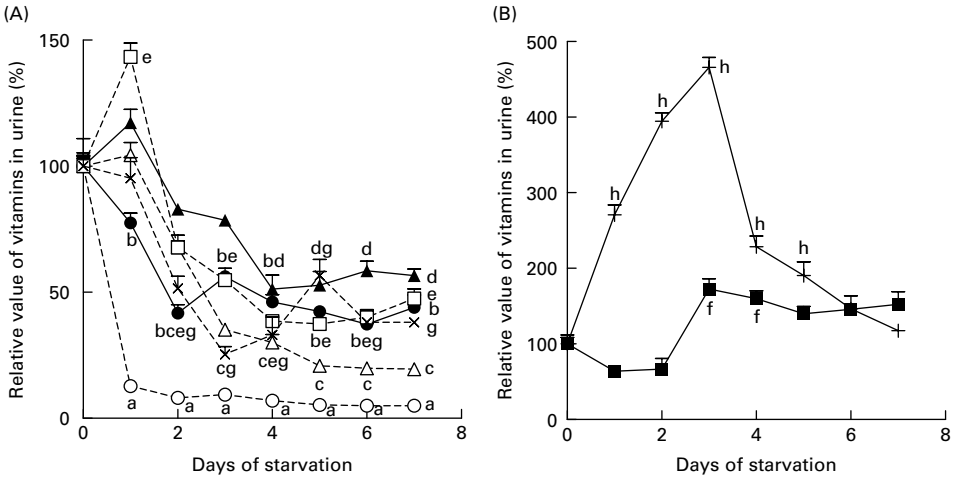

Fig. 3. Relative value of urinary B-group vitamin contents in P3 rats during starvation. Those of vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, nicotinamide and folate are shown in (A), and pantothenic acid and biotin in (B). Thiamin is expressed as vitamin B_1, riboflavin as vitamin B_2, 4-pyridoxic acid as vitamin B_6, and sum of nicotinamide and its catabolites as nicotinamide. Values are reported as means with their standard errors, *n* 5 per d. Values of control rats are expressed as 100 %. P3 is expressed at 8 d of starvation. Means with unlike letters were significantly different from day 0 in [a]vitamin B_1 (○), [b]vitamin B_2 (●), [c]vitamin B_6* (Δ), [d]vitamin B_{12} (▲), [e]nicotinamide (□), [f]pantothenic acid (■), [g]folate (×) and [h]biotin (+; $P<0{\cdot}05$).

which modifies several functional proteins such as histone[39] and some enzymes[40], and in addition, to decrease holoenzymes of carboxylases[41,42]. Acetylation generally activated some enzymes in fatty acid oxidation[40]. The physiologically active form of biotin is covalently attached at the active site of a class of important metabolic enzymes in gluconeogenesis, lipogenesis and amino acid metabolism[41,42]. Accordingly, decreased acetylation and biotin-dependent enzymes lead to reduced fatty acid oxidation and to save fat in the body.

Vitamin B_6 concentrations, expected to be the last vitamin decreased in tissues by starvation[3], decreased in the stomach, skeletal muscle and serum of the S2 rats. Vitamin B_6 in the

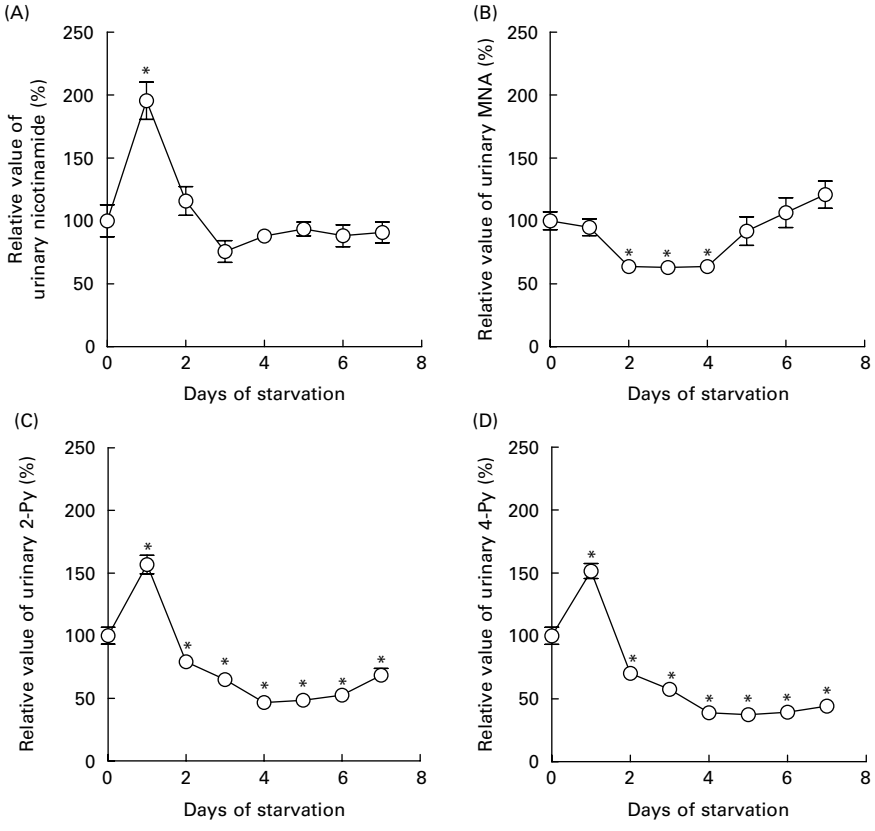

Fig. 4. Relative value of urinary nicotinamide (a) and its catabolites MNA (b), 2-Py (c) and 4-Py (d) contents in P3 rats during starvation. Values are reported as means with their standard errors, *n* 5 per d. * Mean values were significantly different from day 0 determined by one-way ANOVA with Tukey's multiple comparison tests ($P<0{\cdot}05$). MNA, N^1-methylnicotinamide; 2-Py, N^1-methyl-2-pyridone-5-carboxamide; 4-Py, N^1-methyl-4-pyridone-3-carboxamide.

lung and serum decreased in the S6 rats. The differences in the pattern of vitamin B_6 decline may be due to the fat content of each tissue. Nicotinamide concentrations in tissues and blood were unchanged by starvation, despite this vitamin being involved in energy metabolism. Since nicotinamide is biosynthesised from tryptophan[43], nicotinamide concentrations in organs and blood were maintained. Urinary excretion of the sum of nicotinamide and its catabolites was high after 1 d of starvation and subsequently decreased. These results are in agreement with those reported by a previous study[30]. The proportion of nicotinamide, MNA, 2-Py and 4-Py in urine is controlled by enzymes involved in the metabolism of tryptophan to niacin. Starvation or food restriction induces a decline in MNA oxidase activity[44] and an elevation in nicotinamide methyltransferase[44]. This may explain why levels of 2-Py and 4-Py in urine decreased while those of MNA increased. MNA is an inhibitor of nicotinamide methyltransferase[45]. Therefore, an accumulation of MNA inhibits the activity of nicotinamide methyltransferase and leads to an increase in free form of nicotinamide, which inhibits the activities of histone deacetylase[46] and poly(ADP-ribose) synthetase[47]. This control might be suitable for living long during starvation.

To our knowledge, the present study presents the first data on vitamin status during the three phases of starvation. The changes in B-group vitamin concentrations in tissues and blood did not always correspond to metabolic states. The changes in vitamin content can be divided into three groups. First, vitamin B_1, vitamin B_2, nicotinamide and biotin levels declined gradually. Second, vitamin B_6 and vitamin B_{12} levels rapidly decreased after 1 d of starvation and then remained at a steady level. Finally, pantothenic acid and folate initially decreased in the S1 rats, then returned to near basal levels in the next day of starvation, then subsequently decreased again. This might mean that pantothenic acid and folate were mobilised to other tissues. We are unsure why such complicated changes occur. It is clear that further investigation, such as separate measurement of the free forms of the vitamins and of coenzymes, into the changes in the vitamin requirements of starving rats would be useful for the prevention of vitamin deficiency during starvation or for consequent refeeding.

Acknowledgements

The present study was supported by a Research Grant for Comprehensive Research on Cardiovascular and Lifestyle Related Diseases from the Ministry of Health, Labour and Welfare of Japan. The title of the project was 'Studies on Construction of Evidence to revise the Dietary Reference Intakes for Japanese – Elucidation of balance between macronutrient and micronutrient' (Principal Investigator, K. S.). A. M. and T. F. were responsible for designing the study strategies, retrieving the references and writing the manuscript. M. S. participated in retrieving references and critical reading. K. S. directed and designed the study. The authors have no conflict of interests to report.

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NS British Journal of Nutrition

Journal of Gerontology: MEDICAL SCIENCES
Cite journal as: J Gerontol A Biol Sci Med Sci
doi:10.1093/gerona/glr174

A Significant Relationship between Plasma Vitamin C Concentration and Physical Performance among Japanese Elderly Women

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Background. Maintenance of physical performance could improve the quality of life in old age. Recent studies suggested a beneficial relationship between antioxidant vitamin (eg, vitamin C) intake and physical performance in elderly people. The purpose of this study was to examine the relationship between plasma vitamin C concentration and physical performance among Japanese community-dwelling elderly women.

Methods. This is a cross-sectional study involving elderly females residing in an urban area in Tokyo, Japan, in October 2006. We examined anthropometric measurements, physical performance, lifestyles, and plasma vitamin C concentration of participants.

Results. A total of 655 subjects who did not take supplements were analyzed. The mean age (±standard deviation) of participants was 75.7 ± 4.1 years in this study. The geometric mean (geometric standard deviation) of plasma vitamin C concentration was 8.9 (1.5) μg/mL. The plasma vitamin C concentration was positively correlated with handgrip strength, length of time standing on one leg with eyes open and walking speed, and inversely correlated with body mass index. After adjusting for the confounding factors, the quartile plasma vitamin C level was significantly correlated with the subject's handgrip strength (p for trend = .0004) and ability to stand on one leg with eyes open (p for trend = .049).

Conclusions. In community-dwelling elderly women, the concentration of plasma vitamin C related well to their muscle strength and physical performance.

Key Words: Plasma vitamin C—Physical performance—Elderly women—Japanese.

Received January 26, 2011; Accepted August 30, 2011

Decision Editor: Luigi Ferrucci, MD, PhD

PHYSICAL performance and physical ability are the most important indicators of health status in elderly people and are also closely related to the quality of life. Declines in physical performance and physical activity, whether from specific disease, fall, fracture, poor nutrition, or aging itself, are associated with future disability, morbidity, and death (1,2).

In recent years, many studies have examined the roles of diet, protein, and vitamins in physical performance and physical activity(3–5). Several studies have associated low serum albumin concentration with deteriorated muscle strength and function (6,7). Some other studies have examined the relationship between serum vitamin D level and physical performance such as muscle mass, muscle strength, handgrip, walking speed, and functional capacity (8,9). Cesari et al. (3) examined the relationship between antioxidant vitamin intake (vitamin C, vitamin E, β-carotene, and retinol) and physical performance in elderly people and showed significant positive correlations between most antioxidants, especially vitamin C, and higher skeletal muscular strength in this group of people.

There are a number of mechanistic hypotheses about the potential beneficial effects of antioxidant vitamins(10–12). Vitamin C, vitamin E, β-carotene, and retinol are important antioxidants that are not synthesized by humans and, therefore, are mainly supplied via dietary intake. Vitamin C

(ascorbic acid) is a water-soluble antioxidant present in the cytosol and extracellular fluid and can directly react with free radicals such as superoxide ($O_2 \cdot^-$) and hydroxyl radicals (·OH) (13,14). Each one of these oxygen-derived intermediates is considered highly reactive because of their unstable electron configurations, which could attract electrons from other molecules, resulting in another free radical that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid peroxidation, DNA damage, and protein degradation during oxidative stress. Oxidative damage is thought to play an important role in the age-related decline of functional activity in human skeletal muscle (15). Concentration of plasma vitamin C, which has potent antioxidant activity, is known to increase after exercise (4).

An increase in the amount of blood vitamin C content has been used as an indicator of increased oxidative reaction (11). Previous studies have examined the effects of vitamin C supplementation on physical performance and exercise (4,11). Although findings from some of the previous studies do not support any beneficial effect of increased antioxidant intake on physical performance, other studies have shown improved recovery from exercise with antioxidant intake and have also shown a preventive role of antioxidant supplementation against oxidative damage. These studies were carried out on athletes after heavy exercise. So far, however, there has been no study examining the relationship between physical performance and blood levels of vitamin C, which may be a more direct marker of the antioxidative ability of the human body.

The present study, to the best of our knowledge, is the first report that examines the relationship between plasma vitamin C concentration and physical performance in Japanese community-dwelling elderly women.

Subjects and Methods

Study Subjects

The present cross-sectional study was carried out as part of a project involving mass health examination of community-dwelling people ("Otasha-kenshin" in Japanese) aged 70 years and older living in Itabashi-ku, Tokyo. "Otasha-kenshin," which literally means "health examination for successful aging," is a comprehensive health examination program for community-dwelling older adults aimed at preventing geriatric syndromes including falls and fractures, incontinence, mild cognitive impairment, depression, and undernutrition (16).

The eligible subjects were all female residents, aged between 70 and 84 years, living in the Itabashi area, an urban part of Itabashi-ku, Tokyo, Japan in October 2006. The population of women belonging to this age range and residing in the Itabashi area was 5937, and they were recruited by invitation through postal mail. Of them, 1,112 women applied for admission and 957 women ultimately participated in this study. The participants who were taking vitamin C supplements (n = 238) were excluded from the primary analyses for examination of the relationship between plasma vitamin C and physical performance because intake of supplements could strongly influence the plasma vitamin C level. Thus, data from 655 subjects were ultimately used for the primary analysis. However, data from the 238 supplement users were also used for subanalysis to determine whether any relationship exists between vitamin C supplementation and physical performance.

All participants were examined at the Tokyo Metropolitan Institute of Gerontology's hall. Physical performance, blood examinations, lifestyle assessments, and anthropometric measurements were performed as described below (9).

The present study was approved by the ethics review committee of the Tokyo Metropolitan Institute of Gerontology. All subjects gave written informed consent.

Anthropometric Measurements

Height and weight of each participant were measured, and body mass index was defined as weight/height^2 (kg/m^2). Body composition measurements (percent body fat) were obtained by segmental bioelectrical impedance using eight tactile electrodes according to the manufacturer's instructions (In Body 3.0; Biospace, Seoul, Korea). Measurements for the triceps surae muscles were taken between the knee and the ankle, at the level of maximum circumference of the medial and anterior calf of the left leg of each participant at sitting position.

Physical Performance

Physical performance was assessed by muscle strength (handgrip strength), balance capability, and usual and maximal walking speeds, without prior practice before the actual measurements. These assessments are routinely conducted for the elderly community as described previously (9). Handgrip strength (kg) was measured once for the dominant hand with the subjects in a standing position using a Smedley's Hand Dynamometer (Yagami, Tokyo, Japan). Grip devices were calibrated with known weights. Subjects held the dynamometer at thigh level and were encouraged to exert the strongest possible force. Balance capability was measured in terms of the length of time standing on one leg, that is, we asked the subjects to look straight ahead at a dot 1 m in front of them and to stand on the preferred leg with their eyes open and hands down alongside the trunk. The time until balance was lost (or maximum 60 seconds) was recorded. We used the better of two trials in the analysis. To determine the walking speed, participants were asked to walk on a flat surface at their "usual and maximum walking speeds." Two marks were used to delineate the start and end of a 5-m path. The start mark was preceded by a 3-m approach to ensure that the participants achieved their pace of usual or maximum before entering the test path. The participants were also instructed to continue walking past the end of the 5-m path for a further 3 m to ensure that their walking pace was maintained

throughout the test path. The time taken to complete the 5-m walk was measured by an investigator and used for analysis. Walking test at maximum speed was repeated twice, and the faster speed was recorded for the test.

All physical performance tests were performed between 9 AM and 4 PM during the day. We have no data on the reproducibility of the measurements. To reduce interexaminer variation, each test was conducted by the same staff member specifically trained for this study.

Blood Examinations

Blood samples (nonfasting) were collected from the subjects between 9 am and 4 pm during the day. There was no difference in mean plasma vitamin C concentration with regard to the time of collection (data not shown). Venous blood samples were drawn into Ethylene diamine tetraacetic acid tubes. Plasma was then obtained by centrifugation at 3,000 rpm for 15 min at 4°C and subsequently used for biochemical assays. Plasma was treated with Ethylene diamine tetraacetic acid to prevent the spontaneous vitamin C degradation. Next, 100 μl of the plasma was dispensed into storage tubes, to which 450 μl of 3% metaphosphoric acid solution was added, and the mixture was stored at −80°C until further use. Vitamin C concentration was determined by an High performance liquid chromatography-electrochemical detection–based method (17). The analysis was carried out centrally in our laboratory. Serum albumin concentration was measured by the Bromocresol Green method (Special Reference Laboratories Inc., Tokyo, Japan). The coefficient of variation for serum albumin found using this method was less than 1% (9).

Lifestyle Assessment

Information regarding the participants' general health (such as medical history, smoking habits, alcohol drinking habits, regular exercise habits, vegetable intake, fruit intake and use of vitamin C supplement) was collected by interview, and history of medical conditions including hypertension, stroke, heart attack, diabetes mellitus, and hyperlipidemia was self-reported.

Alcohol drinking habits of the subjects were classified as nondrinker, current drinker, or ex-drinker. Smoking habits of the subjects were classified using three categories: never smokers, current smokers, and ex-smokers. The frequency of vegetable and fruit intake was asked using four categories: almost every day, once every two days, once or twice per week, and almost never. Subsequently, for analysis, the categories were summarized as almost every day and others.

Statistical Analysis

Data were summarized as mean and standard deviation or percentage values. The data of plasma vitamin C concentration was logarithmically transformed to approximate a normal distribution and was summarized as the geometric mean and geometric standard deviation.

Table 1. Characteristics of Study Subjects (N = 655)

Characteristic	Mean (*SD*)
Age (y)	75.7 (4.1)
Height (cm)	149.1 (5.7)
Weight (kg)	51.0 (8.3)
Body mass index (kg/m^2)	22.9 (3.4)
Triceps surae muscle (cm)	33.1 (2.8)
Plasma vitamin C (μg/ml)*	8.9 (1.5)
Serum albumin (mg/dL)	4.3 (0.2)
Body composition	
Percent body fat (%)	32.2 (7.0)
Physical performance tests	
Handgrip strength (kg)	18.7 (4.4)
One leg standing with eyes open (s)	35.2 (23.5)
Usual walking speed (m/s)	1.2 (0.3)
Maximal walking speed (m/s)	1.8 (0.4)
	%
Medical history	
Hypertension	50.7
Stroke	6.6
Heart attack	21.2
Diabetes mellitus	9.0
Hyperlipidemia	34.7
Alcohol drinking habit	
Current	25.3
Former	5.0
Never	69.6
Smoking habit	
Current	3.7
Former	5.7
Never	90.7
Regular exercise habit	
Yes	69.2
No	30.8
Vegetable intake	
Everyday	84.2
Others†	15.8
Fruit intake	
Everyday	81.8
Others†	18.2

Notes: Data of vitamin C supplement users were excluded.
*The geometric mean and geometric *SD*.
†Including participants taking vegetables/fruits not everyday or almost never.

The age-adjusted Pearson's correlation coefficient between the plasma vitamin C concentration and other factors were calculated. The least square means and SEs adjusted for potential confounders were calculated and compared between categories by analysis of covariance. To examine the relationship between plasma vitamin C concentration and physical performance, statistical adjustment was done by analysis of covariance for variables (except for other physical performance variables) that were correlated to plasma vitamin C concentration with $p < .20$. The same analyses were repeated for the 238 users of vitamin C supplement. All statistical analyses were performed using the SAS (version 9.0; SAS Institute Inc., NC).

RESULTS

Table 1 summarizes the basic characteristics of the subjects. As shown, the mean age (±standard deviation) of the

Table 2. Correlation between Plasma Vitamin C Concentration and Selected Factors (N = 655)

Factor	Correlation*	
	r	p
Age	−0.004	.91
Height	0.04	.27
Weight	−0.05	.19
Body mass index	−0.08	.054
Triceps surae muscle	0.001	.98
Serum albumin	−0.04	.33
Percent body fat	−0.12	.002
Handgrip strength	0.16	<.001
One leg standing with eyes open	0.15	<.001
Usual walking speed	0.14	<.001
Maximal walking speed	0.09	.036

Notes: Number of subjects is slightly different for the selected factors because of missing values.

*Age-adjusted Pearson's correlation coefficient between logarithm of vitamin C concentration and each factor.

subjects was 75.7 ± 4.1 years. The geometric mean (geometric standard deviation) of plasma vitamin C concentration was 8.9 (1.5) μg/mL. The prevalence of women eating vegetables everyday was 84.2% and those eating fruits everyday was 81.8%.

The age-adjusted geometric mean of plasma vitamin C concentration was significantly lower in subjects who had a medical history of hypertension (8.53 vs 9.22, p = .0015) and diabetes mellitus (7.59 vs 9.00, p = .002) as compared with those who did not. A history of stroke, heart attack, or hyperlipidemia was not associated with plasma vitamin C concentration. Subjects who took fruits every day had a significantly higher concentration of vitamin C than those who did not (9.14 vs 7.78, p < .0001). Vegetable intake, alcohol drinking habit and smoking habit were not related to plasma vitamin C concentration (not shown in table).

Table 2 shows the age-adjusted correlations between the plasma vitamin C concentration and selected factors. As shown, the plasma vitamin C concentration was positively but modestly correlated with handgrip strength, length of time standing on one leg with eyes open, as well as usual walking speed and maximal walking speed, and modestly inversely correlated with body mass index and percent body fat of the subjects.

Table 3 shows the relationship between plasma vitamin C concentration and each physical performance after adjusting for confounding factors. Results obtained after the adjustment for potential confounders confirmed that the plasma vitamin C concentration was correlated with the handgrip strength independently from the other factors (eg, p for trend = .0004 after adjusting for age, body mass index, percent body fat, hypertension, diabetes mellitus, and fruit intake; Table 3). There was also a significant relationship between the plasma vitamin C level and the subject's length of time standing on one leg with eyes open after adjustments for age, body mass index, percent body fat, hypertension, diabetes mellitus, and fruit intake (Table 3; p for trend = .049). We did not observe any significant association between the plasma vitamin C level and the usual or the maximal walking speed of the subjects.

A subanalysis using data from the 238 vitamin C supplement users showed almost null relationship between handgrip strength and plasma vitamin C concentration (data not shown).

Discussion

A previous study has shown an association between higher daily dietary intake of vitamin C and skeletal muscle strength in elderly people (3). Results described in the present study indicated that plasma vitamin C concentration was positively related with muscle and physical performance in community-dwelling elderly women. To the best of our knowledge, this is the first study showing a significant

Table 3. Relationship between Plasma Vitamin C Concentration and Physical Performance Adjusted for Potential Confounder

Physical performance	Quartile of plasma vitamin C level				
	Q1	Q2	Q3	Q4	
	Mean ± *SE*	Mean ± *SE*	Mean ± *SE*	Mean ± *SE*	p for trend
Handgrip strength (kg), *N*	154	159	154	152	
Age adjusted	17.70 ± 0.34	18.75 ± 0.33	18.75 ± 0.34	19.60 ± 0.34	.0001
Multivariate adjusted*	17.83 ± 0.34	18.83 ± 0.32	18.89 ± 0.33	19.60 ± 0.33	.0004
One leg standing with eyes open† (s), *N*	162	163	164	161	
Age adjusted	31.44 ± 1.71	33.98 ± 1.70	37.70 ± 1.70	37.83 ± 1.71	.003
Multivariate adjusted*	33.39 ± 1.74	34.08 ± 1.67	37.63 ± 1.67	37.50 ± 1.70	.049
Usual walking speed (m/s), *N*	146	154	145	147	
Age adjusted	1.13 ± 0.02	1.19 ± 0.02	1.23 ± 0.02	1.21 ± 0.02	.008
Multivariate adjusted*	1.18 ± 0.02	1.19 ± 0.02	1.22 ± 0.02	1.21 ± 0.02	.23
Maximal walking speed (m/s), *N*	146	154	154	147	
Age adjusted	1.70 ± 0.03	1.76 ± 0.03	1.82 ± 0.03	1.76 ± 0.03	.15
Multivariate adjusted*	1.76 ± 0.03	1.77 ± 0.03	1.80 ± 0.03	1.75 ± 0.03	.94

Notes: Values are least squares mean and *SE* adjusted for the factors by analysis of covariance. Q1–Q4: first to fourth quartile groups of plasma vitamin C concentration, respectively.

*Adjusted for age, body mass index, percent body fat, hypertension, diabetes mellitus and **fruit intake**.

†Length of time standing on one leg with eyes open.

correlation between plasma vitamin C concentration and handgrip strength and ability to stand on one leg with eyes open. We, however, were unable to find any relationship between skeletal muscle mass and plasma vitamin C concentration. Handgrip strength has been found to correlate well with the strength of other muscle groups and is thus a good indicator of overall strength (18). Consistent with this idea, handgrip strength was found to be a strong and consistent predictor of all-cause mortality and morbidity of Activities of Daily Living in middle-aged people (19). The handgrip test is considered an easy and inexpensive screening tool to identify elderly people at risk of disability. Handgrip strength, an indicator of overall muscle strength, is thought to predict mortality through mechanisms other than underlying disease that could cause muscle impairment (18,19). The one leg standing test is one of the balance tests (20). The test is a clinical tool to assess postural steadiness in a static position by quantitative measurement. Many studies have shown that the decreased one leg standing time is associated with declines in Activities of Daily Living and increases in other morbidities including osteoporosis and fall (20).

Our findings suggest that vitamin C may play an important role in maintaining physical performance and thereby may help to improve healthy life expectancy in the elderly. However, the usual and maximal walking speeds did not relate to plasma vitamin C concentration. Walking speed test may be an efficient tool in screening older persons with higher risk of mortality and may easily identify high-risk groups in the community (21). Walking is a rhythmic, dynamic, and aerobic activity of the large skeletal muscles that confers multifarious benefits with minimal adverse effects. Muscles of the legs, limbs, and lower trunk are strengthened, and the flexibility of their joints are preserved (22). One of the reasons why walking speed was not related to vitamin C concentration may be because walking requires coordinated movements of arms, legs, and many parts of the body rather than a simple muscle and balance function. Previous reports showed that walking balance function did not correlate with standing balance function (23). Although we did not find any clear association between walking and plasma vitamin C concentration in this study, vitamin C may still have effects on relatively simple strength and balance functions.

One of the possible explanations for the observed relationship between vitamin C and physical performance, especially handgrip strength and the ability to stand on one leg with eyes open, may be the potential protective effects of the antioxidant vitamins against muscle damage (4,11). Vitamin C is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not in humans (12). Vitamin C is an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized (12). Thus, vitamin C readily scavenges reactive oxygen and nitrogen species, thereby effectively protects other substrates from oxidative damage (10,24). Although habitual exercise reduces systemic inflammation and oxidative stress as the production of endogenous antioxidants are enhanced, acute exercise increases the generation of oxygen-free radicals and lipid peroxidation (4,25). Strenuous physical performance can increase oxygen consumption by 10- to 15-folds over the resting state to meet the energy demands and results in muscle injury (26). Prolonged submaximal exercise was shown to increase the amount of both whole-body and skeletal muscle lipid peroxidation by-products; in the case of the former, the increase was indicated by greater exhalation of pentane but not of ethane (4,27,28). Supplementation with vitamin C was shown to decrease the exercise-induced increase in the rate of lipid peroxidation (27,28). Several studies suggested that oxidative damage may play a crucial role in the decline of functional activity in human skeletal muscle with normal aging (15). Consistent with this idea, several studies showed significantly lower plasma vitamin C level in the elderly population than in the younger adult population (29–31). Because the plasma vitamin C levels in these apparently healthy elderly persons rose markedly after an oral dose of vitamin C, their initially low plasma levels can be attributed to the low intake rather than to an age-related physiological defect.

In fact, the relationship between handgrip strength and plasma vitamin C concentration was significantly different between supplement users and nonusers, that is, an almost null relationship in the former and a positive relationship in the latter (data not shown). This finding suggested that vitamin C supplementation did not have any beneficial effect on the physical performance and muscle strength despite the increased plasma level of vitamin C. A number of studies reported that vitamin C supplement users had significantly higher blood vitamin C concentration than non-users (29, 32, 33). Several studies have examined the effects of exercise on changes in the serum vitamin C concentration (34–36). Some other experimental studies have shown that vitamin C supplementation can reduce symptoms or indicators of exercise-induced oxidative stress (37–40). However, the results regarding vitamin C supplementation are equivocal, and most well-controlled intervention studies report no beneficial effect of vitamin C supplementation on either endurance or strength performance (41,42). Likewise, vitamin C restriction studies showed that a marginal vitamin C deficiency did not affect the physical performance (43). Although evidence from a number of studies show that vitamin C is a powerful antioxidant in biological systems in vitro, its antioxidant role in humans has not been supported by currently available clinical studies.

Vitamin C is especially plentiful in fresh fruits and vegetables. Plasma vitamin C concentration may be merely a marker for intake of other nutrients that are abundant in fruits and vegetables. However, the statistical adjustment for fruit intake did not attenuate the relationship between plasma vitamin C and physical performance (Table 3), suggesting that vitamin C did have some beneficial effects

independently of other nutrients. A number of biochemical, clinical, and observational epidemiologic studies have indicated that diets rich in fruits, vegetables, and vitamin C may be of benefit for the prevention of chronic diseases such as cardiovascular disease and cancer (44,45). Several cohort studies have examined associations between plasma vitamin C concentration and mortality from stroke or coronary heart disease (30,46,47). The effects of vitamin C supplementation are, however, still unclear. A pooled study suggested reduced incidences of coronary heart disease events with higher intake of vitamin C supplement (48), while another study showed that a high intake of vitamin C supplement is associated with an increased risk of mortality due to cardiovascular diseases in postmenopausal women with diabetes (49). A randomized placebo controlled 5-year trial, however, did not show any significant reduction in the mortality from, or incidence of, any type of vascular disease or cancer (50). These studies, in fact, have failed to demonstrate any benefit from such supplementation.

There are a number of potential weaknesses in our study that should be mentioned here. The subjects used in this study were not selected randomly from the study population, and they may be relatively healthy elderly women who were able to come to the health examination hall from their homes. A previous study assessed the correlation of antioxidants with physical performance and muscular strength (3) and demonstrated that a higher daily intake of vitamin C and carotene associated with skeletal muscle strength. However, we have no data regarding the presence of other dietary antioxidants in blood such as vitamin E, retinol, and carotene. In our questionnaire, participants were asked to respond "Yes" or "No" to whether they took supplements, and not about the frequency and quantity of intake of the supplements. Thus, we were unable to examine the reason why plasma vitamin C was not related to the handgrip strength in the supplement users by considering the dose of vitamin C they took.

This study was a cross-sectional study and, therefore, does not provide cause/effect relationships, although we demonstrated a significant correlation between physical performance and concentration of plasma vitamin C. Therefore, longitudinal follow-up studies and controlled clinical trials are necessary to confirm the role of plasma vitamin C and physical performance of the elderly women. These limitations should be considered in future studies.

In conclusion, we found a strong correlation of a higher plasma vitamin C concentration with handgrip strength and one leg standing time in community-dwelling elderly women. Although the elderly are prone to vitamin C deficiency, and they appear to have a higher dietary requirement for vitamin C, the beneficial effects of vitamin C supplementation to maintain physical performance in elderly people are equivocal and thus, need further in-depth studies.

Funding

Ministry of Education, Culture, Sports, Science and Technology, Japan (grant-in-aid 16390187 for Scientific Research [B] and grant-in-aid [21790591] for Young Scientists [B]).

Acknowledgment

We offer special thanks to Dr. Satoshi Sasaki for his suggestions and useful comments to this paper; we are indebted to Dr. Shoji Sinkai, Dr. Yoshinori Fujiwara, Mr Toshiro Tango, Mrs Sachiko Imai, Mrs Turuko Maekawa, Miss Erika Hosoi and the staff of the Otasha-kenshin who participated in this study.

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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]

$=387-7.31\times$age [y]+physical activity coefficient [1.00 for sedentary, 1.14 for low active, 1.27 for active, and 1.45 for very active]$\times$(10.9$\times$body weight [kg]+660.7$\times$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 hody 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.

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Japanese Clinical Medicine

ORIGINAL RESEARCH

Improvement of Quality of Life (QOL) in Osteoporotic Patients by Elcatonin Treatment: A Trial Taking the Participants' Preference into Account

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Abstract: Osteoporosis is associated with compromised quality of life (QOL), to which pain has the most important contribution. Elcatonin, a derivative of calcitonin, is widely used in the treatment of osteoporosis in two ways. One is as the inhibitor of osteoclastic bone resorption. The other is for osteoporosis-related pain based on the unique analgesic effects of elcatonin. Since pain is subjective in nature, and QOL is the only clinical outcome representing the patients' subjective perception of health status, pain associated with osteoporosis would be best evaluated based on QOL assessment. Evidence based medicine gives the highest remarks to the double-blinded, randomized controlled trial, which, however, cannot be free from methodological problems on some occasions. For example, it is practically impossible to remain blinded in the trial of a potent analgesia, which in turn causes biases. Thus, the significance of taking the patients' preference into account is increasingly acknowledged. In this study, 45 osteoporotic patients were given brochures describing the pros and cons on the three treatment choices; calcium and alfacalcidol, additional use of elcatonin, and additional use of bisphosphonate. Those who favored elcatonin were older, had more vertebral fractures, and lower QOL scores. QOL was evaluated before and three months after the treatment using SF-8; the most widely used generic questionnaire, and RDQ; a lumbago-specific measure. Elcatonin treatment improved physical function, general health, and vitality of SF-8, and RDQ score. Although this is a preliminary study, our results suggest that patients with vertebral fracture(s) have impaired QOL and more likely to favor elcatonin treatment expecting analgesia.

Keywords: osteoporosis, elcatonin, patient preference trial, quality of life

Japanese Clinical Medicine 2012:2 9–14

doi: 10.4137/JCM.S8291

This article is available from http://www.la-press.com.

Introduction

Calcitonin is a hormone secreted from the parafollicular cells of the thyroid gland. It is involved in calcium homeostasis by inhibiting osteoclast-mediated bone resorption and decreasing serum calcium levels. It is clinically used in various clinical conditions. For example, it is prescribed to osteoporotic patients,[1–3] especially those with enhanced bone resorption. Additionally, it is a therapeutic drug in the treatment of malignancy associated hypercalcemia.[4]

Elcatonin is a derivative of calcitonin[5] with potent analgesic action.[6] Therefore, it is clinically administered to osteoporotic patients with pain.[7,8] Japanese guidelines for the prevention and treatment of osteoporosis have given grade A, the highest possible mark, to the analgesic action of elcatonin.[9]

Of the various measurement scales clinically available, QOL (Quality of Life) is the only clinical outcome representing the patients' subjective perception of health.[10] Pain is subjective in its nature, and could be evaluated only by subjective index: QOL. It follows that the analgesic effect of elcatonin would be best evaluated with QOL as the outcome. A theoretical problem arises, however, if one attempts to evaluate the analgesic effects of a certain drug based on QOL scores.

Recently, the concept of evidence based medicine (EBM) has been advocated in which, a hierarchy of evidence exists, with double-blinded randomized controlled trial (hereafter in this paper, abbreviated as DB-RCT) given the highest remark. DB-RCT is considered to be least affected by various biases. In some types of trials, however, double-blindness or randomization cannot be strictly guaranteed with DB-RCT, and even DB-RCT cannot be free from such biases. As will be detailed in the "Discussion, some forms of bias" to skew the results is known to take place in studies in which blindness or randomization is disrupted.[11,12] Thus in this paper, we have attempted to study the effects of elcatonin treatment on the QOL of osteoporotic patients taking the patients' preference into account.

Subjects and Methods

Subjects

Forty-five (42 females, 3 males) osteoporotic patients with back pain or lumbago were encouraged to participate in the study. The diagnosis of osteoporosis was made based on the diagnostic criteria for primary osteoporosis in Japan.[13] The purpose of the study was explained, and written consent was obtained. Ethical approval was obtained for the use of humans in this research

The exclusion criteria were as follows. Patients receiving bone-active drugs were excluded unless they were drug free for 48 weeks for bisphosphonates and for 8 weeks for other drugs. Patients under sustained analgesic treatment were also excluded unless the analgesics were halted for at least 2 weeks. Patients were also excluded when the attending physician made a judgment that the patient was not eligible for entry based on the clinical condition.

Vertebral fractures were diagnosed based on a plain roentgenogram. The diagnosis of compression fracture was made by one of the authors (KY) before obtaining the information on QOL.

Intervention Protocol

The study design was an open-labeled one based on the patients' preference, further details of which will be described below. There were three treatment groups; group (1): calcium lactate 1 g or calcium aspartate and 1 μg of alacalcidol daily, group (2): once-weekly injection of elcatonin in addition to the regimen in group (1), and group (3): alendronate 5 mg daily or risedronate 2.5 mg daily in addition to the regimen in group (1). Patients were given brochures describing the pros and cons of each treatment, and asked to select the treatment of their preference. Six, twenty-seven, and twelve patients chose group (1), group (2), and group (3) treatment, respectively.

QOL (Quality of Life) Evaluation

QOL was evaluated with two questionnaires; SF-8 and RDQ (Roland Morris Disability Questionnaire). SF-36 is a generic (non-disease specific) QOL questionnaire, and one of the most widely used worldwide. SF-8 is an abridged edition of SF-36. According to the authorized instruction, the data were transformed to the deviation value adjusted by Japanese national norms.[14,15] Eight subscales were obtained: PF (physical function), RP (role physical), BP (bodily pain), GH (general health), VT (vitality), SF (social function), RE (role emotional), and MH (mental health). These scores are further summarized into two summary scores: PCS (physical component summary) and MCS (mental component summary). These subscales

and summary scores are interpreted as follows: 50 corresponding to the national norms, and 40 indicating one standard deviation lower than the norm.

RDQ is a questionnaire specifically targeted to lumbago. It is composed of 24 questions. The subjects are asked to give "yes" or "no" to each question. Total number of questions given "yes" is calculated. Thus higher number is associated with more severe lumbago.[16,17]

QOL evaluation was made before and three months after initiating the intervention.

Statistical Analyses

Data were analyzed using SPSS 17.0J for Windows. Comparison of three independent groups was done with one-way analysis of variance (ANOVA) followed by Tukey's test as the post-test. Contingent tables were analyzed by the chi-square test. Statistical significance was judged based on $P < 0.05$.

Results

Table 1 shows patients characteristics. Patients in group (2) were significantly older than those in other groups, but there was no significant difference in their height or weight. All patients in group (1) and most in group (3) had no vertebral fractures. In contrast, most patients in group (2) had vertebral fracture(s). These differences were statistically significant based on chi-square test.

Baseline QOL scores are shown in Table 2. There was a significant difference between the three groups in four of the eight subscales of SF-8; RP, BP, GH, and VT and total scores of RDQ. Patients in group (2) had worse QOL scores. Although not statistically significant, those in group (2) had worse QOL scores in practically all scales.

In Table 3 is shown the post-intervention changes in the QOL scores. Elcatonin treatment markedly improved in several subscales of QOL score.

Discussion

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture including vertebral, hip and wrist fractures.[18] Hip fracture is associated with high mortality and markedly impaired activity of daily living (ADL). It is not only a tragedy for the elderly individual, but also a great burden to society.[19] Vertebral fracture is the most prevalent osteoporosis-related fracture.[20] Recent studies have clarified that it is a fracture of great clinical importance associated with increased mortality, co-morbidity, and compromised ADL.[21–23] Nevertheless, vertebral fracture has not received much attention until recently. One of the reasons for the ignorance of the importance of vertebral fracture is that approximately two-thirds of patients are without overt clinical signs. Its lack, however, does not necessarily mean that the patients are symptom free or subjectively well. Since QOL is the only index representing the patients' subjective status, QOL would be quite suitable in the evaluation of patients with vertebral fracture.

Recently, many questionnaires for QOL evaluation have become available. They are classified into two major categories; generic and disease-targeted. Generic ones, by their definition, only consist of questions related to the subjects' general status, and

Table 1. Baseline characteristics of participants in three treatment groups.

	Group (1)	Group (2)	Group (3)	*P*	
N	6	27	12		
Age	72.3 ± 9.8	77.6 ± 7.0*	69.4 ± 9.8*	0.017	(2–3)
Height	144.2 ± 6.5	147.7 ± 7.9	149.2 ± 6.6	0.536	
Weight	49.2 ± 6.0	50.7 ± 7.3	47.3 ± 6.8	0.458	
Vertebral fracture number					
0	5 (+2.7)	5 (–4.2)	8 (+2.6)	0.001	
1	0 (–1.4)	10 (+2.8)	0 (–2.1)		
≥2	0 (–1.6)	11 (+1.9)	2 (–0.9)		

Notes: The treatment groups were as follows; group (1): calcium and alfacalcidol, group (2) elcatonin, group (3): bisphosphonate. Age, height, and weight were compared by ANOVA followed by Tukey's test. Vertebral fracture number in each group was analyzed by chi-square test. Figures in the parentheses represent the adjusted residual; those above +1.96 denotes that more subjects were distributed in the cell than expected, and those below –1.96 less subjects than expected.

Table 2. QOL scores at entry.

	Group (1)	Group (2)	Group (3)	Total	*P*	
PF	41.4 ± 13.9	37.4 ± 8.2	43.6 ± 3.9	39.6 ± 8.8	0.123	
RP	42.0 ± 12.4	36.4 ± 10.1	45.4 ± 6.1	39.6 ± 10.2	0.037	(2–3)
BP	47.2 ± 7.7	36.8 ± 7.5	42.3 ± 6.1	39.8 ± 8.1	0.003	(1–2)
GH	47.8 ± 7.7	40.4 ± 7.9	47.9 ± 5.9	43.5 ± 8.1	0.009	(2–3)
VT	50.7 ± 9.9	42.9 ± 7.3	48.1 ± 5.3	45.4 ± 7.9	0.023	(1–2)
SF	48.5 ± 12.8	41.1 ± 11.6	48.1 ± 5.6	44.0 ± 11.0	0.107	
RE	43.1 ± 15.0	38.0 ± 14.0	48.7 ± 6.9	41.5 ± 13.3	0.077	
MH	49.8 ± 8.5	43.9 ± 9.6	50.9 ± 7.5	46.6 ± 9.4	0.063	
PCS	41.9 ± 11.3	34.9 ± 7.6	41.2 ± 5.3	37.7 ± 8.3	0.032	
MCS	49.6 ± 10.1	44.0 ± 12.4	51.8 ± 7.0	46.9 ± 11.3	0.127	
RDQ	6.3 ± 5.7	14.0 ± 5.8	9.5 ± 4.4	11.7 ± 6.1	0.003	(1–2)

Notes: The treatment groups were as follows; group (1): calcium and alfacalcidol, group (2) elcatonin, group (3): bisphosphonate. Age, height, and weight were compared by ANOVA. The results from post-test by Tukey's test are shown in the parentheses. For example, (2–3) indicates the statistically significant difference between groups (2) and (3).

do not include questions related to the features which are specific to a certain disease. Therefore, they are applicable to such studies as comparing the impact of various diseases on QOL, or even to the evaluation of healthy subjects. In contrast, disease-targeted ones include items specific to a certain disease. They can be more sensitive than the generic ones in detecting the QOL impairment closely related to a certain disease state, but are not applicable to the evaluation of patients with other diseases. The most widely used generic QOL questionnaire is SF-36 and its abridged form, SF-8.[14]

Roland-Morris Disability Questionnaire (RDQ) is rather different from the above-mentioned questionnaires, and unique in that it is specific to lumbago.[16]

We have recently shown that patients with vertebral fracture(s) had compromised quality of life (QOL) and elcatonin treatment remarkably improved it.[7] In the course of the study, we have noticed a challenging problem. QOL evaluation could be done by giving the questionnaire to the subjects and asking them to mail it back. In this study, however, the patients' QOL was evaluated by the interviewers considering that most subjects are elderly osteoporotic patients. The attending physician only obtained the consent to participate in the study without participating in the interview. The interviewers were blinded about the treatment regimen for each patient to minimize bias.

Table 3. Incremental QOL scores after the intervention.

	Group (1)	Group (2)	Group (3)	*P*	
PF	–9.3 ± 17.4	5.3 ± 10.7	2.8 ± 6.9	0.043	(1–2)
RP	3.4 ± 5.1	4.8 ± 11.9	3.0 ± 7.6	0.912	
BP	–1.6 ± 7.2	9.6 ± 11.2	7.2 ± 7.5	0.098	
GH	–3.8 ± 5.3	7.4 ± 8.9	1.4 ± 8.1	0.021	(1–2)
VT	–6.6 ± 6.4	6.5 ± 8.4	2.1 ± 3.7	0.004	(1–2)
SF	–6.9 ± 6.7	3.1 ± 12.3	–4.9 ± 10.4	0.102	
RE	–10.0 ± 14.8	6.6 ± 15.8	3.0 ± 6.9	0.079	
MH	–3.8 ± 5.5	5.7 ± 11.3	2.6 ± 10.9	0.197	
PCS	–1.7 ± 5.1	6.6 ± 10.7	4.0 ± 7.5	0.221	
MCS	–7.8 ± 10.4	4.4 ± 14.0	–0.4 ± 10.1	0.161	
RDQ	0.4 ± 2.3	–5.6 ± 5.7	–2.7 ± 1.8	0.042	(1–2)

Notes: The treatment groups were as follows; group (1): calcium and alfacalcidol, group (2) elcatonin, group (3): bisphosphonate. Age, height, and weight were compared by ANOVA. The results from post-test by Tukey's test are shown in the parentheses. For example, (1–2) indicates the statistically significant difference between groups (1) and (2).

Nevertheless, the blindness could not be maintained, since elcatonin treatment markedly relieved pain in some patients, whereas such marked improvement occurred in none of the subjects in the control groups.

Recently, such phenomena have been reported to be a challenging problem to DB-RCT, and the importance of patients' preference has been recognized.[11,24,25] Clinical studies are performed based on the assumption that subjects both in intervention and control arms are randomly sampled from a single population. In RCT, patients are conceptualized as relatively passive recipients of intervention. In reality however patients are far from passive, and in fact are quite active participants in the research. Therefore, patients often have preferences for a certain intervention, and will prefer one over the others where they are given the opportunity to choose.

In RCT, the possible interference is minimized by blinding. In some cases, however, participants know which intervention they have received. Then it is possible that patients not allocated to the intervention of their choice have lower compliance to the therapy, which will impair the validity of the study. Several theoretical frameworks have been developed to take the patients' preference into account.[24]

In this paper, we have attempted to study the analgesic effects of elcatonin considering the participants' preference. When the patients were given brochures describing the benefit and possible side effects of each treatment, and asked which treatment regimen they would like to receive, those who preferred elcatonin were significantly older and more likely to have vertebral fracture(s). Those in the calcitonin group had lower SF-8 scores and higher RDQ scores, indicating more compromised QOL. Thus subjects with vertebral fracture(s) have impaired QOL, and are more likely to prefer elcatonin treatment expecting its analgesic effects.

Elcatonin treatment resulted in more pronounced improvement in the subjects' QOL scores, although statistically significant in only some of the scores probably due to the limited number of patients studied.

This is a preliminary study to identify the analgesic effects of elcatonin taking the patients' preference into account. Despite the limitations described above, patients with vertebral fracture(s) have compromised QOL, and are more likely to prefer elcatonin treatment, and more likely to be benefited by its treatment due to its potent analgesic effects.

Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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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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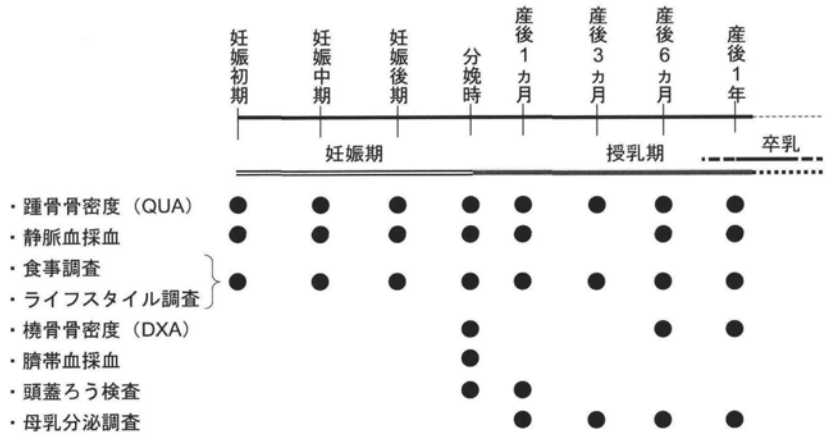

図 1　SKY（Saitama, Kobe, Yokohama Pregnant Cohort）Study のデザイン

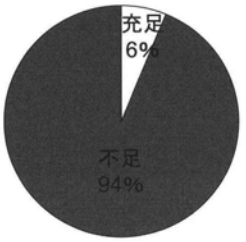

図 2　食物摂取頻度調査におけるカルシウム不足者の割合

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。

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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。

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（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

Trace Nutrients Research 29 : 67－71 (2012)

原　著

兵庫県の都市部在住の乳幼児に対する自家製離乳食のミネラル含有量の評価

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Evaluation of mineral contents in homemade baby foods prepared for infants and toddlers living in an urban area of Hyôgo Prefecture

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Summary

Twenty-five duplicate diet samples of homemade baby foods prepared for infants and toddlers aged 8 to 16 months were collected from their mothers living in an urban area of Hyôgo Prefecture in Japan and their mineral (sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, manganese, iodine, selenium, chromium and molybdenum) contents were determined. Mineral contents obtained were expressed as values per 1000 kcal and compared with the adequate intake (AI) for infants aged 6 to 11 months or the estimated average requirement (EAR) for toddlers aged 1 to 2 years described in Dietary Reference Intakes for Japanese, 2010 (DRI-J).

All mineral contents in the homemade baby foods were almost met the reference values in DRI-J. In particular, contents of magnesium, phosphorus, manganese, selenium, chromium and molybdenum in baby foods prepared for 6 to 11 months babies were markedly higher than the AI. Iodine contents in the baby foods were remarkably varied from near 0 to more than 1000 μg/1000 kcal. These results indicate that 1) mineral contents in the homemade baby foods collected fell within the suitable range, 2) intakes of magnesium, phosphorus, manganese, selenium, chromium and molybdenum increase with the progress of weaning, and 3) an intermittent high iodine intake is important to satisfy the iodine requirement in infants and toddlers.

わが国の食事摂取基準では，6か月未満乳児に対して，母乳中のミネラル含有量と哺乳量の積にもとづいてミネラルの目安量を設定している[1]。一方，6か月以降乳児に対しては，6か月未満乳児に対する目安量を体重比の0.75乗で外挿することによって目安量を設定している。この設定法はミネラル以外の栄養素も同様である。つまり6か月以降乳児の目安量は生後1年間母乳のみを摂取する場合を想定したものといえる。

しかし，現実には多くの乳児が生後6か月以降に離乳食を摂取しており，現行の目安量がこの時期の乳児の栄養素摂取量を反映しているかは不明である。たとえば，離乳食と母乳との間で含有量が大きく異なる栄養素では，離乳食の導入に伴ってその摂取状況が大きく変化するため，目安量と現実の摂取量との間に大きな差が生じることになる。目安量は栄養素不足のリスクを予防するのに十二分な摂取量と考えられるので，現実の摂取量が目安量を下回ったとしてもただちに問題になるわけではないが，目安量の数値がフォローアップミルクなどの調製に参照されていることから，この時期の乳児の栄養素摂取量を調べることは必要といえよう。離乳食からの栄養素摂取量を調べた大規模な研究として中埜らの調査がある[2]。しかしこの調査は栄養素摂取量を五訂食品成分表から算定しているため，ヨウ素，セレン，クロム，モリブデンは対象とされていない。またその他のミネラル類に関しても調理に伴う損耗が指摘されていることから[3,4]，実測によって離乳食からのミネラル摂取量の推定を行うことが必要と考える。

本研究では，兵庫県下の子育て支援施設を通して母親手作りの離乳食を収集し，そのミネラル含有量を測定することにより，離乳食からのミネラル摂取量の推定と評価を行った。

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実験方法

1. 試料の収集

兵庫県阪神地域にある子育て支援施設の協力のもと，同施設を利用し，調査の趣旨を十分理解した母親25名（年齢30.4±3.8歳）から手作り離乳食1日分を収集した。対象とした乳幼児の月齢は8から16か月（平均±標準偏差：10.9±2.6月），男女の内訳は，男児14名，女児11名である。収集した離乳食は1日分すべてを凍結乾燥後，ミルで均一に粉砕した。

2. 主要栄養素の分析

たんぱく質濃度は，含有窒素をケルダール法で分析し，窒素係数6.25を用いて算出した。水分は105℃恒量法で測定した。灰分は550℃での乾式灰化法で測定した。脂質はジエチルエーテルを用いたソックスレー抽出法により測定した。たんぱく質，脂質，水分，灰分以外の成分は炭水化物とみなした。エネルギー量はアトウォータのエネルギー換算係数を用いて算定した。

3. ミネラル類の分析[5]

乾燥試料約1gを硝酸10mlと過塩素酸2mlを用いて灰化し，蒸留水で10mlにメスアップした。調製した試料溶液中の鉄，亜鉛，銅，マンガンを原子吸光光度計，セレンとモリブデンを誘導結合プラズマ質量分析器(ICPMS)，リンをバナドモリブデン酸吸光光度法で定量した。

乾燥試料約500mgを電気炉中550℃で一晩灰化し，得られた残渣を0.1M硝酸5mlに溶解した。この試料溶液中のクロムをICPMSで定量した。

乾燥試料約400mgに0.1M塩酸50mlを加え，十分に振とうして含有されるナトリウム，カリウム，カルシウム，マグネシウムを抽出し，適宜希釈後，原子吸光光度計を用いて定量した。

乾燥試料約200mgに0.5%テトラメチルアンモニウムヒドロキシドを40ml加え，室温で一晩放置した。さらに，60℃に5時間放置後，遠心と濾過を行い得られた抽出液中のヨウ素をICPMSで定量した。

なおICPMS分析においては，内部標準元素として，ヨウ素とセレンにはテルル，モリブデンとクロムにはロジウムを用いた。

結果と考察

Table 1に，収集した1日分の離乳食から摂取されるエネルギー，たんぱく質，および脂質量をまとめ，中埜らが行った全国調査の平均値[2]と比較して示した。エネルギー，たんぱく質ともに月齢の増加とともに離乳食からの摂取量は増加した。エネルギーとたんぱく質の摂取量を中埜らの全国調査と比較した場合，12か月未満の対象者において明らかに低い値だった。このことは今回対象とした乳児の離乳が全国平均よりもやや遅れていることを示している。

脂質エネルギー比は全対象者において，全国平均よりかなり低かった。つまり，今回収集した離乳食は，そのほとんどがかなりの低脂質食だったといえる。このような低脂質の離乳食が家庭で調製されているのは，世間一般に低脂質であることが健康の維持・増進にとって好ましい食事というイメージが浸透しているためと思われる。低脂質食は成人におけるメタボリックシンドローム対策において奨励されるものであり，成長期のように体重あたりのエネルギー必要量が高い場合に推奨すべきものではない。したがって，母親に対しては，乳幼児期には適度の脂質摂取が望ましいことを伝える必要があると思われる。

Table 2は，収集した離乳食に関して，エネルギー1000 kcalあたりのたんぱく質とミネラルの含有量を8～11か月児と12～16か月児に分けて示したものである。脂質エネルギー比は月齢の高い対象者が高い値だったが，たんぱく質とミネラル含有量に関しては，セレンを除いて月齢による差を認めなかった。

今回の調査では，離乳食以外の母乳や調製乳の摂取量を正確に調べていないため，栄養素等の1日総摂取量を算定できない。そこで，離乳食のエネルギーあたりミネラル含有量をTable 2に併記したエネルギー1000 kcalあたりに換算した食事摂取基準の数値と比較することにより評価を試みることにした。

Table 1 Estimated intake of energy, protein and lipid from baby foods in subjects

Age (month)	Sex	Energy[a] (kcal/d)	Protein[a] (g/d)	Lipid[a] (energy %)	Mean values in a nationwide survey[b]		
					Energy (kcal)	Protein (g)	Lipid (energy %)
8	Male (n=6)	129 ± 59	5.9 ± 4.4	8.4 ± 4.5	252	9.5	18.7
	Female (n=1)	93	4.6	13.2		(n=197)	
9-11	Male (n=4)	321 ± 80	12.6 ± 2.8	7.9 ± 3.6	450	17.0	21.0
	Female (n=5)	231 ± 85	7.4 ± 4.0	8.0 ± 5.7		(n=576)	
12-16	Male (n=4)	606 ± 219	22.5 ± 7.9	13.5 ± 6.9	704	24.8	22.4
	Female (n=5)	623 ± 168	21.2 ± 5.0	14.2 ± 3.1		(n=568)	

[a] Values are means ± SD.
[b] Values are quoted from the report by Nakano et al.[2]; values of infants aged 9-11 months are mean of 9, 10 and 11 months; values of toddlers aged 12-16 months are mean of 12, 13-14, and 15-16 months.

6～11か月児に関して，食事摂取基準の数値よりも明らかに大きな数値を与えたのは，マグネシウム，リン，マンガン，セレン，クロム，モリブデンだった。食事摂取基準における6～11か月児の目安量の多くは母乳からの摂取を前提としていることから，算定値が目安量よりも大きいことは，離乳食の導入によって摂取が増加することを意味する。つまり，これらのミネラルは離乳食中濃度が母乳中濃度よりも高いため，離乳を進めることによって摂取量が増加するといえる。なお，算定値が目安量よりも小さければ離乳食導入によって摂取が低下することを意味するが，そのようなミネラルは存在しなかった。ところで，鉄はミネラルの中で唯一6～11か月児に対して目安量ではなく推定平均必要量・推奨量が示されている。先にわれわれは，市販離乳食の鉄濃度がきわめて低いことから，フォローアップミルクを利用しないかぎり，この基準を充足させることは難しいことを指摘した[6]。しかし，今回収集した離乳食の平均鉄濃度は推定平均必要量にほぼ一致しており，この時期の鉄需要を充足しうるものであった。他のミネラル濃度の平均値はいずれも食事摂取基準の目安量を上回っていたことから，今回収集した6から11か月児の離乳食のミネラル含量は適正なものと判断できる。

12～16か月児に関しても，カルシウムとリンを除いて，含有量の平均値は食事摂取基準の数値を超えていた。また，食塩の目標量である4g/日未満もほぼすべての離乳食が達成できていた。カルシウムとリンに関しても基準値との差がわずかであることを考えると，今回収集した12から16か月児の離乳食もそのミネラルの含有量はほぼバランスのとれたものといえる。

わが国の食事摂取基準では，セレンとヨウ素は乳幼児に対しても耐容上限量を設定している[1]。これらの耐容上限量をエネルギーあたりに換算すると，セレン（1～2歳）は53 μg/1000 kcal，ヨウ素は370 μg/1000 kcal（6～11か月）と263 μg/1000 kcal（1～2歳）となる。Table 2で明らかなように，セレンとヨウ素の離乳食中濃度の平均値はいずれもこれらの数値を上回っていた。ヨウ素に関しては別に考察するので，ここではセレンについて述べる。12～16か月児が食していた離乳食（セレン濃度68 μg/1000 kcal）を2000 kcal摂取した場合，セレン摂取量は日本人成人の平均的摂取量[7]よりも少し多い136 μg/日となる。このことは，今回収集した離乳食のセレン濃度は成人が日常的に食べる食事と大差ないことを示している。幼児に対するセレンの耐容上限量は成人の耐容上限量を体重比で外挿したものであるが[1]，この方式で耐容上限量を設定すると，成人のセレン摂取量と耐容上限量との差が小さいため，体重あたりの食事量が多い1～2歳児では普通の食事を摂取していてもセレン摂取量が耐容上限量を超える可能性が高くなる。成人のセレンの耐容上限量は糖尿病発生率の増加を考慮して設定されたものであり，高血圧予防を念頭においた食塩の目標量と同等の意味を持つものといえる。わが国において，食事性セレン中毒の報告は乳幼児を含めて皆無である。したがって，今回の結果は，幼児期のセレン過剰摂取に対する注意喚起ではなく，幼児期のセレンの耐容上

Table 2 Mineral contents in homemade baby foods consumed by subjects

	Contents in baby food [a]		DRI-J [b]	
	Aged 8 to 11 months (n=16)	Aged 12 to 16 months (n=9)	AI for 9 to 11 months [c]	EAR for 1 to 2 years [d]
Energy (kcal/d)	206 ± 106	615 ± 179***	675	950
Protein (g/kcal)	38.1 ± 12.5	35.9 ± 3.7	37.0	15.4
Total lipid (energy%)	8.5 ± 4.5	13.9 ± 4.5**	40	20～30
Minerals				
Salt (g/1000 kcal)	2.7 ± 1.5	2.6 ± 0.7	2.2	< 4.2 [e]
Potassium (g/1000 kcal)	1274 ± 400	1195 ± 318	1037	895 [f]
Calcium (mg/1000 kcal)	435 ± 227	329 ± 121	370	368
Magnesium (mg/1000 kcal)	189 ± 52	178 ± 53	89	63
Phosphorus (mg/1000 kcal)	625 ± 221	595 ± 46	385	632 [f]
Iron (mg/1000 kcal)	4.9 ± 2.1	5.6 ± 1.9	5.2 [g]	3.2
Zinc (mg/1000 kcal)	4.8 ± 0.8	4.8 ± 1.1	4.4	4.2
Copper (mg/1000 kcal)	0.8 ± 0.2	0.8 ± 0.2	0.4	0.2
Manganese (mg/1000 kcal)	2.1 ± 0.5	2.0 ± 0.6	0.7	1.6 [f]
Iodine (μg/1000 kcal)	436 ± 721	283 ± 418	193	37
Selenium (μg/1000 kcal)	92 ± 31	68 ± 11*	22	11
Chromium (μg/1000 kcal)	13 ± 6	14 ± 5	1.5	–
Molybdenum (μg/1000 kcal)	257 ± 132	194 ± 121	4	–

[a] Values are means ± SD. Significant difference was observed between aged 8 to 11 and 12 to 16 months at $p<0.05$ (*), $p<0.01$ (**) or $p<0.001$ (***).
[b] Values are calculated from estimated energy intakes and reference values of protein and mineral in Dietary Reference Intakes for Japanese, 2010 and expressed as means of values for male and female.
[c] Adequate intake for infants aged 6 to 11 months.
[d] Estimated average requirement for toddlers aged 1 to 2 years.
[e] Tentative dietary goal for preventing life-style related diseases.
[f] Adequate intake.
[g] Estimated average requirement.

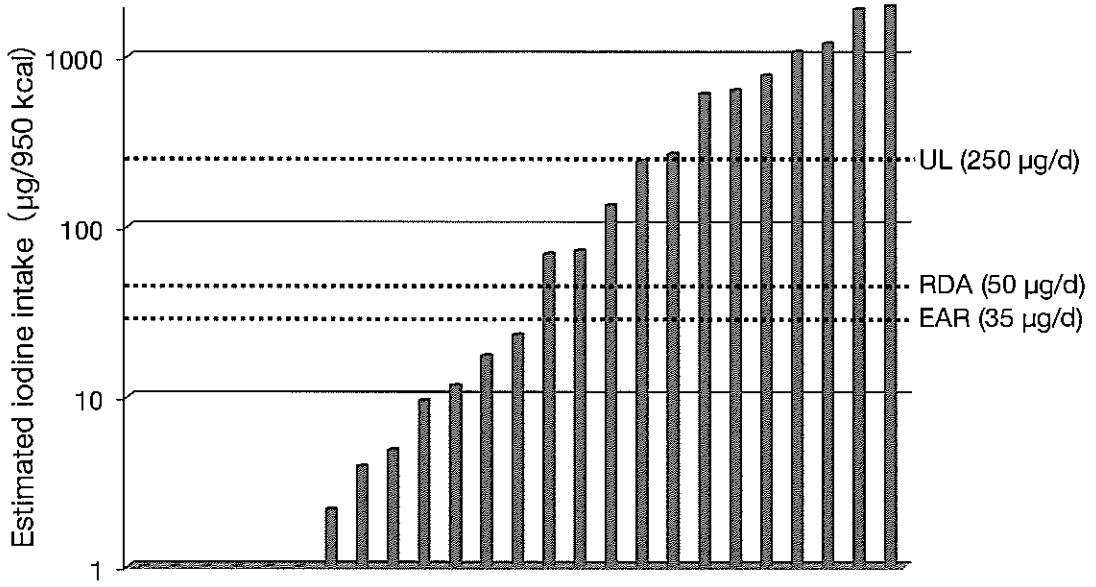

Fig. 1 Estimated iodine intake in consuming each baby food at 950 kcal

限量の再考が必要なことを意味すると思われる。

ヨウ素含有量は月齢やエネルギー含有量と無関係に大きな変動を示した。Fig. 1は，収集離乳食を1～2歳児の推定エネルギー必要量相当（男女平均で950 kcal/日）摂取した場合のヨウ素の摂取量を算定し，個人ごとに表示したものである。25食中，1～2歳児のヨウ素の推定平均必要量（EAR）である35 μg/日を充足できないものが13食あり，うち6食ではヨウ素を検出できなかった。逆に，1～2歳児のヨウ素の耐容上限量（UL）である250 μg/日を超えるものも8食あった。つまり，ヨウ素では，推奨量（RDA）と耐容上限量との間の摂取量を与えるものは25食中4食しかなかった。われわれは，このようなヨウ素濃度の大きな変動を市販離乳食においても認めている[8]。これらのことは，幼児においても，献立中のヨウ素含有量は大きく変動しており，耐容上限量を超える高ヨウ素含有量の食事を間欠的に摂取することによって必要なヨウ素を確保していることを意味している。乳幼児期の高ヨウ素摂取は間欠的であっても甲状腺機能低下を起こす可能性があるので注意すべきだという指摘[9]もあるが，間欠的高摂取は幼児の適切なヨウ素摂取にとって必要なものと考えられる。

なお，ヨウ素摂取量が高値（500 μg/d以上）である離乳食（5食）は，昆布出汁を使用した煮物または味噌汁，ヒジキが献立に含まれており，昆布またはヒジキがヨウ素の供給源であると推定された。これに対してヨウ素を検出しなかった6食は，野菜の煮物や野菜入りの雑炊などの出汁を使った献立を含んでいたが，いずれも洋風のコンソメスープや鰹出汁の使用であった。これらのことは，先に市販離乳食に関して報告したのと同様に[8]，昆布出汁やヒジキを使用した場合には高ヨウ素濃度となるが，使用しなければ極端な低ヨウ素濃度になることを意味している。

今回収集した乳幼児の食事の中には同じ品目がいくつか存在した。今回の離乳食が低脂質，低食塩であり，かつ鉄濃度が比較的高値であったことをあわせて考えると，育児支援ステーション内で母親同士が離乳食に関して情報交換を行い，工夫して離乳食を調製していると思われる。したがって，育児支援ステーションのような母親同士のコミュニケーションの場は，健全な育児にとってきわめて有用といえる。ただし，乳幼児に対して，極端な低脂質食を調製していることから，乳幼児の栄養素摂取に対して正しい助言を行えるような体制をとる必要があるといえる。

今回の検討は兵庫県都市部の特定地域のわずか25食を対象としたものである。したがって，今回の測定値が母親手作りの離乳食の代表的な数値とはいいきれない。今後，より多くの食事を分析することによって値の信頼性を高める必要があると考えられる。

本研究は平成24年度関西大学大学院理工学研究科高度化推進研究費によるものである。

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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？

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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。

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微量ミネラルの食事摂取基準：ヨウ素、セレン、クロム、モリブデン

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はじめに

わが国では、栄養素の不足もしくは過剰のリスクを予防するため、健康なヒトを対象にしてエネルギーと栄養素の適切な摂取範囲を示した食事摂取基準を策定している[1]。ミネラルに関しては、多量ミネラル５種（ナトリウム，カリウム、カルシウム、マグネシウム、リン）と微量ミネラル８種（鉄、亜鉛、銅、マンガン、ヨウ素、セレン、クロム、モリブデン）について食事摂取基準を策定している。しかし、これらの中で、ヨウ素以下の４種の微量ミネラルは食品成分表に数値の記載がなく、献立からの摂取量評価などができない状態であった。2010年の秋に刊行された食品標準成分表2010[2]においては、約500の主要な食品に対してこれら４種の微量ミネラルの含有量が収載されており、ようやく摂取量評価などが行える状況となった。

本稿では、これら４種の微量ミネラルの摂取基準の概要と日本人における摂取の実態について解説する。なお、摂取基準の個々の指標設定に用いた文献については、摂取基準をまとめた成書[1]を参照いただきたい。

1. 食事摂取基準における指標の意味

食事摂取基準では各栄養素に対して、不足もしくは過剰から直接生じる健康障害のリスクを予防するため、原則として推定平均必要量（Estimated average requirement: EAR）、推奨量（Recommended dietary allowance: RDA）、および耐容上限量（Tolerable upper limit of intake: UL）を設定し、EARとRDAが情報不足などの理由で策定できない場合には目安量を設定している。また、一部の栄養素については生活習慣病予防の観点から目標量（Tentative dietary goal for prevention of lifestyle-related disease: DG）を定めている。

EARは実験的に求められた栄養素の最少必要量の平均的な値を意味し、この値ぴったりの量を摂取している場合の不足のリスクは理論上50%である。RDAはEARに一定の係数（1.2～1.4）を乗じたものであり、この値ぴったりの量を摂取している場合の不足のリスクは2.5%である。個人や集団の摂取量を評価する場合はEARと比較する。EARよりも摂取が少ない（集団の場合はEARよりも摂取の少ない人の割合が半数を超える）場合は摂取不足のリスクが50%以上あると判断する。これに対してRDAは栄養指導や献立作成の場合にめざすべき摂取量である。たとえば、摂取量評価において摂取不足のリスクが一定以上であれば、RDAをめざすような指導をすることになる。ULはこれを超えると過剰摂取のリスクが出現する摂取量である。したがって「ここまで摂取してもよい」という意味ではなく、「近づいてはいけない」値である。DGは達成できていなくても、すぐに直接的な健康障害が起こるわけではないが、将来の生活習慣病予防のためには可能な限り達成してほしい文字どおりの目標量である。

2. ヨウ素の食事摂取基準と日本人のヨウ素摂取の実態

ヨウ素は甲状腺ホルモンを構成する微量ミネラルである。ヨウ素の摂取不足は甲状腺機能低下を招き、重症であ

れば良性の腫瘍である甲状腺腫を引き起こす。成人のヨウ素欠乏は可逆的でありヨウ素投与でほぼ完全に回復するが、胎児期から乳幼児期におけるヨウ素欠乏は死産率の上昇、身体･精神的な発育阻害、知的機能の発達阻害など、不可逆的な障害を起こす。ヨウ素は海産物、とくに海藻類に多く含まれており、これらの摂取の少ない地域では摂取不足が問題となる。ヨウ素不足は現在でも世界レベルの栄養問題であり、いくつかの国では食卓塩にヨウ素を添加して摂取不足を予防している。

一方、過剰のヨウ素は甲状腺ホルモンの生成を低下させ、不足時と同様の甲状腺機能低下と甲状腺腫の発生を招く。ただし過剰ヨウ素摂取を繰り返すと一種の慣れが生じ、甲状腺機能低下は起こりにくくなる。日本人は高ヨウ素の食品である昆布を幼少時から摂取しているため、多くの人でこの慣れが成立しており、過剰ヨウ素による甲状腺機能低下は起こりにくいといわれる。

ヨウ素を適切量摂取しているヒトを対象とした実験では、甲状腺のヨウ素濃度は一定であり、1日に新たに甲状腺に蓄積するヨウ素は約 95 μg と見積もられている。これよりわが国の食事摂取基準では、欧米と同様に成人のヨウ素の EAR を 95 μg/ 日、RDA を EAR に 1.4 を乗じた 130 μg/ 日としている。一方、欧米では米国で行われた実験をもとにヨウ素の UL を 1～1.5 mg/ 日としている。この値は、アフリカおよび中国における井戸水からのヨウ素曝露と甲状腺腫発生に関する調査成績と整合している。しかし、後述のように日本人の平均的ヨウ素摂取はこの欧米の UL を明らかに上回っているが、過剰ヨウ素による甲状腺機能低下はほとんど認められない。そこで、わが国の食事摂取基準では北海道で行われた疫学調査の結果にもとづき、ヨウ素の UL を欧米のほぼ 2 倍である 2.2 mg/ 日としている。

日本人のヨウ素摂取は世界的に見てきわめて特殊である。これは日本人がヨウ素を特異的に高濃度に含む昆布を常食するためである。昆布のヨウ素濃度は乾燥重量あたり数 mg/ 日に達する。また昆布だしにも大半のヨウ素が溶出する。このため乾燥昆布を数十 mg、または昆布だしを数 mL 摂取するだけで RDA を超えるヨウ素摂取が達成できる。日本人のヨウ素摂取量は、尿中ヨウ素排泄量、陰膳収集献立の分析、昆布消費量の三面から推定できる。いくつかの報告を総合すると、個人内で 100 μg/ 日未満から 10 mg/ 日の範囲で変動があり、平均摂取量は 1～2 mg/ 日と考えられる。また平均摂取量が日本の UL を超えるケースも稀ではないと考えられる。このようなヨウ素大量摂取にも関わらずヨウ素中毒が起こらない真の理由は不明であるが、先に述べたヨウ素に対する慣れに加え、間欠的高摂取であること、および大豆中イソフラボンのようなヨウ素吸収阻害物質を高頻度で摂取することなどが関連すると思われる。

次回の日本の食事摂取基準の改訂においては、現在の日本人のヨウ素摂取の現状、および日本人にヨウ素過剰摂取を原因とした甲状腺機能低下がほとんどないことを前提として、ヨウ素の UL をより現実的な値に変更することが必要かもしれない。その場合、私見ではあるが、連続的摂取としては現状どおり、摂取平均値としては 5 mg/ 日、間欠的高摂取の上限としては 10 mg/ 日程度が目安になると思われる。

3. セレンの食事摂取基準と日本人のセレン摂取の実態

セレンはグルタチオンペルオキシダーゼ（GPx）などのセレン含有たんぱく質（SeP）として生体内に存在し、機能している。これらの SeP 内でセレンはセレノシステイン（Sec）残基としてペプチド鎖中に存在し、この Sec という特殊なアミノ酸をペプチド鎖に組み込むための特異的な塩基配列も知られている。このためヒトゲノムの解読によって、

ヒトに存在するSePは25種類であることが明らかにされている。セレンが不足するとSePの生合成量が低下し、様々な健康障害が発生する。とくにGPx活性の低下による過酸化物処理能力の低下は様々な病的状態を引き起こし、ヒトでは致命的な心筋障害を起こす。中国東北部の風土病として知られていた克山病は食事性のセレン欠乏を原因とした心筋障害だと考えられている。

セレン摂取量が一定範囲内である場合、SePの生合成量との間に直線的関係が成立する。セレンのEAR設定には、血清GPx活性とセレン摂取量との間の回帰式が応用されている。ただし、克山病を予防する場合、血清GPx活性は飽和値の3分の2で十分といわれる。そこで日本の摂取基準では、成人のセレンのEARを血清GPx活性の飽和値の3分の2の値を与える摂取量（20～25 μg/日）とし、RDAはEARに1.2を乗じた25～30 μg/日としている。

セレンの摂取が極端に少ないと、種々の疾患にかかるリスクが高まる。なかでも、がん発生とセレンの関連についてはよく研究されており、セレン摂取量がおおよそ50 μg/日よりも少ないと、肺がんなど、様々ながんにかかるリスクが高くなる。しかし、平均的なセレン摂取量がおおよそ50 μg/日を超えている集団では、セレン摂取量とがん発生との間の関係は認められない。つまり、セレン摂取量がきわめて少ないとがんにかかるリスクが高まるが、必要以上にセレンを摂取してもがん予防にはつながらない。次回の摂取基準の改訂では、発がんのリスクを高めないという観点から50 μg/日をセレンのDGとすることも考えられる。

セレンを多く含む食品は魚介類である。穀物など植物性食品のセレン濃度は生育土壌のセレン濃度の影響を受ける。米国中央部に土壌セレン濃度が高い地域があるため、米国産小麦に由来するパンとパスタ類もセレン濃度が高い。さらに日本は家畜の飼料も米国に依存するため、肉や卵のセレン濃度も高い。世界各国のセレン摂取量は水産物の摂取量と米国産穀物・飼料への依存度によってほぼ決まる。セレン摂取量が100 μg/日に達するのは米国、カナダ、日本、逆に少ないのは欧州、オセアニア、アフリカである。欧州とオセアニアではがん発生のリスクが高まるラインである約50 μg/日を下回ることが多く、アフリカの一部では現在でも欠乏のリスクがある20 μg/日程度の摂取である。現在の欧州やオセアニアのセレンの摂取水準であれば、がん予防を目的としてセレンサプリメントを利用することに現実的意味があるといえる。

わが国では、セレン摂取量がおおよそ300 μg/日を超えると2型糖尿病の発生率が有意に増加するという調査研究にもとづき、成人のセレンのULを210～300 μg/日としている。しかし、このULは高血圧症予防のための食塩とカリウムのDGと同じ意味を持つ。つまり現在のセレンのULはDG的なものである。他の栄養素と整合させるには、爪の異常などの慢性セレン中毒を念頭においたUL（約450 μg/日）にすべきかもしれない。

がんと糖尿病の発生リスクを小さくするという観点に立てばセレンの適正な摂取範囲は50から250 μg/日である。きわめて狭い範囲であるが、日本の場合、主要栄養素が適切に摂取できる献立であれば、セレンの摂取量も自然に適切な範囲に収まることを強調しておきたい。

4. クロムの食事摂取基準と日本人のクロム摂取の実態

特殊な酵母をラットに与えたときに発生した耐糖能異常が3価クロム投与によって改善したこと、および糖代謝異常の人に200から1000 μg/日の3価クロムを投与すると空腹時血糖値などが若干改善したという報告がいくつかあることなどを根拠として、3価クロムは糖代謝を正常に維持するのに必要な「栄養素」として扱われている。クロム

を含む生体内機能性分子は知られていなかったが、最近になってクロムを投与した動物の臓器から発見された３価クロムを含む低分子ペプチド（クロモデュリン）にインスリン増強作用のあることが示された。しかし、ごく最近、クロモデュリンの発見者は「クロムは必須の栄養素ではない」という論文を発表した[3]。

クロムを栄養素の列に加えたメルツは、ある元素を必須の栄養素とするには、①体内にその元素を含んだ機能性物質が存在する、②欠乏症が存在する、③その元素を与えると欠乏症が治癒する、の三条件が必要とした。クロモデュリンの発見者は、クロムに関して②が証明できていないと指摘している。すなわち、耐糖能異常をクロム欠乏とするには、クロムがほとんど含まれていない飼料を動物に与えて耐糖能異常が起こることを示す必要があるが、実験的にクロム欠乏動物を作成して耐糖能異常を観察した例はない。すなわち、かつてラットに起こった耐糖能異常の原因はクロム欠乏以外にあり、クロムは「栄養素」ではなく「薬」として耐糖能異常を改善したに過ぎないと解釈することもできる。

食事摂取基準における成人のクロム摂取のRDAは出納試験の結果にもとづき25～40 μg/日とされている。糖代謝異常の人に投与されたクロムは多くの場合RDAの10倍以上であるが、クロムが栄養素として作用したのか、それとも薬として作用したのか微妙な投与量である。一方、食事摂取基準ではクロムのULを設定していない。これは３価クロムが安全という意味ではなく、情報不足で数値設定ができなかっただけである。動物実験では、３価クロムであっても、体内蓄積量は摂取量とともに増加することが観察されている[4]。したがって、糖代謝異常ではない健康なヒトが推奨量の10倍以上ものクロムをサプリメントから摂取することを薦めることはできない。

食品標準成分表2010によればクロム含有量の高い食品はいずれも加工食品であり、穀物、豆類、野菜、魚介類、肉類などの主要な食品のクロム含有量はきわめて少ない。このため、献立からのクロム摂取量を算定すると、EARをはるかに下回る約10 μg/日にしかならない。しかし、陰膳収集献立を分析するとRDAをやや上回る程度のクロム摂取量となる[5]。計算値と実測値の乖離、さらに加工食品にクロム含有量が高い事実からは、クロムが調理や分析の過程で紛れ込んでいる可能性が浮かび上がる。すなわち、クロムについては分析の信頼性から検討すべきだと考えられる。ただし、日本において、一般の食事からのクロムの摂取不足に起因する健康障害は発生していない。したがって、成分表からの計算値と摂取基準の数値との間の乖離に食生活上の意味はないといえる。計算値と摂取基準の数値を単純に比較して「日本人はクロム摂取不足である」と判断しないようにしていただきたい。

5. モリブデンの食事摂取基準と日本人のモリブデン摂取の実態

モリブデンはアルデヒド酸化酵素、キサンチン酸化酵素、亜硫酸酸化酵素という３種の酵素において活性発現に必須の補酵素（モリブドプテリン補欠因子）として存在する。亜硫酸酸化酵素には先天的な欠損症が存在し、新生児期に対応を誤ると重篤な症状を来すことがある。理論上、モリブデン摂取不足はこれらのモリブデン含有酵素の活性低下を招くため、モリブデンは必須の微量ミネラルと考えられてきた。しかし、モリブデンは穀物や豆類に豊富に含まれており、実際の食生活で不足を起こす可能性はほぼゼロである。また実験動物用精製飼料においても、でんぷんやたんぱく質源にモリブデンの混入があるため、モリブデン欠乏動物の作成は成功していない。

1980年代のはじめに報告された唯一のモリブデン欠乏の症例は、高カロリー輸液を長期間投与されていた。この症例では、亜硫酸酸化酵素欠損症に類似した症状（けいれんや昏睡など）が認められ、１日300 μgのモリブデン投与で症状が改善している。血液中モリブデン濃度の測定などは行われていないが、300 μg/日という投与量が一

般人の1日摂取量の範囲であることから、この症例におけるモリブデンの効果は薬理効果ではなく、栄養素としての作用であろうと考えられている。

食事摂取基準では、出納試験の結果をもとに成人のモリブデンのRDAを20～30 μg/日、ULを動物実験のデータをもとに450～600 μg/日としている。モリブデンはコメをはじめとする穀物や大豆などの豆類に高濃度に含まれているが、臓物以外の動物性食品のモリブデン含有量はわずかである。穀物と豆類がモリブデンの主な供給源であるため、日本人のモリブデン摂取量はRDAの約10倍に相当する150～350 μg/日に達している。穀物や豆類を多く食べる菜食主義者ではULを超えるモリブデン摂取を示す事例があるが、とくに問題は起こっていない[6]。米国のULが2000 μg/日であること、日本の菜食主義者の中にULを超えるモリブデン摂取量の人が存在するが健康問題を起こしていないことを考えると、現在の日本のULはやや厳しすぎるかもしれない。

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The Japanese Clinical Nutrition Association

特集「日本食品標準成分表 (2010) に新たに収載されたビタミン・ミネラルの活用と課題」

ミニレビュー

セレンとモリブデンの生理機能と適切な摂取量の範囲

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Vitamins (Japan), **86** (10), 548-557 (2012)

The Physiological Functions of Selenium and Molybdenum and the Range of Their Adequate Intake

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The physiological functions of selenium and molybdenum and the range of their adequate intake have been described. Dietary selenium is incorporated to selenocysteine residues in selenoprotein via a unique metabolic process and manifests several physiological functions. Twenty-five species of selenoproteins including glutathione peroxidase family have been identified by genome analysis in humans. The range of adequate intake of selenium is estimated to be 50 to 250 μg/day. Since most Japanese people take selenium at about 100 μg/day mainly from fishes and shells, meats and eggs and wheat products, they should not take additional selenium in a supplement form. Molybdenum exists in three molybdoenzymes and functions as a cofactor for the enzymes. Since a congenital deficiency of molybdoenzymes is fatal, molybdenum is assumed to be an essential trace element. The range of adequate intake of molybdenum is estimated to be 25 to 1000 μg/day. Most Japanese people take molybdenum at the amount of 150 to 350 μg/day mainly from cereals and beans. It is possible to calculate the daily intake amounts of selenium and molybdenum from daily menu using the Standard Tables of Food Composition in Japan 2010 because the calculated values were close to the chemically analyzed values.

Key words: selenium, molybdenum, adequate intake range, selenoprotein, molybdoenzyme

Ⅰ．セレン

1．セレンに対する認識の変化

マルコ・ポーロの東方見聞録は，現在の中国甘粛省付近に家畜の蹄を落とすほどの毒草があると記述している[1]．家畜の慢性セレン中毒症では蹄が落ちることから，東方見聞録はセレン中毒を記述した最初の文献といわれる．急性毒性という点でも，代表的なセレン化合物である亜セレン酸ナトリウムのげっ歯類に対する LD_{50} は 5 ～ 13 mg/kg であり[2]，シアン化カリウムや亜ヒ酸ナトリウムとほぼ同水準である．このようにセレン化合物は毒性がきわめて強く，法律上も毒物である．

一方，1957 年に米国の Schwartz らは，トルラ酵母投与ラットに発生する栄養性肝臓壊死をセレン化合物が予防することを示した[3]．この研究は「セレン＝毒」の

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イメージを「セレン＝栄養素」に変化させたとして有名である．肝臓壊死の予防には含硫アミノ酸とビタミンEも必要であるため，セレンはビタミンEの代替・節約をしたに過ぎないといわれたが，1969年にセレンはビタミンEとは独立した必須栄養素であることが立証された[4]．その後，ウシやヒツジの白筋病，ヒヨコの浸出性素因と膵臓繊維症，七面鳥の砂嚢障害など，家畜の様々なセレン欠乏症が明らかとなった．さらに1980年前後には，中国東北部の風土病である克山病がセレン欠乏と密接に関連することや[5]，セレンをほとんど含まない高カロリー輸液を投与された患者に心筋障害の発生が報告され[6]，セレンはヒトの必須微量ミネラルであることが確定した．その後，1970年代には，がん患者の低血清セレン濃度[7]，および様々な部位のがんの死亡率とセレン摂取量との間の逆相関が示された[8]．そして，1980年代以降，低血清セレン濃度が複数の部位のがん発生にとってリスク要因であることが明確に示された[9)-11)]．さらに動物実験においても，セレン化合物，もしくは意図的にセレンを蓄積させた食品が化学発がんを抑制するという報告が相次いだ[12)-14)]．

現在では「セレン＝毒」よりも「セレン＝抗腫瘍物質」のイメージが優勢であり，がん予防をにおわすセレンサプリメントが販売されている．しかし，セレンがシアン化カリウムや亜ヒ酸に匹敵する毒物であること，1日に1 mg近い量を摂取し続けると，爪の変形を主訴とする慢性セレン中毒が出現すること[15]は疑いもない事実である．「セレン＝抗腫瘍物質」というイメージを先行させ，意図的にセレン摂取を勧めることは避けるべきである．

2. セレンの機能

(1) セレノメチオニンとセレノシステイン

セレンはイオウの同族元素であり，多くのイオウ化合物にイオウ原子がセレン原子に置換したセレンアナログが存在する．代表的なのは，セレン酸，亜セレン酸，セレノメチオニン，セレノシステインである．セレノメチオニンはメチオニンと性質が類似し，タンパク質のアミノ酸配列中のメチオニン残基の位置に誤って挿入される．しかし，セレノメチオニンのアミノ酸配列への誤挿入はタンパク質の立体構造に影響を及ぼさず，セレノメチオニン残基を含むタンパク質に特別な機能はない．最近では，タンパク質のX線解析において，意図的にメチオニンの配列位置にセレノメチオニンを挿入して目印にすることが行われている[16]．

これに対してセレノシステインとシステインは性質が大きく異なる．たとえば生理的pHにおいて，システインのチオール基の水素原子は解離しないが，セレノシステインのセレノール基(-SeH)の水素原子は解離する．このため，アミノ酸配列中にセレノシステインを含むタンパク質は特異な機能を有する．生体におけるセレンの機能の大半は，セレノシステイン残基を含むタンパク質の機能であり，このようなタンパク質をselenoprotein(含セレンタンパク質)と呼ぶ．

(2) 含セレンタンパク質の生合成

含セレンタンパク質のアミノ酸配列中の特異的な位置にセレノシステインを挿入するプロセスは分子生物学上の大きな謎だったが，現在では図1のように大半が解明されている[17]．セレンの主要な分子種であるセレノメチオニン，セレノシステイン，および亜セレン酸はいずれもセレン化物イオン(HSe^{2-})に変換され，ATPと反応してセレノリン酸となる．一方，含セレンタンパク質中のセレノシステイン残基をコードするDNA上の塩基配列はTGAであり，これを転写したmRNAにはUGAコドンとステムループ構造の特徴的なセレノシステイン挿入配列が存在する．アミノ酸プール中のセリンのごく一部はこのUGAコドンに対応するtRNAと結合し，生じたセリルtRNA中のセリンの水酸基がセレノリン酸と反応してセレノール基に変化してセレノシステイニルtRNAが生成する．tRNAに結合したセレノシステインは前述のmRNAのセレノシステイン挿入配列と複数の機能性分子を介してアミノ酸配列中の所定の位置に挿入される．

(3) 含セレンタンパク質の種類と機能

セレノシステインをアミノ酸配列の特異的な位置に挿入する仕組みは，含セレンタンパク質をコードする遺伝子が特徴的な塩基配列であることを意味しており，ゲノム解析により含セレンタンパク質をコードする遺伝子が同定できる．現在，ゲノム解析によってヒトでは表1に示す25種類の含セレンタンパク質が明らかになっている[17]．もっともよく知られるグルタチオンペルオキシダーゼ(GPX)ファミリーはいずれも過酸化物の処理に重要であり，ヒトや家畜のセレン欠乏症との関わりは大きい．ノックアウトマウスを用いた研究ではGPX4欠損のみが致死であると示されるが[18)19)]，生体内に広くかつ高濃度に存在するGPX1の欠損がパラコート急性毒性に対する感受性の増加[20]やコクサッキーウイルス感染時の死亡率増加[21]を起こすことから，外因性刺激に対する防御システムとしてのGPX1の重要性は大きいといえる．

3. 食品中含有量と化学形態

表2にいくつかの食品のセレン含有量を食品標準成

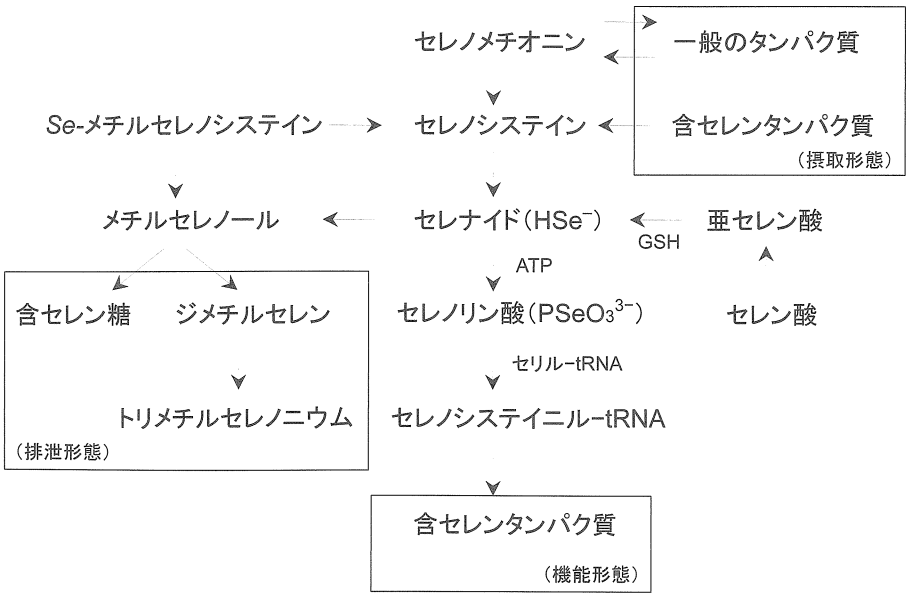

図 1. ヒトにおけるセレン化合物の代謝

一般の食物中のセレンはタンパク質のアミノ酸配列中にメチオニンと誤って取り込まれたセレノメチオニン残基，または含セレンタンパク質中のセレノシステイン残基として存在している．Se-メチルセレノシステインは意図的にセレンを蓄積させた野菜類の主形態である．食物を介して無機セレンを摂取することはほとんどない．いずれのセレン化合物も，効率に差はあるが，セレナイドに変換後，セレノリン酸，セレノシステイニル tRNA を経て，含セレンタンパク質のセレノシステイン残基として機能を発現する．セレンの主排泄経路は尿である．セレン摂取量が日常レベルであれば，含セレン糖，やや多くなればトリメチルセレノニウムとして排泄される．中毒レベルのセレン摂取の場合は呼気中にジメチルセレンとして排泄される．

分表 2010 から抜き出した．100 g あたり 10 μg 以上のセレンを含むのは，魚介類，畜肉・卵，一部の小麦および大豆製品であり，これらが主要なセレン供給源である．植物性食品のセレン濃度が生育土壌のセレン濃度の影響を受けるため，小麦および大豆製品では，原料原産地が高セレン土壌地域の北米大陸中央部であるパン，パスタ，納豆が高セレン濃度となる[22)23)]．わが国は家畜飼料も米国に依存するため，畜肉や卵のセレン濃度も高い．ラム肉は低セレン土壌地域であるニュージーランドからの輸入品なのでセレン濃度が低い．

意図的にセレンを蓄積させた場合を除き，食品中セレンの多くはタンパク質に結合している．動物は含セレンタンパク質を生成するので，動物の筋肉や内臓を摂取した場合，含有セレンの大半は含セレンタンパク質中のセレノシステイン残基，もしくはメチオニンと誤って一般のタンパク質に取り込まれたセレノメチオニン残基と考えられる．ただし，セレノシステインは不安定なので，調理加工中に別の形態に変化しているかもしれない．

植物には含セレンタンパク質が存在しない．植物は無機セレンからセレノシステインを生成するが，これを積極的に利用せずにセレノメチオニンに変換し，タンパク質中のセレノメチオニン残基として含有する．事実，通常のセレン濃度の大豆タンパク質の分解物からセレノメチオニンが検出されており[24)]，穀物や大豆を摂取した場合，含有セレンの大半はタンパク質中のセレノメチオニン残基と考えられる．また，セレンを意図的に蓄積させた高セレン酵母中のセレンも大半がタンパク質中のセレノメチオニン残基である[25)26)]．

セレノシステインは反応性が高く，生体内に高濃度に存在すれば有害である．動物はセレノシステイン-γ-リアーゼによって遊離のセレノシステインを分解するが[27)]，植物にこの酵素は存在しない．このため，高セレン環境下で植物を栽培すると，セレノシステインからセレノメチオニンへの代謝系が飽和し，セレノシステインは特殊な含セレンアミノ酸に変換される．含セレンアミノ酸の分子種としては，Se-メチルセレノシステインが大半を占めるが[28)-30)]，植物の種類によってはセレノホモランチオニン[31)32)]，γ-グルタミル

表1. ヒトに存在する含セレンタンパク質[17)]

略号	名称
GPX1	古典的(Classical)グルタチオンペルオキシダーゼ
GPX2	胃腸グルタチオンペルオキシダーゼ
GPX3	血漿グルタチオンペルオキシダーゼ
GPX4	リン脂質ヒドロペルオキシドグルタチオンペルオキシダーゼ
GPX6	グルタチオンペルオキシダーゼ6
DI 1	ヨードチロニン5'-脱ヨウ素酵素1(Type I DI)
DI 2	ヨードチロニン5'-脱ヨウ素酵素2(Type II DI)
DI 3	ヨードチロニン5'-脱ヨウ素酵素3(Type III DI)
TRR1	チオレドキシン還元酵素1
TRR2	チオレドキシン還元酵素2
TRR3	チオレドキシン還元酵素3
SPS 2	セレノリン酸合成酵素2
SELP	血漿セレノプロテインP
SELW	筋肉セレノプロテインW
SELV	セレノプロテインV
SEP15	15 kDのセレノプロテイン
SELR	メチオニン-R-スルホキシド還元酵素
SELT	セレノプロテインT
SELM	セレノプロテインM
SELN	セレノプロテインN
SELH	セレノプロテインH
SELI	セレノプロテインI
SELK	セレノプロテインK
SELO	セレノプロテインO
SELS	セレノプロテインS

表2. 主な食品のセレン，およびモリブデン含有量(μg/100 g)

食品	セレン	モリブデン	食品	セレン	モリブデン
精白米(水稲)	2	69	まいわし	54	Tr
薄力粉(1等)	4	12	かつお	100	Tr
中力粉(1等)	7	9	まさば	64	0
強力粉(1等)	39	26	まだい	38	0
じゃがいも	0	4	くろまぐろ	110	0
小豆(乾)	1	210	あさり	38	9
国産大豆(乾)	5	260	くるまえび	35	1
米国産大豆(乾)	28	300	するめいか	42	1
ごま(乾)	10	92	牛肉(和牛, リブロース, 赤肉)	11	1
なす	0	10	牛肝臓	50	94
にんにく	1	15	豚肉(ロース, 赤肉)	25	1
ほうれんそう	3	5	ラム肉	4	Tr
温州みかん	0	0	鶏肉(もも, 皮なし)	16	2
りんご	0	0	卵黄	56	14
しいたけ	4	3	卵白	21	1
真昆布(素干し)	2	12	牛乳(ホルスタイン)	3	4

いずれも日本食品標準成分表2010より転載した.

-Se-メチルセレノシステイン[32,33]なども存在する．

魚類も含セレンタンパク質を生成するので，魚肉摂取はセレノシステイン残基の摂取につながる．しかし，魚肉中セレンは高濃度なので，すべて含セレンタンパク質由来とは考えにくい．魚の筋肉や内臓中セレンに関しては様々な形態が提唱・報告されている．マグロなどの大型回遊魚がセレンとともに水銀を蓄積することから，セレンと水銀の複合体の存在が示唆されている[34]．しかし，クロマトグラフィーにおいてセレンと水銀が同じ位置に溶出されるといった報告にとどまっている[35]．マグロなどの血合肉，血液，内臓には，低分子セレン化合物の存在が示唆されていたが[36]，最近，その一部が図 2 に示すセレノネインであると同定された[37]．しかし，この化合物は筋肉には検出されないので，魚肉中セレンの主形態とはみなせない．

4. 摂取量

(1) 食品標準成分表 2010 を用いたセレン摂取量の算定

筆者らは，食品標準成分表 2010 記載のセレン含有量をもとに献立からのセレン摂取量を算定し，実測値と比較した[38]．すなわち，病院または介護施設の一般食または介護食を 8 日分収集し，成分表記載の数値からセレン摂取量を算定して実測値と比較した．なお，献立に使用された食品の約半数は成分表にセレン含有量の記載がなかったため，近縁食品の数値の転用，または属する食品群のセレン含有量の平均値を代用などの方法で数値を当てはめた．表 3 に示すように，病院一般食からのセレン摂取量の実測値（90 ～ 150 μg/ 日）は，これまでの日本人のセレン摂取量の推定値（約 100 μg/ 日）[39]に近似していた．実測値が計算値の 2 倍近い場合もあるが，この程度の乖離は微量栄養素においてしばしば認められる．すなわち，セレン含有量表示のない食品に対して数値を当てはめるという問題はあるが，食品標準成分表 2010 を用いてセレン摂取量を推定することはおおむね可能といえる．

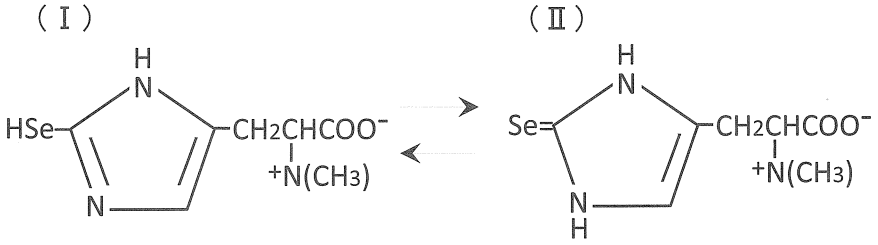

図 2．セレノネインの構造[37]

ジクロルメタンなど非極性溶媒中において，−20℃であればⅠの構造であるが，室温下ではⅠが酸化された二量体（ジセレニド（-Se-Se-））となる．水，メタノール，アセトニトリルなどの極性溶媒中ではⅡの構造をとる．

表 3．セレン，およびモリブデン摂取量の計算値と実測値の比較[39]

食事	セレン（μg/日）		モリブデン（μg/日）	
	計算値	実測値	計算値	実測値
病院普通食1	108	101	242	302
病院普通食2	146	114	269	289
病院普通食3	73	90	253	247
病院普通食4	120	125	223	177
病院普通食5	82	151	218	333
病院普通食6	86	146	267	480
介護施設普通食1	58	59	157	230
介護施設介護食1	17	24	65	106

介護施設の食事での摂取量が低いのは食事量そのものが少ないためである．

(2) 適正な摂取範囲

食事摂取基準2010年版における成人のセレン摂取の推奨量(25～30 μg/日)[40]は，体格差を考慮しても欧米(55～75 μg/日)より低い．この違いは，欧米の推奨量が血清GPX活性の飽和に必要なセレン摂取量にもとづくのに対して[41]，日本の推奨量の策定根拠が血清GPX活性の飽和値の3分の2値を維持するのに必要なセレン摂取量であること[40]に起因する．日本の策定根拠は血清GPX活性が飽和値の3分の2であれば克山病は発症しないというWHOの報告[42]に従っている．推奨量は欠乏症予防のための指標なので日本の推奨量に大きな問題はない．欧米が血清GPX活性の飽和にこだわるのは，平均セレン摂取量が50～60 μg/日を下回る集団では低セレン摂取ががんの発症リスクを高めることが疫学的に証明されているからである[9)-11)]．50～60 μg/日は血清GPX活性の飽和に必要なセレン摂取量にほぼ一致するだけでなく，血清GPX以外の含セレンタンパク質の生合成量をも飽和する摂取量である．低セレン摂取ががん発症リスクを高める機構は不明だが，25種類の含セレンタンパク質のどれかが関連すると推定できることから，がん予防を念頭におく場合，50～60 μg/日のセレンを摂取してすべての含セレンタンパク質の生合成量を飽和水準まで高めるのは妥当かもしれない．摂取基準では生活習慣病予防を念頭においた指標を目標量と定義しているので，50～60 μg/日は目標量の下限値とすべきである．

一方，日本の成人のセレン摂取の耐容上限量(210～300 μg/日)[40]は欧米(約400 μg/日)に比較して厳しい値である．この差は，欧米が爪の変形を主症状とする慢性セレン中毒予防を念頭におくのに対して[41]，日本は継続的な300 μg/日程度のセレン摂取が2型糖尿病発症リスクを増加させるという報告[43]を上限量策定の根拠としたためである．しかし，耐容上限量は慢性中毒予防のための指標なので，次回の改訂では欧米の策定根拠を採用すべきであろう．すなわち現在の日本のセレンの耐容上限量は，糖尿病という生活習慣病の発症リスクを念頭においているので，目標量の上限値といえる．

以上より，成人のセレン摂取に関する食事摂取基準としては，推奨量を現行どおり25～30 μg/日，耐容上限量を食事摂取基準2005年版が採用していた350～450 μg/日とし，新たに50から250 μg/日の範囲を目標量とするのが適切と判断できる．図3に，世界各国のセレン摂取量をこれらの摂取の指標と比較して示した[44)-56)]．なお，フィンランドやニュージーランドは低セレン摂取を解消するための対策を講じているが，

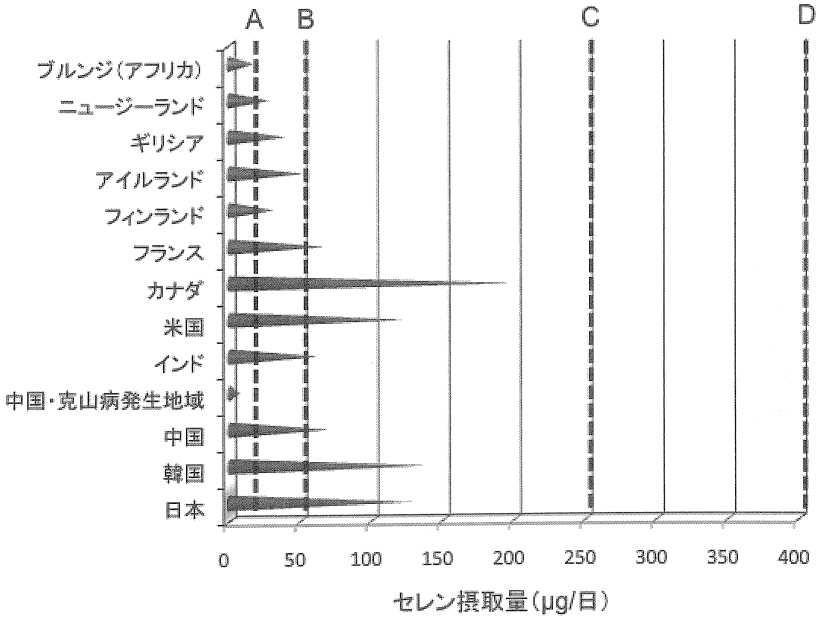

図3．世界各国の平均的なセレン摂取量[44)-56)]

A：セレン欠乏を予防するための推奨摂取量

B：がん発生リスクを高めないための目標摂取量の下限

C：糖尿病発生リスクを高めないための目標摂取量の上限

D：慢性セレン中毒を避けるための耐容上限量

図にはあえて対策以前の数値を用いた．セレン摂取が適正範囲に収まるのは米国，カナダ，日本，適正範囲より少ないのは欧州，オセアニア，アフリカである．すなわち，欧州とオセアニアの一部ではがん予防のための目標量を下回り，アフリカの一部では現在でも欠乏症の生じる危険性がある．すなわち，食品へのセレン強化やセレンサプリメントに意味があるのは欧州，オセアニア，アフリカであり，日本では普通に食事を摂取するかぎりセレンを意図的に摂取する必要はないことを強調したい．

Ⅱ．モリブデン

1．生理機能と欠乏症

ヒトを含む高等動物にはモリブデンを含む酵素が3種存在する．すなわち，キサンチン酸化酵素，アルデヒド酸化酵素，亜硫酸酸化酵素であり，モリブデンはモリブドプテリン補因子として存在する．ヒトには遺伝的にモリブデン含有酵素を合成できない欠損症[57]とモリブドプテリン補因子が合成できず複数のモリブデン含有酵素が機能しない欠損症[58]が存在する．とくに亜硫酸酸化酵素の機能欠損は，亜硫酸の蓄積が中枢神経障害を引き起こすため，新生児の段階で死に至ることがほとんどである．モリブデン含有酵素である亜硫酸酸化酵素の機能欠損が死につながることは，モリブデンがヒトの生存に必須であることを意味する．しかし，ヒトの必須微量ミネラルとしてのモリブデンの地位は，モリブデン非添加の高カロリー輸液を長期間投与された患者において，上記の亜硫酸酸化酵素欠損症に類似した中枢神経症状が出現し，栄養水準のモリブデン酸投与によって回復したというわずか一例の症例報告[59]によってようやく確定した．

モリブデンは表2に示すように穀物と豆類に高濃度で存在し，ヒトでの食事性欠乏の報告例はない．動物実験では，モリブデン非添加 AIN76 飼料（モリブデン濃度 25 ng/g）を用いてモリブデン含有酵素の低下を引き起こした研究が一例存在する[60]．しかし，筆者らの測定によれば，飼料用カゼインとデンプンのモリブデン濃度は，それぞれ 120 ～ 237 ng/g，および 50 ～ 71 ng/g であり，さらに大豆タンパク質や小麦グルテンにはより高濃度のモリブデンが混入しているため，一般的には食品タンパク質とデンプンを用いてモリブデン濃度 50 ng/g 未満の飼料を調製するのは難しい．事実，筆者の研究室で作成したモリブデン非添加 AIN93G 飼料のモリブデン濃度は 80 ng/g であり，これを低モリブデン基本食とした場合，表4のように血清モリブデン濃度のみがモリブデン投与量に依存して変化するだけで，臓器中モリブデン濃度とキサンチン酸化酵素活性はモリブデン投与量とは無関係に一定だった[61]．このように食事性モリブデン欠乏を作成するのが困難であるため，モリブデンと化学的挙動が類似するタングステンを投与してモリブデン含有酵素活性を低下させることが試みられている[62]．

2．吸収と排泄

食品中モリブデンの化学形態に関する報告はきわめて少ない．モリブデンがリン酸と高い親和性を有することからモリブドリン酸として存在すると考えられるが[63]，十分な証明はない．筆者らは，出納実験によってモリブデン摂取量 150 ～ 320 μg/ 日の範囲で食事中モリブデンの吸収を 90％以上と算定した[64]．また，主排泄経路は尿であり，摂取量と尿排泄量との間に強い相関が成立する．一般人を対象とした研究でも，モリブデン摂取量と 24 時間尿中排泄量との間に有意な関連が認められる[65]．一方，米国で行われた出納実験では，約 20 ～ 1500 μg/ 日のモリブデン摂取範囲で出納値ゼロが維持されること[66]，および血清モリブデン濃度が摂取量と強く相関することが報告されている[67]．すなわち，モリブデンは広い摂取範囲において高い吸収率であるが，速やかに尿に排泄されるので必要以上の臓器蓄積は生じないといえる．

表4．飼料中モリブデン濃度が臓器モリブデン濃度と肝臓キサンチン酸化酵素に及ぼす影響[61]

飼料へのモリブデン添加量（μg/g）	モリブデン濃度（ng/g）			肝臓キサンチン酸化酵素活性 (unit/mg protein)
	肝臓	腎臓	血清	
0	839 ± 24 [a]	478 ± 9 [a]	5.7 ± 0.2 [a]	0.16 ± 0.01 [a]
0.1	949 ± 32 [a]	508 ± 24 [a]	6.5 ± 1.3 [a]	0.13 ± 0.02 [a]
0.5	893 ± 44 [a]	496 ± 17 [a]	12.4 ± 2.1 [b]	0.12 ± 0.02 [a]

4 週齢の Wistar 系雄ラットを 4 週間飼育．基本飼料はモリブデン非添加 AIN93G 飼料（モリブデン濃度，80 ng/g）．
値は平均値 ± 標準誤差．共通の添字のない群間には有意差（$p<0.05$）がある．

3. 摂取量

日本人の食事摂取基準では，成人のモリブデン摂取の推奨量を，20 μg/日の摂取でもゼロ出納が維持されること[66]にもとづき，20～25 μg/日としている[40]．モリブデンは穀物と豆類に豊富に含まれ，日本人の食事からの摂取量は欧米人よりもやや多い150～350 μg/日と推定される[64)68)]．つまり日本人は，日常的に推奨量の約10倍に相当するモリブデンを摂取しており，通常の食生活で不足が起こることはない．成人のモリブデンの耐容上限量はヒトでの情報が少ないため，ラットの健康障害非発現量にもとづき450～600 μg/日とされている[40]．厳密な菜食習慣を持つ場合，穀物や豆類の消費が多くなるのでモリブデン摂取量が日常的に耐容上限量を上回ることがあるが，健康障害は認められない[69]．米国のモリブデン摂取の耐容上限量が2000 μg/日[70]であることを考慮すると，1000 μg/日程度まで耐容上限量を高めてもいいかもしれない．

表3に，セレンと同様に食品標準成分表2010に記載されたモリブデン含有量をもとに食事からのモリブデン摂取量を算定し，実測値と比較した結果を示す[39]．計算値と実測値はおおむね一致している．モリブデンの供給源は穀物，および豆製品であり，献立における使用量把握が容易であることから，食事調査においてモリブデン摂取は比較的正確に見積もることが可能と思われる．

おわりに

モリブデンは推奨量の100倍近く摂取しても健康障害は生じないと思われ，推奨量の10倍を超える日本人のモリブデン摂取量を問題視する必要はない．一方，セレンの望ましい摂取範囲は50～250 μg/日と考えられるが，ほとんどの日本人の平均的な摂取量はこの範囲に収まっている．米国において，前立腺がん発生に対する200 μg/日の付加的なセレン摂取の影響を調べる大規模な疫学研究が実施されたが，予防的な効果は認められなかった[71]．日常の食事において適切なセレン摂取を達成している一般の日本人がセレンサプリメントを使用することは，目標量の上限を超える可能性があり，健康の維持・増進にとって何のメリットもないことを重ねて強調しておきたい．

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J Nutr Sci Vitaminol, 57, 383–393, 2011

Review

The Optimal Dietary Fat to Carbohydrate Ratio to Prevent Obesity in the Japanese Population: A Review of the Epidemiological, Physiological and Molecular Evidence

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(Received July 13, 2011)

Summary 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. According to the Japanese Dietary Reference Intakes (RDA) for 2010, the optimal fat intake is 20–30% of energy for ages 1–29 y and 20–25% for ages 30 y and over. Upper boundary values of this recommendation were the median of the percentage of energy from dietary fat in Japanese. In a systematic review to estimate the optimal dietary fat to carbohydrate ratio, it was found that obese subjects with hyperinsulinemia (or insulin resistance) lost more weight on a mild low-carbohydrate (LC) (or low-glycemic load diet; 40% carbohydrate, 30–35% fat) than on a low-fat (LF) diet (55–60% carbohydrate, 20% fat), whereas those without hyperinsulinemia showed the opposite. In non-obese primarily insulin-sensitive subjects, decreasing fat rather than carbohydrate intake is generally more effective to prevent obesity. Physiological and molecular evidence supports this conclusion. Increased carbohydrate intake, especially in high-glycemic food, leads to postprandial hyperglycemia and hyperinsulinemia, which are exaggerated in obese insulin-resistant subjects. Even in an insulin-resistant state, insulin is able to stimulate fatty acid synthesis in liver, activate lipoprotein lipase, and prevent lipolysis in adipose tissues, which all facilitate adipose tissue enlargement. Optimal dietary fat to carbohydrate ratio may differ in populations depending on their prevalence for obesity. Because the prevalence of overweight/obesity in Japanese is low, a LF diet is recommended in the general population.

Key Words low-carbohydrate diet, low-fat diet, RDA, insulin resistance, obesity

Obesity in the United States and in much of the westernized world has increased dramatically over the past several decades: 64.5% of adults in the United States are overweight (body mass index [BMI]≥25 kg/m^2 and <30 kg/m^2) or obese (BMI≥30 kg/m^2) (*1*). Overweight/obesity (BMI≥25 kg/m^2) was the most important predictor of diabetes. In the Nurses' Health Study, during 16 y of follow-up, 3,300 new cases of type 2 diabetes were observed in the baseline population of 84,941 female nurses. The relative risk of diabetes was 38.8 for women with a BMI of 35.0 kg/m^2 or higher, 20.1 for women with BMI of 30.0 to 34.5 kg/m^2, and 7.59 for women with BMI of 25.0 to 29.9 kg/m^2, as compared with women who had a BMI of less than 23.0 kg/m^2 (*2*).

In Japan, the prevalence of overweight/obesity (BMI≥25 kg/m^2) in adults is very low compared with the United States: 30.4% in men and 20.2% in women in 2007, according to Japanese cross-sectional nationwide surveys (*3*). However, a strong positive association between baseline BMI and the incidence of diabetes in the follow-up period was observed similar to that in the United States. In a Japanese cohort of healthy men (n=16,829) and women (n=8,370) followed for 7.4 y, new cases of diabetes were documented in 869 men and 224 women (*4*). The relative risk of diabetes was 5.55 for men with a BMI of 25.2 to 26.3, compared with men who had a BMI of 15.0 to 19.7, and the relative risk of diabetes was 5.70 for women with a BMI of 24.4 to 25.9, compared with women who had a BMI of 14.9 to 19.1. Therefore, in Japan also, the prevention of overweight/obese subjects is a major public issue.

The role of dietary fat and carbohydrate in the obesity epidemic has been a hotly debated topic for decades and remains unresolved. To reduce the incidence of obesity in general populations, public statements on optimal ratios of dietary fat to carbohydrate have been issued. Health organizations have recommended diets that are low in total and saturated fat and high in carbohydrates obtained from vegetables, fruits, and whole grains or fiber-rich foods (*5–7*). Dietary guidelines for Americans published in 2005 emphasized the importance of the amount of energy consumed rather than the proportions of protein, fat, and carbohydrate in the diet, pro-

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vided that the macronutrients are within the AMDR, the acceptable macronutrient distribution range: 10–35% of energy from protein, 45–65% from carbohydrate, and 20–35% from fat (*8*). Dietary reference intakes for Japanese issued by the Ministry of Health, Labour, and Welfare in 2010 indicated that optimal fat intake is 20–30% for ages 1–29 y and 20–25% for ages 30 y and over. Upper boundary values of this recommendation were a median of the percentage of energy from dietary fat in Japanese, a recommendation that most Japanese are able to follow.

The present review was conducted to determine the optimal dietary fat to carbohydrate ratio to prevent obesity in the Japanese population. As a result, it was suggested that a mild low-carbohydrate (LC) diet was effective in reducing body weight in obese subjects with hyperinsulinemia (or insulin resistance), whereas a low-fat (LF) diet favored prevention of obesity in non-obese subjects or treatment of obese subjects without hyperinsulinemia. In addition, to elucidate the molecular mechanisms of obesity in response to a carbohydrate-rich diet, several aspects of insulin actions, namely lipogenesis in the liver, activation of lipoprotein lipase (LPL), and lipolysis under insulin-resistance state were also reviewed.

Methods of Review and Definitions

Selection of publications of epidemiological studies. For epidemiological studies, key words "(Diet, Fat-Restricted [MESH]) AND (dietary OR intake OR consumption) AND ((randomized controlled trial [PTYP] OR random [WORD]) OR (cohort studies [MESH] OR risk [MESH] OR (odds [WORD] AND ratio [WORD]) OR (relative [WORD] AND risk [WORD]) OR case control [WORD] OR case-control studies [MESH]))" with a limitation of "humans" were used in PubMed to select all publications through June 1, 2011 (n=1,004), initially to review the effects of dietary fat on mortality and mobility reported therein. From these publications, those related to changes in body weight were selected and reviewed. Other important topics, such as the effects of dietary fat subtypes, i.e., saturated, mono-unsaturated, n-6, and n-3 fatty acids, on obesity, are not discussed in this review. Because several reviews and meta-analyses have been published since the original search date, publications that appeared after this date are presented in this study with comments relating their findings to those of the previous reviews and meta-analyses. To show a visual representation of the results of the review, findings from representative publications are presented here in figures.

Current body weight is the result of the accumulated daily balance of energy intake and expenditure over previous days. Therefore, the causes of obesity are multifactorial, including such factors as physical activity level, energy intake, and food availability. It is difficult to assess these factors, and there are strong limitations to examining the effects of dietary macronutrients on obesity in cross-sectional and prospective studies (confounding factors may not be measured adequately). For this reason, carefully conducted intervention studies in which dietary fat to carbohydrate ratios were changed were mostly selected for this review.

Selection of publications of physiological and molecular studies. In a review of the mechanism of lipogenic action of insulin (covered later in this review), key words "insulin AND obesity AND ((lipogenesis AND liver) OR LPL OR (lipolysis and adipose tissues))" were used initially in PubMed to select appropriate publications, including reviews. Additional publications, which were necessary to describe the effects of insulin in an insulin-resistance state, were included from citations obtained from review articles and personal reference lists.

Definitions of LF and LC diets. The term LF diet is used relative to that of a high-fat diet in the literature; therefore, the absolute amounts of fat were diverse. In general, a high-fat diet means fat intake provides more than 30% of energy and a LF diet means less than 30%. The LC diet has been used in two different types of diet: a very LC diet (ketogenic diet) and a mild LC diet (low-glycemic load diet). Glycemic load is the mathematical product of glycemic index and carbohydrate amount. In the ketogenic diet, carbohydrate intake is less than 40 g/d (*9*), whereas in the low-glycemic load diet, the total amount of carbohydrate is decreased by 10–20% of energy, and foods containing carbohydrate with lower glycemic index were used. In Japanese, median intake of energy in adults was 1,856 kcal/d, and median intakes of carbohydrate, fat, and protein were 258 g/d (56% of energy), 51 g/d (24.8%), and 68 g/d (15%), respectively, according to The National Health and Nutrition Survey in Japan, 2007 (*3*). In this review, these two types of LC diets are reviewed separately.

Results and Discussion

A LF diet prevents obesity in general populations

In a meta-analysis of general populations under free-living conditions, weight loss was positively and independently associated with a reduction in the percentage of energy as fat (0.37 kg/%, p<0.005) (*10*). Another meta-analysis of intervention studies also supports this conclusion (*11*). For every 1% decrease in energy from fat, there was a 0.28-kg decrease in body weight.

A large randomized intervention trial including 48,835 post-menopausal women in the United States (The Women's Health Initiative Dietary Modification Trial) also supports a LF diet for the prevention of obesity (*12*). This intervention included group and individual sessions to promote a decrease in fat intake and did not include weight loss or energy restriction goals. Energy from fat was decreased from 38.8% to 29.8% in the intervention group, whereas there was no alteration of fat intake in the control group (from 38.8% to 38.1%). Concomitantly, energy from carbohydrate was increased from 44.5% to 52.7% in the intervention group, whereas there was no alteration of carbohydrate intake in the control group (from 44.5% to 44.7%). Women in the intervention group lost weight in the first year and maintained a lower weight than the control

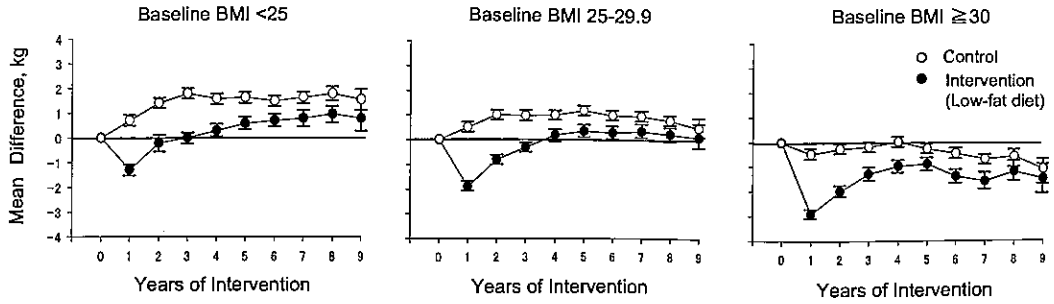

Fig. 1. Differences in body weight by body mass index (BMI) at screening in response to a low-fat (LF) diet. A large randomized intervention trial including 48,835 post-menopausal women during an average 7.5 y of follow-up supports a LF diet (energy from fat decreased from 38.8% to 29.8%) but not energy intake for the prevention of obesity. Women in the intervention groups lost weight in the first year and maintained lower weight than did women in the control groups. No tendency toward weight gain was observed in the intervention groups, whereas body weights in the control groups gradually increased. Error bars indicate 95% confidence intervals. Patient numbers at baseline for the intervention and control groups by BMI: BMI <25 kg/m^2, 5,072 and 7,585; BMI 25–29.9 kg/m^2, 6,940 and 10,446; and BMI≧30 kg/m^2, 7,442 and 11,126, respectively. Reproduced with permission (*12*).

women over an average 7.5 y of follow-up (Fig. 1). No tendency toward weight gain was observed in the intervention group, whereas body weights in the control group gradually increased. In both groups, weight loss was greatest among women who decreased their percentage of energy from fat. Weight loss in response to fat reduction was also slightly greater in subjects with a baseline BMI of <25 kg/m^2.

Several mechanisms for body fat increase in response to a high-fat intake have been proposed (*13, 14*). Fat is the most energy-dense of the macronutrients and is palatable. Fat produces less of a thermogenic effect than does carbohydrate (*15, 16*), and fat intake is not regulated, whereas carbohydrate intake is regulated for combustion of carbohydrate substrates (*17*). A prompt increase in glucose oxidation occurs after ingestion of carbohydrate-containing meals, whereas fat oxidation is reduced after food consumption, even when meals provide substantial amounts of fat (*18*). These findings indicate that when energy intake is not intentionally restricted, a LF diet prevents body weight increase in the general population.

A very LC diet (ketogenic diet) decreases body weight in obese subjects

Intervention studies to compare the efficacy of LF and very LC diets to reduce body weight in obese subjects have been conducted and summarized in several meta-analyses (*19–22*). All analyses revealed that a very LC diet is more effective than a LF diet in reducing body weight in obese subjects. In a recent meta-analysis performed by Hession et al., studies comparing the weight loss effects of a very LC diet (less than 60 g/d carbohydrate without intentional energy restriction) against a LF diet with energy restriction (less than 30% fat with 600 kcal/d energy restriction) of more than 6 mo were included (*21*). Among 9 studies analyzed (n=690 in total), 6 studies (*23–28*) showed greater reduction in body weight by LC diet than by LF diet, whereas 3 studies (*29–31*) reported no differences between LC and LF diets in the decrease of body weight when measured at 6 mo of intervention.

However, several adverse effects were observed in a very LC diet. A meta-analysis showed an increase in LDL cholesterol (*22*). Increased blood ketone productions showed unfavorable effects, such as hyperuricemia and orthostatic hypotension (*32*). Recently, even under energy restricted conditions, it was reported that a very LC diet (60% fat/5% carbohydrate) for 6 wk (*33*) or a very LC diet (60% fat/4% carbohydrate) for 1 y (*34*) reduced endothelium-dependent flow-mediated dilation of brachial arteries. A relatively very LC diet (60% fat/20% carbohydrate) worsened the aortic augmentation index (*35*). These adverse effects might be mediated by a large amount of dietary fat. Therefore, a very LC diet was not recommended in the general population.

Mixed evidence that a mild LC diet (low-glycemic diet) decreases body weight in obese subjects

In a Cochrane review, a low-glycemic-index or low-glycemic load diet was compared with a high-glycemic-index or high-glycemic-load diet on different indices of body fat in 6 studies (*36*). Pooled data from 4 studies (*37–40*) showed that weight loss was significantly greater in participants (n=163 in total) receiving the low-glycemic diet (−1.1 kg of difference, p<0.05). Other studies reported a favorable percent change in body mass (*41*) or a favorable change in BMI on a low-glycemic diet (*39, 42*).

However, two recent intervention studies suggested that reduced-calorie diets resulted in meaningful weight loss, regardless of macronutrient balance. In one study, a total of 34 healthy overweight adults ate a high-glycemic load diet (20% fat, 20% protein, and 60% carbohydrate) or a low-glycemic load diet (30% fat, 30% protein, and 40% carbohydrate) under 30% energy-restricted conditions (*43*). There was no significant change in body weight between the two groups: percentage weight change at 12 mo was −8.04±4.1% in the high-glycemic load diet group and −7.81±5.0% in the low-glycemic load diet group. In the other study, a total of 811 overweight adults (BMI>25 kg/m^2) ate one of four diets for 2 y (*44*). The targeted percentages of energy derived from fat, protein, and carbohydrate in

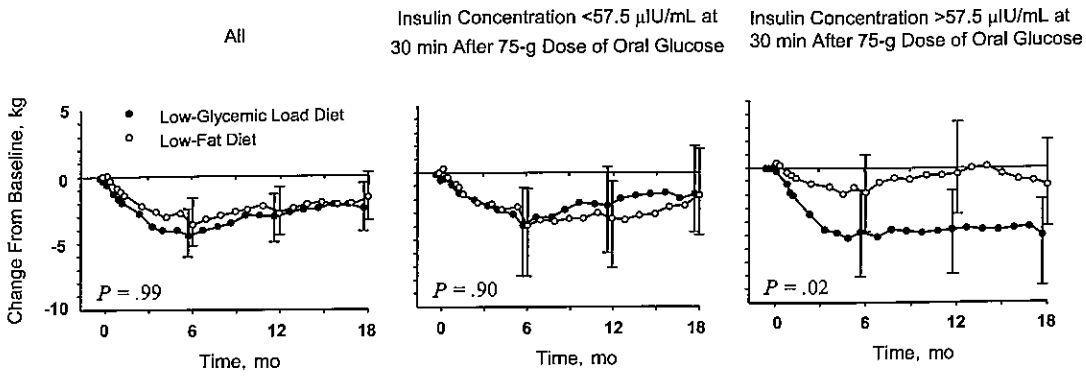

Fig. 2. Changes in body weight in insulin-sensitive and -resistant obese subjects. Obese nondiabetic insulin-sensitive (insulin concentration ≤57.5 μU/mL at 30 min after 75-g dose of oral glucose, $n=28$) and obese nondiabetic insulin-resistant (insulin concentration >57.5 μU/mL at 30 min after 75-g dose of oral glucose, $n=28$) young adults were randomized to either a low-fat diet (55% carbohydrate of energy, 20% fat, and 25% protein) or a low-glycemic load diet (or a low-carbohydrate diet; 40% carbohydrate, 35% fat, and 25% protein) for a 6-mo intervention and a 12-mo follow-up period. In the insulin-resistant groups, a low-glycemic load diet produced a greater decrease in weight than did the low-fat diet at 18 mo. Reproduced with permissiou (47).

the four diets were 20%, 15%, and 65% (LF/low protein [LP] diet); 20%, 25%, and 55% (LF/high protein [HP] diet); 40%, 15%, and 45% (LC/LP diet); and 40%, 25%, and 35% (LC/HP diet). At 2 y, weight loss remained similar in those who were assigned to a diet with 15% or 25% protein (3.0 and 3.6 kg, respectively), in those assigned to a diet with 20% fat or 40% fat (3.3 kg for both groups), and in those assigned to a diet with 65% carbohydrate or 35% carbohydrate (2.9 and 3.4 kg, respectively). There were no differences in reduction of body weights between groups when measured at 6, 12, and 18 mo. When considering the results of recent intervention studies, it is not conclusive that a mild LC diet is preferable for obese subjects.

A mild LC diet preferentially reduces body weights in obese subjects with hyperinsulinemia (insulin resistance)

The studies described above comprised mixed populations of insulin-sensitive and insulin-resistant obese subjects. However, when only the publications that separately examine the effects of LF and mild LC diets on body weight decrease in insulin-sensitive and insulin-resistant subjects were selected, a clear picture appeared. In obese subjects with hyperinsulinemia and insulin resistance, a mild LC diet was more likely than was a LF diet to reduce body weight under energy-restricted conditions (45–47).

In the first intervention study, obese nondiabetic insulin-sensitive (fasting insulin <10 μU/mL, $n=12$) and obese nondiabetic insulin-resistant (fasting insulin >15 μU/mL, $n=9$) women were randomized to either a LF diet (60% carbohydrate, 20% fat, and 20% protein) or a mild LC diet (40% carbohydrate, 40% fat, and 20% protein) for 16 wk under a 400-kcal energy deficit/d (45). A marked difference was observed in body weight reduction. Insulin-sensitive women on the LF diet lost 13.5±1.2% ($n=6$) of their initial body weight, whereas those on the mild LC diet lost 6.8±1.2% ($n=6$). In contrast, among the insulin-resistant women, those on the mild LC diet lost 13.4±1.3% ($n=5$) of their initial body weight as compared with 8.5±1.4% ($n=4$) lost by those on the LF diet. Differences in resting metabolic rate, physical activity, or energy intake between the two dietary groups were not observed (45).

In the second intervention study, obese (BMI 25–29.9 kg/m^2) insulin-sensitive (insulin concentration ≤66 μU/mL at 30 min after 75-g dose of oral glucose, $n=16$) and obese nondiabetic insulin-resistant (insulin concentration >66 μU/mL at 30 min after 75-g dose of oral glucose, $n=16$) adults were randomized to either a LF diet (or high-glycemic diet; 60% carbohydrate, 20% fat, and 20% protein) or a mild LC diet (or low-glycemic diet; 40% carbohydrate, 30% fat, and 30% protein) for 6 mo at 30% calorie restriction compared to baseline individual energy needs (46). In the insulin-resistant groups, the mild LC diet produced a greater decrease in weight (−10.2 vs −6.2 kg) than did the LF diet at 6 mo. There were no significant differences in weight decrease between the mild LC and LF diets in the insulin-sensitive groups.

In the third intervention study, obese nondiabetic insulin-sensitive (insulin concentration ≤57.5 μU/mL at 30 min after 75-g dose of oral glucose, $n=28$) and obese nondiabetic insulin-resistant (insulin concentration >57.5 μU/mL at 30 min after 75-g dose of oral glucose, $n=28$) young adults were randomized to either a LF diet (or high-glycemic diet; 55% carbohydrate, 20% fat, and 25% protein) or a mild LC diet (or low-glycemic diet; 40% carbohydrate, 35% fat, and 25% protein) for a 6-mo intervention and 12-mo follow-up period (47). Although both the mild LF- and LC-diet groups decreased energy intake similarly by 400 kcal/d, effects of LF and LC diets on body weight reduction were markedly different between the insulin-sensitive and -resistant groups. In the insulin-resistant groups, the mild LC diet produced a greater decrease in weight (−5.8 vs −1.2 kg) and body fat percentage (−2.6 vs −0.9%) than did the LF diet at 18 mo (Fig. 2). There were no significant differences in decreases in weight and body

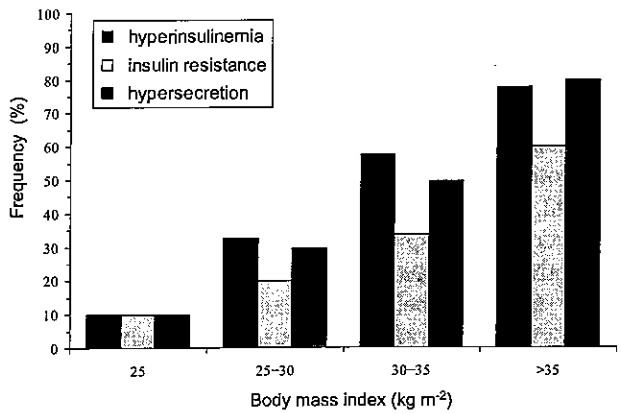

Fig. 3. Prevalence rates of insulin resistance, hyperinsulinemia, and insnlin hypersecretion (all defined as the top decile of the respective distributions in lean subjects) as a function of the body mass index (BMI). Black bars, hyperinsulinemia; light gray bars, insulin resistance; dark gray bars, hypersecretion. Reproduced with permission (*51*).

fat between the mild LC and LF diets for any subjects or in the insulin-sensitive group.

Metabolic syndrome is closely associated with hyperinsulinemia (*48*). A recent study examining the effects of LF and mild LC diets in subjects with and without metabolic syndrome under 500-kcal/d energy deficit conditions indicated that a LF diet is preferable in insulin-sensitive obese subjects (*49*). In this study, 202 obese subjects were randomized to either a LF diet (55–60% carbohydrate, less than 30% fat, and 15% protein) or a mild LC diet (or low-glycemic diet; 30–35% carbohydrate, 35–40% fat, and 25–30% protein) for a 12-mo follow-up period. In the subjects with metabolic syndrome, both the mild LC and LF diets were equally effective in reducing waist circumference, whereas in subjects without metabolic syndrome, the LF diet was preferable to that of the mild LC diet: the change in waist circumference was -7.8 ± 7.1 cm in the LF diet group versus -3.8 ± 5.0 cm in the mild LC diet group.

Thus, these four studies suggest that a mild LC diet preferentially reduces body weight in obese subjects with hyperinsulinemia (insulin resistance), whereas a LF diet preferentially reduces body weight in obese subjects without hyperinsulinemia.

Physiological aspects of a mild LC diet making it preferable in obese, insulin-resistant subjects to reduce body fat

It is known that not all obese subjects show insulin resistance (*50*, *51*). In a European study of insulin resistance in the obese, hyperinsulinemia, insulin resistance, and insulin hypersecretion were found to increase linearly with an increase in BMI (Fig. 3) (*51*). In this study, hyperinsulinemia was defined as the upper 10% of fasting plasma insulin concentrations in the lean groups. Insulin resistance was defined as the bottom 10% of glucose disposal estimated by euglycemic insulin clamp technique in the lean groups, and insulin hypersecretion was defined as the upper 10% of the distribution of posthepatic insulin delivery rate. According to these criteria, roughly one-half of the obese subjects (BMI>30 kg/m^2) were insulin resistant. The frequency of insulin resistance was 20% in subjects with a BMI of 25–30 kg/m^2, 34% in subjects with a BMI of 30–35 kg/m^2, and 60% in subjects with a BMI of >35 kg/m^2, relative to 10% in subjects with a BMI of 25 kg/m^2 (*51*). Similar trends were observed in regard to hyperinsulinemia and insulin hypersecretion.

Insulin resistance in liver and skeletal muscles elevates glucose concentrations, by which insulin secretion is increased. Moreover, pancreatic beta cells can acutely assess the body's sensitivity to insulin and translate this information into an insulin response that is precisely balanced to offset the severity of insulin resistance (*52*). In patients with insulin resistance, the increment of insulin secretion from β-cells in response to a fixed amount of glucose is greater than that in normal subjects (*53*). Therefore, the sensitivity of glucose to an increased blood insulin level is augmented in obese subjects. Diets with higher glycemic load resulted in higher postprandial insulin concentration in a dose-dependent manner in lean young adults (*54*). It is well known that obese subjects show hyperinsulinemia after oral glucose tolerance testing (glucose is a substance of high glycemic load) (*55*, *56*). Postprandial hyperglycemia and hyperinsulinemia augmented by an increase in dietary carbohydrate intake in obese subjects may further promote fat cell enlargement (*57*).

Increased blood insulin stimulates the synthesis of fatty acid in liver and the preferential uptake of fatty acids in adipose tissues to store fat and prevents lipolysis in adipose tissues, all of which facilitate adipose tissue enlargement. Furthermore, these lipogenic effects of insulin are not impaired in obese subjects, whereas the glucose-lowering effects of insulin (inhibition of gluconeogenesis/glycolysis in the liver and stimulation of glucose uptake in skeletal muscles) is severely impaired. Recently, it was shown that hyperinsulinemia is associated with increased production of intestinal apoprotein B-48, which is one of the causes of postprandial hypertriglycemia (*58*). This effect of insulin also indirectly promotes obesity. In the following sections, the mechanisms of insulin-mediated increases in lipid synthesis and fat accumulation in the insulin-resistant state are reviewed.

Insulin-induced lipogenesis in liver is not impaired in insulin-resistant animals or humans

The insulin signaling pathway is thought to proceed through receptor-mediated tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and/or IRS-2. This leads to activation of phosphoinositide 3-kinase (PI3K) and activated Akt (also known as protein kinase B). In activating hepatic lipogenesis, insulin increases transcription of genes encoding acetyl-CoA carboxylase, fatty acid synthase, and others. These actions are caused by an insulin-induced increase in sterol regulatory element-binding protein-1c (SREBP-1c) mRNA (*59*).

To examine the insulin signaling pathway and lipogenesis in the insulin-resistant state, two different ani-

mal models of insulin resistance and hyperinsulinemia, those of lipodystrophy induced by overexpression of the aP2-SREBP1c transgene in adipocytes and obesity induced by mutational disruption of the leptin gene (ob/ob mice) were investigated (*60*). Both animal models showed a reduction of IRS-2 mRNA and protein and increased gluconeogenesis in livers, whereas they showed an increase in SREBP-1c mRNA and lipogenesis. IRS-1 mRNA in the liver was not altered in these animal models. In addition, prolonged insulin treatment in isolated rat hepatocytes led to a fall in IRS-2 mRNA and protein and an increase in SREBP-1c transcript, suggesting that chronic hyperinsulinemia promotes gluconeogenesis in the liver and hyperglycemia, whereas it stimulates fatty acid synthesis in the liver and hypertriglycemia (*60*). It was shown with IRS-1 and IRS-2 liver knockout mice that IRS-1 could convey signals to increase SREBP-1c mRNA and lipogenesis (*61*, *62*). The complete blockage of insulin signaling observed in liver insulin receptor knockont mice showed a decrease in the expression of SREBP-1c (*63*), suggesting that selective insulin resistance may occur in animal models of insulin resistance (*64*). Recently, a branch point in the insulin signaling pathway that may account for selective insulin resistance (in which insulin loses its ability to block glucose production but retains its ability to stimulate lipogenesis) was identified (*65*). In rat hepatocytes, subnanomolar concentrations of rapamycin, an inhibitor of the mammalian target of rapamycin complex 1 (mTORC1), blocked insulin induction of SREBP-1c but had no effect on insulin suppression of phosphoenolpyruvate carboxylase (PEPCK), suggesting that the kinase complex designated mTORC1 was a branch point in the insulin signaling pathway. Therefore, the IRS-1/Akt/mTORC1 pathways are thought to mediate the increase of lipogenesis in the insulin-resistant state.

The finding that insulin-induced lipogenesis in the liver was not impaired in the insulin-resistant state in animal studies could apply to humans. The pattern of stored energy distribution derived from a high-carbohydrate meal is different in young, lean, insulin-resistant individuals (fasting insulin concentration of 12.1 ± 1.2 μU/mL) compared with young, lean, insulin-sensitive individuals (fasting insulin concentration of 7.6 ± 0.6 μU/mL) (*66*). In contrast to the insulin-sensitive subjects, who stored most of their ingested energy in the liver as glycogen, the insulin-resistant subjects had a marked defect in muscle glycogen synthesis and diverted much more of their ingested energy into hepatic de novo lipogenesis, as assessed by incorporation of deuterated water into plasma triglyceride, resulting in increased liver and plasma triglycerides (TGs). Increasing very-low-density lipoprotein-TG secretion from the liver may lead to increased fat accumulation in adipose tissue (*67*). Therefore, insulin activation of the liver IRS-1/Akt/mTORC1 pathway in the insulin-resistant state may lead to obesity.

An increase in lipoprotein lipase (LPL) activity in adipose tissue in response to insulin is not impaired in obese subjects

LPL, located on the capillary endothelium of tissues, catalyses the rate-limiting step in the hydrolysis of TGs from circulating chylomicrons and very-low-density lipoproteins. Most LPL is found in adipose tissues and skeletal muscles, where some of the liberated free fatty acids are taken up and are either stored or oxidized, respectively (*68*). In healthy humans, a combination of stable isotope labeling and arteriovenous difference measurements in adipose tissues showed that in postprandial periods, there is preferential uptake of fatty acids released from chylomicrons by LPL in adipose tissues and also a release of LPL-derived fatty acids into plasma (*69*). Therefore, an increase in LPL activity in adipose tissues may promote fat cell enlargement via increased uptake of fatty acids into adipocytes, in addition to an increased supply of fatty acids to muscle and liver.

Regulation of LPL activity is complex and is controlled by several modulators, such as apoproteins and angiopoietin-like proteins ANGPTL3 and ANGPTL4 (*70*). LPL is active as a dimer, whereas its monomer is inactive. ANGPTL4 inhibits LPL activity by promoting the conversion of active LPL dimers into inactive LPL monomers. Insulin not only increases the level of LPL mRNA but may also regulate LPL activity through both posttranscriptional and posttranslational mechanisms (*71*). The fact that feeding increases active dimeric LPL from inactive monomeric LPL in adipose tissues suggests that insulin may stimulate dimer formation of LPL by an unknown mechanism (*72*). Glucose also increases adipose tissue LPL activity and enhances the stimulatory effects of insulin, possibly by the glycosylation of LPL (*73*).

In humans, feeding or insulin/glucose infusion stimulates LPL activity in adipose tissues, whereas its activity decreases in skeletal muscles (*74*). This divergent response would serve to direct lipoprotein TG-derived fatty acids away from muscle to adipose tissue for storage. A high-carbohydrate diet for 16 d in normal-weight subjects increased postprandial LPL activity in adipose tissue, with elevation of blood glucose and insulin concentrations after meals, relative to a high-fat diet (*75*). Therefore, increased insulin and glucose from a high-carbohydrate diet may promote obesity via activation of LPL in adipose tissues.

The LPL activity in adipose tissues in response to insulin during maintenance of euglycemia was examined in 22 obese and 8 normal-weight subjects (*76*). Basal levels of LPL activity per g of fat tissue in the obese and control groups were 18.7 ± 2.0 and 9.6 ± 2.7 nEq/g/min, respectively. When the responses of LPL in absolute change from basal values were compared between the obese and control groups, no significant differences were found. However, because of the higher baseline LPL activity in the obese subjects, the percent increase in LPL from the basal value was significantly blunted in obese subjects. Basal LPL activity expressed per 10^6 cells correlated positively with cell size, and both the

obese and normal-weight subjects were found to respond similarly to insulin. These data suggest that insulin activates LPL in adipose tissues in obese subjects, irrespective of insulin resistance.

Inhibition of lipolysis in adipocytes in response to insulin is not impaired in insulin-resistant subjects

The concentration of blood free fatty acids (FFA) is determined primarily by their rate of appearance from adipose tissues (lipolysis) and also by their rate of disappearance from plasma. Blood FFA concentrations are elevated during fasting and decreased after feeding. Lipolysis is stimulated by catecholamines during fasting and inhibited by insulin after feeding. If the antilipolytic effect of insulin in obese subjects were impaired due to insulin resistance, fat mass would be smaller in obese subjects. However, most of the studies suggested that insulin resistance is not observed at this step in obese subjects (see following paragraph), although the resistance of insulin to increased glucose oxidation in enlarged adipocytes was clearly shown and is due to a marked decrease in GLUT4 in adipocytes (*77, 78*).

The antilipolytic effects of insulin on fat cells of different sizes were examined in the 1970s by measuring glycerol release. Basal lipolysis was larger in larger cells (*79*). The antilipolytic effects of insulin on noradrenalin-stimulated lipolysis were more pronounced in the large cells at all tested concentrations (*80, 81*). Responsiveness and sensitivity to insulin was not altered in adipose tissues of either control or obese subjects (*82*). Rather, a marked resistance to the lipolytic effect of noradrenalin was observed in isolated adipocytes from obese subjects (*83*).

In vivo studies also show that the antilipolytic effect of insulin is not impaired in obese subjects. Both antilipolytic and antiketotic actions occurred at lower insulin concentrations (<90 μU/mL) than those required for hypoglycemic activity (>1,000 μU/mL) (*84*), suggesting that marked insulin resistance might be required to reduce antilipolytic action in adipose tissues. Decreases in blood FFA and glycerol observed during oral glucose tolerance tests were not impaired in obese subjects (*85*). Insulin and glucose infusion rapidly produced antilipolysis in obese and normal groups, as evidenced by large falls in FFA at 20 min after insulin infusion, where FFA was 47% of the basal level in the obese subjects and 31% of the basal level in the normal subjects (*76*).

Triglycerides in tissues are hydrolyzed in a sequential process involving different lipases. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are necessary for proper hydrolysis of tri- and diglycerides, respectively. The last step in lipolysis is performed by monoglyceride lipase (MGL), which hydrolyzes monoglycerides to form glycerol and fatty acids (*86*). The activity of ATGL and HSL is tightly regulated by catecholamines and insulin. β-Adrenergic stimulation of the G-protein-coupled receptor activates adenylate cyclase to increase cellular cAMP levels. The antilipolytic action of insulin is mediated by lowering cAMP levels via activation of phosphodiesterase 3B (*87*). The IRS-1/PI3K/PDE3IK (an insulin-stimulated protein serine kinase) signaling pathway is involved in PDE3B activation (*88*). cAMP binding to protein kinase A (PKA) induces phosphorylation of HSL and perilipin, a protein coating the lipid droplet. PKA phosphorylation of HSL causes HSL translocation from the cytosol to the lipid droplet, whereas phosphorylation of perilipin by PKA alleviates the barrier function of this protein and promotes lipolysis (*89*). ATGL is phosphorylated on two conserved serine residues (Ser 404 and 428), although PKA does not phosphorylate ATGL (*90*). However, insulin treatment downregulates ATGL mRNA levels in adipocytes (*91, 92*). To my knowledge, it has not been shown that decreases in cAMP concentration or ATGL mRNA in adipocytes in response to insulin are blunted in adipocytes from obese subjects.

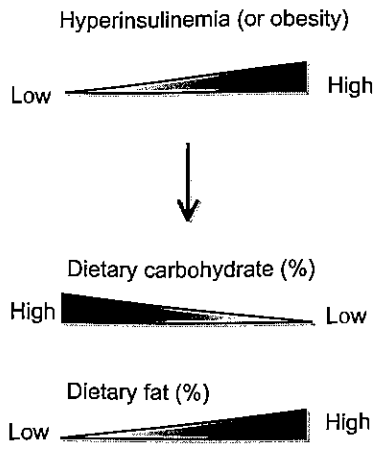

Fig. 4. A proposed model of optimal dietary fat to carbohydrate ratio according to the degree of hyperinsulinemia (or obesity). A key to macronutrient balance in the reduction of body weight is the state of hyperinsulinemia (insulin resistance or obesity); thus, optimal dietary fat to carbohydrate ratios may differ between prevention and treatment of obesity. A mild low-carbohydrate diet (40% carbohydrate) is preferable for obese, hyperinsulinemic, insulin-resistant subjects, whereas a low-fat diet (20–25% fat) is preferable for normal-weight, normoinsulinemic, insulin-sensitive subjects.

Shift from a mild LC diet to a LF diet during obesity treatment (hypothesis)

When a mild LC diet is given to obese subjects, body weights might decrease with improvement in hyperinsulinemia and insulin resistance. Data from the National Weight Control Registry of people who were successful in losing weight and maintaining reduced body weight show that despite wide variation in the methods used to lose body weight, there was remarkable similarity in how they maintained the weight loss, including a diet that was, on average, 24% fat (*93*). Therefore, fat intake might be gradually decreased with a concomitant increase in carbohydrate intake with improvement in obesity (Fig. 4).

Conclusions

In terms of epidemiological, physiological, and molecular aspects, the optimal dietary fat to carbohydrate ratio varies due to the amount of body fat present and

to hyperinsulinemia (insulin resistance). No evidence was found that the lipogenic effects of insulin were impaired in subjects with insulin resistance. In general, in non-obese subjects, most of whom are insulin sensitive, decreasing fat intake is more effective than decreasing carbohydrates to prevent obesity. However, for obese subjects with insulin resistance, a mild LC diet favors a reduction in body weight. The optimal dietary fat to carbohydrate ratio may differ depending on whether the goal is prevention or treatment of obesity, and public guidelines on macronutrients should either be based on the prevalence of obesity in the target society or individualized.

Acknowledgments

This work was supported by research grants from the Japanese Ministry of Health, Labor and Welfare (Tokyo, Japan).

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J Nutr Sci Vitaminol, 57, 418–425, 2011

An Evaluation of Protein Intake for Metabolic Demands and the Quality of Dietary Protein in Rats Using an Indicator Amino Acid Oxidation Method

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(Received June 27, 2011)

Summary Currently, protein requirements are generally determined based on nitrogen balance studies, but there are a variety of limitations associated with this method. The indicator amino acid oxidation (IAAO) method, with a theoretical base that differs widely from the nitrogen balance method, was developed as an alternative method for humans. The objective of the present study was to evaluate protein intakes for metabolic demands and protein quality, using protein itself, in rats employing the IAAO technique with L-[1-^{13}C]phenylalanine. Male Wistar/ST rats (5–6 wk old) received a graded casein (4.3, 8.6, 12.9, 17.2, 21.5, 25.8%), or a wheat gluten (7.2, 10.8, 14.4, 18.0, 21.6, 25.2%) diet, along with L-[1-^{13}C]phenylalanine. An isotopic plateau in breath was achieved 210 min after the start of the ^{13}C ingestion. The protein intakes for metabolic demands were calculated by applying a mixed-effect change-point regression model to breath $^{13}CO_2$ data, which identified a breakpoint at minimal breath $^{13}CO_2$ in response to graded protein intake. The protein intakes for metabolic demands determined by the IAAO method were 13.1 g/kg BW/d for casein and 18.1 g/kg BW/d for wheat gluten, showing a tendency similar to that determined by the nitrogen balance method. These results demonstrated that the IAAO method could be employed to evaluate not only the protein intakes for metabolic demands, but the dietary protein quality in freely living rats, suggesting that this method might be viable in a clinical setting.

Key Words protein metabolic demand, protein quality, indicator amino acid oxidation, rats

The nitrogen balance method is normally employed to determine protein requirements, as specified in the 2007 WHO/FAO/UNU (*1*). However, the limitations of the nitrogen balance method, which can result in considerable error in the prediction of balance (*2*, *3*), have been well described (*4–6*). In the nitrogen balance method, after the diet has been changed, a period of time is usually allowed for adaptation to be complete during the first 5–7 d (*7*). Therefore, employing the nitrogen balance method, the metabolic demand for protein cannot be assessed in patients with a widely varying metabolic demand. The indicator amino acid oxidation (IAAO) method was originally employed to study amino acid requirements in pigs (*8*), and thereafter it has been widely used for studies on pigs (*9–11*) and humans (*12–17*). Since the IAAO method does not require prior dietary adaptation (*18*) to each of the varying protein intake levels, it could be available when an assessment of the metabolic demand for protein is required for post-operative patients or patients with injuries or infections.

In 2007, Humayun et al. (*19*) applied the IAAO method and conducted a reevaluation study on the protein requirements in healthy young men by feeding the subjects graded protein intake as a crystalline amino acid mixture and measuring changes in the oxidation of orally administered L-[1-^{13}C]phenylalanine. However, no studies have previously been conducted on determining the protein requirement using protein itself in animals or humans employing the IAAO method. Therefore, sufficient evidence has not been gathered showing that the IAAO method is viable for measurements of the protein requirement, and it has not been sufficiently validated in studies employing experimental rats up to the present. We should consider that the mechanism of the assimilation of the amino acid mixture differed from that of the protein. Amino acid mix-

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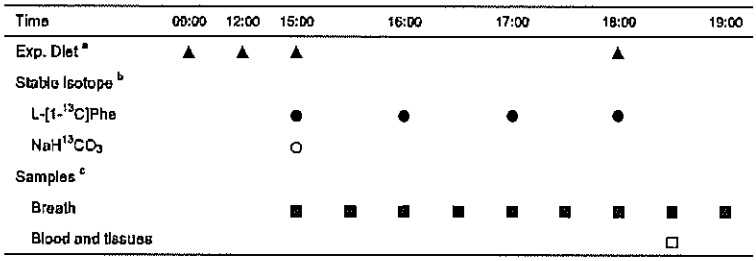

Fig. 1. The protocols employed for each IAAO study day. [a]The experimental diet was either a 4.3% or 17.2% casein diet. The diet was provided every 3 h (9:00–18:00). Each meal represented one-eighth of each rat's daily intake. [b]Isotope: Priming doses of L-[1-^{13}C]phenylalanine and $NaH^{13}CO_3$ were started with the third meal at 15:00, and the infusion of L-[1-^{13}C]phenylalanine was continued hourly until the end of the study. [c]Sample collection: Baseline breath sample was collected before the isotope protocol began. Nine breath samples were collected every 30 min after the initiation of the isotope protocol. Samples of blood, liver, and gastrocnemius muscle were collected at 18:30.

tures will be absorbed very rapidly, and protein utilization will show a higher efficiency, compared with slow proteins such as casein (*20*). Incidentally, a previous study by Moehn et al. (*21*) evaluated the metabolic availability of amino acids in peas, and they indicated the applicability of using IAAO for intact protein sources.

Measurements of the quality and quantity of the dietary protein employed can be used to facilitate adjustments to the diet to ensure that the metabolic demands for protein can be met sufficiently. Poor protein quality compromises the nutritional status and increases the protein requirement. In the 1991 FAO/WHO/UNU report (*22*), the protein digestibility corrected amino acid score (PDCAAS) value for casein is 1.00, compared with 0.25 for wheat gluten. Therefore, the protein requirement calculated for rats fed a wheat gluten diet is higher than that for rats fed a casein diet. In a clinical setting, the adequate quality and quantity of protein or amino acid for each disease might be estimated using the IAAO method.

The objective of the present study was to establish whether or not the IAAO method is viable for determining the metabolic demand for protein and to evaluate protein quality using protein itself, employing casein and wheat gluten as protein sources in experimental diets and using the IAAO method with L-[1-^{13}C]phenylalanine.

MATERIALS AND METHODS

Animals. This study was performed in accordance with the guidelines for animal experimentation at Kyoto Prefectural University, Japan. Male Wistar/ST rats (4 wk old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The rats were housed in individual mesh cages under controlled temperature (22±2°C) and lighting (lights on from 08:00 to 20:00) conditions. The rats were given free access to water and a 17.2% casein maintenance diet, and they were allowed to adapt to the laboratory environment for at least 1 wk before starting the experiment. After adaptation, 5- to 6-wk-old rats (initial BW=130.1±2.3 g) were used for the experiment. The amount of feed available and any feed not eaten were recorded for each rat for 3 d before the first study day, and the total daily intake for each rat, equivalent to the 24-h dietary intake, was calculated on the basis of the average intake during the previous 3 d.

Experiment 1. The objective of Experiment 1 was to examine the effect of L-[1-^{13}C]phenylalanine administration on breath $^{13}CO_2$ enrichment, and to evaluate whether the protein metabolism could be measured by the IAAO method in rats consuming different protein level diets. All of the eight rats were included in two IAAO studies, consuming both 4.3% and 17.2% casein diets (N×6.38) (*23*) with a time period of more than 2 d between the studies. The 17.2% casein maintenance diet employed for all of the studies was provided for at least 24 h. Then, the rats fasted overnight for 13 h from 20:00 on the day before the study day, but had free access to drinking water. The study protocol for all of the IAAO studies is depicted in Fig. 1. On the study day, the rats were weighed in the morning before feeding. Then, they received either 4.3% or 17.2% casein diets (Table 1). The study-day diet was provided in 4 isoenergetic, isonitrogenous diets, and each meal accounted for one-eighth of the rat's total daily intake. Specifically, the casein diet was consumed beginning at 09:00 and continued at each 3-h interval until 18:00 for a total of 4 meals. The rats were allowed free access to drinking water during the experiment period. The rats were fed the remaining half of the daily ration in the evening. The tracer protocol was started with the third meal at 15:00 to measure the phenylalanine kinetics with the use of L-[1-^{13}C]phenylalanine, and continued hourly until 18:00. The rats were placed in the chamber immediately after the oral administration of the ^{13}C substance. Breath samples were collected, and the $^{13}CO_2$ level in breath CO_2 was measured at 30-min intervals from 15:00 to 19:00. Baseline breath samples were collected before the isotope protocol began at 15:00. On a later day, the rats were dissected at 18:30; blood, liver and gastrocnemius muscle samples were collected for subsequent analysis of amino acid concentration in plasma and tissues.

Experiment 2. The protein intake for metabolic demands was measured using the IAAO method for rats fed the casein diets, and also for rats fed diets based on

Table 1. Composition of experimental diets.

Protein	Casein diet						Wheat gluten diet					
	4.3%	8.6%	12.9%	17.2%	21.5%	25.8%	7.2%	10.8%	14.4%	18.0%	21.6%	25.2%
	g/kg diet						g/kg diet					
Casein[1,2]	50	100	150	200	250	300	—	—	—	—	—	—
Wheat gluten[3,4]	—	—	—	—	—	—	100	150	200	250	300	350
Cornstarch[1]	557	523	490	457	423	390	527	498	470	440	411	383
Sucrose[1]	278	262	245	228	212	195	265	250	235	221	206	190
Rapeseed oil[5]	35	35	35	35	35	35	31	27	22	18	14	9
Soy bean oil[6]	15	15	15	15	15	15	12	10	8	6	4	3
Vitamins[1,7]	10	10	10	10	10	10	10	10	10	10	10	10
Minerals[1,8]	35	35	35	35	35	35	35	35	35	35	35	35
Cellulose[1]	20	20	20	20	20	20	20	20	20	20	20	20
L-Phenylalanine[9]	11	9	7	5	2	—	9	7	5	3	1	—
L-Tyrosine[10]	13	10	8	5	3	—	13	11	10	9	8	6
Energy (kJ/g)	15.4	15.4	15.5	15.5	15.5	15.6	15.5	15.5	15.5	15.5	15.6	15.6

[1] Oriental Yeast Co., Ltd., Japan.

[2] Protein, 86.2% (N×6.38). Amino acid (mg/100 g Casein): L-alanine, 2,700; L-arginine, 3,300; L-aspartic acid, 6,300; L-cysteine, 430; L-glutamic acid, 19,000; L-glycine, 1,600; L-histidine, 2,700; L-isoleucine, 4,900; L-leucine, 8,400; L-lysine, 7,100; L-methionine, 2,600; L-phenylalanine, 4,500; L-proline, 10,000; L-serine, 4,600; L-threonine, 3,700; L-tryptophan, 1,100; L-tyrosine, 5,000; L-valine, 6,000; total, 93,930.

[3] Weston Bioproducts Ltd., Queens land, Australia.

[4] Protein, 72.0% (N×5.70). Amino acid (mg/100 g wheat gluten): L-alanine, 2,100; L-arginine, 2,700; L-aspartic acid, 2,700; L-cysteine, 1,600; L-glutamic acid, 29,000; L-glycine, 2,700; L-histidine, 1,800; L-isoleucine, 3,000; L-leucine, 5,400; L-lysine, 1,400; L-methionine, 1,300; L-phenylalanine, 4,100; L-proline, 11,000; L-serine, 3,600; L-threonine, 2,000; L-tryptophan, 780; L-tyrosine, 2,500; L-valine, 3,300; total, 80,980.

[5] Nisshin Oillio Ltd., Japan.

[6] Wako Pure Chemical Industries, Ltd., Japan.

[7] AIN-76™ vitamin mixture (per g mixture): vitamin A, 400 IU; vitamin D_3, 100 IU; vitamin E, 5 mg; vitamin K_3, 0.005 mg; vitamin B_1, 0.6 mg; vitamin B_2, 0.6 mg; vitamin B_6, 0.7 mg; vitamin B_{12}, 0.001 mg; D-biotin, 0.02 mg; folic acid, 0.2 mg; calcium pantothenate, 1.6 mg; nicotinic acid, 3 mg; choline chloride, 200 mg; sucrose, 0.968 g.

[8] AIN-76™ mineral mixture (g/kg mixture): calcium phosphate dibasic, 500.0; sodium chloride, 74.0; potassium citrate, 220.0; potassium sulfate, 52.0; magnesium oxide, 24.0; manganese carbonate, 3.5; ferric citrate, 6.0; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.0066; chromium potassium sulfate, 0.55; sucrose, 118.03.

[9] L-Phenylalanine content was kept constant at 13,500 mg/kg diet in all diets, except the 25.2% wheat gluten diet (14,350 mg/kg diet).

[10] L-Tyrosine content was kept constant at 15,000 mg/kg diet in all diets.

wheat gluten instead of casein to determine whether it was important to consider the effects of the source of the protein in the diet. Sixteen rats were used, and even when they were measured for the wheat gluten diets, the 17.2% casein diet was provided as a maintenance diet for the 2 d before the study day for all of the IAAO studies. On the study day, eight rats received, in random order without repeats, one of six levels of the casein (4.3, 8.6, 12.9, 17.2, 21.5, 25.8%) diet (N×6.38) (*23*), and the other eight rats received one of six levels of the wheat gluten (7.2, 10.8, 14.4, 18.0, 21.6, 25.2%) diet (N×5.70) (*23*). The tracer protocol employed was the same as that employed in Experiment 1, and ^{13}C substance administration was performed for a total of four times at 15:00, 16:00, 17:00, and 18:00. However, breath samples were collected and the $^{13}CO_2$ level in the breath was measured only twice at 15:00 and 18:30. The experimental design was a completely randomized crossover design. Eight rats consumed the casein diet at all six levels, and the other eight rats consumed the wheat gluten diet at all six levels. Each IAAO study day was separated by 2 d, and the six IAAO studies were completed within 2 wk. Except for these points, all of the protocols were the same as those employed in Experiment 1.

Tracer administration protocol. L-[1-^{13}C]Phenylalanine (Cambridge Isotope Laboratories, Andover, MA) and $NaH^{13}CO_3$ (Cambridge Isotope Laboratories) were used as tracers. Labeled compounds were dissolved in saline and stored at 4°C. Isotopic solutions were prepared and administered in a volume of 2.5 mL/kg BW. Oral priming doses of 0.88 mg/kg BW $NaH^{13}CO_3$ and 7.92 mg/kg BW $NaHCO_3$ were given with the third meal at 15:00. An oral dosing protocol of 3.3 mg/kg BW L-[1-^{13}C]phenylalanine and 29.7 mg/kg BW phenylalanine was commenced simultaneously with the third meal, and administration of 6.0 mg/kg BW L-[1-^{13}C]phenylalanine and 54.0 mg/kg BW phenylalanine

was performed hourly until the end of the study.

Experimental diets. The composition and source of the powdery experimental casein and wheat gluten diets are shown in Table 1. Casein and wheat gluten provided the sole source of protein in the casein and wheat gluten diets, respectively. The compositions of the amino acids in the casein and wheat gluten are shown in the footnote to Table 1 (*23*). L-Phenylalanine and L-tyrosine were added to the diets to achieve an equal content of these amino acids in all diets. In the present study, L-phenylalanine (13.5 g/kg diet) and L-tyrosine (15.0 g/kg diet) were consumed in excess of these amino acid requirements for rodents (L-phenylalanine, 8.8 g/kg diet; L-tyrosine, 9.3 g/kg diet) (*24*), in order to minimize the net hydroxylation of phenylalanine to tyrosine. Each casein diet with varying protein content was kept at an identical energy level by varying the levels of sugar and starch. The oil levels in the wheat gluten diet were decreased because the energy level of wheat gluten is higher than those of casein. Thus, all of the diets had a similar energy level (15.4–15.6 kJ/g).

Breath sample collection and analysis. The instruments used for the collection of breath samples in the rats consisted of an acrylic chamber (10.6 L) fitted with a drinker, an aspiration pump (Columbus Instruments, Columbus, OH) and an air flow meter (Columbus Instruments). The chambers were continuously charged with fresh room air through the aspiration tube by a pump. The rats were moved outside the chamber for the administration of the ^{13}C substance, and thereafter moved back into the same chamber. Because the chambers filled with expired air were necessary in order to collect the breath samples, rats were placed in separate compartments for 30 min before the collection of the breath samples.

Breath samples of 200 mL volume drawn into a 200 mL syringe were injected into breath-sampling bags (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The $^{13}CO_2$ concentration in the expired air was measured by attaching the breath-sampling bags to the sampling joint of an infrared spectrometer (POCone; Otsuka Electronics Co., Ltd., Tokyo, Japan). Using the measurement system provided by POCone, the concentration of CO_2 in the aspirated air in the breath sampling bags was at least more than 0.5%. Therefore fresh room air was drawn through the system at comparatively low rates of approximately 0.4 L/min, and the CO_2 concentration within the chamber was stabilized at 0.8–1.2%. The $^{13}CO_2$ rate was measured as the $^{13}CO_2/^{12}CO_2$ ratio, and followed by a pulse of mixed gas composed of 5% CO_2, 12% O_2 and the rest of the mixture was N_2 for the control. Isotopic abundances were expressed relative to the international Vienna Pee Dee Belemnite standard (‰) as over the baseline ($\Delta - \Delta_0$) value, further normalized by each rat's weight.

Blood and tissue samples collection and analysis. Blood samples drawn from the inferior vena cava were collected in tubes with heparin, and plasma was separated from the blood samples by centrifugation at 1,500 $\times g$ for 5 min. The plasma was stored at −20°C until it was analyzed. The liver and gastrocnemius muscle were rapidly removed and snap-frozen in liquid nitrogen and stored at −80°C for analysis. Approximately 0.5 g of liver and muscle were homogenized in 4.5 mL of saline, centrifuged at 1,000 $\times g$ for 10 min.

A 100 μL plasma sample and the supernatant of liver and muscle obtained as described above were deproteinized with 300 mL ethanol and centrifuged at 1,500 $\times g$ for 10 min. A 200 mL sample of the supernatant fluid was cleared of contamination by using a strong cation exchanger (AG 50W-X8, Bio-Rad Laboratories, Hercules, CA), dried under a vacuum, derived to its 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) derivative using the Waters AccQ, Fluor Reagent Kit (Waters Corp., Milford, MA) and dried. Then the supernatant fluid was reconstituted in 200 μL of 0.1% formic acid. Phenylalanine and tyrosine concentrations were measured by an HPLC system. The individual amino acids were separated by an Inertsil ODS-3 column (250×4.6 mm, GL Sciences, Tokyo, Japan) with a binary LC gradient (0–60% aqueous acetonitrile containing 0.1% formic acid). The areas under the peaks were integrated using Peak Net 5.1c (Dionex Corp., Osaka, Japan). L-[1-^{13}C]Phenylanine and L-[1-^{13}C]tyrosine enrichment in the plasma and tissue samples was analyzed with a MS (LCQ Fleet, Thermo Scientific, Waltham, MA) coupled to the HPLC system. Selected ion chromatograms were obtained by monitoring ions *m/z* 336 and 337 for L-phenylalanine and L-[1-^{13}C]phenylalanine, *m/z* 352 and 353 for L-tyrosine and L-[1-^{13}C]tyrosine, respectively.

Statistical analysis. Data analysis was performed using Statcel2 software (Oms Publishing Inc., Tokyo, Japan). All results were presented as the mean±SE. Values of $p<0.05$ were considered statistically significant. Student's *t* test was used to analyze differences between two different groups, such as the protein intake. Statistical analysis for multiple comparisons was performed using one-way analysis of variance (ANOVA) with repeated measures followed by a Tukey-Kramer post hoc test.

The protein intake for metabolic demands was derived by applying a mixed-effect change-point model to breath $^{13}CO_2$ data (*25*), and the regression oxidation rate of the dietary protein contents. The first regression line showed a downward slope and the second line was horizontal with minimal or no slope. The breakpoint, the protein intake with a plateau in oxidation, was regarded as the protein intake for metabolic demand.

RESULTS

Experiment 1

The rats were given free access to a 17.2% casein diet as a maintenance diet for 3 d before the first study day, and the total daily intake for each rat was 16.5±0.5 g/d (calorie, 255.9±7.8 kJ/d; protein, 2.8±0.1 g/d). The body weights for the rats used for the 4.3% and 17.2% casein diet experiments were 144.1±5.7 g and 143.5±5.0 g, respectively.

Complete data sets of 9 breath samples were obtained

in only 7 of the rats fed the 17.2% casein diet. One rat did not consume its feed completely at 18:00, which affected the $^{13}CO_2$ values thereafter. Regardless of the protein intake and the 4.3% or 17.2% casein diets, breath $^{13}CO_2$ enrichment gradually increased after the initiation of the isotope protocol (Fig. 2). The plateau breath samples were collected during the isotopic steady state every 30 min during the period from 16:30 to 19:00 in rats fed the 17.2% casein diet, and from 17:30 to 19:00 in rats fed the 4.3% casein diet. This isotope protocol had been shown to achieve a satisfactory isotopic steady state 2.5 h after the start of L-[1-^{13}C]phenylalanine isotope administration. In addition, when the 4.3% casein diet was employed, the enrichment of breath $^{13}CO_2$ was greater than that achieved with the 17.2% casein diet, and during the period from 17:30 to 19:00, significant differences were shown between the 4.3% and 17.2% casein diets on breath $^{13}CO_2$ enrichment at 18:30 ($p<0.01$) and 19:00 ($p<0.01$).

The amino acid concentrations of plasma, liver and gastrocnemius muscle obtained at 18:30 on the IAAO study day are shown in Table 2. In both phenylalanine and tyrosine, ^{13}C-amino acid concentrations, ^{12}C-amino acid concentrations, and the total of these concentrations in the plasma and tissues of rats fed the 4.3% casein diet were similar to those of rats fed the 17.2% casein diet, and there were no significant differences.

Experiment 2

The rats were given free access to a 17.2% casein maintenance diet for 3 d before the first study day. The total daily intake for each rat used for the casein and wheat gluten diet experiments employing the IAAO method were 16.7±0.3 g/d (calorie, 258.9±4.3 kJ/d; protein, 2.9±0.1 g/d) and 17.3±0.5 g/d (calorie, 268.2±7.0 kJ/d; protein, 3.0±0.1 g/d), respectively. The body weights for the rats used for the 4.3, 8.6, 12.9, 17.2, 21.5, and 25.8% casein diet experiments were 149.4±5.7, 141.0±9.9, 155.2±10.8, 147.4±3.5, 158.0±2.4, and 184.7±3.1 g, respectively. The body weights for the rats used for the 7.2, 10.8, 14.4, 18.0, 21.6, and 25.2% wheat gluten diet experiments were 130.7±3.5, 148.8± 4.8, 157.1±5.4, 160.8±10.0, 134.4±2.8, and 142.4±3.0 g, respectively.

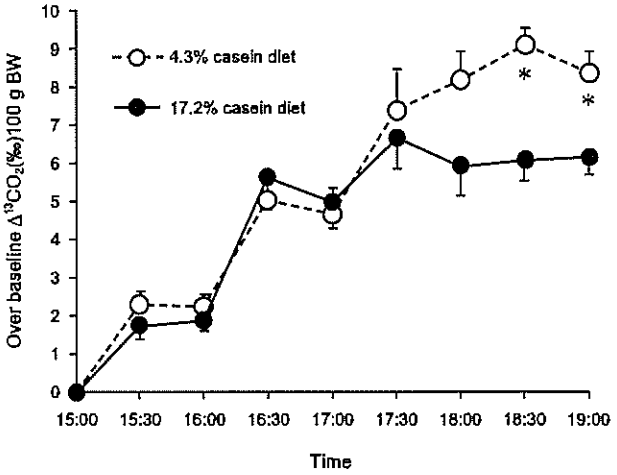

Fig. 2. The effect of L-[1-^{13}C]phenylalanine infusion on $^{13}CO_2$ enrichment of the breath. Values are mean±SE for the 4.3% ($n=8$ per mean) and 17.2% ($n=7$ per mean) casein diets. On the study day, the rats received either a 4.3% or 17.2% casein diet every 3 h from 09:00. Each meal represented one-eighth of the rat's total daily intake. The administrations of L-[1-^{13}C]phenylalanine were performed at 15:00, 16:00, 17:00, and 18:00. The establishment of a plateau in the breath samples on the basis of no significant differences among the timed samples was confirmed using repeated-measures ANOVA. The isotopic steady state was confirmed at 16:30–19:00 in rats fed the 17.2% casein diet, and at 17:30–19:00 in rats fed the 4.3% casein diet. *The asterisk marks shown significant differences ($p<0.01$) between the 4.3% and 17.2% casein diets at 18:30 and 19:00.

Table 2. The concentrations of phenylalanine and tyrosine in the plasma, liver and gastrocnemius muscle in rats fed 4.3% or 17.2% casein diets.

Diet	Phenylalanine			Tyrosine		
	^{13}C-Phe	^{12}C-Phe	Total	^{13}C-Tyr	^{12}C-Tyr	Total
			Plasma (nmol/mL)			
4.3% casein	13.2±2.9	47.2±4.3	60.4±7.0	7.5±2.0	113.0±29.4	120.6±30.7
17.2% casein	12.1±2.5	50.8±10.0	62.9±11.8	8.5±1.4	119.8±15.2	128.3±16.2
			Liver (nmol/g)			
4.3% casein	10.6±0.4	40.9±5.1	51.5±4.9	7.4±1.3	99.4±32.0	106.9±33.2
17.2% casein	10.4±2.1	43.1±10.5	53.6±12.3	8.8±2.8	92.5±7.5	101.4±9.2
			Gastrocnemius muscle (nmol/g)			
4.3% casein	13.0±1.7	46.6±4.5	59.6±5.7	8.4±0.8	91.9±8.7	100.3±8.5
17.2% casein	11.6±1.9	48.2±2.5	59.8±3.6	7.0±1.1	84.7±5.8	91.7±5.0

Values are shown as mean±SE for the 4.3% ($n=5$) and 17.2% ($n=5$) casein diets. Student's t test was performed to assess the effect of protein intake. No significant differences were demonstrated in the plasma and tissues phenylalanine or tyrosine concentrations between the 4.3% and 17.2% casein diets.

^{13}C-Phe, L-[1-^{13}C]phenylalanine; ^{12}C-Phe, L-[1–^{12}C]phenylalanine; ^{13}C-Tyr, L-[1-^{13}C]tyrosine; ^{12}C-Tyr, L-[1–^{12}C]tyrosine.

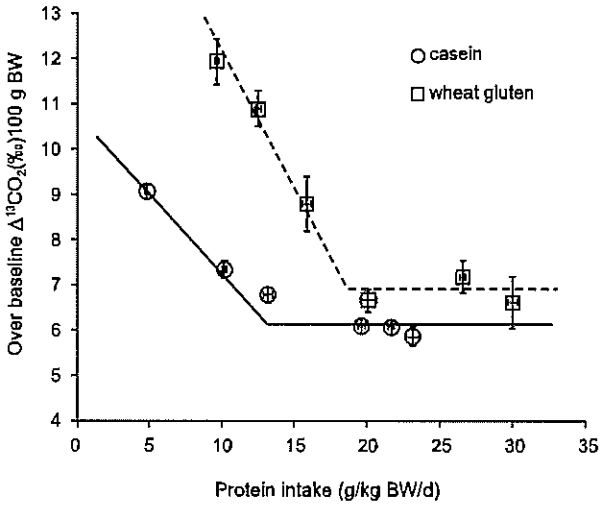

Fig. 3. The relationship between the intake of various proteins and the production of $^{13}CO_2$ from the oxidation of L-[1-^{13}C]phenylalanine when the rats were fed a casein diet ($n=8$ per mean) or a wheat gluten diet ($n=8$ per mean). Values are shown as mean±SE. The breakpoint estimates the mean protein intake for metabolic demands. The linear regression equation for the estimated protein intake for metabolic demands for the casein diet is as follows: $y=10.73-0.35x$ and $y=6.17$, for the wheat gluten diet is as follows: $y=18.87-0.66x$ and $y=6.92$, for the downward slope of the line and the level part of the line, respectively. The protein (%) included in the casein and wheat gluten diets was converted into protein intake (g) per day, and further normalized according to each rat's body weight. The mean protein intakes for metabolic demands for the casein and wheat gluten diets were estimated to be 13.1 g/kg BW/d and 18.1 g/kg BW/d, respectively.

Figure 3 shows the mean breakpoints illustrated in the breath $^{13}CO_2$ data, which were representative of the mean protein intake for metabolic demands. As the protein intake increased, breath $^{13}CO_2$ decreased steadily until the breakpoints were reached. There was no further decrease in breath $^{13}CO_2$ with the increase in protein intake. The protein (%) included in the casein and wheat gluten diets was converted into protein intake (g) per day, and further normalized according to each rat's body weight. Application of a mixed-effect change-point regression models to the breath $^{13}CO_2$ data resulted in the identification of a breakpoint at a dietary casein intake of 13.1 g/kg BW/d and a dietary wheat gluten intake of 18.1 g/kg BW/d. The enrichment of breath $^{13}CO_2$ was consistently higher in rats fed the wheat gluten diet, compared with the casein diet.

DISCUSSION

In the current IAAO study on rats, the protein intake for metabolic demands was estimated to be covered by a 13.1 g/kg BW/d for casein. This result was similar to the value recommended by the AIN-93G diet for laboratory rodents (a purified 20% casein (≅85% protein)), which was developed based on nitrogen balance studies. According to procedures recommended by the AIN, values were converted to dietary content by assuming a dietary intake of 15 g/rat/d for growing rats, and also for the rats fed 16.7±0.3 g/rat/d in the present study. This is the first study conducted that employed the IAAO method to determine the protein intake for metabolic demands using protein itself in rats, and the determined the protein intake for metabolic demands should be considered provisional.

Temperature, age, and physical activity influence the energy requirements of rats. It is difficult to estimate the energy requirement for growth due to variations in the composition of weight gain (*26–30*) and variations in the energetic efficiency of net protein and fat synthesis. However, it has been suggested that rats will generally consume enough food to meet their energy requirements (*31, 32*). The AIN-93 specifications indicate that a diet containing at least 15.0 kJ/g will meet the energy requirement for maintenance and growth if the rats are allowed free access to food and the diet is not deficient in other nutrients. In the present study, the rats accepted a 15.5 kJ/g diet containing 17.2% casein as a maintenance diet. Furthermore, the rats were given free access to this diet and the 24-h dietary intake was regarded as an individual rat's energy requirement.

Humayun et al. (*19*) reevaluated the protein requirement in young men employing the IAAO method, and the protein source of the experimental diet was consumed hourly in small meals consisting of a crystalline amino acid mixture. In the present study, casein and wheat gluten were employed as the protein source, and therefore, as the rats consumed the experimental diet at 3-h intervals, it can be considered that the mechanism of assimilation differed from that of the amino acid mixture. Amino acid mixtures will be absorbed very rapidly, and protein utilization will show a lower efficiency, compared with slow proteins such as casein (*20*).

The phenylalanine and L-[1-^{13}C]phenylalanine concentrations in the plasma, liver and gastrocnemius muscle were not affected by the amount of protein intake in the 4.3% or 17.2% casein diets, suggesting that the precursor pool for indicator oxidation did not change in size in response to the test protein intake. After phenylalanine is hydroxylated, conversion to tyrosine takes place, so the tyrosine concentration was also examined. In comparison with the ratio of L-[1-^{13}C]phenylalanine to the total phenylalanine concentration, only a trace of L-[1-^{13}C]tyrosine to the total tyrosine occurred in the plasma and tissues, regardless of the protein intake, suggesting that there was no tyrosine deficiency. In previous studies, the loss of the ^{13}C into the protein-bound tyrosine pool or tyrosine metabolites was minimized by providing a high-tyrosine diet before the study (*19*).

$^{13}CO_2$ breath tests are normally performed in the presence of a large background of naturally occurring isotope of approximately 1.1% ^{13}C (*33*). The ^{13}C rate of any unlabeled substrate ingested during a $^{13}CO_2$ breath test must be considered in order to eliminate artifacts that may reduce the sensitivity of the breath test and produce erroneous results (*33*). In our preliminary

examination, the stable rate of $^{13}CO_2$ production in breath was achieved between 5 h and 6 h and maintained until the end of the study. These results suggested that two meals received every 3 h were required to achieve constant $^{13}CO_2$ enrichment, and that the effect of the ^{13}C infusion could be evaluated correctly after the third meal at 15:00.

Experiment 1 demonstrated a similar pattern and a latter steady state ~2.5 h after the start of the stable isotope protocol (Fig. 2), so breath samples for the measurement of the protein metabolism were collected 210 min after the administration of the stable isotope began. Moreover, the protein intake level, the 4.3% or 17.2% casein diets, had a significant effect on breath $^{13}CO_2$ concentration at 18:30, showing that this protocol could detect differences in protein metabolism. These results reflected the supposition that if one indispensable amino acid (limiting) was deficient for protein synthesis, then all other indispensable amino acids (including the indicator amino acid, [^{13}C]phenylalanine) would be oxidized. Therefore, when the rats were fed a low protein diet, the 4.3% casein diet, most of amino acids were oxidized, and the $^{13}CO_2$ concentration in breath increased. By increasing the protein intake with the 17.2% casein diet, the intake of the limiting amino acid also increased, and the values produced by the IAAO method decreased, reflecting the increasing incorporation into protein.

The mean protein intakes for metabolic demands determined by the IAAO method were 13.1 g/kg BW/d for the casein and 18.1 g/kg BW/d for the wheat gluten. Therefore, the protein intakes for metabolic demands based on wheat gluten was higher than that based on casein. The differences between the casein and wheat gluten diets will be a function of the limiting amino acid in the respective protein source. This limiting amino acid will be dependent on both the amino acid profile and the digestibility of the protein. These results also conformed with our hypothesis, that the protein requirement will decrease with good quality (amino acid scoring pattern) protein intake, and increase with poor quality protein intake, validating the concept that the IAAO method could be employed to evaluate the quality of protein.

In regard to the measured phenylalanine oxidation, the enrichment of breath $^{13}CO_2$ differed between the rats fed the casein and wheat gluten diets. The enrichment of breath $^{13}CO_2$ was consistently higher in rats fed the wheat gluten diet, compared with rats fed the casein diet, even at the plateau line with a protein intake more than the metabolic demand for protein. According to intake of protein, specifically, the limiting amino acid, the indicator amino acid is partitioned between incorporation into proteins and oxidation. The quality of the protein also affected the $^{13}CO_2$ volume in the breath. Future extensions of this study to other protein sources will be necessary in order to confirm this relationship.

Hegsted (*34*) suggested the necessity of taking account of adaptation in their nitrogen balance methods, arguing that prior adaptation is required. The IAAO method can be conducted in short time periods because no period of adaptation to each intake is employed (*35*). Therefore, the IAAO method could be employed to evaluate the metabolic protein demand for all age groups (infants, children, adolescents, adults, and the elderly), as well as for post-operative patients or patients with injuries or infections that have specific metabolic conditions, such as a widely varying metabolic demand. In a clinical setting, the adequate quality and quantity of protein or amino acid for each specific condition could be estimated using the IAAO method.

The results of this study demonstrated that the IAAO method can be employed to evaluate not only the protein intake for metabolic demands, but the dietary protein quality in freely living rats. Further studies are necessary to assess the viability of the IAAO method in a clinical setting.

Acknowledgments

We would like to thank Professor Kenji Sato for technical support. This study was supported in part by a Health and Labor Sciences Research Grant entitled "Studies on the Dietary Reference Intakes (Recommended Dietary Allowance) for Japanese" from the Ministry of Health, Labor and Welfare, Japan; Grant-in Aid for Scientific Research (C) (23617016) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. None of the authors have any conflicts of interest.

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Original Article

Association between 24 hour urinary α-tocopherol catabolite, 2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman (α-CEHC) and α-tocopherol intake in intervention and cross-sectional studies

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The objective is to determine the association between the 24 hour urinary α-tocopherol catabolite, 2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman (α-CEHC) and α-tocopherol intake in an intervention and a cross-sectional studies. In the 4-weeks intervention study, Japanese men (n = 10) consumed the test diet in week 1, and the test diet plus varying amounts of α-tocopherol in the three subsequent weeks: 21 μmol/d α-tocopherol in week 2, 63 μmol/d in week 3, and 125 μmol/d in week 4. A significant association between α-tocopherol intake and urinary α-CEHC was observed in this strictly controlled experiment (r = 0.99, p<0.001). In the cross-sectional study, all foods consumed over 4 consecutive days were recorded in 76 free-living young subjects (18-33 years). The association was weak, but a significant relationship was observed (r = 0.29, p<0.05) even in the cross-sectional study. In the cross-sectional study adults, mean estimated α-tocopherol intake calculated by urinary α-CEHC and the excretory ratio was 91% of their mean intake over the 4 days. The results show that urinary α-CEHC level reflected recent α-tocopherol intake in free-living young Japanese adults, and could be used as a measure of intake during the previous few days, both for group means and for individual rankings within a group.

Key Words: α-tocopherol, catabolism, CEHC, urine, biomarker

INTRODUCTION

Measurements of food intake are widely used for surveys of nutritional assessment. However, there are limitations in assessing only information from food surveys,[1] and methods that measure biological parameters can reveal new information. Urine, which is a noninvasive bio-sample, might overcome the limitations of nutritional assessment by food survey. For example, 24-hour urinary nitrogen level is established as a marker for protein intake,[2] urinary potassium level as a marker for potassium intake,[3] and urinary sugar level as a marker for sugar intake.[4,5] In previous studies, we investigated the relationship between water-soluble vitamin intake and their urinary excretion of these nutrients. We clarified that urinary water-soluble vitamin levels are strongly correlated with their intake.[6-9] These studies [6-9] have indicated that 24-h urinary excretion of water-soluble vitamins is a potential biomarker for recent vitamin intake in both intervention and cross-sectional studies.

Generally, fat-soluble vitamins are not excreted in urine. However, In 1995, Schultz *et al*,[10] reported that a catabolite of α-tocopherol, 2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman (α-CEHC), which is a metabolite with an intact chroman ring, is excreted in urine. Previously, α-CEHC was proposed as a potential excretion product of α-tocopherol in 1965 by Schmandke *et al*,[11] but had not been described again until Schultz's report. Schultz *et al*,[10] suggested that α-CEHC excretion indicates the saturated binding capacity of α-tocopherol in the plasma, and thus may be considered to be a marker of optimum α-tocopherol intake. This proposal was strengthened by Shuelke *et al*,[12] who found that α-CEHC was excreted into the urine of patients with α-tocopherol binding protein defects regardless of the plasma α-tocopherol concentration, whereas it was not excreted by healthy subjects until the plasma α-tocopherol concentration

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Manuscript received 7 February 2011. Initial review completed 2 June 2011. Revision accepted 12 July 2011.

surpassed 40 μmol/L. These findings indicate that the urinary content of α-CEHC in the Japanese is almost below detection, because the average plasma concentration of α-tocopherol in the Japanese is around 20 μmol/L.[13] However, as Schultz *et al*,10 pointed out in their paper, their results were obtained with only seven participants, and therefore, further research is necessary to allow the results to be applied generally.

In 2003, Morinobu *et al*,[14] reported a straightforward and reliable method of determining α-CEHC, which was later modified by Stahl *et al*[15] and Lodge *et al*[16] Morinobu *et al*[14] also reported that α-CEHC was detected in healthy adult male Japanese volunteers (n = 14). Therefore, it is probable that α-CEHC is excreted into urine in Japanese individuals who take an ordinary amount of α-tocopherol.

The aim of the present study was to determine the possibility that α-CEHC can be used as a biomarker of α-tocopherol status in young Japanese adults. We examined the association between 24 h urinary α-CEHC levels and the intakes of α-tocopherol in strictly controlled-living and in free-living participants.

MATERIALS AND METHODS

Both studies were reviewed and approved by the ethics committee of The University of Shiga Prefecture. 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.

Subjects and experimental design

Intervention study group

We recruited students from a registered dietician department. All subjects (male Japanese college students, n = 10) were housed in the same facility and given the same diet. The experimental period was 4 weeks. They did not have regular use of medications or dietary supplements, or habitual alcohol or cigarette consumption. Age, body weight, height, and body mass index (mean ± SD) were 22.1 ± 2.3 years (18–25 years), 63.6 ± 5.2 kg, 174 ± 5 cm, and 21.0 ± 1.6 kg/m^2, respectively.

Cross-sectional study group

A total of 102 healthy, free-living Japanese females, aged 18-33 years, voluntarily participated in this study. The exclusion criteria were: presence of cold or influenza, and use of multivitamin supplements at least once during the previous month. In addition, we excluded participants whose 24-hour urine collection or dietary records were considered as incomplete, with a collection time outside the 22-26 h range, a urine volume of <250 ml, creatinine excretion in relation to body weight outside the 10.8-25.2 mg/kg range,[17,18] or extremely low or high energy intake (<2,090 or >16,700 kJ/d).[19] After these exclusions, 76 of the 102 female students were found to be eligible and were enrolled into the group.

Dietary records

Intervention study group

The diet given to the participants consisted of a breakfast of bread, margarine, ham, yoghurt, tomato, lettuce, and milk; a lunch of rice, toasted and seasoned laver (a type of seaweed), luncheon meat, boiled egg, raw cabbage, miso soup, and Japanese tea; and an evening meal of rice, soy sauce, seasonal Pacific saury (a type of fish), tofu (soybean curd), boiled spinach leaves, kiwi fruit and Japanese tea, with a midnight snack of cheese and jelly fruit mix. The daily intakes of energy and nutrients form the basal diet are shown in Table 1. Nutrients were calculated by using Standard Tables of Food Composition in Japan, (fifth revised and enlarged edition).[20] The subjects ate this diet on days 1-5 of each week over the experimental period of 4 weeks and were free to eat what they wanted on days 6 and 7. In the latter three weeks, the participants took α-tocopherol acetate in addition to the diet: 21 μmol/d in week 2, 63 μmol/d in week 3, and 125 μmol/d in week 4.

Table 1. Daily intake of energy and nutrients form the basal diet in intervention study

Energy and nutrients[1]	Amount
Energy (kJ)	11,100
Protein (g)	97.5
Fat (g)	86.7
Carbohydrates (g)	361
α-Tocopherol (mg)	8.7 (20.2 μmol)

[1]Nutrients were calculated from the Standard Tables of Food Composition in Japan.[20]

Cross-sectional study group

This group underwent a 4-day dietary assessment in which the participants were living freely in the university and consuming their normal diet. The 4-day assessment began on a Monday (day 1) and ended on Thursday (day 4). All food consumed during the 4-day period was recorded using a weighed food recording method.[21] A digital cooking scale capable of weighing in 1 g increments (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. In the dietary record, the ingested food was described (eg "raw", "boiled", "cooked", "skin present", "a part of cooking ingredients", or "with or without seasoning"), and coded according to the Standard Tables of Food Composition in Japan (fifth revised and enlarged edition) as for the intervention group.[20] The participants took photographs with a disposable camera of the dishes 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 on the digital scales and was deducted from the dietary record. Food, nutrient and energy intakes were calculated using SAS statistical software (version 6.12; SAS Institute, Cary, NC, USA), based on the Standard Tables of Food Composition in Japan.

Collection of 24 hour urine sample

For the intervention study group, the 24 hour urine samples were collected from the second urination on day 4 to the first urination after 06:30 hours (wake-up time) on day 5 in each week**.**

For the cross-sectional group, a single 24-hour urine sample was collected on day 4 to measure the α-

tocopherol metabolite, α-CEHC. In the morning, participants were asked to discard the first specimen and to record the time on the sheet. The following morning, participants were asked to collect a specimen at the same time as the discarded specimen from the previous morning and to record the time on the sheet.

After the urine samples were collected, the sample volumes were measured, and aliquots of the urine were stabilized to avoid destruction of α-CEHC. All treated urine samples were then stored at –20°C until analysis.

Chemicals

α-CEHC was purchased from Cayman Chemical Co., Ltd (Ann Arbor, Michigan, USA). β-Glucuronidase derived from *Escherichia coli* was obtained from Nacalai Tasque Co., Ltd (Kyoto, Japan). All other chemicals used were of the highest purity available from commercial sources.

Analysis of α-CEHC

The concentrations of α-CEHC in urine were measured by high performance liquid chromatography with electrochemical detection (HPLC-ECD), as described by Morinobu *et al.*[14] β-Glucuronidase (25,000 units) was dissolved in 2.5 ml of 0.1 mol/L sodium acetate-acetate buffer (pH 4.5) on ice and used to hydrolyze the conjugate immediately after preparation. Urine (1 ml) was placed into a tube with 1,000 U (100 µl) of β-glucuronidase, 100 µl of 57 mmol/L ascorbic acid, and 20 µl of 0.015% dibuthylhydroxytoluene (BHT). After which the mixture was incubated for 4 hours at 37°C to achieve hydrolysis, 50 µl of 6 mol/L HCl and 2 ml of diethylether were added to stop the reaction. After mixing by vortex and separating by centrifugation at 1,800×*g* for 10 min, 1 ml of the diethylether layer was collected and evaporated to dryness. The residue was dissolved in 200 µl of 0.015% BHT, and a 20 µl aliquot was injected into the HPLC-ECD.

Statistics

SPSS software (version 16 for Windows; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Values were presented as means ± SD. The daily measurements of urinary α-CEHC and the dietary α-tocopherol intakes were not normally distributed, therefore, the data were converted logarithmically. Pearson correlation coefficients were calculated to determine the association between urinary and dietary measurements. The value of the urinary excretory ratio (%) was calculated as follows: [α-CEHC excretion in the fourth day (µmol/d)/the average α-tocopherol intake during 4-days (µmol/d)]×100. An analysis of variance (ANOVA) random effects model was used to quantify inter- and intra-individual percentage coefficient of variance (% CV), which was used to estimate the variability in α-tocopherol intake.

RESULTS

Relationship between the intake of α-tocopherol and the urinary excretion of α-CEHC

Intervention study group

Figure 1 shows the relationship between the intake of α-tocopherol and the urinary excretion of α-CEHC. A strong significant association was observed ($r = 0.99$, $p = 0.0043$). The average urinary α-CEHC concentration at baseline (week 1) was 0.74 µmol/d, which increased to 0.94, 1.89, and 3.34 µmol/d after supplementation at the doses of 21, 63 and 125 µmol/d, respectively. The excretory ratio of α-CEHC was 3.7 ± 1.3, 2.3 ± 0.7, 2.3 ± 1.1, and 2.3 ± 0.6% for α-tocopherol intakes of 20, 41, 83 and 146 µmol/d, respectively.

Cross-sectional study group

The basic characteristics of the 76 young women are presented in Table 2. Each values were similar to those reported for young adults female aged 18–22 years.[19] In brief, the participants were considered as typical female university students in Japan, characterized by relatively low BMI (20.2 kg/m^2), and low intake of fat (28.4%). During the experimental period, all participants were living freely, and none of the participants were drinking or smoking. Average intake of α-tocopherol in participants was 13.7 ± 3.8 µmol/d, and daily intake of α-tocopherol

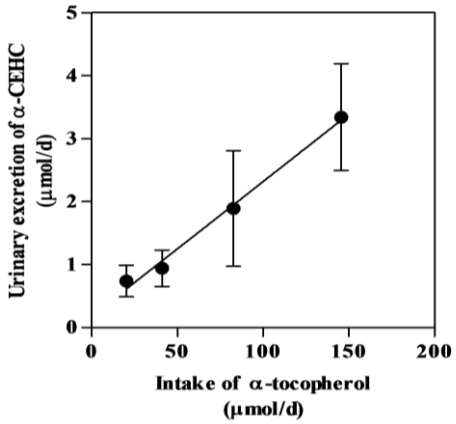

Figure 1. Relationship between urinary excretion of α-CEHC and the intake of α-tocopherol in strictly controlled participants (intervention study). Individual average intake of α-tocopherol is plotted on the *x*-axis, and the 24-h urinary excretion of α-CEHC, a catabolite of α-tocopherol, is plotted on the *y*-axis. In total, 10 healthy male Japanese college students aged 18-25 years were enrolled. Values are mean ± SD. A significant correlation ($r = 0.99$, $p = 0.0043$) was obtained. Regression line: $y = 0.0214 (\pm 0.0014) x + 0.180 (\pm 0.122)$.

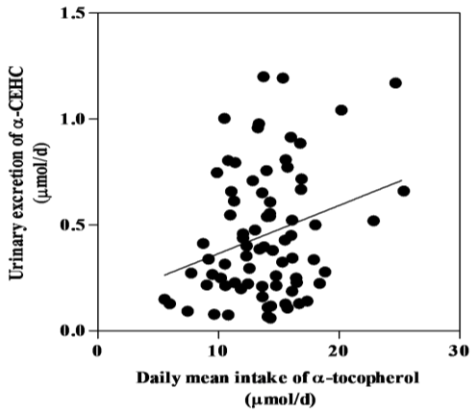

Figure 2. Relationship between urinary excretion of α-CEHC and intake of α-tocopherol in young adults' female (cross-sectional study). Measurements were taken of food intake on 4 consecutive days. The urine samples were collected at day 4. Individual average intake of α-tocopherol is plotted on the *x*-axis, and the urinary excretion of α-CEHC on day 4 is plotted on the *y*-axis. In total, 76 healthy, free-living, female college students aged 18–33 years were enrolled. A significant correlation ($r = 0.29$, $p = 0.0147$) was obtained. Regression line: $y = 0.0228 (\pm 0.0091) x + 0.137 (\pm 0.130)$.

Table 2. Characteristics and dietary intakes of female subjects

Variables	Young adult female (n = 76)
Anthropometric variable	
Age (years)	20.1 ± 2.3
Body height (cm)	158 ± 5
Body weight (kg)	50.6 ± 5.4
Body mass index (kg/m^2)	20.2 ± 1.8
Dietary mean intake at days 1-4	
Total energy (kJ/d)	6950 ± 1260
Protein (% of energy)	13.8 ± 2.2
Fat (% of energy)	28.4 ± 4.5
Carbohydrate (% of energy)	56.5 ± 4.9
α-Tocopherol intake (μmol/d)	13.7 ± 3.8
Inter-individual variations on the vitamin E intake (% CV)[1]	41.5
Intra-individual variations on the vitamin E intake (% CV)[1]	55.3
Urinary α-CEHC (μmol/d)	0.444 ± 0.292
Excretory ratio[2] of vitamin E (%)	3.58 ± 3.39

[1]% CV, percentage coefficient of variance.
[2]Calculated by the formula [α-CEHC excretion on day 4 (μmol/d) / the average α-tocopherol intake over the 4 days (μmol/d)] × 100.

Table 3. Mean dietary α-tocopherol intake and 24-hour urinary α-CEHC, excretory ratio, and estimated mean α-tocopherol intake in young adult females

	Young adult female (n = 76)
Mean α-tocopherol intake[1] (μmol/d)	13.7 ± 3.8
Excretory ratio[2] (%)	3.58 ± 3.39
Estimated mean α-tocopherol intake[3] (μmol/d)	12.4 ± 8.2
% ratio[4]	91

[1]Calculated by average α-tocopherol intake over the 4 days for each individual.
[2]Calculated by the formula [α-CEHC excretion on day 4 (μmol/d) / the average α-tocopherol intake over the 4 days (μmol/d)] × 100.
[3]The estimated α-tocopherol intake in young adult was calculated by the individual value of the 24-h urinary excretion of α-CEHC and the excretory ratio of 3.58 %, and the estimated mean α-tocopherol intake was calculated by the resulting estimated α-tocopherol intake.
[4]%Ratio between the mean and the estimated mean α-tocopherol intake.

was similar to adequate intake for Japan. [20] Urinary excretion of α-CEHC was twofold higher in elderly subjects than healthy peoples. The value of the excretory ratio of vitamin E in elderly subjects was 1.4-fold higher than in healthy individuals. Intra and inter-individual variations in α-tocopherol intakes, the values were both around 50% (Table 2).

Correlations between 24-hour urinary excretion of α-tocopherol metabolite, α-CEHC on day 4 and the average α-tocopherol intake on 4 consecutive days are shown in Figure 2. A significant association was observed in young adult females.

Estimated mean α-tocopherol intake calculated by urinary excretion of α-CEHC and the excretory ratio in the **cross-sectional study**

Mean dietary α-tocopherol intake and 24-hour urinary α-CEHC, excretory ratio, and estimated mean α-tocopherol intakes in young women are shown in Table 3. The excretory ratio was determined from the urinary excretion of α-CEHC and the average α-tocopherol intake over 4 days. The individual estimated α-tocopherol intake was calculated by average excretory ratio and the individual urinary α-CEHC value. The estimated mean α-tocopherol intake in young women was 91% of the real mean α-tocopherol intake over 4 days in young adult females.

DISCUSSION

Alpha-CEHC, a urinary metabolite of α-tocopherol, was described in 1995 by Schultz *et al*[10] Excretion of α-CEHC is considered to reflect saturation of α-tocopherol in the body, because it is not a metabolite of the α-tocopherol consumed for antioxidant defense. In other words, the detection of α-CEHC in urine generally indicates a better α-tocopherol nutritional status. Therefore, it is generally considered that the content of α-CEHC is below the limit of detection under an ordinary dietary habitant. Morinobu *et al*,[14] however, reported that α-CEHC was detected in healthy adult male Japanese volunteers. Therefore, it is probable that α-CEHC is excreted into urine in Japanese individuals who consume an ordinary amount of α-tocopherol. But, the data are based on only seven persons. Further research is necessary to allow the results to be applied generally.

In the present first study, the intervention study was performed to determine whether urinary α-CEHC excretion correlates with intake of α-tocopherol. We found a significant positive correlation between urinary α-CEHC and intake of α-tocopherol in healthy young male Japanese adults who consumed a strictly controlled diet with doses of α-tocopherol ranging from 20 to 145 μmol/d.

In the second experiment, to determine the usefulness of urinary α-CEHC as a biomarker for α-tocopherol nutritional status, a cross-sectional study was performed on free-living subjects. A weak but significant correlation was found between urinary α-CEHC and the mean α-tocopherol intake. These results indicate that α-CEHC levels in 24-h urine reflect dietary α-tocopherol intakes over the past few days, and suggest that α-tocopherol intake can be estimated from urinary α-CEHC values in free-living subjects.

This phenomenon might be only applicable for the Japanese and East-Asian populations. The subjects ate a lot of vegetable and were of relatively low BMI and had low intake of fat. The requirement of α-tocopherol is dependent on the intakes of polyunsaturated fatty acids. The average intakes of the polyunsaturated fatty acids were around 10 g/d. The optimum ratio of α-tocopherol (mg/d) to polyunsaturated fatty acids (g/d) is reported to be 0.60.[22] Its average ratio was about 0.60 in the present subjects. This is a reason why a significant association was observed even with low intake of α-tocopherol for the Japanese.

Metabolism of α-CEHC from α-tocopherol is somewhat different between Japanese, America and European populations. For example, the metabolic activity of α-tocopherol to α-CEHC might be higher in the Japanese than in the Americans and Europeans. The precise regulatory mechanism of post-absorption α-tocopherol elimination is not clear. The current knowledge is only of a pathway involving cytochrome P450-mediated ω-hydroxy lation of the α-tocopherol phytyl side chain, followed by stepwise removal of two or three carbon molecules such as acetyl-CoA or propionyl-CoA, ultimately yielding the α-CEHC that is excreted in urine.[23] Low fat intakes in the Japanese might bring about surplus ability to the β oxidation system, which results in increased activity of α-tocopherol to α-CEHC. Sesamin increases tissue α-tocopherol concentration by inhibiting the α-tocopherol oxidation pathway;[24] sesame oil, which contains sesamin, is often used in Japanese cooking, which might bring about the saving effect of α-tocopherol. Compared to Caucasian, Asian population had significantly lower plasma vitamin E levels in the same environment.[25] However, there are no data regarding its metabolism or immune functions among two populations. In Asian populations, they may be easily excreted into urine as α-CEHC compared to Caucasians. This is also a reason why the significant association was observed even with low intake of α-tocopherol for the Japanese.

The limiting factor of the transport capacity appears to be plasma lipid concentration. In general, subjects with a low total plasma lipid concentration did not accumulate as high concentration of α-tocopherol, as those with a higher total lipid content; and they started to excrete α-CEHC earlier[10]. If the urinary excretion of α-CEHC is related to the α-tocopherol content of plasma lipid, the range of thresholds of excretion is much wider and the correlation between plasma concentration and urinary excretion becomes more pronounced. In the present cross-sectional study, we did not measure plasma lipid concentration, and how plasma lipid affected urinary excretion of α-CEHC is unclear.

In terms of the completeness of the dietary assessment in the present study, there are several limitations in terms of using a weighed food record method. To reduce errors associated with self report, several dietitians reviewed the collated records along with the photographs. The selection of participants from a dietetics course also contributed to reduce reporting errors, as they had nutritional knowledge and were well trained. Another limitation exists in the present food composition table developed for Japan. In a dietary assessment of free-living people, potential errors caused by the quality of this table, such as defects in food composition, are inevitable. These limitations might cause the relatively low correlation in the free-living experiment population compared with that of the intervention study.

In conclusion, a significant relationship between the α-tocopherol intake and urinary excretion of α-CEHC was observed in young Japanese adults.

ACKNOWLEDGMENTS

We thank all volunteers who participated in the present study. This study represents a part of the results of "Studies on the construction of evidence to revise the dietary reference intake for Japanese people – elucidation of the balance of micronutrients and major elements" (principal investigator, Katsumi Shibata), which was supported by a research grant for Comprehensive Related Diseases from the Ministry of Health, Labour and Welfare of Japan.

AUTHOR DISCLOSURES

None of the authors had any financial or personal conflicts of interest associated with this manuscript.

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Original Article

Association between 24 hour urinary α-tocopherol catabolite, 2,5,7,8-tetramethyl-2(2'-carboxyethyl)-6-hydroxychroman (α-CEHC) and α-tocopherol intake in intervention and cross-sectional studies

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24 小時尿液 α-生育醇代謝產物 2,5,7,8-tertranetgyl-2 (2'carboxyethyl)-6-hydroxychroman(α-CEHC)與 α-生育醇攝取之介入及橫斷研究之相關

本研究的目的為評估在介入及橫斷研究中，24 小時尿液的 α-生育醇代謝產物 2,5,7,8-tertranetgyl-2(2'carboxyethyl)-6-hydroxychroman (α-CEHC) 與 α-生育醇攝取量之相關性。在四週的介入性研究，日本男性 (n=10) 在第一週攝取測試飲食，後續三周攝取添加不同量 α-生育醇的測試飲食：第二週為 21 μmol/dα-生育醇、第三週為 63 μmol/dα-生育醇與第四週為 125 μmol/dα-生育醇。在這個嚴格控制的實驗中，觀察到 α-生育醇的攝取量與尿液中的 α-CEHC 有顯著的相關 (r=0.99, p<0.001)。在橫斷性研究，76 名年輕一般研究對象 (18-33 歲) 紀錄連續四天攝取的所有食物。在橫斷性研究這個相關性儘管不強，但是顯著的 (r=0.29, p<0.05)。在橫斷性研究中的成年人，以尿液 α-CEHC 計算其平均估計 α-生育醇攝取量，其排泄率為四日平均攝取量的 91%。無論組別平均值或是組別中個體的排序結果，都顯示尿液中的 α-CEHC 量可反映年輕日本一般成年人近期的 α-生育醇攝取量，且可以當作過去幾天的攝取量測量方法。

關鍵字：α-生育醇、代謝產物、CEHC、尿液、生物標記

J Nutr Sci Vitaminol, 57, 203–208, 2011

Vitamin Contents in Rat Milk and Effects of Dietary Vitamin Intakes of Dams on the Vitamin Contents in Their Milk

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(Received October 8, 2010)

Summary Studies of factors that affect milk vitamin contents are important. We investigated the vitamin contents in rat milk and the effects of dietary vitamin intakes of dams on the vitamin contents in their milk. A low-vitamin diet (0.2%) and a high-vitamin diet (4.0%) based on a diet containing 1% AIN-93-VX (normal diet) was given to female rats from pregnancy to lactation. Regarding the effects of the vitamin intakes, the concentrations of vitamins B_1, B_2, B_6, B_{12} and E were decreased with the low-vitamin diet, but were not increased with the high-vitamin diet. The concentrations of niacin, pantothenic acid and biotin were not decreased with the low-vitamin diet, but were increased with the high-vitamin mixture diet. The folate concentration remained constant regardless of the intake of folate. These findings clearly indicate that the levels of certain vitamins in milk are easily affected by the dietary vitamin intakes.

Key Words content, lactation, milk, rat, vitamin

It is generally believed that milk contains all of the nutrients for the proper development of infants. However, this is not the case, at least with regard to vitamins. Therefore, studies of factors that affect milk vitamin contents are important. Kirchgessner et al. (*1*) reported that the vitamin B_1 content in rat milk was lower in rats fed with a low-vitamin B_1 diet than in rats fed with a sufficient vitamin B_1 diet. Duerden and Bates (*2*) reported that the vitamin B_2 concentration in rat milk was extremely low when dams were fed a vitamin B_2-restricted diet compared with control dams. Regarding the vitamin B_6 content in rat milk, Kirksey and Susten (*3*) reported that the vitamin B_6 level was a more sensitive indicator than liver or muscle of chronically low intakes of vitamin B_6 by dams, while other investigators also reported that the level of vitamin B_6 in the milk of dams changed according to their intake of vitamin B_6 (*3–6*). Two groups reported that the concentration of vitamin B_{12} in milk was affected by the dietary intake of vitamin B_{12} (*7*, *8*). Meanwhile, O'Connor et al. (*9*) reported that the folate content of rat milk was increased according to increases in dietary folate. These reports clearly indicate that the levels of certain vitamins in rat milk are easily affected by the dietary vitamin intakes. For other vitamins, there is no available information.

In this study, we investigated nine kinds of vitamin contents in rat milk, the changes in the vitamin contents during lactation, and the effects of dietary vitamin intakes of the dams on the vitamin concentrations in their milk.

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MATERIALS AND METHODS

Chemicals. Vitamin-free milk casein, sucrose and L-methionine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Corn oil was purchased from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, a mineral mixture (AIN-93-M-MX) (*10*) and a vitamin mixture (AIN-93-VX containing chorine bitartrate) (*10*) were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan).

Thiamin hydrochloride (vitamin B_1, $C_{12}H_{17}ClN_4OS$-HCl=337.27), thiamin diphosphate chloride ($C_{12}H_{19}ClN_4O_7P_2S$=460.77), riboflavin (vitamin B_2, $C_{17}H_{20}N_4O_6$=376.37), cyanocobalamin (vitamin B_{12}, $C_{63}H_{88}CoN_{14}O_{14}P$=1,355.40), nicotinamide ($C_6H_6N_2O$ =122.13), calcium pantothenate (PaA-Ca, $C_{18}H_{32}N_2O_{10}$-Ca=476.54), folic acid ($C_{19}H_{19}N_7O_6$= 441.40), D(+)-biotin ($C_{10}H_{16}N_2O_3S$=244.31), (±)D-α-tocopheryl acetate ($C_{31}H_{52}O_3$=472.74), pyridoxal 5′-phosphate monohydrate ($C_8H_{10}NO_6P$-H_2O=265.169) and pyridoxal hydrochloride ($C_8H_9NO_3$-HCl=203.62) were purchased from Wako Pure Chemical Industries.

Nembutal (2.5 g/50 mL) was obtained from Dainippon Sumitomo Pharma (Osaka, Japan). Oxytocin (50 IU/mg) and lumiflavin ($C_{13}H_{12}N_4O_2$=256.3) were obtained from Sigma-Aldrich Japan K.K. (Tokyo, Japan).

All other chemicals used were of the highest purity available from commercial sources.

Animals and diets. Male and female rats of the Wistar strain (8 wk old) were obtained from CLEA Japan, Inc. (Tokyo, Japan). The rats were immediately placed in individual cages and fed a 20% casein diet containing 1% vitamin mixture (Table 1) for 1 wk to

Table 1. Compositions of the experimental diets.

	0.2% VX[1] (%)	1.0% VX (%)	4.0% VX (%)
Casein	20	20	20
L-Methionine	0.2	0.2	0.2
Gelatinized cornstarch	46.9	46.9	46.9
Sucrose	24.2	23.4	20.4
Corn oil	5	5	5
Mineral mixture (AIN-93-MX)	3.5	3.5	3.5
Vitamin mixture (AIN-93-VX)	0.2	1	4
(mg/100 g of diet)			
All-*trans*-retinyl palmitate (500,000 IU/g)	0.16	0.8	3.2
Cholecalciferol (400,000 IU/g)	0.05	0.25	1
All-rac-α-tocopheryl acetate (500 IU/g)	3	15	60
Phylloquinone	0.015	0.075	0.3
Thiamin-HCl	0.12	0.6	2.4
Riboflavin	0.12	0.6	2.4
Pyridoxine-HCl	0.14	0.7	2.8
Cyanocobalamin	0.0005	0.025	0.01
Nicotinic acid	0.6	3	12
Ca pantothenate	0.32	1.6	6.4
Folic acid	0.04	0.2	0.8
D-Biotin	0.004	0.02	0.08
Choline bitartrate	5	25	100
Sucrose	up to 200	up to 1,000	up to 4,000

[1] VX: vitamin mixture.

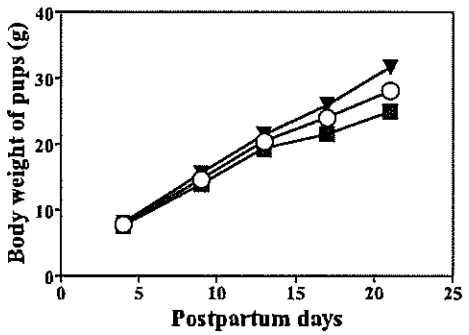

Fig. 1. Effect of feeding with the three levels of vitamin mixture diets to dams on the body weight gains of pups. ■, 0.2% vitamin mixture diet; ○, 1.0% vitamin mixture diet (normal diet); ▼, 4.0% vitamin mixture diet. Values are expressed as means±SE for 5–7 rats.

Table 2. The vitamin contents in rat milk.

Vitamins	Values	*n*
Vitamin B_1 (μg/mL)	0.204±0.082	16
Vitamin B_2 (μg/mL)	4.67±0.78	16
Vitamin B_6 (μg/mL)	1.49±0.23	13
Vitamin B_{12} (μg/mL)	0.032±0.008	16
Niacin (μg/mL)	7.02±2.28	16
Pantothenic acid (μg/mL)	15.2±6.6	16
Folate (μg/mL)	2.91±0.38	16
Biotin (μg/mL)	0.154±0.047	13
Vitamin E (μg/mL)	15.3±0.55	16

The values are means±SD for postpartum days 9, 13, 17, and 21.

allow them to acclimatize to their new circumstances. The female rats were then divided into three groups and fed one of three experimental diets containing 0.2, 1.0 or 4.0% vitamin mixture (Table 1) during mating, gestation and lactation. After the female rats delivered their pups, the pups that the mother rat brought up was adjusted to six. The day of postpartum was designated day 1. For collection of milk, the mother rat was separated from the litter at 09:00. At 15:00, the dam was anesthetized with 0.1 mL of nembutal (2.5 g/50 mL) and intraperitoneally injected with 2.5 IU of oxytocin. After confirming the effectiveness of the anesthesia, milk (about 2 mL) was collected using a special milking machine (Automatic Milker WAT-2001; Little Leonard Co. Ltd., Tokyo, Japan). The milk collection was carried out on days 4, 9, 13, 17 and 21. The collected milk was diluted by 1 : 2 by the addition of saline (0.85% NaCl), and the diluted milk was stored at −20°C until analysis.

The animal room was maintained at a temperature of about 22°C and a humidity of about 60% with a 12-h light (06:00–18:00)/12-h dark (18:00–06:00) cycle. The body weight of each pup was measured when it was separated from the mother. The care and treatment of the experimental animals conformed to The University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

Analyses. Vitamin B_1 (*11*), vitamin B_2 (*12*), vitamin B_6 (*13*), vitamin B_{12} (*14*), nicotinamide (*15*), pantothenic acid (*16*), folate (*17*), biotin (*18*) and vitamin E (*19*) in milk were measured as described in the cited references.

Statistical analysis. Each value was expressed as the mean±SE. The statistical significance of differences was determined by ANOVA and subsequent Tukey-Kramer multiple-comparison tests. Differences with values of $p<0.05$ were considered to be statistically significant. Prism version 5.0 (GraphPad Software Inc., San Diego, CA, USA) was used for all analyses.

RESULTS

Effects of feeding the three vitamin diets to the dams on the body weight gains of pups

The low-vitamin (0.2%) and high-vitamin (4.0%) diets based on the AIN-93 diet (*10*) (1.0% vitamin diet; normal diet) were given to the female rats from pregnancy to lactation. Figure 1 shows the effects of feeding the three vitamin diets to the dams on the body weight gains of the pups. The weights of the pups were almost the same among the three groups.

Vitamin contents in milk of dams fed the normal diet

The reference values of rat's milk vitamin contents were assumed to be the average of the values on the days 9, 13, 17, and 21, because there were quite different values for vitamin B_1, vitamin B_2, and vitamin E on day 4. Table 2 shows the average vitamin contents in

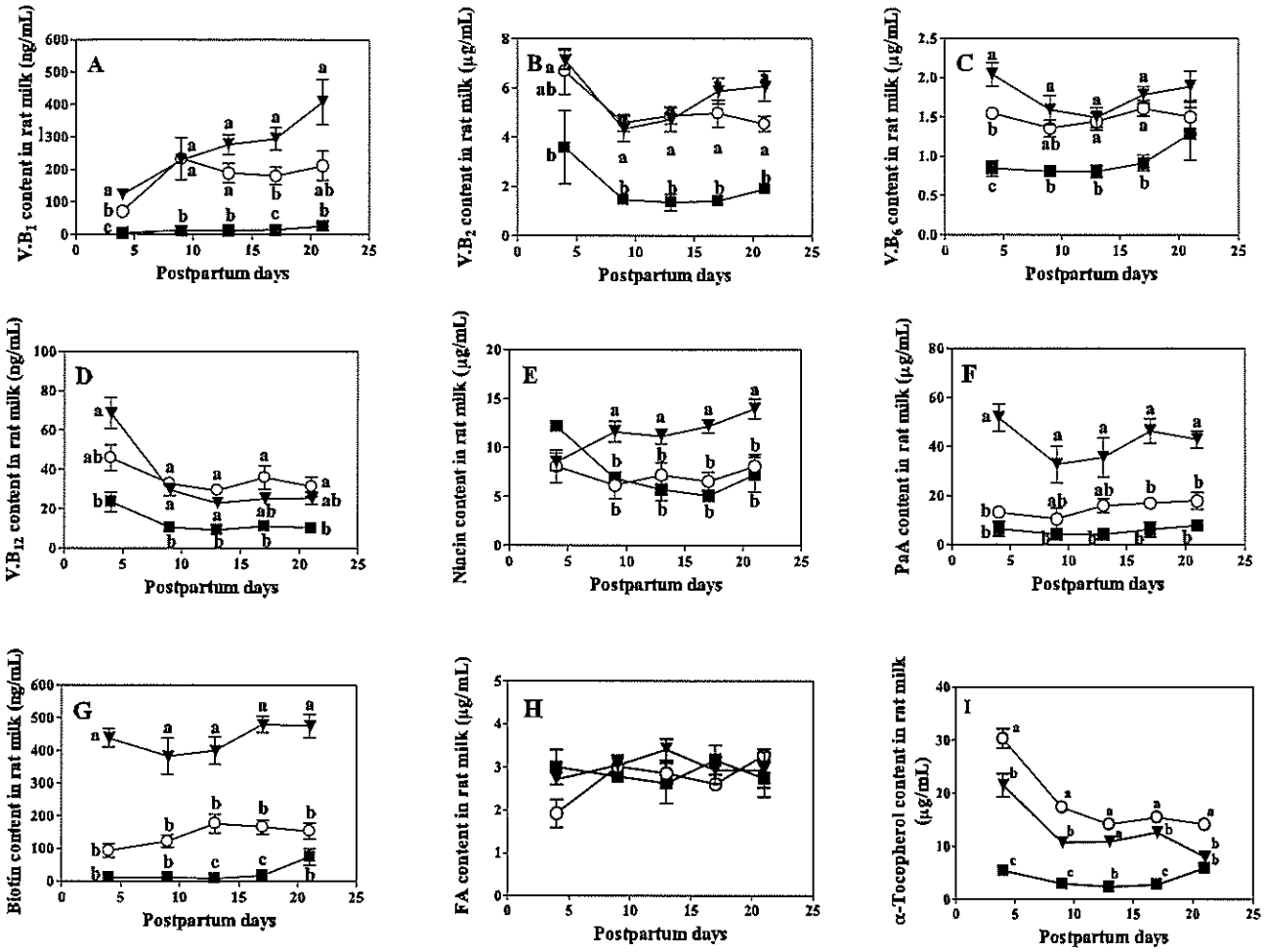

Fig. 2. Effect of feeding with the three levels of vitamin mixture diets to dams on the milk contents of vitamin B_1 (A), vitamin B_2 (B), vitamin B_6 (C), vitamin B_{12} (D), niacin (E), pantothenic acid (PaA) (F), biotin (G), folate (H), and vitamin E (I) contents in rat milk. ■, 0.2% vitamin mixture diet; ○, 1.0% vitamin mixture diet (normal diet); ▼, 4.0% vitamin mixture diet. Values are expressed as means±SE for 3~5 rat milks. Different letters on the symbols denote statistically significant differences (as determined by Tukey-Kramer multiple-comparison tests, $p<0.05$) in milk vitamin content among the groups of the three vitamin mixture diets in the same postpartum day.

rat milk collected on postpartum days 9, 13, 17 and 21 when the dams were fed the normal diet (1% vitamin mixture diet).

Changes in the vitamin contents during lactation of the dams fed the normal diet

Figure 2 shows the changes in the vitamin contents on specific postpartum days. The vitamin B_1 (Fig. 2A) content was lower on postpartum day 4 than on the other days. On the other hand, the contents of vitamins B_2 (Fig. 2B), B_{12} (Fig. 2D) and E (Fig. 2I) were higher on postpartum day 4 than on the other days. The contents of vitamin B_6 (Fig. 2C), niacin (Fig. 2E), biotin (Fig. 2G) and folate (Fig. 2H) remained relatively constant during lactation.

Effects of feeding the three vitamin diets to the dams on the vitamin contents in their milk

Figure 2 also shows the effects of the intake of the dietary vitamins on the vitamin contents in the rat milk during lactation. Although the concentrations of vitamins B_1 (Fig. 2A), B_2 (Fig. 2B), B_6 (Fig. 2C) and B_{12} (Fig. 2D) did not increase with the high-vitamin diet compared with the normal vitamin diet, these vitamin concentrations decreased with the low-vitamin diet.

The concentrations of niacin, pantothenic acid and biotin were not decreased by feeding the low-vitamin diet to the dams, although these vitamin concentrations were increased by feeding the high-vitamin mixture diet to the dams (Fig. 2E, 2F and 2G, respectively).

The folate concentration remained constant regardless of the amount of folate intake (Fig. 2H). The concentration of vitamin E was decreased with the low-vitamin diet, but was not increased with the high-vitamin diet compared with the normal vitamin diet (Fig. 2I).

DISCUSSION

The body weight gains of the pups were almost the same among the three groups regardless of whether the dams were fed diets containing low (0.2%), normal (1.0%) or high (4%) levels of the vitamin mixture. The present findings mean that the milk of lactating rats fed the low-vitamin diet (0.2%) was able to maintain normal growth of the pups. This finding is likely to have arisen because the AIN-vitamin mixture (*10*) contains

around five-fold higher vitamin contents than the required amounts.

The first purpose of the present study was to analyze the milk vitamin concentrations of rats fed the normal diet. As summarized in Table 2, we measured 9 kinds of vitamins, namely vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin, pantothenic acid, folate, biotin and vitamin E.

We did not measure the contents of vitamins A, D and K in rat milk. The reported values for vitamins A and D in rat milk are around 800 ng/mL (*20*) and 2 ng/mL (*21*), respectively. We could not find any data for the vitamin K content in rat milk.

The concentration of vitamin B_1 in the milk of dams fed the normal diet was around 200 ng/mL (Fig. 2A and Table 2). There is one previous report about the vitamin B_1 content in rat milk. Kirchgessner et al. (*1*) reported that the content increased according to the postpartum days, with values of 840, 1,600 and 2,500 ng/mL on days 2, 6 and 13, respectively, when the dams were fed a diet containing 0.67 mg/100 g diet (the same concentration in the 1% vitamin mixture diet as the normal diet). These values were about 10-fold higher than the present data for the 1% vitamin mixture diet. Kirchgessner et al. (*1*) used Sprague-Dawley rats as the experimental animals and fed a relatively high-fat diet (8.7% fat), while we used Wistar rats and fed a 5% fat diet. These differences may be reasons why the milk vitamin B_1 concentrations were so different.

The concentration of vitamin B_2 in the milk of dams fed the normal diet was around 5,000 ng/mL (Fig. 2B and Table 2). There is one previous report about the vitamin B_2 content in rat milk. Duerden and Bates (*2*) reported that the content was around 8,000 ng/mL when the dams were fed a diet containing 1.5 mg/100 g diet. The concentration of dietary vitamin B_2 was 2.5-fold higher than that in the present normal diet, and the content of vitamin B_2 in the milk was 1.6-fold higher than that in the present study. Duerden and Bates (*2*) also reported that the vitamin B_2 content in milk was significantly lower when the dams were fed a vitamin B_2-restricted diet. In the present study, the vitamin B_2 content in milk was lower in the dams fed the low-vitamin diet than in the dams fed the normal and high-vitamin diets.

The concentration of vitamin B_6 in the milk of dams fed the normal diet was around 1,500 ng/mL (Fig. 2C and Table 2). In previous reports, a constant value for the vitamin B_6 content in rat milk was not obtained. Thomas and Kirksey (*6*) reported that the vitamin B_6 content was 500 ng/mL when the dams were fed a diet containing 0.3 mg pyridoxine-HCl/100 g diet (this concentration is three-sevenths of that in our normal control diet). Felice and Kirksey (*5*) reported that the content was around 900 ng/mL when the dams were fed a diet containing 1.0 mg pyridoxine-HCl/100 g diet (this concentration is 1.4-fold higher that the concentration in our normal control diet). Debes and Kirksey (*4*) reported that content was around 500 ng/mL when the dams were fed a diet containing 2.0 mg pyridoxine-HCl/100 g diet (this concentration is 2.85-fold higher than the concentration in our normal control diet). In the present study, the vitamin B_6 content increased according to the change in diet from the low-vitamin (0.14 mg pyridoxine-HCl/100 g diet) to the normal diet (0.7 mg pyridoxine-HCl/100 g diet). However, the concentration did not increase when the dietary vitamin intake was increased from the normal diet to the high-vitamin diet (2.8 mg pyridoxine-HCl/100 g diet) (Fig. 2C). This finding is consistent with those in Kirksey and Susten (*3*) and Pang and Kirksey (*22*). They fed five levels of pyridoxine-HCl (0.12, 0.24, 0.48. 0.96 and 1.92 mg/100 g diet) to female rats, and found that the vitamin B_6 concentration in the milk reached a plateau of around 300 ng/mL with the 0.48 mg pyridoxine-HCl/100 g diet.

The concentration of vitamin B_{12} in the milk of dams fed the normal diet was around 30 ng/mL (Fig. 2D and Table 2). Regarding previous reports, the vitamin B_{12} content in rat milk was dramatically increased according to the intake of vitamin B_{12} of the dams. When the dams were changed from a diet containing 0.2 μg/100 g diet to a diet containing 20 μg/100 g diet, the concentration of vitamin B_{12} increased from about 7 ng/g milk curd to 120 ng/g milk curd (*22*). When the dams were fed a vitamin B_{12}-deficient diet, the content was around 5 ng/mL (*8*).

Regarding previous reports of the folate content in rat milk, values of 150 ng/mL milk (*9*) and 440 ng/mL milk (*23*) were observed when the dams were fed a diet containing 0.2 mg/100 g diet. In the present study, the folate concentration in the milk of dams fed the normal diet was around 3 μg/mL (Fig. 2H and Table 2). This value was about 10-fold higher than the previously reported values (*9*, *23*). The previously reported values were obtained using Sprague-Dawley rats as the experimental animals, while we used Wistar rats. This difference may be the reason why the folate concentrations in the milk were so different.

No data for the contents of niacin, pantothenic acid, biotin and vitamin E in rat milk have been reported. The concentration of niacin in the milk of dams fed the normal diet was around 7 μg/mL (Fig. 2E and Table 2). The concentration of pantothenic acid in the milk of dams fed the normal diet was around 15 μg/mL (Fig. 2F and Table 2). The concentration of biotin in the milk of dams fed the normal diet was around 150 ng/mL (Fig. 2G and Table 2). The concentration of vitamin E in the milk of dams fed the normal diet was around 15 μg/mL (Fig. 2I and Table 2).

The second purpose of the present study was to evaluate the changes in the vitamin contents during lactation. The content of vitamin B_1 was remarkably increased from day 9 (Fig. 2A). A similar phenomenon has already been reported by Kirchgessner et al. (*1*). On the other hand, the contents of vitamin B_2 (Fig. 2B) and vitamin E (Fig. 2I) remarkably decreased from day 9. These phenomena would be associated with the vitamin requirement in the pups and regulated through the expression of carrier proteins for the vitamins in the

mammary glands. The other vitamins remained at relatively constant concentrations during lactation. Regarding vitamin B_{12}, Williams and Spray (*8*) already reported a similar phenomenon to the present study. However, Felice and Kirksey (*5*) reported that the content of vitamin B_6 was significantly higher on day 21 than earlier in the lactation period. For vitamin B_2, niacin, pantothenic acid, biotin, folate and vitamin E, the changes in the vitamin contents during lactation are reported here for the first time.

The final purpose of the present study was to clarify the effects of dietary vitamin contents on the milk vitamin contents in rats. In previous reports, the milk contents of vitamins B_1 (*1*) and B_2 (*2*) were decreased when the dams were fed on corresponding vitamin-restricted diets, and the vitamin B_6 (*3*) and B_{12} (*7*) contents in the rat milk reflected the intakes of the respective vitamins. In the present study, the concentrations of vitamins B_1, B_2, B_6, B_{12} and E were decreased with the low-vitamin diet, but were not increased with the high-vitamin diet. The present findings for vitamin B_1 are similar to the findings of Kirchgessner et al. (*1*), who also found that the vitamin B_1 content was decreased by feeding a low-vitamin B_1 diet, but was not increased by feeding an excess vitamin B_1 diet. The concentrations of niacin, pantothenic acid and biotin were not decreased with the low-vitamin diet, but were increased with the high-vitamin diet. These results indicate that the concentrations of niacin, pantothenic acid and biotin in milk are not easily decreased, even with low intake, while the concentrations of vitamins B_1, B_2, B_6, B_{12} and E in milk are affected by their intakes. The folate concentration remained constant regardless of the folate intake. It is known that there is a well-developed epithelial folate transport system for the regulation of normal folate homeostasis (*24*, *25*). Therefore, the concentrations of the vitamins could also be well-regulated by transport systems in intestinal absorption and in secretion to the milk. However, the present findings suggest there is a specific regulation mechanism for each of the vitamins to maintain the milk vitamin contents. Regarding the vitamin E concentration in human milk, its concentration is associated with the total fat intake by mothers, while the vitamin E intake seems to have no effect (*26*).

Acknowledgments

This investigation is a part of a study entitled "Studies on construction of evidence to the revised Dietary Reference Intakes for Japanese—Elucidation of balance of dietary intake between micronutrients and macro elements—(principal investigator, Katsumi Shibata)," which was supported by The Ministry of Health, Labor and Welfare. The authors would like to thank Ema Sugimoto, Alato Okuno, Eri Imai, Atsushi Shimizu, Kei Takahashi, Miki Terakata, Aya Moriya, Keiko Miki, Tomoyo Chiba, Masako Otsubo and Akemi Kawai for technical assistance.

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Biosci. Biotechnol. Biochem., 75 (1), 185–187, 2011

Communication

Fluorometric Determination of 2-Oxoadipic Acid, a Common Metabolite of Tryptophan and Lysine, by High-Performance Liquid Chromatography with Pre-Chemical Derivatization

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Received October 8, 2010; Accepted December 8, 2010; Online Publication, January 7, 2011
[doi:10.1271/bbb.100723]

2-Oxoadipic acid, a key metabolite of tryptophan and lysine, reacted with 1,2-diamino-4,5-methylenebenzene in an acidic solution to produce a fluorescent derivative. The reaction product was separated using a Tosoh ODS-80Ts column with 20 mmol/L of KH_2PO_4–K_2HPO_4 buffer (pH 7.0) containing 26% methanol at a flow rate 0.8 mL/min. The excitation wavelength of detection was 367 nm, and the emission wavelength was 446 nm. The limit of quantification was 1 pmol per injection, sufficiently sensitive for the determination of 2-oxoadipic acid in human and experimental animal urine.

Key words: 2-oxoadipic acid; 1,2-diamino-4,5-methylenebenzene; tryptophan; lysine; urine

The nutritional factors affecting the metabolism of tryptophan-niacin have been studied,[1–3] and we have confirmed that the reaction of ACMS to α-aminomuconate-ε-semialdehyde, the branch point of tryptophan catabolism that leads to niacin production, is inversely related to the amount of niacin synthesis that occurs in the liver cell.[4,5]

2-OAA is a common catabolic metabolite of the essential amino acids tryptophan and lysine. We are interested in 2-OAA because excess dietary lysine might lead to a build-up of 2-OAA, which in turn would inhibit the catabolism of ACMS to 2-OAA, thus shunting ACMS (from tryptophan) toward niacin biosynthesis. In addition, we are interested in the relationship between dietary intake of tryptophan and/or lysine and the formation of 2-OAA. We have confirmed that urinary excretion of tryptophan catabolites such as kynurenic acid and xanthurenic acid reflect the intake of tryptophan.[6] Hence, we did quantitative analyses of 2-OAA, and learned that 2-oxoadipic aciduria, a rare congenital condition, was present, as first reported in 1975 by Przyrember *et al.*[7] Since that report, there have been seven reported cases,[8] found mainly by employing organic solvent extraction and GC-MS techniques,[9] but the GC-MS technique is not a practical measurement method for the detection of 2-OAA.

Nakamura *et al.*[10] reported an attractive chemical derivatization method for 2-oxo acids with DMB. Although no examination of the reaction between 2-OAA and DMB was described in their report, we succeeded in producing a fluorescent compound by reacting 2-OAA with DMB, and we developed a separation method based on this fluorescent compound by HPLC.

Here, we describe a new assay for measuring 2-OAA in the urine by HPLC with pre-chemical derivatization.

2-OAA was purchased from Sigma-Aldrich Chemicals (St. Louis, MO). DMB was purchased from Dojinkagaku Labs (Kumamoto, Japan). All the other chemicals and solvents used were of reagent grade.

A DMB solution was made by mixing the following in order: 8.7 mL of H_2O, 0.049 g of sodium hydrosulfite, 0.7 mL of 2-mercaptoethanol, 0.58 mL of concentrated HCl, and 0.016 g of DMB. The DMB solution was usable for at least 1 month when stored in a refrigerator. 2-Mercaptoethanol and sodium hydrosulfite were added to stabilize DMB during the reaction.

The 2-OAA was derivatized by the method of Nakamura *et al.*[10] The reaction is shown in Fig. 1. A total of 0.1 mL of the DMB solution was added to 0.1 mL of the 2-OAA solution or to a urine sample suitably diluted in a microtube with a sealed cap. The reaction was carried out by immersing the microtube in a boiling water bath for 45 min, and the microtube was cooled in ice water for at least 5 min. The resulting reaction mixture was filtered through a 0.45-μm filter (Millipore, Bedford, MA), and the filtrate (5 μL) was injected directly into the HPLC system. The fluorescent compound in the final mixtures was stable for at least 24 h when exposed to room light at room temperature.

Separation of the fluorescent product of 2-OAA in urine was carried out using a Tosoh ODS-80Ts (4.6 i.d. × 250 mm) column (Tosoh, Tokyo). The mobile phase consisted of a 20-mmol/L KH_2PO_4-K_2HPO_4 buffer (pH 7.0) containing 26% methanol. A flow rate of 0.8 mL/min was used, and the column temperature was maintained at 40 °C. Fluorometric detection was done at an excitation wavelength of 367 nm and an emission wavelength of 446 nm.

Stock solutions of 2-OAA were made of concentrations of up to 100 μmol/L with water, and were stored at −20 °C. Working standard solutions were diluted from the stock solutions to produce a series of concentrations (0.2 μmol/L, 0.3 μmol/L, 0.5 μmol/L, 1.0 μmol/L, 3.0 μmol/L, and 5.0 μmol/L). A total of 0.1 mL of each

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Abbreviations: 2-OAA, 2-oxoadipic acid; DMB, 1,2-diamino-4,5-methylenebenzene; ACMS, α-amino-β-carboxymuconate-ε-semialdehyde

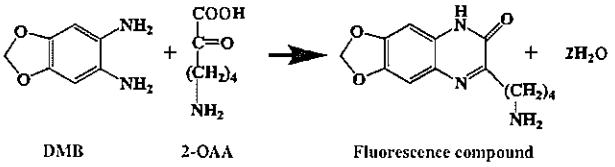

Fig. 1. Reaction of 2-OAA with DMB.

concentration was then reacted with 0.1 mL of the DMB solution, 5 μL of the reacted mixture containing 0.5, 0.75, 1.25, 2.5, 7.5, and 12.5 pmol of 2-OAA was injected into the HPLC system, and fluorescent intensities corresponding to 2-OAA were measured.

The linearity of the calibration curve was determined by plotting the peak areas (y) of the fluorescent intensity against the standard 2-OAA concentrations (x). The correlation coefficient was greater than 0.99, confirming that the calibration curve was linear over a concentration range of 0.5 to 12.5 pmol per injection for the 2-OAA standard. The typical standard curve can be represented by y (fmol) $= 21 + 118x$ ($r = 0.999$). The limit of detection was 0.5 pmol (approximately 80 pg) per injection at a signal-to-noise ratio of 5:1. The limit of quantification was 1 pmol (approximately 160 pg) per injection, sufficiently sensitive for the determination of 2-OAA in human, rat, and mouse urine.

Spot urine samples collected from three healthy young Japanese women was used for validation of the method. The three urine samples were mixed. We removed 9 mL from the mixed urine sample, and then 1 mL of 1 mol/L HCl was added to the urine samples to stabilize 2-OAA. The acidified sample was compared with the optimal basal conditions in the HPLC system, which served as the quality control (QC) sample.

Short-term stability was determined by maintaining the QC urine at room temperature for 24 h, middle-term stability was evaluated by storing the QC urine at 4 °C for 7 d, and the long-term stability of 2-OAA was assessed at −20 °C for 30 d. The freeze-thaw stability of 2-OAA was determined over three cycles of thawing at 4 °C for 12 h and refreezing for 12 h. For each storage condition, five replicates were analyzed in each batch. The 2-OAA concentration after each storage period was related to the initial concentration determined for the samples, which were freshly prepared and processed immediately. The range of change was calculated by the following equation:

$$\text{Range of change (\%)} = (\text{concentration under each condition} / \text{concentration of fresh preparation}) \times 100.$$

The stability of 2-OAA over the short-term, the long-term, and the freeze-thaw cycles was found to be +2, −3, and −2% change respectively as compared to the value for fresh urine, which was taken to be 100%. Under all conditions, 2-OAA in urine was stable.

Within-run precision was calculated by analyzing five replicates of the QC urine on the same day. Between-run precision was determined by triplicate analysis of the QC urine on three separate occasions, and the value on each occasion was calculated by analyzing five replicates. The coefficient of variation (CV) was used to measure the precision:

$$\text{CV (\%)} = \{\text{standard deviation (SD)/mean}\} \times 100.$$

The CVs of the within- and between-run precision were 0.73% and 0.94% respectively.

Within-run accuracy was measured in different experiments to calculate precision, and was evaluated in the same experiment to ascertain the recovery percentages. Accuracy was expressed as relative error (RE) and determined by the following equation:

$$\text{RE (\%)} = \{(\text{observed concentration} - \text{added concentration}) / \text{added concentration}\} \times 100.$$

The accuracies as shown by RE were 1.4%, −2.1%, and 2.6% at concentrations of 1.25, 3.75, and 6.25 pmol per 5 μL of sample respectively.

These data indicate that the assay was reproducible, accurate, and reliable.

Recovery was calculated using the following formula:

$$\text{Recovery (\%)} = (\text{observed concentration} / \text{added concentration}) \times 100.$$

Three additional concentrations (1.0 μmol/L, 3.0 μmol/L, and 5.0 μmol/L of 2-OAA, which contained 50, 150, and 250 pmol/0.05 mL of standard 2-OAA), were added to 0.05 mL of the QC urine and then reacted with 0.1 mL of the DMB solution. All analyses were performed in triplicate. Recovery was 101 ± 3, 97 ± 1, and 96 ± 3% respectively.

A typical chromatogram of the reference 2-OAA derivative is shown in Fig. 2A, and the 2-OAA derivative eluted at approximately 14.5 min. When standard 2-OAA was reacted with the DMB-free solution (removing only DMB of the DMB solution), the peak was not detected, as shown in Fig. 2E.

Healthy young Japanese women (21–23 years old, n = 14) were recruited for this experiment. A 24-h urine sample was collected from the second passage of urine on the first day to the first passage on the next day. The urine sample volumes were measured, and 1 mL of 1 mol/L HCl was added to 9-mL urine samples to stabilize 2-OAA. The acidified urine samples were stored at −20 °C until needed. This study was reviewed and approved by The Ethical Committee of The University of Shiga Prefecture.

Male rats of the Wistar strain (6 weeks old) and female mice of the ICR strain (6 weeks old) were obtained from CLEA Japan (Tokyo) and immediately placed in individual metabolic cages. The animals were fed *ad libitum* for 21 d on a niacin-free 20% casein diet.[11] Urine samples (24 h; 10:00 AM–10:00 AM) on the last day were collected in amber bottles containing 1 mL of 1 mol/L HCl, and were stored at −20 °C until needed. The care and treatment of the experimental animals conformed to the University of Shiga Prefecture guidelines for the ethical treatment of laboratory animals.

The chromatograms of derivatized urine sample from a human, a rat, and a mouse are shown in Fig. 2B, C, and D respectively. The 2-OAA derivative in the sample was characterized on the basis of its retention time and the entire excitation and emission spectra between 320 and 500 nm. Figure 2F, G, and H are chromatograms of

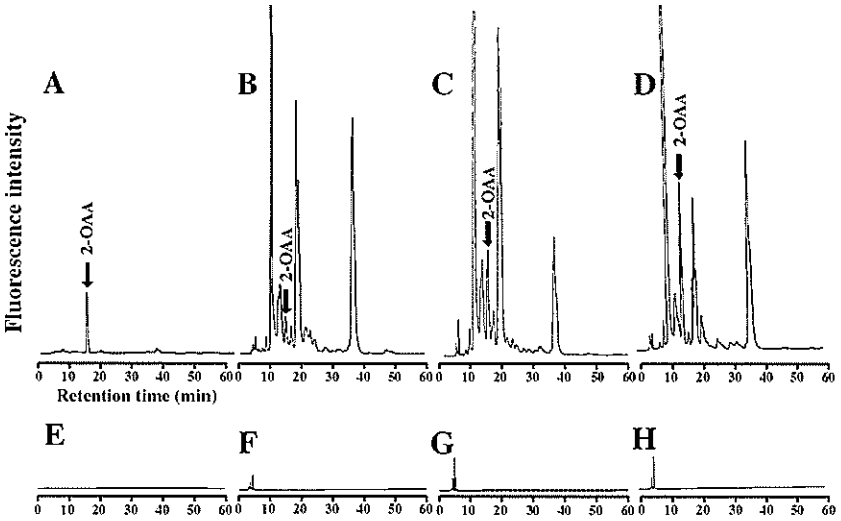

Fig. 2. Chromatograms of the 2-OAA Derivatives.

Conditions: column, Tosoh ODS 80 Ts (4.6 i.d. × 250 mm); mobile phase, 20 mmol/L KH_2PO_4–K_2HPO_4 buffer (pH 7.0) containing 26% methanol; flow rate, 0.8 mL/min; excitation wavelength, 367 nm; emission wavelength, 446 nm; column temperature, 40 °C. The chromatograms shown in upper level are of standard 2-OAA (A) (2.5 pmol/5 μL), a human urine sample (B) (1.1 pmol/5 μL), a rat urine sample (C) (3.6 pmol/5 μL), and a mouse urine sample (D) (6.6 pmol/5 μL) reacted with the DMB solution. The chromatograms in lower level are those of standard 2-OAA (E), a human urine sample (F), a rat urine sample (G), and a mouse urine sample (H) reacted with the DMB-free solution.

a human urine sample (F), a rat urine sample (G), and a mouse urine sample (H) reacted with DMB-free solution. The total HPLC analysis time was approximately 60 min.

There were many reactive compounds in the urine, indicating that the urine samples also contained several measurable 2-oxo acids. In future, we intend to develop simultaneous determination of the other 2-oxo acids in the urine.

The daily urinary excretion levels of 2-OAA in humans, rats, and mice were 14.6 ± 2.8 μmol/d (mean ± SEM, n = 14), 2.9 ± 0.8 μmol/d (mean ± SEM, n = 5), and 0.7 ± 0.1 μmol/d (mean ± SEM, n = 5) respectively. This is the first report on the urinary excretion of 2-OAA in healthy (non 2-OAA aciduria) people, rats, and mice.

The present method can be applied to study tryptophan and lysine metabolism.

Acknowledgments

This report is part of a larger project: Development of an index of "metabolic upper intake level" instead of "tolerable upper intake level" of tryptophan for humans (principal investigator, Katsumi Shibata). The investigation was supported by a grant from the International Council on Amino Acid Science Research Funding.

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Public Health Nutrition: 14(2), 327–333 doi:10.1017/S1368980010001904

Twenty-four-hour urinary water-soluble vitamin levels correlate with their intakes in free-living Japanese schoolchildren

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Submitted 15 December 2009: Accepted 10 May 2010: First published online 25 June 2010

Abstract

Objective: To examine the association between 24 h urinary water-soluble vitamin levels and their intakes in free-living Japanese schoolchildren.
Design: All foods consumed for four consecutive days were recorded accurately by a weighed food record. A single 24 h urine sample was collected on the fourth day, and the urinary levels of water-soluble vitamins were measured.
Setting: An elementary school in Inazawa City, Japan.
Subjects: A total of 114 healthy, free-living, Japanese elementary-school children aged 10–12 years.
Results: The urinary level of each water-soluble vitamin was correlated positively to its mean intake in the past 2–4 d (vitamin B_1: $r = 0{\cdot}42$, $P < 0{\cdot}001$; vitamin B_2: $r = 0{\cdot}43$, $P < 0{\cdot}001$; vitamin B_6: $r = 0{\cdot}49$, $P < 0{\cdot}001$; niacin: $r = 0{\cdot}32$, $P < 0{\cdot}001$; niacin equivalents: $r = 0{\cdot}32$, $P < 0{\cdot}001$; pantothenic acid: $r = 0{\cdot}32$, $P < 0{\cdot}001$; folic acid: $r = 0{\cdot}27$, $P < 0{\cdot}01$; vitamin C: $r = 0{\cdot}39$, $P < 0.001$), except for vitamin B_{12} ($r = 0{\cdot}10$, $P =$ NS). Estimated mean intakes of water-soluble vitamins calculated using urinary levels and recovery rates were 97–102 % of their 3 d mean intake, except for vitamin B_{12} (79 %).
Conclusions: The results show that urinary levels of water-soluble vitamins, except for vitamin B_{12}, reflected their recent intakes in free-living Japanese schoolchildren and could be used as a potential biomarker to estimate mean vitamin intake.

Keywords
Urinary water-soluble vitamin
Biomarker
Free-living
Japanese schoolchildren

Since vitamin deficiencies cause various disorders in the growth of schoolchildren, a method to evaluate vitamin status easily and accurately is desired for early screening at a primary preventive stage. Methods using biomarkers for assessing vitamin intakes offer an effective approach to evaluate vitamin status in individuals. Many preceding studies have investigated urinary excretion as a biomarker for vitamin intake[1–3]. We have also reported recently that 24 h urinary levels of water-soluble vitamins correlate highly with their intakes for Japanese college students in a strictly controlled environment[4,5]. Performing a study under a free-living environment without any interventions is the next step to confirm the applicability of the biomarker method. In the present study, we examined the association between 24 h urinary excretion of water-soluble vitamins and their dietary intakes for free-living schoolchildren to confirm the validity of the findings obtained in the controlled environment.

To capture dietary intake and calculate nutrients under a free-living environment, we used a weighed food record for four consecutive days. Although a weighed food record can provide relatively precise information regarding dietary intake compared with other dietary assessment methods[6], it is difficult for schoolchildren to complete a weighed food record without support. Few studies have reported this kind of assessment for free-living schoolchildren[7], while many studies have reported using a 24 h recall[8], a dietary diary[9] or an FFQ[10]. To overcome the difficulty of using a weighed food record for schoolchildren, we formed a close and cooperative relationship not only with the children but also their parents and teachers in the target elementary school before starting the study, through supporting the prolonged dietary education programme provided by the school board.

Methods

Participants

A total of 132 healthy, free-living schoolchildren aged 10–12 years voluntarily participated in the present study.

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The purpose and protocol were explained to all participants, as well as their parents, before joining the study, and written informed consent was obtained from each parent because all participants were less than 20 years old. We excluded participants diagnosed with the common cold or influenza, and those who had taken multivitamin supplements at least once during the previous month. In addition, we excluded participants whose 24 h urine collection or dietary records were considered incomplete, with a collection time outside the range of 22–26 h, urine volume <250 ml, creatinine excretion in relation to body weight outside the range of 10·8–25·2 mg/kg[11,12] or extremely low or high energy intake (<2092 or >16 736 kJ/d)[13]. After these screenings, 114 schoolchildren (sixty-seven boys and forty-seven girls) were found to be eligible. The study was reviewed and approved by The Ethical Committee of The University of Shiga Prefecture.

Dietary records

This was a 4 d dietary assessment in which the participants were living freely and consuming their normal diet. The assessment was performed at one of the elementary schools in Inazawa City (population >130 000) in Aichi Prefecture, Japan, in June 2007 and June 2008. The first day (Monday) of the experimental period was defined as day 1, the second day as day 2, the third day as day 3, and the fourth day as day 4. All foods consumed during the 4 d period were recorded using a weighed food record[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 in the dietary record, the status of food at oral intake was identified as 'raw', 'boiled', 'cooked', 'the presence of skin', 'a part of cooking ingredients' 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 with support from their parents took photographs with the disposable camera of the dishes before and after eating. Several experienced dietitians used the photographs to check the records, asking participants or their parents to resolve any discrepancies or to give further information when needed. The food that remained after eating was measured with a digital scale and was deducted from the dietary record. For school meals, the registered dietitians completed the records on behalf of the participants. Nutrient and energy intakes were calculated using the SAS statistical software package version 6·12 (SAS Institute Inc., Cary, NC, USA), based on the current *Standard Tables of Food Composition in Japan*[15]. For vitamins, the intakes of eight water-soluble vitamins –vitamins B_1, B_2, B_6, B_{12}, niacin, pantothenic acid, folic acid and vitamin C – were calculated, except for biotin which is not designated in the current *Standard Tables of Food Composition in Japan*. Since niacin is synthesized from tryptophan, the amount of niacin equivalents was handled separately from niacin. Since 1 mg nicotinamide is synthesized from 60 mg tryptophan[16], niacin equivalents was calculated as the sum of niacin and 1/60 tryptophan intakes. For calculating mean vitamin intakes, the 2 d mean intake corresponds to average intakes on days 3 and 4. Similarly, the 3 d mean intake corresponds to average intakes on days 2–4, and the 4 d mean intake corresponds to average intakes on days 1–4.

24 h urine sampling

A single 24 h urine sample was collected on the fourth day to measure urinary levels of water-soluble vitamins and their metabolites. It was collected from the second passage of urine on the fourth day to the first passage on the fifth day. The participants were asked to record all the times of urination on the sheet. After the total urine sample was collected, the volume was measured. Aliquots of the urine were stabilized to avoid destruction of water-soluble vitamins and their metabolites, and then stored at −20°C until analysis.

Urinalysis

Urinary thiamine was determined by post-HPLC labelled fluorescence[17]. Urinary riboflavin was determined by HPLC[18]. Urinary vitamin B_6 metabolite, 4-pyridoxic acid, was determined by HPLC[19]. To measure urinary vitamin B_{12}, urine samples were added to 0·2-mM acetate buffer (pH 4·8), vitamin B_{12} was converted to cyanocobalamin by boiling for 30 min with 0·0006 % w/w potassium cyanide at acidic pH, and cyanocobalamin was determined by a microbioassay using *Lactobacillus leichmanii* ATCC 7830[20]. Urinary N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-3-carboxamide[21] and N^1-methylnicotinamide[22] were determined by HPLC, and the sum of these compounds was determined as nicotinamide metabolites. Urinary pantothenic acid was determined by a microbioassay using *Lactobacillus plantarum* ATCC 8014[23]. Urinary folic acid was determined by a microbioassay using *Lactobacillus casei* ATCC 2733[24]. Urinary reduced and oxidized ascorbic acid and 2,3-diketogluconic acid were determined by HPLC[25].

Statistical analysis

To exclude extraordinarily abnormal urinary vitamin levels which might be caused by taking unexpected fortified foods, participants in the upper 5 % limit in terms of urinary excretion for each vitamin were removed from the 114 eligible participants, and a total of 108 samples were identified to be valid for data analysis for each water-soluble vitamin. Similar to a previous free-living study[2], males and females were not separated for analysis. The SPSS for Windows statistical software package version 16 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Values are presented as means and standard deviations. Since measurements of urinary and dietary water-soluble vitamins were not distributed normally, the data were converted logarithmically. Pearson correlation

Table 1 Characteristics of the participants: 114 eligible Japanese elementary-school children aged 10–12 years

	Total (*n* 114)		Boys (*n* 67)		Girls (*n* 47)	
Variable	Mean	SD	Mean	SD	Mean	SD
Anthropometric variables						
Age (years)	10·8	0·7	10·7	0·7	11·0	0·7
Body height (cm)	144·0	7·7	142·2	7·7	146·5	7·0
Body weight (kg)	36·7	8·3	34·6	7·2	39·8	8·9
Rohrer index (kg/cm^3×10^7)	122·0	17·9	119·3	15·9	125·7	20·1
Obesity index (%)	−4·01	3·8	−6·5	13·3	0·4	13·8
Dietary intake†						
Total energy (kJ/d)	8489	1298	8665	1409	8238	1086
Protein (% of energy)	14·9	2·5	14·9	2·6	14·8	2·1
Fat (% of energy)	29·0	5·8	29·1	6·0	28·8	5·5
Carbohydrate (% of energy)	54·8	8·7	54·7	9·3	55·1	7·7
% Energy intake‡						
Breakfast	21·3		21·7		20·8	
Lunch	32·7		32·1		33·6	
Supper	31·1		31·4		30·8	
Snacks	14·8		14·8		14·9	

†Dietary intake assessed from the consecutive 4 d dietary records.
‡Average starting time of each meal: breakfast, 06.50 hours; lunch, 12.30 hours; supper, 18.40 hours.

coefficients were calculated to determine the association between urinary and dietary measurements, and between dietary and estimated water-soluble vitamin intakes. $P < 0{\cdot}05$ was considered statistically significant. An ANOVA random-effects model was used to quantify inter- and intra-individual CV (%CV), which was used to estimate variability in vitamin intake.

Results

The characteristics of the 114 eligible participants are presented in Table 1. Since each value was almost the same as those reported for children aged 10–11 years in the *Dietary Reference Intakes for Japanese* in 2005[13], the participants were considered as typical elementary-school children in Japan. During the experimental period, all participants were living freely. Inter- and intra-individual variations in dietary intake of water-soluble vitamins for the consecutive 4 d period are shown in Table 2. For intra-individual variations, %CV was 25–45 %, except for vitamin B_{12} and vitamin C. For inter-individual variations, vitamin B_1, vitamin B_{12}, folic acid and vitamin C exceeded 50 %.

The correlations between 24 h urinary excretion of water-soluble vitamins and their intakes are shown in Table 3. For all vitamins except for vitamin B_{12}, a significant positive correlation was found between urinary excretion and dietary intake on day 4. For all vitamins except for pantothenic acid, the correlations on day 4 were higher than those on other days.

To examine the influence of dietary intake during the past few days on 24 h urinary excretion, we calculated the correlations between 24 h urinary excretions and mean dietary intakes, which are shown in Table 4. For all vitamins except for B_{12}, niacin equivalents and folic acid, the correlations between the urinary excretion (column 2 in Table 3) and the 3 d mean intake (column 5 in Table 4) were higher than those based on daily intake shown in Table 3 (columns 6, 9, 12 and 15). Because the most significant correlations were found between the urinary excretion and the 3 d mean intake, recovery rates (column 11 in Table 4) were derived from the urinary excretions (column 2 in Table 3) and the 3 d mean intakes (column 5 in Table 4), which are also shown in Table 4. Estimated mean intakes of water-soluble vitamins (column 13 in Table 4) were calculated using these recovery rates and urinary excretions. Estimated mean intakes, except for vitamin B_{12}, niacin equivalents and folic acid, correlated with 3 d mean intakes and were 97–102 % of the 3 d mean intake, except for vitamin B_{12} (79 %).

Table 2 Inter- and intra-individual variations in the dietary intake of water-soluble vitamins measured for the consecutive 4 d experimental period: eligible Japanese elementary-school children aged 10–12 years

	%CV (*n* 108)†	
Vitamin	Inter-individual variations	Intra-individual variations
Vitamin B_1	71·0	31·1
Vitamin B_2	28·8	29·5
Vitamin B_6	5·7	32·1
Vitamin B_{12}	166·8	95·0
Niacin	30·4	33·1
Niacin equivalents	8·8	25·2
Pantothenic acid	42·7	25·0
Folic acid	87·4	45·0
Vitamin C	62·2	65·5

†A total of 108 samples were valid for data analysis after removing the upper 5 % limit in terms of urinary excretion for each vitamin.

Discussion

In the present study we found a significant positive correlation between the urinary excretion and the dietary

Table 3 Measured values for 24 h urinary excretion collected on day 4 and daily vitamin intake for each water-soluble vitamin, and correlation between 24 h urinary excretion and daily vitamin intake (*n* 108), among eligible Japanese elementary-school children aged 10–12 years

	24 h urinary vitamin excretion†		Vitamin intake at day 4			Vitamin intake at day 3			Vitamin intake at day 2			Vitamin intake at day 1		
Vitamin	Mean	SD	Mean	SD	*r*‡	Mean	SD	*r*‡	Mean	SD	*r*‡	Mean	SD	*r*‡
Vitamin B_1 (μmol/d)	0·766	0·383	3·13	1·01	0·41***	2·90	0·85	0·25**	2·60	0·74	0·22*	2·75	0·92	0·07
Vitamin B_2 (μmol/d)	0·290	0·209	3·47	0·94	0·36***	3·75	1·13	0·36***	3·59	1·00	0·33***	3·60	1·17	0·23*
Vitamin B_6 (μmol/d)	2·36	0·92	5·93	1·86	0·42***	5·96	1·65	0·32***	5·97	1·69	0·36***	6·00	2·41	0·17
Vitamin B_{12} (nmol/d)	0·0256	0·0147	3·15	1·97	0·18	4·85	5·93	0·14	4·76	4·29	−0·02	4·64	3·37	0·11
Niacin (μmol/d)	–		97·0	32·3	0·28***	101·7	38·2	0·11	105·3	31·3	0·21*	101·4	32·5	0·23*
Niacin equivalents (μmol/d)	65·6	27·6	214	56	0·28**	218	56	0·23**	218	52	0·16	218	56	0·25**
Pantothenic acid (μmol/d)	11·6	5·5	27·6	6·9	0·23*	30·1	7·4	0·20*	27·0	6·3	0·31***	28·7	7·8	0·25**
Folic acid (nmol/d)	16·8	6·6	575	170	0·27**	615	423	0·12	491	123	0·18	532	164	0·24*
Vitamin C (μmol/d)	161	221	477	225	0·35***	448	313	0·23*	403	289	0·26**	445	328	0·18

†Urinary excretion for each vitamin corresponds to: thiamin for vitamin B_1; riboflavin for vitamin B_2; 4-pyridoxic acid for vitamin B_6; the sum of nicotinamide, N^1-methylnicotinamide, N^1-methyl-2-pyridone-5-carboxamide and N^1-methyl-4-pyridone-3-carboxamide for niacin equivalents; the sum of reduced and oxidized ascorbic acid and 2,3-diketogluconic acid for vitamin C.
‡*r* indicates the correlation between urinary excretion and dietary intake of the vitamin; significance of the correlation: **P* < 0·05, ***P* < 0·01, ****P* < 0·001.

Table 4 Summary of values derived from measured values (daily vitamin intake and 24 h urinary excretion in Table 3), i.e. mean dietary intakes and their correlations with 24 h urinary excretion, recovery rates and estimated mean intakes (*n* 108), among eligible Japanese elementary-school children aged 10–12 years

	2 d mean vitamin intake† (day 3–day 4)			3 d mean vitamin intake (day 2–day 4)			4 d mean vitamin intake (day 1–day 4)			% Recovery‡		Estimated mean vitamin intake§			
Vitamin	Mean	SD	*r*‖	Mean	SD	*r*‖	Mean	SD	*r*‖	Mean	SD	Mean	SD	*r*¶	% Ratio††
Vitamin B_1 (μmol/d)	3·02	0·77	0·42***	2·88	0·63	0·42***	2·85	0·58	0·35***	27·6	12·2	2·83	1·42	0·37***	100
Vitamin B_2 (μmol/d)	3·61	0·85	0·41***	3·60	0·79	0·43***	3·60	0·78	0·42***	7·9	5·2	3·66	2·63	0·26**	102
Vitamin B_6 (μmol/d)	5·94	1·41	0·45***	5·95	1·29	0·49***	5·96	1·35	0·43***	39·8	14·0	5·90	2·30	0·41***	100
Vitamin B_{12} (nmol/d)	4·00	3·14	0·19*	4·25	2·55	0·10	4·35	2·10	0·10	0·7	0·6	3·72	2·14	0·06	79
Niacin (μmol/d)	99·4	26·0	0·24*	101·3	21·7	0·29**	101·4	20·4	0·32***	–		–		–	–
Niacin equivalents (μmol/d)	216	48	0·29**	217	43	0·29**	217	39	0·32***	30·7	12·6	215	91	0·20*	99
Pantothenic acid (μmol/d)	28·8	6·0	0·26**	28·2	5·6	0·32***	28·3	5·7	0·32***	41·4	19·5	28·1	13·3	0·27**	99
Folic acid (nmol/d)	595	236	0·23*	560	174	0·24*	553	147	0·27**	3·1	1·3	536	211	0·09	97
Vitamin C (μmol/d)	462	200	0·39***	442	183	0·39***	443	170	0·39***	36·4	50·3	447	613	0·39***	100

†Mean dietary intake was calculated using daily dietary intake (Table 3).
‡% Recovery rate was derived from 24 h urinary excretion (Table 3)/3 d mean intake ×100.
§Estimated mean intake was calculated using 24 h urinary excretion (Table 3) and recovery rate.
‖*r* indicates the correlation between 24 h urinary excretion (Table 3) and mean intake; significance of the correlation: **P* < 0·05, ***P* < 0·01, ****P* < 0·001.
¶*r* indicates the correlation between 3 d mean dietary intake and estimated intake; significance of the correlation: **P* < 0·05, ***P* < 0·01, ****P* < 0·001.
††% Ratio indicates the ratio between 3 d mean intake and mean estimated intake.

intake of seven water-soluble vitamins, except for vitamin B_{12}, in free-living Japanese schoolchildren aged 10–12 years. The correlation between the urinary excretion and the dietary intake on the same day as urine collection was highest, except for pantothenic acid, compared with the correlations on other days. Moreover, the correlations between the urinary excretion and the mean dietary intakes during the past 2–4 d showed higher correlations, except for vitamin B_{12} and folic acid, than those for daily intakes. These findings show that urinary levels of water-soluble vitamins are affected by not only their dietary intakes on the same day as urine collection, but also their intakes over the past few days.

The earlier intervention study showed extremely high positive correlations between urinary levels of water-soluble vitamins and their intakes[4]. In the earlier study, participants comprised college students and they consumed exactly the same defined diets, with or without synthesized water-soluble vitamin mixtures, for 4 weeks. In the present study, the dietary assessment for schoolchildren using a weighed food record was performed for four consecutive days without intervention. Assuming the dietary assessment protocol in the present study contributed best to reduce the errors in the dietary records, the similar results from the different groups and protocols indicate that the urinary levels of water-soluble vitamins are closely associated with vitamin intakes, and that this is true even for free-living schoolchildren.

Correlation coefficients between the urinary excretions and the 3 d mean intakes ranged from 0·24 to 0·49 with a mean of 0·36, except for vitamin B_{12}, which showed a lower level than reported in our earlier study[4]. The considerable inter- and intra-individual variability for vitamin intakes in a free-living environment might affect these modest correlations. In addition, several factors are also known to affect water-soluble vitamin metabolism. For example, carbohydrate and physical activity are known to affect vitamin B_1 metabolism[26–28], the bioavailability of pantothenic acid in food is half that of free pantothenic acid[29], and the single-nucleotide polymorphism of the methylenetetrahydrofolate reductase gene affects folic acid metabolism[30]. These factors might also affect the modest correlations.

The dietary habits of the schoolchildren who participated in this study were well disciplined. They had regular breakfast (before 07.00 hours), school lunch (around 12.30 hours) and supper (around 18.40 hours), with few snacks. The daily distributions of energy intakes were 21 % at breakfast, 33 % at lunch, 31 % at supper and 15 % for snacks, which is thought to be well balanced compared with that reported in a previous study: 24 % at breakfast, 30 % at lunch, 23 % at supper and 23 % for snacks[31]. Fifty-five per cent of energy intake was obtained from carbohydrates, 30 % from fats and 15 % from protein, which fits with the *Dietary Reference Intakes for Japanese*[13]. These data show that the participants had regular dietary habits with well-balanced nutrition.

In terms of the completeness of the dietary assessment in the present study, there are several limitations of using a weighed food record method. One of the limitations is the reliance on self-report. In the present study, to reduce errors associated with self-report, several dietitians reviewed the collated records along with the photos. Another limitation exists in the present food composition table in Japan. In a dietary assessment for free-living people, potential errors caused by the quality of the food composition table are inevitable, such as defects in food composition. For example, the composition of Japanese tea may vary depending on whether the extract of tea was made personally or whether it was a bottled tea beverage, because the present Japanese food composition table cannot differentiate such products. Such restrictions may lower the accuracy of the data obtained from a weighed food record. However, identifying the food status at oral intake and coding the intake according to the food composition table should contribute to increase the accuracy of the records.

In terms of completeness of 24 h urine collection, we used the INTERMAP criteria[11] as already described. Because the *p*-aminobenzoic acid (PABA) method requires intervention by taking PABA tablets orally and would be difficult for schoolchildren, we did not use that method to avoid any interventions. Because the participants in the present study were well motivated for the study, the proportion of them with incomplete urine samples was presumed to be small[32].

We have recently reported the intra-individual variations of urinary water-soluble vitamins in young Japanese, and our intervention study showed that the collection of 24 h urine samples for 1–5 d was required to estimate those values within 20 % of the true mean[33]. Indeed, correlation between the 30 d mean urinary thiamin excretion and 30 d mean thiamin intake was higher than that between daily excretion and daily intake[1]. In the present study, urinary water-soluble vitamins were measured based on a single 24 h urine sample. Thus the urinary vitamin contents have potential for data inaccuracy from variability, and the results should be interpreted cautiously. However, recent findings also suggest that using several days of 24 h urine sample would improve the relationships between urinary excretion and intake of water-soluble vitamins.

A significant correlation was not found between urinary vitamin B_{12} and dietary intake in this or a previous study[4]. This is consistent with studies showing that urinary vitamin B_{12} increased by only 1·5 to 2 times when 1 mg of vitamin B_{12}, which is 300 times higher than usual intake, was administered orally, and by 2–3 times when 0·45 mg was injected intramuscularly[34,35]. Foods including vitamin B_{12} were so limited that its intake showed an extremely high inter- and intra-individual variation in the present study.

Estimated mean intakes of water-soluble vitamins calculated using the urinary levels and recovery rates correlated

well with the 3 d mean intakes, except for vitamin B_{12} and folic acid, and the estimated mean intakes agreed exactly with the 3 d mean intakes. These findings suggest that urinary levels of water-soluble vitamins can be used as a biomarker to assess their estimated mean intakes. As training schoolchildren to collect urine samples is easier than completing weighed food records, a nutritional assessment for water-soluble vitamins using urine samples and recovery rates is expected to be one of the applications of the present study.

In conclusion, for free-living Japanese schoolchildren aged 10–12 years, we found that 24 h urinary levels of water-soluble vitamins, except for vitamin B_{12}, correlated with their recent intakes, and can be used as a biomarker to assess, compare and validate estimated mean intakes of water-soluble vitamins.

Acknowledgements

Source of funding: This study represents the results of 'Studies on the construction of evidence to revise the Dietary Reference Intake for Japanese people – Elucidation of the balance of micronutrients and major elements' (Principal Investigator: Katsumi Shibata), which was supported by a research grant for Comprehensive Related Diseases from the Ministry of Health, Labour and Welfare of Japan. *Conflict of interest:* The authors have no conflict of interest to declare. *Author responsibilities:* T.T. designed the study, performed experiments, completed the statistical analysis and prepared the manuscript. T.F. helped design the study, performed experiments and assisted with data analysis. S.S. reviewed the study and assisted with data analysis. K.S. contributed to the study design and supervised the study. All authors critically reviewed the manuscript. *Acknowledgements:* We thank all the schoolchildren and their families who supported this assessment. We also thank the teachers in Orizu Elementary School and the staff of the school board in Inazawa City, who expressed understanding and cooperated with this assessment.

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Vol.3, No.11, 672-676 (2011) **Health**
doi:10.4236/health.2011.311113

Estimation of mineral and trace element intake in vegans living in Japan by chemical analysis of duplicate diets

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Received 10 September 2011; Revised 5 October 2011; Accepted 21 October 2011.

ABSTRACT

Thirty-six daily duplicate diet samples were collected from 12 healthy female Japanese vegans and sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, manganese, iodine, selenium, chromium and molybdenum in the diets were measured to estimate mineral and trace element intake by Japanese vegans. Significantly higher intake of potassium, magnesium, phosphorus, iron, copper, manganese and molybdenum was observed in vegans than in general Japanese women, but no difference was observed in sodium, iodine, selenium and chromium intake. Vegan calcium intake tended to be low compared to that of general women but the difference was not significant. Since high potassium, magnesium and iron intakes cannot be achieved by general Japanese diets and high intake of potassium and magnesium may prevent hyperextension and cardiovascular disease in vegans, there are few problems with Japanese vegan diets regarding mineral and trace element intake, except for calcium intake, which is low as it is in the general Japanese people.

Keywords: Vegan; Mineral intake; Trace Element Intake; Duplicate Diets; Japan

1. INTRODUCTION

Vegetarian diets, essentially excluding animal foods, have become increasingly popular in developed countries [1]. These diets are classified according to the types of animal foods consumed, and strict vegetarians consuming no foods of animal origin are known as vegans. Although vegan diets cause lower serum cholesterol, lower blood pressure and a reduced risk of cardiovascular diseases, eliminating all animal foods from the diet increases the risk of several micronutrient deficiencies, including vitamin B_{12}, vitamin D and n-3 fatty acids [2]. Regarding the intake of minerals and trace elements, vegetarians, including vegans, show low intakes of calcium, zinc and selenium because the main sources of these micronutrients are animal foods in Western diets [3,4].

Traditional Asian diets are predominately plant-based, differing from Western diets. In Japan, although the consumption of meat and dairy products has increased along with the Westernization of society, more than three quarters of the energy intake still depends on plant foods [5]. Accordingly, it is thought that the effect of adopting a vegan diet on the nutrient intake pattern is different between the West and Japan. However, little research has examined the nutrient intake of vegetarians in Japan [6], and research on the intake of minerals and trace elements by Japanese vegans is scarce. In the present study, to evaluate mineral and trace element intake by Japanese vegans, duplicate diet samples were collected from Japanese vegans, and concentrations of sodium, potassium, calcium, magnesium, phosphorus, iron, zinc, copper, manganese, iodine, selenium, chromium and molybdenum were measured.

2. SUBJECTS AND METHODS

2.1. Subjects and Duplicate Diet Sampling

In the present study, vegans were defined as people eating food of plant origin only. Twelve healthy female vegans were recruited through a vegetarian food shop located in Chiba Prefecture, Japan. The characteristics of the subjects are described in **Table 1**. Duplicate meals, beverages and between-meal snacks were collected over 24 h period; 36 duplicate diets from 12 subjects were sampled for 3 consecutive days between September and November 2010. All subjects gave informed consent for the use of their personal information in this study.

Table 1. Characteristics of vegan subjects (n = 12).

	Mean ± SD	Median
Age (y)	48.4 ± 12.9	47.5
Duration of vegan diet (y)	20.7 ± 14.5	12.0
Height (cm)	156.4 ± 7.7	157.0
Weight (kg)	49.1 ± 8.9	48.5
Body mass index (kg/m^2)	19.9 ± 2.4	19.7

2.2. Treatment of Samples

The daily duplicate diet sample was freeze-dried, homogenized and milled. Approximately 1 g of the dried sample was mixed with 200 mL of 1% HCl, shaken for 30 min and centrifuged. The supernatant was filtrated with 0.45-μm membrane filter. Filtrate thus obtained was used for the determination of sodium and potassium. Another 1 g of the dried sample was heated with 10 mL metal-free HNO_3 until the disappearance of insoluble components, and then, 2 mL metal-free $HClO_4$ was added to the digestion mixture, which was further heated until the appearance of white vapor of $HClO_4$. The volume of the digest was made up to 10 mL with pure water. Diluted digest thus obtained was used for the determination of calcium, magnesium, phosphorus, iron, zinc, copper, manganese, selenium and molybdenum. For the analysis of chromium, approximately 1 g of the dried sample was heated in an electric furnace (F-B1414M; As One, Osaka, Japan) at 550˚C for 16 h [7]. After dry incineration, the remaining ash was dissolved in 10 mL of 0.1 M HNO_3. Iodine in the dried samples was extracted with 0.5% tetramethylammonium hydroxide (TMAH) [8]. Two hundred milligrams of the dried samples was mixed with 40 mL of 0.5% TMAH and left overnight. The mixture was heated at 60˚C for 6 h and centrifuged. The supernatant was filtrated through a 0.45-μm membrane filter.

2.3. Analysis

Sodium, potassium, calcium, magnesium, iron, zinc, copper and manganese were measured using atomic absorption spectrometer (AA-6300; Shimadzu, Kyoto, Japan). Iodine, selenium, chromium and molybdenum were determined by inductively coupled plasma mass spectrometry (ICPMS) with direct nebulization. The ICPMS operating conditions were as follows: instrument, ICPM-8500 (Shimadzu); forward power, 1200 W; coolant gas flow rate, 7.0 L/min; auxiliary gas flow rate, 1.5 L/min; nebulizer gas flow rate, 0.58 L/min; sampling depth, 5.0 mm; integration time, 2.0 s; number of run, 20; mode of analysis, pulse; isotopes monitored, ^{52}Cr, ^{82}Se, ^{95}Mo, ^{97}Mo, ^{98}Mo and ^{127}I. Rhodium (^{103}Rh) and tellurium (^{126}Te, ^{128}Te and ^{130}Te) were used as internal standards. Phosphorus was determined with vanadomolybdate absorption spectrometry [9]. Protein, total lipid and energy were analyzed by a commercial service system (Japan Functional Food Analysis and Research Center, Fukuoka, Japan).

2.4. Statistical Analysis

For each subject, mean daily intake was calculated from the analytical results of duplicate diet samples from 3 consecutive days. The mean and median of the daily intake for 12 subjects were then calculated. For iodine, the mean and median were also calculated when each value was logarithmically transformed because values highly varied. Mean daily intake for 12 subjects was statistically compared with the mean daily intake by general Japanese women aged 30 to 49 y described in the National Health and Nutritional Survey in Japan (NHNSJ) [10] by calculation of the *Z*-score; in which women aged 30 to 49 y in NHNSJ, 2008 (n = 1053) were regarded as a population.

3. RESULTS AND DISCUSSION

In **Table 2**, daily intake of major nutrients, minerals and trace elements by 12 Japanese female vegans was summarized and compared with those by general Japanese women and several criteria in the Dietary Reference Intakes for Japanese (DRIJ) [11]. For the intake of energy, protein and total lipids, no difference was observed between vegans and general women.

Among major mineral intake, calcium intake by vegans was below the estimated average requirement (EAR) and tended to be low compared to that by the general population. In several Western researches, calcium intake by vegans was markedly lower than that by omnivores [12] and lacto-vegetarians [13]. In the present analysis, vegan calcium intake was somewhat low but was not significantly lower than in the general Japanese calcium intake. Since calcium intake by general Japanese people is always low due to the low consumption of dairy products, the low calcium intake of Japanese vegans may be inconspicuous.

Phosphorus intake by vegans was markedly higher than by general women. In Western research, a vegan diet contains low phosphorus and is appropriate for patients with renal failure [14]. In the West, because the major source of phosphorus in general diets is dairy products, vegan phosphorus intake is comparatively low; however, Japanese people ingest phosphorus mainly from plant foods [5]. The difference in the source of

Table 2. Intake of energy, protein, lipids, minerals and trace elements in Japanese vegans.

	Vegans (n = 12)		NHNSJ, 2008[1)]		DRIJ, 2010[2)]				
	Mean ± SD	Median	Mean ± SD	Median	EAR	RDA	AI	DG	UL
Energy (kcal)	1847 ± 141	1840	1682 ± 469	1645	1750	-	-	-	-
Protein (g)	56.2 ± 8.1	58.4	60.2 ± 19.0	58.7	40	50	-	-	-
Lipids (% energy)	20.8 ± 7.3	21.0	24.5 ± 14.1	22.6	-	-	-	20 - 25	-
Sodium (mg)	3649 ± 1719	3029	3696 ± 1415[3)]	3538 [3)]	590 [3)]	-	-	<2950[3)]	-
Potassium (mg)	3610 ± 1272*	3217	1983 ± 777	1891	-	-	2000	2800	-
Calcium (mg)	361 ± 122	389	440 ± 224	406	550	650	-	-	2300
Magnesium (mg)	494 ± 112*	462	214 ± 80	204	240	290	-	-	-
Phosphorus (mg)	1225 ± 311*	1197	854 ± 284	830	-	-	-	-	3000
Iron (mg)	13.0 ± 2.4*	12.2	6.9 ± 3.0	6.5	9.0	11.0	900	-	40
Zinc (mg)	8.3 ± 1.6	9.1	7.1 ± 2.4	6.9	8	9	-	-	35
Copper (mg)	1.75 ± 0.37*	1.66	1.00 ± 0.35	0.96	0.6	0.7	-	-	10
Manganese (mg)	7.5 ± 2.2	7.9	-	-	-	-	3.5	-	11
Iodine (μg)	1865 ± 1934	1158	-	-	95	130	-	-	2200
	788 (255 - 2441)[4)]	746[5)]							
Selenium (μg)	87 ± 34	76	-	-	20	25	-	-	230
Chromium (μg)	27 ± 8	28	-	-	25	30	-	-	-
Molybdenum (μg)	540 ± 207	563	-	-	20	25	-	-	500

*Significant difference from NHNSJ data was observed at $p < 0.001$ by calculation of *Z*-score; [1)]Values for general Japanese women aged 30 to 49 y (n = 1053) quoted from the National Health and Nutrition Survey in Japan, 2008 [10]; [2)]Criteria for Japanese women aged 30 to 49 y in Dietary Reference Intakes for Japanese, 2010 [11]; EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake; DG, tentative dietary goal for preventing lifestyle-related diseases; UL, tolerable upper intake level; [3)]Calculated from the values for salt; [4)]Geometrical mean with SD range in parentheses; [5)]Median calculated after logarithmic transformation of data for each daily duplicate diet sample.

phosphorus may contribute to the difference in phosphorus intake between Western and Japanese vegans. In addition, phytate may contribute to the high phosphorus intake in vegans because whole grains and beans contain it at a high level.

No difference was observed between vegans and general women in sodium intake. On the other hand, vegan potassium intake was markedly higher than by general women and far exceeded the tentative dietary goal for preventing lifestyle-related diseases (DG) in DRIJ. Similarly, markedly higher magnesium intake was observed in vegans than in general women. This high intake of potassium and magnesium is probably due to the high consumption of vegetables and fruit.

Among trace element intake, significantly higher iron and copper intake was observed in vegans than in general women. Similarly, manganese and molybdenum intake by vegans was markedly higher than by general Japanese, as described in several reports [15,16]. Intake of these four trace elements far exceeded the recommended dietary allowance (RDA) or the adequate intake (AI) in DRIJ. High intake of copper and manganese is also reported in Western researches [17], probably, because the high consumption of whole grains and beans results in high intake of these trace elements. The mean and median of vegan molybdenum intake exceeded the tolerable upper intake level of this element in DRIJ. This is also caused by high consumption of cereals and beans since they particularly soybean, contain molybdenum at a high level [16].

Although vegan zinc intake has been reported to be low [12], there was no difference between vegans and general women; however, because it has been reported that the serum zinc level in Japanese vegetarians tends to be low [18], it is necessary to examine whether phytate and/or dietary fiber, which are contained in whole grains and beans at a high level, decrease the bioavailability of zinc in Japanese vegan diets.

Since the main sources of selenium in general Japanese diets are fish, meats and eggs [19], the low sele-

nium intake by Japanese vegans is concern; however, selenium intake by Japanese vegans was comparable to that by general Japanese described in several previous reports [19-21]. Japanese vegans may ingest selenium from imported wheat and soybeans, which contain selenium at a high level [22]. Similarly to selenium intake, iodine and chromium intake by vegans was also comparable to general Japanese people described in the literature [20,23].

In conclusion, Japanese vegans are estimated to ingest high potassium, magnesium, phosphorus, iron, copper, manganese and molybdenum compared to general Japanese people. In particular, high potassium, magnesium and iron intake cannot be achieved by ingesting general Japanese diets. High intake of potassium and magnesium may lead to the preventing of hyperextension and cardiovascular disease in vegans [24]. Accordingly, there are few problems with Japanese vegan diets regarding mineral and trace element intake, except for calcium intake, which is low as it is in general Japanese people.

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.

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Original Article

High prevalence of hypovitaminosis D and K in patients with hip fracture

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Although hip fracture is considered to be associated with hypovitaminosis D and K, few reports have previously studied both of them. We have studied the vitamin D- and K-status as well as the general nutritional status in ninety-nine patients with hip fracture. Mean serum concentration of 25hydroxy-vitamin D (25OH-D) in female fractured patients was only approximately 9 ng/mL, suggesting severe vitamin D deficiency. There was no significant difference between the two groups in serum concentration of intact parathyroid hormone in both genders and serum 25OH-D levels in the male subjects. Plasma concentrations of phylloquinone (vitamin K_1; PK) and menaquinone-7 (MK-7) were significantly lower in the fractured group than in the control group in both genders. Logistic regression analysis indicated that circulating concentrations of albumin, PK and 25OH-D were the significant and independent determinants of fracture risk, with their higher concentrations associated with decreased fracture risk. Finally, principal component analysis (PCA) was performed to summarize the clinical parameters into smaller numbers of independent components. Three components were obtained, each representing the overall nutritional status, the vitamin D status, and the vitamin K status. In conclusion, our study has shown that patients with hip fracture have vitamin D and K deficiency independent of general malnutrition.

Key Words: hypovitaminosis D, hypovitaminosis K, patients with hip fracture, general malnutrition, principal component analysis

INTRODUCTION

Hip fracture is the most serious consequence of osteoporosis. In addition to the high mortality rates after fracture, even the survivors suffer from functional impairment and limited daily activities.[1] With increased percentage of the elderly in the society, the incidence of hip fracture is constantly increasing in Japan, as in other countries.[2] Hip fracture is also considered to be a great burden to the society because of costly medical expenditure.[3]

Among the various risk factors of hip fracture so far reported are the nutritional ones including poor vitamin D and K status. "Vitamin deficiency" causes various disorders with phenotypic abnormalities, such as osteomalacia and rickets by vitamin D deficiency, and clotting abnormality by vitamin K deficiency. Recently, however, it is known that inadequate supply of vitamins, even in the milder form, causes increased susceptibility to various diseases, and is called vitamin insufficiency.[4] For example, vitamin D insufficiency, through decreased calcium absorption and negative calcium balance, is associated with decreased bone mineral density (BMD) and increased risk of fracture. The prevalence of hypovitaminosis D has been reported to be quite high in patients with hip fracture in various countries.[5-7]

The most essential role of vitamin K is to act as the coenzyme in the γ-carboxylation of glutamic acid residue (glu) to γ-carboxyglutamin acid (gla) residue, through which four of the clotting factors acquire calcium binding capacity. It has long been held that the sole physiological action of vitamin K is the γ-carboxylation of these clotting factors in the liver. Recently, however, extrahepatic action of vitamin K has come to receive much attention.[8] For example, mice devoid of the matrix gla protein (MGP) gene, which is a gla-containing protein present in the skeleton and vasculature, died of severe arterial calcification.[9] Although mice lacking the osteocalcin gene had apparently higher bone mineral density than the control ones, they were more susceptible to bone loss after ovariectomy than their normal littermates, suggesting the compromised bone quality in these mice.[10] There also have been clinical observations to show the association between vitamin K inadequacy and hip fracture. For example, high intake of vitamin K was associated with

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Manuscript received 2 August 2010. Initial review completed 8 November 2010. Revision accepted 27 January 2011.

decreased risk of hip fracture,[11] and high serum concentration of undercarboxyled osteocalcin (ucOC), which is a sensitive indicator of insufficient vitamin K action in the skeleton, was a significant risk factor of hip fracture independent of BMD.[12]

Despite these observations, there have been few reports to evaluate the status of these two bone-active vitamins in hip fractured patients.[13] Thus in the current study, we have studied the serum concentration of these two bone-active vitamins in patients with hip fracture and age-matched controls.

MATERIALS AND METHODS

Subjects

Consecutive patients with hip fracture transferred to Tamana Central Hospital were studied. The duration of the enrollment was 6 months. Written informed consent was obtained in 99 cases from the patients or a family member when obtaining the patients' approval was practically impossible because of their poor general condition. Age-matched nursing home residents in close proximity to the hospital in Tamana City served as the control. Those without severe liver or kidney dysfunction, or those receiving bone-active drugs or supplementation with vitamin D or K, were encouraged to participate in the study, and the consent was obtained in 48 cases.

Informed consent was similarly obtained in 48 cases. Their background profiles are shown in Table 1. The study protocol was approved by the Ethical Committee of Tamana Central Hospital.

Laboratory data

Blood was drawn within 24 hours following the fracture. After centrifugation, plasma and serum were stored under dark condition at –30C until assay. Serum concentration of 25 hydroxy-vitamin D (25OH-D) was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA). Serum level of intact parathyroid hormone (PTH) was measured by electro chemiluminescent immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany). Plasma vitamin K_1 (phylloquinone; PK), and K_2 (menaquinone-7; MK-7) levels were determined by high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS) using a HPLC system (Shimadzu, Kyoto, Japan) and API3000 LC-MS/MS System (Applied Biosystems, Foster City, CA) with ^{18}O-labeled vitamin K as the internal standard.[14]

Statistical analyses

Statistical analyses were done with SPSS 17.0J. Comparison of two independent groups was done with Student's t-test or Mann-Whitney test depending on normality. The association between vitamin status and the occurrence of hip fracture was analyzed by logistic regression analysis. The relationship between various nutritional indices and circulating vitamin D- and K-levels was analyzed with principal component analysis (PCA) as previously described.[15]

RESULTS

Blood tests

Baseline characteristics and data from blood examination are shown in Table 1. Serum albumin concentration was significantly lower in the fractured group in both genders, and serum cholesterol concentration and blood hemoglobin level were significantly lower in female patients with fracture. In Table 2 shows the blood concentrations of vitamin D, vitamin K and related molecules. Mean serum concentration of 25OH-D, which most reliably represents

Table 1. Patients' profiles

	Male		Female	
	Control (n=13)	Fracture (n=27)	Control (n=35)	Fracture (n=72)
Age	82.2±9.3	82.6±7.6	84.1±7.8	85.5±7.0
Serum albumin (g/dL)	4.3±0.5	3.5±0.5**	4.4±0.2	3.6±0.4**
Serum cholesterol (mg/dL)	175.4±41.9	156.1±36.6	232.3±37.0	179.4±39.4**
Serum BUN (mg/dL)	24.1±2.2	29.5±26.1	20.6±7.4	20.6±10.2
Hemoglobin (g/dL)	12.4±2.2	11.9±1.9	12.5±1.1	10.8±1.8**
Serum GOT (U/L)	26.2±20.4	32.1±9.4	23.9±7.2	20.9±7.5
Serum GPT (U/L)	19.3±16.2	22.8±21.1	13.7±8.6	14.0±8.4

Data are shown as mean ± SD. The asterisk (**) denotes that the value in fracture group is significantly different from that in control group (p<0.01) by Student's t-test. BUN, GOT, and GPT are abbreviations for blood urea nitrogen, glutamyl oxaloacetic transaminase, glutamyl pyruvate transaminase, respectively.

Table 2. Serum concentrations of vitamin D, vitamin K and related molecules

	Male		Female	
	Control (n=13)	Fracture (n=27)	Control (n=35)	Fracture (n=72)
Serum 25OH-D (ng/mL)	20.7±7.3	19.0±13.0	18.6±6.3	9.1±4.6**
Serum intact PTH (pg/mL)	64.3±53.7	61.4±34.4	56.0±23.2	67.8±33.9
Plasma PK (ng/mL)	0.55±0.31	0.31±0.24*	0.77±0.36	0.46±0.36**
Plasma MK-7 (ng/mL)	4.28±3.75	1.60±1.60**	10.8±7.01	2.67±4.13**

Data are shown as mean ± SD. The asterisk denotes that the value in fracture group is significantly different from that in control group (*; p<0.05, **; p<0.01) by Student's t-test. 25OH-D, PK , and MK-7 are the abbreviations for 25 hydroxy-vitamin D, phylloquinone, and menaquinone-7, respectively.

Table 3. Logistic regression analysis

	Odds ratio (95%CI)	*p* value
Serum 25OH-D (per 10ng/mL increase)	0.246 (0.090-0.673)	<0.001
Plasma PK (per 1ng/mL increase)	0.072 (0.009-0.612)	0.016
Albumin (per 1g/dL increase)	0.003 (0.000-0.054)	<0.001
MK-7 (per 1ng/mL increase)	0.867 (0.747-1.006)	0.061
Hemoglobin (per 1g/dL increase)	1.482 (0.891-2.465)	0.129
Sex (1; Male, 2; Female)	2.464 (0.381-15.95)	0.344

Logistic regression analysis with stepwise method was done. Sex, circulating concentrations of albumin, hemoglobin, 25OH-D, PK, and MK-7 were included for analysis.

Table 4. Principal component analysis of nutrition indices

	Component 1	Component 2	Component 3
Serum Albumin	0.744†	0.481†	-0.028
Serum total Cholesterol	0.824†	0.098	0.157
Hemoglobin	0.538†	0.589†	-0.269
Serum 25OH-D	0.035	0.902†	0.228
Plasma PK	0.191	0.109	0.922†
Plasma MK-7	0.773†	0.009	0.210

Factor loadings to three components after varimax rotation are shown. †Loadings greater than 0.35

the vitamin D status, was approximately 20 ng/mL in all groups, except for the female fracture group where it was approximately 9 ng/mL. In both genders, serum 25OH-D levels were lower than 20 ng/mL in 90% and 61% of subjects, in the fracture and control groups, respectively. It was below 10 ng/mL in 50% and 7% of subjects in the fracture and control group, respectively. Serum concentration of intact PTH, which is a sensitive indicator of vitamin D insufficiency; hence secondary hyperparathyroidism, was not different between control and fracture groups in males. It was slightly higher in the fractured group than in the control group in female, which, however, did not reach statistical significance (p=0.07).

Serum concentrations of PK and MK-7 were significantly lower in the fracture group than in the control group in both genders.

Logistic regression analysis for variables associated with hip fracture

In order to evaluate whether the above-mentioned vitamin insufficiency is related to the occurrence of hip fracture, logistic regression analysis was performed. Of the factors subjected for analysis, circulating concentrations of albumin, PK and 25OH-D were the significant determinants, whereas MK-7, gender or hemoglobin level was not (Table 3). The odds ratio for fracture markedly decreased in accordance with increased concentrations of albumin, PK and 25OH-D.

Principal component analysis (PCA)

Since patients with hip fracture are generally malnourished, we considered it to be important whether the low vitamin D- and K-status as described above simply reflects overall malnutrition. Then PCA was performed with parameters included for analysis being: serum albumin and cholesterol concentrations, blood hemoglobin levels, and plasma 25OH-D, PK and MK-7. Three components were obtained as shown in Table 4. The first component was contributed by high serum albumin, total cholesterol, blood hemoglobin and plasma MK-7. The second component consisted of high serum albumin, blood hemoglobin and serum 25OH-D. The third component was composite of high plasma PK. Each component was interpreted as follows; the first, second, and third component representing overall nutritional status, vitamin D status, and vitamin K_1 status, respectively.

DISCUSSION

In the present study, we have studied the blood concentration of 25OH-D, PTH, PK, MK-7 and other nutritional indices. In 90% of patients with hip fracture, serum 25OH-D level was lower than 20 ng/mL which is a generally accepted cut-off for hypovitaminosis D. In half of the patients, serum 25OH-D concentration fell into the severe hypovitaminosis D range of below 10 ng/mL. Nurmi *et al.* reported that serum 25OH-D level was lower than 15 ng/mL and 8 ng/mL in 53% and 9%, respectively, of the patients with hip fracture in Finland.[16] In a study on Japanese patients with hip fracture, Sakuma *et al*. reported that 62% of the patients had their serum 25OH-D level below 20 ng/ml.[7] Thus, the prevalence of hypovitaminosis D in the present study was compatible with the previous studies, but was even higher.

Serum concentration of 25OH-D in the fracture group was significantly lower than that in the control group in women, but not in men. There have been some reports to show that elderly women are more prone to vitamin D deficiency than elderly men. Hirani *et al*. reported that hypovitaminosis D was more prevalent in women than men with a odds ratio of 2.1.[17] Maggio *et al*. reported that age-related decline of serum 25OH-D was already evident shortly after age 50 in women, whereas in men it started only after age 70.[18] Thus there seems to be a gender dif-

ference that women are more prone to vitamin D inadequacy, for which there is no clear explanation at present.

Lack of significant difference in serum PTH level between fracture and control groups is most likely due to the large standard deviation in serum PTH concentration. However, there still can be alternative explanations. There have been some reports describing the absence of PTH elevation in face of hypovitaminosis D in patients with hip fracture.[19-22] Sahota *et al*. studied the vitamin D status in the post-hip fracture patients. They found that only half of them had elevated serum PTH levels, the rest had normal to low serum PTH levels in face of hypovitaminosis D.[19] As an explanation for this apparently paradoxical observation, they postulated magnesium deficiency as the underlying cause since magnesium deficiency is known to be associated with impaired PTH secretion.[20] Thus the question has now come to our attention whether skeletal impairment in hypovitaminosis D can be explained by secondary hyperparathyroidism alone. A recent paper from Finland also reported that serum PTH level was within the reference range despite hypovitaminosis D in 74.8% of the bedridden geriatric patients.[21] Patients in the lowest quartile of serum PTH level were associated with the history of hip fracture (odds ratio 2.9). Thus it is obvious that hypovitaminosis D is associated with increased risk of hip fracture, although further studies are required to determine whether it is mediated by secondary hyperparathyroidism or due to hypovitaminosis per se.

Compared to vitamin D, far smaller number of papers has been published on the relationship of vitamin K with hip fracture. Epidemiological studied have shown that higher intake of vitamin K is associated with lower risk of hip fracture.[11,23] Among the two vitamin K analogs studied here, PK seems to best represent the vitamin K status of these subjects. Kaneki *et al*. reported that there is a large geographic difference in serum MK-7 concentration in Japan, which could be accounted for by the frequency of consuming natto, which contains extraordinary amount of MK-7.[24] Blood concentrations of PK and MK-7 were consistently lower in fractured patients than control subjects in both genders.

Kawana *et al*. reported that there was no significant alteration in the circulating concentrations of PK and MK-7 in hip fractured patients.[25] In their paper, these concentrations were below the detection limit in the substantial number of subjects. Blood vitamin K levels were reported to be below the detection limit in other papers also.[24,26] In our data using newly developed LC-APCI-MS/MS method for the determination of circulating vitamin K levels, serum concentrations of PK and MK-7 were detectable in almost all subjects.[14] Thus, previous reports using less sensitive assay methods should be interpreted with caution.

In fractured subjects, serum albumin concentration was significantly lower in both genders, and hemoglobin level and serum cholesterol concentration was significantly lower in the females. Thus patients with hip fracture are malnourished. Then it was considered mandatory to analyze the relationship between the overall malnutrition and decreased levels of circulating these vitamins. We have studied it with two analytical procedures; logistic regression analysis and principal component analysis (PCA). Logistic regression analysis revealed that serum concentrations of 25OH-D, PK and albumin were significant contributing factors for fracture risk, and suggested that circulating 25OH-D and PK levels contributed to the increased risk of fracture independent of general malnutrition.

Finally PCA was done. Three components were obtained, representing overall nutritional status, vitamin D status, and vitamin K status, respectively. Since these components are, by their definition, independent of each other, these results strongly suggest that hypovitaminosis D and K in patients with hip fracture is not merely a manifestation of general malnutrition. At present, the reason for the association of MK-7 with the first component, representing the overall nutritional status is not known. We have also recently reported that institutionalized elderly subjects had high prevalence of hypovitaminosis D and K, which is independent of general malnutrition by PCA.[15]

One of the limitations of the current work is that it is a case control study, but not a prospective one. Since the association of hip fracture with the insufficiency of two bone-active vitamins; vitamin D and vitamin K has been scarce, we have done this study as the initial step.

Another limitation is that the nursing home residents adjacent to the hospital were the control subjects. It is unclear whether the control subjects represent the average Japanese elderly population or not. However, it is quite unlikely the nursing home residents have nutritional status far better than the average Japanese elderly. Rather, they are likely to be equal to or worse than the average. Thus, we believe that our finding that the blood levels of these vitamins in fractured patients were even lower than that in nursing home residents has clinical implications.

In summary, patients with hip fracture had lower serum concentration of vitamin K in both genders, and lower serum concentration of vitamin D in female subjects. Since blood samples were obtained within 24 hours after fracture, these data is likely to represent the patients' status before fracture. Lower serum albumin concentration in fractured patients suggests that these subjects are also generally malnourished. Insufficiency of these vitamins as well as the overall malnutrition is likely to predispose elderly people to hip fracture, and intervention study to correct these abnormalities is needed.

ACKNOWLEDGMENTS

This study was supported by Health and Labor Science Research Grant from the Ministry of Health, Labor and Welfare, Japan, and Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (JSPS).

AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

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Original Article

High prevalence of hypovitaminosis D and K in patients with hip fracture

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髖部骨折病患維生素 D 與 K 不足之高盛行率

過去研究顯示髖部骨折與維生素 D 及維生素 K 不足有關，但較少研究將兩者共同納入探討。本研究之對象為 99 位有髖部骨折的病患，檢測其整體營養及體內維生素 D 與維生素 K 的狀態。女性患者血清 25-羥化維生素 D(25OH-D)濃度平均只有約 9 ng/mL，顯示女性患者有嚴重維生素 D 缺乏。男女性患者血清中副甲狀腺素及男性血清 25OH-D 平均濃度與對照組皆沒有顯著差異。然而在男女性髖部骨折患者，其血漿維生素 K_1 及維生素 K_2 濃度都顯著較對照組低。以羅吉斯回歸分析發現，體內白蛋白、維生素 K_1 及 25OH-D 濃度皆為骨折發生風險之顯著獨立預測因子，具呈負相關。最後以主成份分析進行臨床參數統整後，獲得三項代表參數，分別代表整體營養狀態、維生素 D 營養狀態及維生素 K 狀態。總而言之，本研究顯示髖部骨折患者易出現維生素 D 及維生素 K 缺乏，且與整體營養不良無關。

關鍵字：維生素 D 缺乏、維生素 K 缺乏、髖部骨折病患、整體營養不良、主成分分析

Original Article

Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly

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In Japan, γ-carboxylation of blood coagulation factors is the basis for determining adequate intake (AI) for vitamin K in Dietary Reference Intakes (DRIs) issued in 2010. Recently, vitamin K is also known to be essential for preventing fracture. In this study, relative susceptibility of liver and bone to vitamin K deficiency was studied. Thirty-seven elderly institutionalized subjects were evaluated for vitamin K status by measuring serum PIVKA (protein induced by vitamin K absence) –II and ucOC (undercarboxylated osteocalcin) levels, as sensitive markers for hepatic and skeletal vitamin K deficiency, respectively. Serum PIVKA-II and ucOC levels, with their cut-off values in the parentheses, were 20.2±8.9 mAUmL (28 mAU/mL) and 4.7±3.0 ng/mL (4.5 ng/mL), respectively. Median vitamin K intake was approximately 200 μg/day, which is more than 3 times higher than the current Japanese AI. Vitamin K intake was significantly correlated with serum PIVKA-II and ucOC/OC levels, but not with serum ucOC level. Although serum ucOC level is generally a good indicator for vitamin K status, multiple regression analysis revealed that elevated bone turnover marker significantly contributed to serum ucOC level. All subjects had vitamin K intake exceeding AI for vitamin K. Nevertheless, serum PIVKA-II and ucOC concentrations exceeded the cut-off value in 14% and 43% of subjects, respectively. The present findings suggest that vitamin K intake greater than the current AI is required for the skeletal health in the institutionalized elderly.

Key Words: vitamin K, adequate intake, γ-carboxylation, ucOC, PIVKA-II

INTRODUCTION

Gamma-glutamyl carboxylase (GGCX) catalyzes the conversion of glutamyl (Glu) residue into γ-carboxyglutamyl (Gla) residue in certain proteins. The most fundamental role of vitamin K is the one as a cofactor of GGCX.[1] Although GGCX is present in various tissues, its role in the liver has received most attention until recently. In the liver, conversion of Glu residue to Gla residue takes place in four of the blood coagulation factors (II, VII, IX, and X), by which they acquire calcium-binding ability and are activated.[1] Recently, attention have been focused on the physiological roles of vitamin K-dependent proteins in extrahepatic tissues such as bone and blood vessel.[2,3] Osteocalcin is produced by osteoblasts, the most abundant non-collagenous protein in the bone matrix. Through γ-carboxylation, osteocalcin gains hydroxyapatite-binding ability, and regulates bone mineralization.[2] Recent evidences strongly suggest that skeletal vitamin K deficiency increases the risk of hip fracture.[4] Matrix Gla protein (MGP); another vitamin K-dependent protein, is an inhibitor of vascular calcification.[5-7]

In the current Japanese Dietary Reference Intakes (DRIs) issued in 2010, Adequate Intake (AI) for vitamin K in the adult is uniformly 75 μg/day for men and 65 μg/day for women. These values however, carries some problems when applied to the study population.[8] First, they are based on data from America or Europe. Since nutrients intake is greatly dependent on nationality or dietary patterns, vitamin K status in the Japanese must be studied. Second, they are from healthy young volunteers, not from the elderly who are likely to have nutrients malabsorption. This is especially the case with fat-soluble vitamins including vitamin K due to various factors such as decreased secretion of bile acids and pancreatic juice, and reduced dietary fat intake.[8] Finally, AI for vitamin K was determined as the dose sufficient to maintain normal blood coagulation with little mentioning to bone.[8] Serum levels of protein induced by vitamin K absence-II (PIVKA-II) and undercarboxylated osteocalcin (ucOC) are sensitive markers for vitamin K deficiency in the liver and bone, respectively. Vitamin K status in the liver and bone

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Manuscript received 30 May 2010. Initial review completed 4 October 2010. Revision accepted November 2010.

can be separately evaluated by measuring these markers. By employing such methodology, previous studies have shown that much higher doses of vitamin K are needed for the γ-carboxylation of osteocalcin than for that of blood coagulation factors.[9,10]

Thus it is possible that an elderly judged to be vitamin K sufficient based on the current AI has skeletal vitamin K deficiency and increased fracture risk. In this paper, we have measured serum PIVKA-II and ucOC levels, assessed vitamin K intake, and studied the prevalence of vitamin K deficiency in the liver and bone in the institutionalized elderly.

MATERIALS AND METHODS

Subjects

The study subjects were 37 institutionalized elderly (male 8, female 29) in a nursing home, Kayu-Shirakawa. Exclusion criteria were routine medication that has potential interference with bone metabolism and vitamin K status such as warfarin. None had history of hepatic diseases. Detailed information about this study was given and written consent was obtained from the subject or the proxy. The study protocol was approved by the ethical committee in Kyoto Women's University.

Laboratory data

Blood was obtained after overnight fasting. After centrifugation, serum was kept frozen at –30°C until analysis. Serum PIVKA-II and ucOC levels were measured by electro chemiluminescence immunoassay (ECLIA) (Sanko Junyaku, Co, Ltd, Tokyo, Japan) as the markers of hepatic and skeletal vitamin K deficiency, respectively. Serum intact osteocalcin (intact OC) was measured by enzyme immunoassay (EIA) (Mitsubishi Yuka, Tokyo, Japan). The ucOC/OC was calculated as the ratio of ucOC to intact OC. Serum levels of tartrate-resistant acid phosphatase-5b (TRACP-5b) and bone specific alkaline phosphatase (BAP) were measured by EIA (DS Pharma Biomedical, Osaka, Japan) and chemiluminescence enzyme immunoassay (CLEIA) (Beckman Coulter Inc, Tokyo, Japan), respectively. TRACP-5b and BAP are markers of bone resorption and bone formation, respectively. The reference range of serum TRACP-5b was 170-590 mU/dL in male and 120-420 mU/dL in female, and that of serum BAP was 3.7-20.9 μg/L in male and 3.8-22.6 μg/L in female.

Nutrition intake study

Nutrient intake was assessed by food record method. The intake of vitamin K was calculated by multiplying the amount of vitamin K supplied from the institution with the average percentage intake. Based on these records, their intake of vitamin K was calculated using the software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan). Vitamin K intake/kg body weight was also calculated, since 1 μg/kg of vitamin K is considered to be sufficient for maintaining normal coagulation in the adult according to the Japanese DRI 2010.[8]

Statistical analyses

Statistical analyses were performed using the SPSS 17.0 J for Windows (SPSS. Japan Inc, Tokyo, Japan). Association between variables was analyzed by Pearson's or Spearman rank correlation coefficient. Multiple regression analyses with stepwise method were performed to determine independent determinants for serum ucOC and ucOC/OC. Chi-square test was employed for categorical data.

RESULTS

Background profiles of the study subjects

The background profiles and biochemical data are shown in Table 1. Care level is a 5-grade score which is commonly used in the long-term care insurance in Japan with higher number indicating more intensive care needed. It was higher than grade 3 in 78% of subjects, indicating that they had low physical activity level. For example, most of the present subjects required wheelchair for transportation. In 27% of subjects, serum albumin level was lower than 3.5 g/dL, which is a generally accepted cut-off for malnutrition. Overall, nutritional parameters including the biochemical indicators and body mass index (BMI) remained within the reference range for most of the subjects. Thus, despite the elderly population and high level of care needed, the subjects' nutritional status was considered to be generally preserved. Although average serum TRACP-5b and BAP levels were within the reference range as a whole, 20% and 32% of subjects had serum BAP and TRACP-5b level above upper reference range, respectively. Serum PIVKA-II and ucOC levels were 20.2±8.9 mAU/mL and 4.7±3.0 ng/mL, respectively. All subjects were on orally consumed their meals. Although energy intakes were lower than estimated energy requirement (EER) of DRI in all men and 93% of women, the intake of macronutrients such as protein, fat and carbohydrates appeared appropriate for their age and sex. Average vitamin K intake was 194±51 (median; 197)

Table 1. Baseline data of the study subjects

	(M/F; 8/29, n=37)
Age (y)	85.1±8.2（87.0）
Care level	Median; 3 (min-max; 1-5)
Body weight (kg)	45.9±6.1（46.1）
Height (cm)	149.3±9.7（145.3）
BMI (kg/m^2)	20.6±2.5（20.0）
Serum Albumin (g/dL)	3.7±0.3（3.8）
Serum triglyceride (mg/dL)	119±41（118）
Serum total cholesterol (mg/dL)	198±49（191）
eGFR (ml/min./1.73m^2)	65.4±15.8（63.3）
Serum BAP (μg/L)	18.4±9.6（17.6）
Serum TRACP-5b (mU/dL)	365.2±124.9（372.0）
Serum ucOC (ng/mL)	4.7±3.0 (3.8)
Serum total OC (ng/mL)	6.1±3.1 (5.4)
ucOC / intact OC	0.81±0.36 (0.80)
Serum PIVKA-II (mAU/mL)	20.2±8.9 (18.0)
Energy intake (kcal)	1346±129 (1401)
Protein intake (g)	53.2±5.2 (55.4)
Fat intake (g)	35.6±3.6 (36.9)
Carbohydrates intake (g)	193.8±18.7 (199.4)
Vitamin K intake (μg/day)	194±51 (197)
Vitamin K intake/BW (μg/BW kg/day)	3.5±1.1 (3.4)

Data are expressed as mean±SD with the values in parentheses showing the median.

μg/day in the study population, 166±50 (median; 159) μg/day in males and 202±49 (median; 224) μg/day in females. It was approximately 220% and 310% of the AI in DRI in male and female subjects, respectively. All subjects had vitamin K intake exceeding AI. In addition, the vitamin K intake/kg body weight was 3.5±1.1 μg/day in the present study subjects, far exceeding 1μg/kg.

Correlations among vitamin K intake and serum PIVKA-II, OCs

Table 2 shows that vitamin K intake was significantly correlated with serum PIVKA-II and ucOC/OC levels, but not with serum ucOC concentrations. (Table 2)

Correlations among serum OCs and bone turnover markers

Serum TRACP-5b and BAP levels were significantly correlated with serum ucOC concentration, but not with ucOC/OC ratio. (Table 3)

Multiple regression analyses for serum OCs levels

Multiple regression analyses revealed that serum TRACP-5b level was a significant determinant of serum ucOC concentration. Vitamin K intake was a significant predictor for ucOC/OC. (Table 4)

Relative susceptibility of liver and bone to vitamin K deficiency

Serum PIVKA-II level exceeded the cut-off level (28 mAU/mL) in only 14% of the subjects, whereas serum ucOC concentration was above the cut-off value (4.5 ng/mL) in 43% of subjects, which was significantly different by chi-square test (p<0.001). (Table 5)

DISCUSSION

Vitamin status could be evaluated by several ways such as measuring its blood concentration or measuring the markers representing the vitamin status. Recently, we have reported that the prevalence of vitamin D- and K-deficiency is quite high in the institutionalized elderly by measuring plasma levels of 25 hydroxy-vitamin D concentration which is the best indicator of vitamin D status, and plasma vitamin K concentration.[11] Plasma vitamin K concentrations, however, only reflect the vitamin K status as a whole, and do not provide us with information regarding the vitamin K status in various tissues individually. Thus, in this study, we have evaluated the subjects' vitamin K status by measuring their serum levels of PIVKA- II and ucOC rather than their plasma vitamin K levels.

First, we have studied the association between serum levels of PIVKA-II and ucOC, and vitamin K intake. Vitamin K intake was significantly correlated with PIVKA-II and ucOC/OC, but not with ucOC. Similar findings were also reported by Booth *et al* that circulating levels of PIVKA-II and ucOC/OC ratio reflected dietary vitamin K intake, whereas serum ucOC levels did not.[9] Two mechanisms were considered to be responsible for these find-

Table 2. The correlation between vitamin K intake and serum levels of PIVKA-II and ucOC

	ucOC		ucOC/OC		PIVKA-II	
	r	*p*-value	r	*p*-value	r	*p*-value
Vitamin K intake	0.092	0.588	-0.416	0.010	-0.362	0.028

Correlations of vitamin K intake with markers for vitamin K deficiency were analyzed by Spearman rank correlation.

Table 3. The correlation of serum ucOC and uc/OC ration and bone turnover markers

	ucOC		ucOC/OC	
	r	*p*-value	r	*p*-value
Serum TRACP-5b	0.425	0.009	0.014	0.935
Serum BAP	0.517	0.001	0.243	0.147

Correlations of serum OCs with bone turnover markers were analyzed by Spearman rank correlation.

Table 4. Multiple regression analyses for serum ucOC level and ucOC/OC ratio

Dependent variable	R^2	Independent variable	β	*p*-value
ucOC	0.206**	Serum TRACP-5b	0.454	0.005
ucOC/OC	0.134*	Vitamin K	-0.366	0.026

The abbreviations are β for β coefficient. Independent predictor(s) for serum OCs levels were analyzed by multiple regression analyses with stepwise method. Sex, serum TRACP-5b, and vitamin K intake (μg) were included in all analyses.
*; p< 0.05, **; p< 0.01

Table 5. Number of subjects with vitamin K sufficiency and deficiency in the liver and bone

	Vitamin K sufficiency	Vitamin K deficiency
In the bone (serum ucOC concentration)	21 (57%)	16 (43%)
In the liver (serum PIVKA-II concentration)	32 (86%)	5 (14%)

Values represent number of subjects, with percentage of subjects in the parentheses. Vitamin K status in the bone and that in the liver were significantly different by chi-square test (p<0.001).

ings. The first is the different bioavailability of phylloquinone (PK; vitamin K_1) and menaquinones (MKs; vitamin K_2). In the present study, PK was the major form of vitamin K taken as in America or Europe,[12,13] since the subjects had no intake of natto which contains large amount of MK-7 during the study.[14] Recent studies have shown that PK can be utilized for γ-carboxylation in the liver, but can only be utilized in extrahepatic tissues after conversion into MK-4.[15,16]

Second issue is the association of serum ucOC level with bone turnover. Serum levels of BAP and TRACP-5b reflect osteoblastic bone formation and osteoclastic bone resorption, respectively, and are elevated in the high turnover state. Since osteocalcin is produced in osteoblasts,[17] it is conceivable that serum concentration of osteocalcin as well as its subfraction, ucOC level is increased with high turnover. Thus, it is currently under debate whether ucOC alone is satisfactory or measurement of ucOC as well as ucOC/OC is a better indicator of vitamin K status. In the present study, vitamin K intake was a significant predictor for ucOC/OC, but not with ucOC. Therefore, there is a possibility that ucOC/OC is a better index for vitamin K status than serum ucOC concentration. Unfortunately, however, there is no cut-off value published regarding ucOC/OC ratio, while the clinical usefulness of serum ucOC measurement is increasingly acknowledged. Thus, analysis using ucOC/OC could not be done as serum ucOC level in Table 5.

The cut-off value of 4.5 ng/mL for serum ucOC was validated by Shiraki by simultaneously evaluating the subjects' dietary intake of vitamin K, blood levels of vitamin K and ucOC.[18] They also reported that serum ucOC concentration exceeding 5.5 ng/mL was associated with increased risk of fracture. The clinical usefulness of ucOC measurement was previously reported, although with different assay procedure of hydroxy-appatite binding assay. In the European epidemiological study, Vergnaud *et al* reported that subjects in the lowest quartile of femoral neck bone mineral density (BMD) and those in the highest quartile of ucOC had increased hip fracture risk with an odds ratio of 2.4 and 1.9, respectively. These two risk factors were independent of each other, and those with both conditions had a even higher odds ratio of 5.5.[19] Thus, serum ucOC concentration is shown to be a good indicator of skeletal vitamin K deficiency, and a predictor of fracture risk.

In the current study subjects with vitamin K intake far exceeding AI, serum concentration of PIVKA-II and ucOC were within the reference range in 86% and 57% of the subjects respectively, which was significantly different. Thus, their vitamin K intake is sufficient for γ-carboxylation in the liver, but not in the bone, and bone is much more susceptible to vitamin K deficiency than liver. Such difference is likely to arise from the anatomical basis that vitamin K absorbed from the intestine is first transported to liver and preferentially used there, then utilized in extrahepatic organs.[9,10]

Booth *et al* in their depletion-repletion studies, reported that the γ-carboxylation of prothrombin was restored at 200 μg/day of PK, whereas that of osteocalcin was not even at 450 μg/day of PK.[9] Schurgers *et al* also reported that undercarboxylated prothrombin concentration was significantly decreased at supplementary intake of 100 μg/day of PK, whereas ucOC level did not decrease below 300 μg/day of PK.[10] Furthermore, Binkley *et al* reported that supplementation with 1,000 μg/day of vitamin K was optimal for the maximal γ-carboxylation of osteocalcin.[20] These results suggest that at least 300-500 μg g/day of vitamin K intake is required for the sufficient γ-carboxylation in the bone. Our results in the Japanese elderly are compatible with these results from Caucasians, and have additionally provided data on the prevalence of hepatic and skeletal vitamin K deficiency.

We believe that this paper is of importance in considering the AI for vitamin K. The current DRI states that the AI for vitamin K was determined based on its requirement for the γ-carboxylation of blood coagulation factors. The present findings suggest that vitamin K intake greater than the current AI is required for the skeletal health in the institutionalized elderly. Further studies with lager number of subjects and intervention studies are necessary to define the amount of vitamin K necessary for the elderly.

AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

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Original Article

Bone is more susceptible to vitamin K deficiency than liver in the institutionalized elderly

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居住機構老人骨骼比肝臟易受維生素 K 缺乏影響

日本 2010 年發佈的膳食營養素參考攝取量(DRI)中，維生素 K 的足夠攝取量是根據凝血因子的 γ-羧化作用而訂定的。近來，維生素 K 也被視為預防骨折不可或缺的角色。本研究在於比較肝和骨骼對維生素 K 缺乏的敏感性。評估 37 位居住機構的老人之維生素 K 狀況─測量血清 PIVKA–II (因維生素 K 缺乏所產生的蛋白質)和 ucOC (未羧化的骨鈣素)濃度，兩者分別為肝和骨骼在維生素 K 缺乏時的敏感指標。受試者血清 PIVKA-II 和 ucOC 濃度分別為 20.2±8.9 mAU/mL (臨界值 28 mAU/mL)和 4.7±3.0 ng/mL (臨界值 4.5 ng/mL)。維生素 K 攝取量中位數約為 200 μg/day，超過了日本目前所建議的足夠攝取量 3 倍。維生素 K 攝取量與血清 PIVKA-II 和 ucOC/OC 濃度顯著相關，但與血清 ucOC 濃度無相關。雖然血清 ucOC 濃度是體內維生素 K 狀況很好的指標，但複迴歸分析顯示骨骼轉換標記增加，也會影響血清 ucOC 濃度。所有的受試者維生素 K 攝取量皆超過足夠攝取量。然而，分別有 14%和 43%受試者的血清 PIVKA-II 和 ucOC 濃度超過臨界值。本研究結果建議，對於住在機構的老人，為維持骨骼健康，維生素 K 攝取量應超過目前建議的足夠攝取量。

關鍵字：維生素 K、足夠攝取量、γ-羧化作用、未羧化骨鈣素、PIVKA-II

Medical Hypotheses 76 (2011) 336–338

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Body weight divided by squared knee height as an alternative to body mass index

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ARTICLE INFO

Article history:
Received 16 September 2010
Accepted 22 October 2010

ABSTRACT

Weight/height^2 (Quetelet's index) is the basis for defining both underweight and obesity. Height, however, is often not precisely measurable in the elderly due to involutional changes such as spinal deformity. Body volume or body surface area are not proportionately decreased even with height loss. Previous reports have shown that Quetelet's index is overestimated in the elderly with height loss. Then we have made a hypothesis described below.

Maximal height or height at youth would better represent the subjects' nutritional or clinical status. The distinction of these two heights has not been mentioned before. There have been many publications showing the equations to estimate height from the surrogate parameter(s) such as knee height (KH). Most equations published so far are expressed as estimated height = $a + b \times \mathrm{KH} - c \times$ age, where a, b, and c are constants. Negative correction by age is unexceptionally far greater in women than in men. Apparently, previous researchers have estimated current height by their equations.

Maximal height cannot be measurable. It, however, is unaffected by age by its definition. Therefore, maximal height does not have to be corrected by age, and would be almost proportional to KH. Then weight/KH^2 could be a better alternative to the most commonly used weight-height ratio; weight/height^2; the Quetelet's index.

Height is the basis for various clinically important indices such as body surface area (BSA) and energy requirement. Employing current height could lead to the underestimation of BSA or energy requirement in the elderly with height loss. Our hypothesis described here would yield a novel and better indices for the clinical assessment of the elderly.

Introduction

Body weight is one of the most fundamental indices in the clinical evaluation of the subjects. Longitudinal and serial observation of body weight provides us with valuable information concerning the alteration in the subjects' clinical status. In the cross-sectional settings, however, the usefulness of body weight for the clinical evaluation is seriously limited by the fact that it is influenced by the body size. Therefore, weight must be corrected by some parameter(s) representing the body size. For such purpose, body weight divided by squared height (weight/height^2) is commonly employed and called body mass index (BMI) [1]. High BMI and low BMI are the basis for the diagnosis of obesity and emaciation, respectively.

Why is the weight/height^2 the standard weight-height ratio? Since human body is three-dimensionally structured, body volume may be proportional to height^3. In that case, body weight would be proportional to height^3. Then one could argue for weight/height^3 as another weight-height ratio. Indeed, weight/height^2 is not the only weight-height ratio, and might be more properly called Quetelet's index (hereafter abbreviated as QI) [1,2].

Other weight-height ratios have been reported, such as weight/height ratio (weight/height), Khosla-Lowe index (weight/height^3), Ponderal index (weight/height$^{1/3}$), Benn's idex (weight/height^p) where p is a population-specific exponent [3]. Of these, QI is considered to be the most appropriate weight-height ratio, since it fulfills the following requirements.

Thus, a preferred weight-height ratio must be maximally correlated with body mass and minimally correlated with stature [4]. Hereafter in this paper, weight/height^2 will be designated as QI rather than BMI for clarity. The idea of body weight divided by squared height (weight/height^2) as the weight-height ratio was originally developed by a Belgian mathematician, Adolphe Quetelet in the 19th century [2]. World Health Organization (WHO) defined overweight as well as underweight based on QI [5]. In other words, QI is not the theoretically derived standard weight-height ratio *a priori*, but has become the *de facto* standard because of its clinical usefulness.

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doi:10.1016/j.mehy.2010.10.033

Quetelet's index in the elderly

The curve showing the relationship between QI and mortality are considered to be U-shaped. Thus, both overweight or obesity, and underweight are associated with increased mortality. There have been numerous publications, however, to cast doubt that the above theory holds true in the elderly [4,6–8]. Two examples will be given below.

First, many studies have indicated that the optimal QI associated with the lowest mortality differs in the elderly and in the younger generations. Andres reported that QI with the lowest mortality was 22.9 kg/m^2, 25.8 kg/m^2, and, 26.6 kg/m^2 in men aged 40–49 years, 50–59 years, and 60–69 years, respectively. In women, it was 23.2 kg/m^2, 25.2 kg/m^2, and 27.3 kg/m^2, respectively [4]. Matsuo also reported that QI with the lowest mortality was 22.5 kg/m^2 and 24.8 kg/m^2 in men aged 40–59 years and 60–79 years old, and it was 21.9 kg/m^2 and 23.3 kg/m^2 in women aged 40–59 years and 60–79 years old [6]. Thus, it has consistently been demonstrated that QI with the lowest mortality is higher than in the younger generations.

Second, the association between the underweight and the all-cause mortality is much more debated. Excess mortality associated with underweight has been reported to be lower, higher, or unaffected by aging [6].

Height loss in the elderly

Aging is almost inevitably associated with height loss. Various factors contribute to the involutional height loss, the most important cause of which would be the vertebral compression fracture caused by osteoporosis. Osteoporosis is a condition that renders the patients susceptible to fragility fractures, such as spinal, hip, and wrist fractures [9]. Spinal fracture, which is the most common osteoporosis-related fracture, is a compression one in its nature. Thus it causes spinal deformity and height loss. Since post-menopausal estrogen deficiency is the most important cause of osteoporosis, it is quite conceivable that women are at much greater risk for height loss than men. Prospective study with repeated height measurement has confirmed that women indeed lost more height than men [10]. Sorkin et al. have reported that average cumulative height loss from age 30 to 70 was 3 cm for men and 5 cm for women, and that from age 30 to 80 was 5 cm for men and 8 cm for women. Such involutional height loss poses serious problem upon the validity of QI as the weight-height ratio in the elderly. It is already pointed out that there will be an overestimation of QI in the elderly because of shortened height [10]. As described above, an ideal weight-height ratio should be minimally correlated with stature. QI is generally considered to fulfill this requirement, which, however, may not always hold true in the elderly.

Estimation of height using surrogate measurement

Height cannot be exactly measured in the elderly too often, due to various reasons such as vertebral fracture, disc degeneration and frailty. Then height is estimated from the surrogate parameters such as arm span and knee height (KH) [1]. Of these, KH is the most frequently used, since it could be easily measured even in the elderly and minimally affected by the involutional changes. Many equations have been so far published to predict height from KH, the most well known of which is Chumlea's one; height = 64.19 + 2.02 × KH−0.04 × age for men, and 84.88 + 1.83 × KH−0.24 × age for women [11]. In most equations hitherto published, negative height correction by age is much greater in females than in males, the reason of which, however, has not been described in the previous publications.

Hypotheses

Height generally means the distance between the top and bottom of the body. Height loss means the shortening of this distance. We believe that two types of height should be distinguished in the clinical evaluation of the elderly; height A and height B in Fig. 1. Height A is the current height. Height B would be quite close to the height at youth or maximal height. A woman in Fig. 1 has spinal deformity and height loss, but her body volume or body surface area is not proportionally diminished. Then, it is obvious that calculating QI using the current height (height A in Fig. 1) would lead to the significant overestimation.

Although previous authors on establishing the equation to predict height from KH do not seem to have considered the distinction of these two heights, but much greater negative correction by age in women strongly indicate that they meant current height in their equations.

Then we have come to an idea that employing maximal height would yield a better estimate of weight–height ratio in the elderly. Since maximal height cannot be measured in the elderly, it must be estimated. Unlike the current height, maximal height is independent of age by its definition. Therefore, the correction by age would be unnecessary for the estimation of maximal height. KH is little affected by age and would be almost proportional to maximal height.

Then we have made a hypothesis that weight divided by squared KH (BMI–KH) could be a good alternative to usual QI in the clinical evaluation of the elderly. Malnutrition in the elderly is a major health problem, and the significance of nutritional assessment has been stressed [12,13]. Despite many parameters currently available, such as anthropometric and laboratory ones, there is no consensus on what parameter would best predict the nutritional status of the elderly. We believe that BMI–KH could be a promising alternative to usual BMI; Quetelet's index in the elderly.

Clinical implications of BMI–KH

We believe that taking the distinction of two heights could yield a solution to the above-mentioned apparent paradox regarding the QI in the elderly.

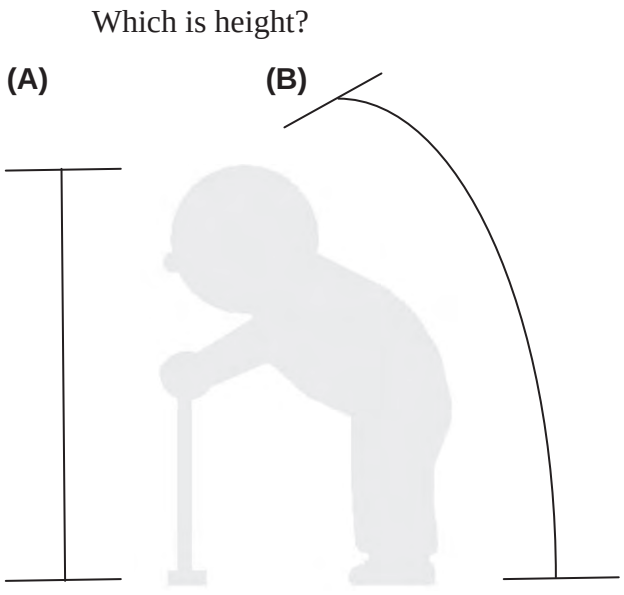

Fig. 1. Two heights are shown. Height (A) is the distance between the ground and the top of the body, and the current height. Height (B) corresponds to the maximal height or height at youth.

As the possible causes of inconsistency in the association between obesity and underweight and mortality in the elderly, Nagai et al. have mentioned various possibilities, such as history of cancer and cardiovascular diseases, inadequate adjustment for several confounders including smoking, alcohol consumption, physical activity, and socioeconomic status [8]. Another factor of importance not mentioned by them is the validity of height measurement. Given the QI calculated with current height, thus overestimated, the relationship between QI and mortality would be obscured. Although higher QI has been reported to be associated with the lowest mortality in the elderly [4,6], "true" QI may not be high.

In a large-scale and long-term cohort study in Japan, multivariate-adjusted relative risk (RR) for all-cause mortality was greatly dependent on the age of the study subjects in women [6]. In those 40–59 years old, RR in the group with QI exceeding 30 kg/m^2 was 2.23 (95% confidence interval; CI 1.46–3.42) compared to that with QI 21.0–22.9 kg/m^2, whereas it was only 1.39 (95% CI 1.14–1.69) 60–79 years old. In contrast, RR in men was not different between age groups. Although the reason for this gender difference is not discussed by the authors, we believe that our hypothesis could yield a possible explanation. Since women are much more likely to lose their height, their QI is quite prone to be overestimated. Thus it is possible that the subjects with QI higher than 30 kg/m^2 in the above-mentioned study is actually not obese, thus was not associated with higher mortality.

Other clinical implications

Height is an essential anthropometric parameter in the patients' evaluation. In addition to BMI, It is also a prerequisite for the calculation of such important indices as body surface area (BSA), and resting energy expenditure (REE) [14,15]. BSA is considered to be superior to body weight as an index of metabolically active mass, since it is less affected by adiposity. BSA could be calculated by various equations such the one by Dubois and Dubois; BSA $(m^2) = 0.007184 \times \text{weight (kg)}^{0.425} \times \text{height (cm)}^{0.725}$ [14]. BSA has various clinical applications with some examples given below. Glomerular filtration rate (GFR) is corrected by BSA [16]. Furthermore, dosage of some therapeutic drugs is determined in terms of BSA [17].

Furthermore, height is needed for determining the patients' energy expenditure. REE is usually calculated using various equations, the well-known of which is the Harris-Benedict equation; BMR = 66 + 13.7 × weight (kg) + 5 × height (cm)−6.76 × age (years) for men and BMR = 655 + 9.6 × weight (kg) + 1.8 × height (cm)−4.7 × age (years) for women [15]. Patients' total energy expenditure (TEE) is then calculated as TEE = REE × activity factor × stress factor. Thus height is necessary in deciding how much energy the patients' need, and essential in the medical nutritional therapy. Then, estimating height using the above-mentioned equations can be problematic. For example, if a dietitian estimates height from KH using Chumlea's equation, calculate REE with Harris-Benedict equation using the height estimated from KH, and determine the TEE, i.e. the energy intake necessary for the elderly subjects, it could be a substantial underestimate. Then the elderly subjects may experience malnutrition due to insufficient energy supplied. Epidemiological studies, favorably the cohort ones, with mortality and morbidity as the clinical outcomes, are to be performed for the comparison of QI and BMI–KH as the clinical useful weight-height ratio in the elderly.

Although detailed consideration on the possible roles of KH in estimating BSA or BEE is beyond the scope of this manuscript, we believe that there is a possibility that these parameters are better predicted by maximal height or KH than the current height.

Conflict of interest

None of the authors have any conflict of interest.

Acknowledgement

We would like appreciate Kiyoaki Tanaka for preparing Figure 1.

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Hindawi Publishing Corporation
Ulcers
Volume 2011, Article ID 594532, 5 pages
doi:10.1155/2011/594532

Clinical Study

Fat Restriction Is Associated with Impaired Quality of Life in Patients with Ulcerative Colitis and Crohn's Disease

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Received 9 August 2010; Accepted 21 September 2010

Academic Editor: Gyula Mozsik

Inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease, is reported to be associated with impaired health-related quality of life (QOL). Although decreased QOL in these subjects has been reported to be associated with various factors, the effect of nutritional therapy, especially nutrients intake on QOL has received less attention. In this study, we evaluated the various factors including nutrients intake on QOL using SF-8 in 64 patients with IBD. Patients with IBD seem to have decreased QOL especially in the mental aspects. The percentage energy intake from fat of total energy fat intake (% energy) of the whole subjects, was lower than those of the annual National Nutrition Survey in Japan. Multiple regression analyses revealed that fat intake (% energy) was a significant predictor for mental component summary. In conclusion, fat restriction contributes to impaired QOL especially in the mental aspects in IBD patients.

1. Introduction

Inflammatory bowel disease (IBD); ulcerative colitis (UC) and Crohn's disease, is reported to be associated with impaired health-related quality of life (HR-QOL). In this paper, HR-QOL will be simply designated as QOL. Decreased QOL in these subjects has been reported to be related to various factors such as age, gender [1, 2], treatment effects [3], disease activity, and social environment [4]. However, the effect of nutritional therapy on the QOL of IBD patients has received less attention, most of which is devoted to the parenteral nutrition therapy, not the nutritional therapy in general [5, 6].

Since excessive fat intake is considered to worsen the inflammation in the intestine, its restriction has traditionally been employed in Japan as the oral nutritional therapy for IBD patients, especially for those with CD, which, however, has its own pros and cons.

Recently, we have studied the possible involvement of hypovitaminosis D and K in the development of osteoporosis in IBD patients [7]. In face of apparently sufficient intake of these vitamins, their plasma levels were quite low in these patients. Paradoxically, plasma concentrations of vitamin D and K were correlated with the fat intake but not with their intake of these vitamins. These results were more prominent in patients with CD than those with UC. Then it was concluded that fat-soluble substances such as vitamin D and K were not effectively absorbed from the intestine without concomitant intake of enough fat.

Through this paper, we were interested in what fat restriction means from the patients' perspectives and studied

the effect of fat restriction on the QOL of IBD subjects in this paper.

2. Subjects and Methods

2.1. Subjects. Study subjects were 64 patients with IBD attending the gastroenterology clinic at the Kyoto University Hospital; 33 with CD (19 men/14 women) and 31 with UC (20 men/11 women). Detailed information was given and written consent was obtained. The study protocol was approved by the ethical committee of the Kyoto Women's University. Almost all patients (27/33 in CD and 28/33 in UC) were receiving 5-aminosalicylic acid. Glucocorticoid therapy was given to four and two patients with CD and UC, respectively. Immunosuppressive drug therapy was performed in 25 and 4 patients with CD and UC, respectively. Eight patients with CD, but none with UC, were on combined therapy of infliximab, synthetic glucocorticoid, and immunosuppressive drug. Fifteen patients with CD and one with UC were on enteral or total parenteral nutrition therapy, respectively.

2.2. Methods

2.2.1. Dietary Information. Dietary information was obtained from food intake records in 2 weekdays by the patients. By calculating these records, their energy and nutrients intakes were obtained by computer software program (Healthy Maker Pro 501, Mushroom soft Corp.).

2.2.2. QOL Measurement. QOL was assessed using the Japanese Short Form Health Survey (SF-8), a widely used generic questionnaire [8]. Eight subscales are obtained; physical function (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social function (SF), role emotional (RE), and mental health (MH). RP and RE refer to the limitations due to physical or emotional reasons, respectively. They are also summarized into two summary scores: physical component summary (PCS) and mental component summary (MCS). Data are transformed to deviation scores based on Japanese norms [8]. Higher scores indicate better QOL, with 50 corresponding to the national norms.

2.2.3. Statistical Analyses. Statistical analyses were performed using SPSS 17.0 J for Windows (SPSS. Japan Inc., Tokyo, Japan). Comparison of data from IBD patients with Japanese norms was done by one-sample t test. The difference between two independent groups was analyzed by unpaired t test or Mann-Whitney test depending on normality. Correlations between two independent variables were analyzed by Pearson's or Spearman's correlations. Multiple regression analysis was performed to determine independent factors for QOL scores in IBD patients.

3. Result

3.1. Background Profiles and Biochemical Indices. The baseline characteristics of the patients are shown in Table 1.

Table 1: Background profiles and results from blood tests in patients with CD and UC.

	CD	UC	P value
Age (y)	35.6 ± 7.3	41.7 ± 17.3	.343[a]
Sex (F/M)	19/14	20/11	—
Disease duration (y)	13.7 ± 7.4	6.8 ± 4.8	<.001[b]
Body mass index (kg/m^2)	19.5 ± 2.3	21.1 ± 3.3	.025[b]
Disease location (involving small bowel/not involving small bowel)	30/2	0/31	—
Glucocorticoid therpy	4	2	—
Immunosuppressive therapy	25	4	—
Immunopotentiating therapy (TNF-α)	8	0	—
Enteral or total parenteral nutrition therapy	15	1	—
C-reactive protein (g/dl)	0.6 ± 1.0	0.3 ± 0.6	.135[b]
Albumin (g/dl)	3.9 ± 0.4	4.3 ± 0.3	<.001[b]
Total cholesterol (mg/dl)	126.9 ± 25.0	177.1 ± 40.3	<.001[b]

Values represent mean ± SD. Comparison of indices between patients with CD and those with UC was done by unpaired t test[a] or Mann-Whitney test[b] depending on normality.

CD patients had significantly longer disease duration and lower BMI than UC patients. While nutritional indices such as serum albumin and total cholesterol were lower in CD subjects, there was no significant difference in C-reactive protein which is an inflammatory parameter between these groups. Most of patients were in remission.

3.2. Energy and Nutrients Intake in CD and UC Patients. Food intake could be evaluated in 62 patients (31 with CD and 31 with UC). Energy and nutrients intake in these patients is shown in Table 2. Fourteen patients with CD were on enteral nutrition, and each one of subjects with CD and UC was on total parental nutrition. Although the energy intake was not significantly different between the two groups, fat intake was significantly lower in CD patients than UC subjects. The annual National Nutrition Survey in Japan (NNS-J) in 2008 showed that in subjects of 30–39 or 40–49, years of age including both genders [9], the daily fat intake (% energy) was 26.5% or 25.6%, respectively. These were significantly higher than those of IBD subjects in this study ($P = .001$; data not shown). Subjects with enteral or parental nutrition had fat intake only approximately half of that in subjects with oral intake (data not shown). The percentage energy intake from protein, fat, and carbohydrates was significantly different between CD and UC subjects.

Table 2: Comparison of nutrient intakes in CD and UC patients.

		IBD ($n = 62$)	CD ($n = 31$)	UC ($n = 31$)	P value
Energy	Intake (kcal)	1816 ± 465 (1804)	1847 ± 392 (1842)	1785 ± 533 (1764)	NS
Protein	Intake (g)	66.0 ± 21.8 (63.5)	71.0 ± 20.6 (67.2)	60.9 ± 22.0 (61.6)	NS
Fat	Intake (g)	44.7 ± 21.6 (43.0)	38.7 ± 17.6 (37.4)	50.6 ± 23.6 (48.1)	$P < .05$
Carbohydrates	Intake (g)	275.4 ± 91.6 (268.6)	298.3 ± 93.1 (275.7)	252.4 ± 85.4 (254.9)	$P < .05$
Protein (% energy)		14.4 ± 2.7 (14.2)	15.0 ± 2.2 (15.6)	13.5 ± 2.9 (13.6)	$P < .001$
Fat (% energy)		22.4 ± 9.6 (24.6)	19.5 ± 8.9 (18.9)	25.2 ± 9.5 (26.8)	$P < .001$
Carbohydrates (% energy)		63.2 ± 9.6 (62.4)	65.2 ± 8.6 (64.0)	56.5±9.5 (60.5)	$P < .001$

Data are expressed as mean ± SD with the values in parentheses showing the median. Comparison of indices between patients with CD and those with UC was done by unpaired t test

Table 3: Dimensional SF-8 scores in patients with CD and UC.

	IBD ($n = 64$)	CD ($n = 33$)	UC ($n = 31$)
PF	50.1 ± 4.7 (53.6)	50.1 ± 4.5 (53.6)	50.0 ± 5.0 (53.6)
RP	*48.2 ± 6.8 (48.5)	48.7 ± 5.3 (48.5)	47.7 ± 8.1 (48.5)
BP	50.8 ± 7.6 (51.8)	50.5 ± 6.8 (51.8)	51.2 ± 8.5 (51.8)
GH	*47.8 ± 7.5 (50.7)	*47.7 ± 6.5 (50.7)	47.8 ± 8.5 (50.7)
VT	49.6 ± 6.5 (54.5)	48.4 ± 5.7 (45.3)	51.0 ± 7.1 (54.5)
SF	**46.2 ± 8.3 (45.2)	*46.9 ± 7.2 (45.2)	*45.5 ± 9.4 (45.2)
RE	*48.3 ± 6.4 (49.1)	48.0 ± 6.5 (49.1)	48.6 ± 6.5 (49.1)
MH	**47.3 ± 6.5 (45.0)	*46.8 ± 7.5 (45.0)	*47.8 ± 5.4 (50.3)
PCS	49.0 ± 6.7 (49.1)	49.2 ± 5.4 (49.0)	48.9 ± 7.9 (50.0)
MCS	***46.1 ± 6.6 (46.5)	**45.7 ± 7.1 (46.6)	**46.6 ± 6.0 (46.5)

Data are expressed as mean ±SD with median in the parentheses. One-sample t test was used for comparison between Japanese norms and scores of CD or UC patients. The asterisk denotes the significant difference (*$P >$ .05; **$P >$ .01; ***$P >$ .001).

3.3. QOL Assessment. In Table 3 is shown the eight subscales and two summary scores of SF-8 in subjects with IBD patients. Since data are expressed as the deviation values normalized by the Japanese normative values, the value "50" correponds to Japanese norm. Subscales such as RP, GH, SF, MH, and MCS were significantly lower than the Japanese norms.

Table 3 shows the comparison between CD and UC subjects. There were no significant differences in the eight subscales and two summary scores except for lower VT in CD patients than in those with UC.

3.4. Correlations between PCS/MCS Scores and Clinical Characteristics, Biochemical Markers, and Nutritients Intakes. We analyzed the correlation between these summary scores and biochemical indices, fat intake expressed as the percentage energy intake from fat of total energy, fat intake (% energy) (Table 4). Fat intake (% energy) was significantly correlated with MCS in CD patients. There was significant but weak, correlation between PCS and serum albumin and MCS and BMI in UC patients. In the whole subjects, BMI was significantly correlated with PCS, and fat intake (% energy) was associated with MCS.

3.5. Multiple Regression Analysis for Variable Associated with PCS/MCS Scores. Then multiple regression analyses were done to study the determinant(s) of the subjects' PCS and MCS (Table 5). Variables included in the analysis were types of disease (CD/UC), BMI, serum concentrations of Alb, and fat intake (% energy). BMI was the significant predictor of PCS score (βcoefficient 0.29, $P = .023$) whereas fat intake was the only significant determinant of MCS score (βcoefficient 0.29, $P = .027$).

4. Discussion

Recently, various questionnaires have been developed for QOL evaluation, both generic and disease targeted [10]. Generic ones, by their definition, only consist of questions related to the subjects' general status and do not include the questions related to the features which are specific to a certain disease. Therefore, they are applicable to such studies as comparing the impact on QOL by various diseases or even to the evaluation of healthy subjects. In contrast, disease-targeted ones include items specific to a certain disease. They can be more sensitive than the generic ones in detecting the QOL impairment closely related to a certain disease state but are not applicable to the evaluation of patients with other diseases. Various disease-targeted questionnaires have been developed for IBD subjects; the most well known of which would be IBDQ (inflammatory bowel disease questionnaire) including many items related to the patients' gastroenterological problems [11]. Since the purpose of our current work was to study the effects of nutritional therapy on the patients' QOL, we considered it more appropriate to evaluate the patients' QOL using the generic questionnaire.

SF-36 is one of the most commonly used generic questionnaires, and SF-8, used in this study, is the shortened one. Eight subscales, two summary scores are obtained, and expressed as the deviation values, which are normalized by the nations' normative value. Many previous papers on the QOL of IBD patients using SF-36 seem to have handled the data improperly [2, 4]. For example, Bernklev and Andersson expressed their data as the 0–100 scale scores [2, 4], which

Table 4: Correlations between PCS/MCS scale scores and clinical characteristics, biochemical markers, and fat intake as proportion of total energy intake.

		IBD ($n = 64$)		CD ($n = 33$)		UC ($n = 31$)	
		PCS	MCS	PCS	MCS	PCS	MCS
Diseaae duration (y)	r	0.012	−0.175	0.070	−0.221	−0.085	−0.066
Body mass index (kg/m^2)	r	0.261*	0.088	0.144	−0.075	0.248	0.415*
C-reactive protein (g/dl)	r	−0.083	0.075	−0.058	0.196	−0.116	−0.045
Albumin (g/dl)	r	0.235	0.082	0.092	0.064	0.424*	0.059
Total cholesterol (mg/dl)	r	0.033	0.196	−0.132	0.169	0.174	0.249
Fat intake(% energy)	r	0.175	0.287*	0.146	0.458***	0.238	0.109

The asterisk denotes the value is significant correlation (*$P < .05$, **$P < .01$, ***$P < .001$) by Pearson's correlation or Spearman's correlation.

Table 5: Multiple regression analyses for the predictor(s) of PCS and MCS scores in IBD patients.

	PCS score		MCS score	
	$r^2 = 0.086$	$P = .023$	$r^2 = 0.081$	$P = .027$
	β	P	β	P
CD/UC (1;CD, 2;UC)	−0.141	.283	−0.059	.657
BMI	0.293	.023	0.069	.594
Alb	0.141	.309	0.024	.855
Fat intake (% total energy)	0.121	.347	0.285	.027

Abbreviations are as follow: β for β coefficient and P for P value. Determinants of independent predictors for PCS/MCS scores were analyzed by multivariate analysis with stepwise method. Variables included were CD/UC, BMI, serum albumin concentration, and fat intake (% total energy)

can be misleading [12]. In the present paper, data were analyzed according to the authorized instruction.

In this study, subscales such as RP, GH, SF, RE, MH, and MCS were significantly lower than the Japanese norms. Decreased RP in face of normal PF is conceivable considering that the patients do not have severe physical impairment but have some limitation in their daily activities by reasons such as the bowel habit problem. Impaired SF would be also conceivable from the similar viewpoint. As a whole, patients with IBD seem to have decreased QOL especially in the mental aspects.

Then, we have analyzed variables associated with PCS and MCS. There were substantial differences in the objective clinical features of patients with CD and UC. For example, CD patients had longer disease duration and lower nutritional status than those of UC subjects. Nevertheless, there were no significant differences in 7 out of 8 dimensions between the two conditions. Namely, QOL which represents the patients' subjective evaluation of their health states seems to be impaired in both CD and UC patients.

Then, we have studied the determinants for PCS and MCS. PCS score was correlated with indices representing their nutritional status such as BMI ($r = 0.261$, $P < .05$) and albumin with marginal significance ($r = 0.235$, $P = .066$). In contrast, none of these factors were significantly correlated with MCS. Thus, it was considered unlikely that disease activities or other clinical features alone could account for the impaired mental aspects of QOL in these subjects. The association of QOL with mental aspects of the subjects has been previously reported. Boye et al. reported that neuroticism was a significant predictor for mental and vitality subscales of SF-36 in IBD patients using multiple regression analyses controlled for gender, age, and clinical disease activity [13]. Martin also reported that QOL was not closely correlated with the clinical features in CD patients [14]. These results, together with our current findings, suggest that mental aspects can more strongly affect QOL than clinical ones in IBD patients.

Theoretically, it is well known that the QOL scores in subjects with disabilities are higher than those anticipated from their objective physical impairment (disability paradox) [15]. This phenomenon is because subjects with long-term disabilities change their internal standard and make the adaptation to their actual status (response shift) [16].

Next, we have made a hypothesis that nutrients intake such as fat restriction may contribute to the impairment of mental aspects of QOL in these subjects. Although CD patients had lower fat intake than UC subjects, fat intake (% energy) of the whole subjects was significantly lower than those of the NNS-J.

Then, we have analyzed the association between these summary scores and their fat intake (% energy). Fat intake (% energy) was significantly associated with MCS, but not with PCS in patients with IBD. When CD and UC patients were separately analyzed, the correlation coefficients was almost the same, but not statistically significant anymore, probably due to the smaller number of study subjects. We then have performed the multivariate analysis. Of the various factors included for analysis types of disease (CD/UC), BMI, serum albumin, fat intake (% energy), BMI, and fat intake (% energy) were the only significant determinants of PCS and MCS, respectively. Since many IBD patients are young, they are quite likely to favor foods rich in fat. Nevertheless, fat

restriction is the common practice in the nutritional therapy for IBD. It is quite conceivable that fat restriction impairs the mental and social aspects of QOL, and enteral nutrition will make the matter even worse. Of interest, but not apparently compatible with our findings, is the report by Kuriyama et al. They reported that enteral nutrition improved the health-related quality of life of Crohn's disease patients with long-term disease duration, and enteral nutrition was an independent factor for bowel symptoms and systemic symptoms [17]. In their study, IBDQ was employed for the assessment of QOL, which is an IBD-targeted questionnaire with many items related to the patients' gastroenterological problems. Thus it is likely that only the physical aspects of QOL were detected, and mental aspects were overlooked in their study.

Two additional considerations might be added to the current finding: decreased QOL in IBD patients and its association with fat restriction. First, considering the response shift, actual detrimental effect of fat restriction on the mental aspects of QOL might be even greater. Second, the adaptation process seems to be only partial. Chronic pain is known to be associated with response shift [18]. However, the association of fat restriction with impaired mental aspects of QOL was obvious in the current study. Since food intake is one of the most fundamental requirements, it is likely that subjects with fat restriction cannot easily adapt to a situation with long-term fat-restricted diet.

In conclusion, fat restriction exerts undesirable effects on IBD patients in two different ways: decreased intestinal absorption of fat-soluble substances such as vitamin D and K and impaired QOL especially in the mental aspects.

Conflict of interests

None of the authors have any conflict of interests.

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Relationship of homocysteine and homocysteine-related vitamins to bone mineral density in Japanese patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: To estimate nutritional risk factors for osteoporosis in patients with type 2 diabetes, bone mineral density, homocysteine level, and intakes and levels of Hcy-related vitamins including folate, vitamin B_6 and vitamin B_{12} were analyzed in a cross-sectional study.

Materials and Methods: Lumbar spine and femoral neck bone mineral density, serum concentrations of vitamin B_6, vitamin B_{12}, and folate and plasma homocysteine levels were measured in 125 Japanese patients with type 2 diabetes. Nutrient intake values were evaluated using a food frequency questionnaire.

Results: Homocysteine was inversely correlated with bone mineral density, and with both dietary intake and serum concentration of folate. Intake of green vegetables was correlated with intake and level of folate and homocysteine levels. When the population was analyzed across the quartiles, bone mineral density, serum folate concentration, folate intake and intake of green vegetables were lowest in the highest homocysteine group.

Conclusions: In patients with type 2 diabetes, the nutritional status of folate might affect the homocysteine level, a putative risk factor for osteoporosis. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00088.x, 2011)

KEY WORDS: Osteoporosis, Homocysteine, Folate

INTRODUCTION

Diabetes is becoming increasingly recognized as a risk factor for osteoporotic fracture. Although fracture risk in patients with type 2 diabetes is increased compared with normal subjects, not only in those with low bone mineral density (BMD) but also in those with normal or high BMD[1–3], decreased BMD is a major determinant of fragility fracture.

Patients with type 2 diabetes often follow a calorie-restricted diet, but few studies have investigated the sufficiency of these nutrients for the maintenance of skeletal health. Generally, nutrient intake increases along with energy intake. *Ad libitum* food intake values obtained from a longitudinal study in institutionalized elderly found that intake values of vitamins increased along with increased energy intake[4]. In contrast, implementation of a low-fat, low-energy diet (1000 or 1500 kcal/day) in patients with overweight and hyperlipidemia has been shown to result in a decrease of the intake of certain nutrients, including B-vitamins[5].

Folate, vitamin B_6 and vitamin B_{12} are important enzymatic cofactors in the synthesis of methionine from homocysteine (Hcy), and an elevation of Hcy can be caused by insufficiency of folate, vitamin B_6 or vitamin B_{12}. Numerous studies have linked high circulating Hcy levels and low concentrations of folate or vitamin B_{12} with increased risk of low BMD in non-diabetic subjects[6–14]. The possibility that elevated Hcy is a risk factor for osteoporosis is suggested by studies of patients with homocystinuria, a rare autosomal recessive disease characterized by markedly elevated levels of plasma Hcy, in which early onset of generalized osteoporosis has occurred[15,16]. The underlying pathophysiological mechanism of osteoporosis in patients with elevated Hcy is not completely understood. Hcy has been reported to interfere with cross-links of newly formed collagen[17,18], and consequently with bone mineralization and strength[19], as well as to stimulate osteoclast formation and activity[20,21]. However, there has been no report on the association of Hcy and Hcy-related vitamins with osteoporosis in patients with diabetes. Furthermore, vitamin insufficiency was evaluated only by serum vitamin concentrations in most of these studies, and there has been no comprehensive investigation of the relationship of dietary intake of nutrients and

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Received 28 July 2010; revised 8 November 2010; accepted 9 November 2010

serum vitamin concentrations with Hcy and BMD among subjects in the same study.

In the present study, to evaluate nutritional risk factors for osteoporosis in patients with type 2 diabetes, BMD, Hcy level, and intakes and levels of Hcy-related vitamins including folate, vitamin B_6 and vitamin B_{12} were analyzed.

MATERIALS AND METHODS

Study Population

A total of 125 Japanese patients with type 2 diabetes admitted between December 2008 and June 2009 to Kyoto University Hospital were sequentially enrolled in the study. Lateral lumbar X-ray was carried out to exclude those with scoliosis, compression fractures and ectopic calcifications. Subjects with bilateral hip fractures or prosthesis and other diseases that might influence bone metabolism including liver disease, renal dysfunction (serum creatinine above 2 mg/dL), hyperthyroidism, hyperparathyroidism, hypercorticoidism, and hypogonadism were excluded. All subjects were free of drugs that influence bone and calcium metabolism including glucocorticoids, bisphosphonates, calcitonin injection, estrogens, selective estrogen receptor modulators, vitamin D, vitamin K, thiazide diuretics, heparin and anticonvulsants. The number of patients treated with thiazolidinedione and metformin was 7 and 28, respectively. The present study was cross-sectional in design, and was approved by The Ethical Committee of Kyoto University Hospital and complies with the Helsinki Declaration. Written informed consent was obtained from all participants.

Measurement of Bone Mineral Density

BMD was measured by dual-energy X-ray absorptiometry (DXA; Discovery; Hologic, Waltham, MA, USA) at the lumbar spine (L1-L4) and femoral neck. The coefficient of variation of the measurements of BMD was 0.39%. BMD (g/cm^2) was expressed as *Z*-score calculated on the basis of the normal reference values of the age- and sex-matched Japanese group provided by the DXA system manufacturer. Because male and female patients of different ages were included in the study, comparison of BMD was made based on *Z*-scores. Fat mass and lean body mass (without bone mineral content) were measured by DXA (Hologic Discovery; Hologic) using whole-body absorptiometry software, and each value was expressed in kilograms.

Biochemical Measurements

Blood samples were obtained after overnight fasting immediately after admission. Glycosylated hemoglobin (HbA_{1c}) was measured by high performance liquid chromatography (HPLC). The value for HbA_{1c} (%) is estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} [Japan Diabetes Society (JDS); %] +0.4%, considering the relational expression of HbA_{1c} (JDS; %) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (NGSP)[22]. Fasting serum C-peptide was measured by ELISA (ST AIA-PACK C-Peptide; Toso Corporation, Tokyo, Japan). Bone-specific alkaline phosphatase (BAP) was measured by enzyme immunoassay (Osteolinks BAP; DS Pharma Biomedical, Suita, Japan), and urine N-terminal cross-linked telopeptide of type-I collagen (uNTx) was measured by ELISA (Osteomark NTx ELISA Urine; Inverness Medical, Waltham, MA, USA). Plasma Hcy levels were determined by HPLC using a thiol-specific fluorogenic reagent, ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfate[23], and the upper limit of Hcy was 13.5 nmol/L. As pyridoxal 5′-phosphate (PLP) is the predominant circulating form of vitamin B_6, serum PLP concentrations were measured by HPLC[24,25] for evaluation of vitamin B_6 status. For vitamin B_{12} measurement, 0.2 mmol/L acetate buffer (pH 4.8) was added to the serum samples, and the vitamin B_{12} was converted to cyanocobalamin by boiling with 0.0006% potassium cyanide at acidic pH. Cyanocobalamin was determined by the microbioassay method using *Lactobacillus leichmanii*, ATCC 7830[24,25]. Serum folate was determined by the microbioassay method using *Lactobacillus casei* ATCC 2733[24,25].

Evaluation of Dietary Nutrient Intake

A food frequency questionnaire (FFQ) validated by Takahashi *et al.*[26,27] was used to calculate nutrient intakes. The FFQ used in the present study included questions on the consumption of various food items over the previous 1 or 2 months. Daily nutrient intake was calculated by multiplying the frequency of consumption of each food by the nutrient content of the portion size and summing the products for all food items. The FFQ is validated against 7-day dietary records and the FFQ-estimated nutrient intake values are 72–121% of those of 7-day dietary records[26]. The reproducibility of the FFQ at intervals of 1–2 months is 93–119% for each nutrient[26]. Correlations of dietary folate intake, serum folate concentration, and plasma Hcy level with intakes of various food groups including grain/rice, potato, green vegetables, other vegetables, fruits, seaweeds, beans/soy products, seafood, meats, egg, milk products and oil/fat were evaluated.

Statistical Analysis

Data were expressed as mean ± SD. SPSS statistical software (version 13.0; SPSS, Chicago, IL, USA) was used for all statistical analyses. Pearson's correlation coefficient was calculated as a measure of association by adjusting for age and sex where appropriate. Stepwise multiple linear regression analyses were carried out to determine independent factors for plasma Hcy levels including (i) dietary vitamin B_6, vitamin B_{12} and folate intake values; and (ii) serum PLP, vitamin B_{12} and folate concentrations as independent variables. The relationship between BMD with Hcy and Hcy-related vitamins was further explored using a quartile-based analysis. Statistical differences among the groups were evaluated using analysis of covariance (ANCOVA) adjusted for age and sex, and Dunnett's multiple comparison tests by comparison with the highest Hcy group. $P < 0.05$ was considered significant.

RESULTS

Clinical characteristics, laboratory data and nutrient intake of subjects are shown in Table 1. The average serum vitamin B_{12} concentration was 1.45 ± 0.45 pmol/mL (Table 1) and there was no difference between patients taking metformin (1.52 ± 0.49 pmol/mL, $n = 97$) and those without (1.43 ± 0.49 pmol/mL, $n = 28$). Nutrient intake values were significantly positively correlated with total energy intake (Table 2). Dietary vitamin B_6, vitamin B_{12} and folate intake values were positively correlated with serum vitamin B_6, vitamin B_{12} and folate levels, respectively (Table 2). Plasma Hcy levels were negatively correlated with both dietary intake and serum concentration of folate (Table 2). Only vitamin B_6 intake and not vitamin B_6 concentration showed a weak negative correlation with Hcy; the influence of vitamin B_{12} on Hcy elevation was unclear (Table 2). Stepwise multiple linear regression analyses were carried out to determine independent factors for plasma Hcy levels. Dietary folate intake was a significant predictor of Hcy when dietary vitamin B_6, vitamin B_{12} and folate intake values were included as independent variables ($R^2 = 0.088$, β-coefficient = −0.297, $P < 0.001$), and serum folate concentration also was a significant predictor of Hcy when serum PLP, vitamin B_{12} and folate concentrations were included as independent variables ($R^2 = 0.121$, β-coefficient = −0.347, $P < 0.001$). We then evaluated the correlations of folate intake and the concentrations of folate and Hcy with intake of the various food groups determined by FFQ. Dietary folate intake and serum folate concentration were significantly associated with intakes of certain food groups including potato, green vegetables, other vegetables and fruits. Only intake of green vegetables was significantly correlated with the plasma Hcy level (Table 3).

Bone mineral density of lumbar spine (SP-BMD) and femoral neck (FN-BMD) were positively correlated with body mass index (BMI) and fat mass, although no significant correlations were found in diabetes-related parameters including fasting plasma glucose, HbA_{1c} and diabetes duration (Table 4). Both SP-BMD and FN-BMD were positively correlated with fasting serum C-peptide, but these correlations were cancelled when adjusted for BMI. Urinary NTx, a marker of bone resorption, was negatively correlated with FN-BMD. As nutrient intake significantly increases with energy intake, nutrition intakes were also evaluated by adjusting for calories. As a result, calorie-adjusted folate intake was positively correlated with SP-BMD, although the association between calorie-adjusted folate and FN-BMD did not reach statistical significance. There were no significant associations between BMD of both sites and serum concentrations of vitamin B_6, vitamin B_{12} and folate. The plasma Hcy concentration was negatively correlated with both

Table 1 | Background characteristics of the study subjects

Characteristic	
No. subjects	125
Male/female	79 (63.2%)/46 (36.8%)
Age (years)	61.2 ± 12.4
Duration of diabetes (years)	11.2 ± 9.4
Diabetes treatment (diet/OHA/Ins/Ins + OHA)	27 (21.6%)/62 (49.6%)/28 (22.4%)/8 (6.4%)
BMI (kg/m^2)	24.9 ± 4.9
Fat mass (kg)	16.5 ± 9.8
Lean body mass (kg)	45.9 ± 9.3
Fasting plasma glucose (mg/dL)	160.2 ± 48.6
HbA_{1c} (%)	9.6 ± 2.2
Fasting serum C-peptide (ng/mL)	1.71 ± 0.89
Serum BAP (U/L)	23.5 ± 8.7
uNTx (nMBCE/mmol Cr)	35.6 ± 19.8
Energy intake (kcal/day)	2073.2 ± 582.5
Protein/fat/carbohydrate intake (g/day)	73.6 ± 19.7/64.4 ± 23.7/278.7 ± 80.2
Calcium intake (mg/day)	596.0 ± 213.6
Vitamin D intake (μg/day)	9.21 ± 4.48
Vitamin B_6 intake (mg/day)	1.22 ± 0.34
Vitamin B_{12} intake (μg/day)	8.81 ± 4.65
Folate intake (μg/day)	287.4 ± 100.5
Serum PLP concentration (pmol/mL)	61.3 ± 29.1
Serum vitamin B_{12} concentration (pmol/mL)	1.45 ± 0.45
Serum folate concentration (pmol/mL)	27.5 ± 10.3
Plasma homocysteine concentration (nmol/mL)	11.2 ± 5.1

Data are number of patients (categorized data) or mean ± SD (quantitative data).
BAP, bone-specific alkaline phosphatase; BMI, body mass index; Ins, insulin; OHA, oral hypoglycemic agents; PLP, pyridoxal 5′-phosphate; uNTX, urine N-terminal cross-linked telopeptide of type-I collagen.

Table 2 | Correlations among dietary nutrient intake values, serum concentrations and plasma homocysteine levels adjusted for age and sex

	r	*P*
Correlations of total energy intake with various nutrient intakes		
Vitamin B_6 (mg)	0.521	<0.001
Vitamin B_{12} (μg)	0.253	0.005
Folate (μg)	0.331	<0.001
Correlations of intake values with serum concentrations		
Vitamin B_6	0.192	0.034
Vitamin B_{12}	0.336	<0.001
Folate	0.400	<0.001
Correlations of plasma Hcy levels with B vitamins		
Vitamin B_6 intake (mg)	−0.207	0.022
Vitamin B_{12} intake (μg)	−0.001	0.988
Folate intake (μg)	−0.328	<0.001
Serum PLP concentration (pmol/mL)	0.002	0.982
Serum B_{12} concentration (pmol/mL)	0.001	0.993
Serum folate concentration (pmol/mL)	−0.369	<0.001

Hcy, homocysteine; PLP, pyridoxal 5′-phosphate.

Table 3 | Correlations of dietary folate intake, serum folate concentration and plasma homocysteine level with various food groups

	Dietary folate intake		Serum folate concentration		Plasma Hcy level	
	r	*P*	*r*	*P*	*r*	*P*
Grain/rice	−0.076	0.399	−0.086	0.341	−0.056	0.538
Potato	0.470	<0.001	0.220	0.014	0.012	0.895
Green vegetables	0.843	<0.001	0.361	<0.001	−0.207	0.020
Other vegetables	0.620	<0.001	0.197	0.027	0.077	0.390
Fruits	0.338	<0.001	0.206	0.021	0.018	0.839
Seaweeds	0.322	<0.001	0.072	0.426	0.071	0.435
Beans/soy products	0.390	<0.001	0.156	0.083	0.016	0.856
Seafood	0.313	<0.001	0.075	0.407	−0.017	0.848
Meats	0.065	0.474	0.042	0.643	−0.070	0.435
Egg	0.278	0.002	0.068	0.450	−0.056	0.538
Milk products	0.108	0.230	0.113	0.208	−0.035	0.698
Oil/fat	0.145	0.107	0.161	0.073	−0.112	0.214

Hcy, homocysteine.

Table 4 | Correlations of bone mineral density of lumbar spine and femoral neck with diabetes-related parameters, bone turnover markers and B vitamin status

	SP-BMD		FN-BMD	
	r	*P*	*r*	*P*
BMI (kg/m^2)	0.288	0.001	0.463	<0.001
Fasting plasma glucose (mg/dL)	−0.149	0.098	−0.113	0.210
HbA_{1c} (%)	0.098	0.194	0.053	0.556
Diabetes duration (years)	0.082	0.366	0.057	0.528
Fasting serum C-peptide (ng/mL)	0.182	0.045	0.285	0.001
BAP (U/L)	0.112	0.218	−0.061	0.499
uNTx (nMBCE/mmol Cr)	−0.138	0.084	−0.183	0.042
Vitamin B_6 intake (mg)	−0.032	0.727	−0.053	0.559
Vitamin B_6 intake (mg/100 kcal)	0.113	0.211	0.005	0.959
Vitamin B_{12} intake (μg)	0.012	0.899	0.166	0.065
Vitamin B_{12} intake (μg/1000 kcal)	0.054	0.554	0.148	0.102
Folate intake (μg)	0.103	0.256	0.112	0.216
Folate intake (μg/1000 kcal)	0.198	0.027	0.153	0.090
Serum PLP concentration (pmol/mL)	−0.062	0.497	−0.007	0.936
Serum B_{12} concentration (pmol/mL)	0.023	0.799	0.058	0.524
Serum folate concentration (pmol/mL)	0.104	0.248	0.114	0.205
Plasma Hcy concentration (nmol/mL)	−0.278	0.002	−0.201	0.025

BAP, bone-specific alkaline phosphatase; BMI, body mass index; FN-BMD bone mineral density of femoral neck; Hcy, homocysteine; PLP, pyridoxal 5′-phosphate; SP-BMD, bone mineral density of lumbar spine; uNTX, urine N-terminal cross-linked telopeptide of type-I collagen.

SP-BMD and FN-BMD, showing that hyperhomocysteinemia is clearly associated with low BMD in patients with type 2 diabetes (Figure 1).

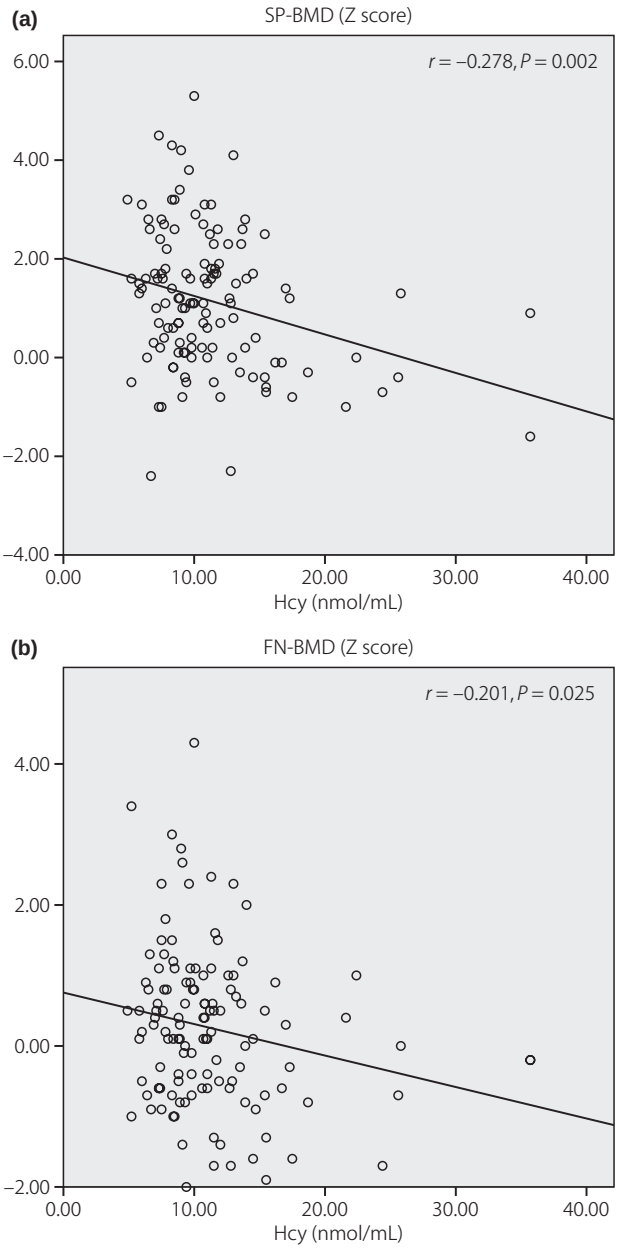

Figure 1 | The relationship between homocysteine (Hcy) and bone mineral density of lumbar spine (SP-BMD) and femoral neck (FN-BMD).

As hyperhomocysteinemia derived from folate insufficiency has been suggested to be involved in low BMD, we compared clinical characteristics of the study population across the quartiles of Hcy (quartile 1, n = 31, Hcy < 8.3 nmol/mL; quartile 2, n = 32, Hcy 8.3 to <9.9 nmol/mL; quartile 3, n = 32, Hcy 9.9 to <12.8 nmol/mL; quartile 4, n = 30, Hcy > 12.8 nmol/mL). There were no significant differences across the quartiles in general clinical characteristics including age, BMI, diabetes-related parameters, energy intake, and vitamin B_6 and vitamin B_{12} status (Table 5). However, SP-BMD and FN-BMD were significantly lower in patients in the highest quartile of Hcy than

Table 5 | Comparison of clinical characteristics according to homocysteine quartiles adjusted for age and sex

Hcy concentration (nmol/mL)	Quartile 1 (4.9–8.0)	Quartile 2 (8.1–9.9)	Quartile 3 (10.0–12.8)	Quartile 4 (12.8–35.7)	ANCOVA *P*
Male/female	17/14	21/11	23/9	18/12	
Age (years)	59.3 ± 13.8	58.1 ± 12.6	63.9 ± 8.7	64.0 ± 13.4	0.212
BMI (kg/m^2)	25.0 ± 4.4	25.8 ± 5.0	25.0 ± 5.6	23.8 ± 4.5	0.461
Fasting plasma glucose (mg/dL)	158.8 ± 44.8	162.0 ± 45.8	155.6 ± 50.0	164.6 ± 55.4	0.721
HbA_{1c} (%)	10.1 ± 2.3	9.9 ± 2.5	9.1 ± 1.8	9.4 ± 2.1	0.378
Diabetes duration (years)	9.5 ± 8.4	10.2 ± 9.7	12.6 ± 8.6	12.4 ± 9.0	0.183
SP-BMD (Z score)	1.34 ± 1.43*	1.24 ± 1.38*	1.39 ± 1.24*	0.50 ± 1.18	0.037
FN-BMD (Z score)	0.45 ± 0.99**	0.32 ± 1.23*	0.26 ± 0.96*	−0.27 ± 1.03	<0.001
Energy intake (kcal/day)	2161 ± 543	2145 ± 565	2069 ± 563	1910 ± 650	0.260
Vitamin B_6 intake (mg)	1.31 ± 0.35	1.26 ± 0.36	1.21 ± 0.32	1.09 ± 0.29	0.136
Vitamin B_{12} intake (μg)	8.59 ± 3.44	8.86 ± 4.64	9.49 ± 5.24	8.27 ± 5.21	0.798
Folate intake (μg)	323.5 ± 92.2**	287.2 ± 108.0*	305.0 ± 91.8**	231.7 ± 89.1	0.001
Intake of green vegetables (g/day)	101.9 ± 65.3*	86.1 ± 60.6	89.3 ± 47.5	68.9 ± 49.2	0.043
Serum PLP concentration (pmol/mL)	65.0 ± 33.1	60.0 ± 24.6	58.9 ± 32.9	61.4 ± 26.2	0.943
Serum B_{12} concentration (pmol/mL)	2.39 ± 0.88	2.90 ± 1.61	2.50 ± 0.73	2.53 ± 0.92	0.419
Serum folate concentration (pmol/mL)	33.6 ± 11.5**	26.9 ± 7.6*	26.9 ± 9.0*	21.7 ± 8.7	<0.001
Plasma Hcy concentration (nmol/mL)	6.9 ± 0.9**	9.1 ± 0.5**	11.3 ± 0.8**	17.8 ± 6.1	<0.001

BMI, body mass index; FN-BMD bone mineral density of femoral neck; Hcy, homocysteine; PLP, pyridoxal 5′-phosphate; SP-BMD, bone mineral density of lumbar spine. Mean ± SD, *P < 0.05, **P < 0.01 relative to the highest homocysteine quartile group.

those in patients in the other quartiles. Furthermore, patients in the highest Hcy quartile showed significantly decreased dietary folate intake, serum folate concentration and intake of green vegetables compared with those in the lower Hcy quartiles. Because the caloric intake was similar across the quartiles, the quality of the diet might be poor in the highest Hcy group. Quartile analysis revealed that the highest Hcy group showed the lowest BMD, the lowest serum folate concentration, the lowest folate intake and the lowest intake of green vegetables.

DISCUSSION

In the present study, hyperhomocysteinemia was found to be clearly associated with low BMD in type 2 diabetes patients, as it has been reported to be in non-diabetic subjects[6–14]. Furthermore, folate insufficiency might be one of the important factors in hyperhomocysteinemia, as plasma Hcy levels were negatively correlated with both dietary intake and serum concentration of folate.

Osteoporosis is a multifactorial disease, a major health problem characterized by low BMD, deterioration of bone microarchitecture and increased risk of fracture. Elevation of Hcy is one of the important risk factors for osteoporosis[28,29], and can be caused by insufficiency of Hcy-related vitamins, such as folate, vitamin B_6 and vitamin B_{12}[6–14]. Because dietary risk factors can be improved when recognized, sufficiency of Hcy-related vitamins and its relationship to osteoporosis in patients with type 2 diabetes is of primary concern.

Elevation of Hcy can be caused by insufficiency of folate, vitamin B_6 or vitamin B_{12}, and the plasma Hcy level is considered to be a fairly sensitive index of folate metabolic status compared with that of the other factors in non-diabetic subjects. Previous studies reported hyperhomocysteinemia was observed in 86% of subjects with clinically expressed folate deficiency[30]; folate is a major determinant of Hcy levels in healthy people[31,32] and vitamin B_{12} influences Hcy levels less than folate does[33,34]. Folate, vitamin B_6 and vitamin B_{12} are water-soluble vitamins, which are in general not readily stored and consistent daily intake is important. Usually, folate and vitamin B_6 deficiency develops within a month of insufficient intake. In contrast, it is known that patients with complete loss of intrinsic factor require 3–5 years to become overtly vitamin B_{12} deficient[35]. Vitamin B_{12} is a unique water-soluble vitamin, and because 80% of the 2.5 mg average whole body stock of vitamin B_{12} is reserved in the liver and vitamin B_{12} excreted in the bile and is effectively reabsorbed in the intestine, clinical signs of vitamin B_{12} deficiency take a long time to appear and progress slowly[36]. Some patients in the present study were taking metformin, which is known to inhibit absorption of vitamin B_{12}[37], but there was no difference between the patients taking metformin and those not taking metformin. As to vitamin B_6, only a weak negative correlation between vitamin B_6 intake and Hcy was not enough to conclude that vitamin B_6 is a nutritional risk factor for osteoporosis, and there have been no other studies showing the effect of vitamin B_6 on BMD.

Leafy green vegetables, such as spinach and broccoli, are rich sources of folate. Folate is also contained in a variety of foods including fruits, beans, seaweeds, liver and egg yolk. To investigate the cause of folate insufficiency, we focused particularly on dietary sources of folate. We evaluated the association of dietary folate intake, serum folate concentration, and plasma Hcy level

with various food groups, and found that intake of green vegetables correlated well with folate status and Hcy levels. Furthermore, it was revealed by the quartile analysis that the highest Hcy group showed the lowest BMD, the lowest serum folate concentration, the lowest folate intake and the lowest intake of green vegetables. This analysis suggests that insufficient intake of green vegetables, but not insufficient caloric intake, causes folate insufficiency in the group with the highest Hcy.

The strength of the present study is that it is the first study to show that nutritional status of folate might affect the homocysteine level, a putative risk factor for osteoporosis, in Japanese patients with type 2 diabetes. The present study is also meaningful in promoting awareness of the importance of diet quality, because patients with diabetes are at high risk of developing osteoporosis. In contrast, the present study has some limitations. First, the sample size was not large enough for conclusions regarding marginal insignificant *P*-values. We estimated sample size using a correlation coefficient obtained from a previous cross-sectional study assessing the relationship between BMD and plasma Hcy[8]. The correlation coefficient of femoral BMD with Hcy was −0.18 and the sample size was estimated to be $n = 153$ (two-sided $\alpha = 0.1$, $\beta = 0.2$), while we analyzed 125 patients. Second, we only analyzed patients with type 2 diabetes and comparison with non-diabetic subjects is necessary. An unanswered question is whether diabetes modulates the effects of nutritional state of folate on Hcy metabolism, and the effects of Hcy levels on BMD. Finally, a longitudinal study is required to examine the effects of Hcy on rate of BMD loss and risk of fracture for a longer duration in patients with type 2 diabetes. It is also necessary to determine whether encouraging patients with higher Hcy levels to eat more green vegetables is useful as a dietary intervention to improve Hcy levels and BMD.

In conclusion, the present study shows that BMD inversely correlates to plasma Hcy levels in Japanese patients with type 2 diabetes, and that dietary intake and the serum concentration of folate are determinant factors of Hcy levels. When our group was analyzed across quartiles, BMD, serum folate concentration, folate intake and intake of green vegetables were lowest in the highest Hcy group. Taken together, in Japanese patients with type 2 diabetes, a diet low in green vegetables rather than a calorie-restricted diet might be the more important factor in the declining nutritional status of folate that increases the Hcy level, a putative risk factor for osteoporosis.

ACKNOWLEDGEMENTS

This study was supported in part by Diabetes Masters Conference and Japan Diabetes Foundation. The authors declare no conflicts of interest.

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Clinical Nutrition 30 (2011) 261

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Overestimated serum albumin levels in patients with hip fracture

Hip fracture is associated with high mortality and morbidity. Malnutrition has been reported to an important predictor of clinical outcomes. Serum albumin level and blood total lymphocyte count (TLC) are considered to be the simple, but reliable markers indicating the patients' nutritional status, and often employed in various clinical settings including hip fracture.[1] Recently O'Daly et al.[2] in the recent issue of Clinical Nutrition, studied the significance of these two markers as the predictors of outcome in hip fractured patients. They defined protein energy malnutrition (PEM) as serum albumin level below 3.5 g/dl and TLC below 1500/mm^3. Patients with both parameters below the cut-off had higher one year mortality than those with both values above the cut-off (odds ratio; 4.6). Cox regression analysis revealed that serum albumin level (hazard ratio; 0.932) and age were independent prognostic factors of mortality. Lee et al.[3] also reported that serum albumin was associated with increased post-operative complications (odds ratio; 6.23) after adjusting for various confounders. Although these findings are of clinical interest, measurement method for serum albumin is not described. Serum albumin level can be measured with various ways, each having its own pros and cons. BCG (bromocresol green) binding assay is the most frequently used one, but has the disadvantage that BCG also binds to α1 and α2 globulins, which are acute phase proteins and increased in inflammation. Thus, serum albumin level can be overestimated with BCG assay. Then, we have measured serum albumin level in 86 patients with hip fracture by BCG assay (albumin-BCG) and nepherometry (albumin-N), which is based on antigen–antibody interaction and free from the interference by globulins. Written consent was obtained from the subjects or the proxy. Albumin-BCG (3.86 $\pm$ 0.42 g/dl) was significantly higher than albumin-N (3.51 $\pm$ 0.43 g/dl) by paired t-test ($p < 0.001$). Next, we have compared the number of subjects with their serum albumin level below or above the cut-off of 3.5 g/dl (Table 1). All subjects with albumin-BCG below 3.5 g/dl had also albumin-N below 3.5 g/dl. In contrast, approximately half of the subjects with albumin-BCG above 3.5 g/dL had albumin-N below 3.5 g/dl. Our results show that the measurement of serum albumin level by BCG assay could overestimate the serum albumin level, and underestimate the percentage of subjects with PEM in hip fractured patients. Thus, the method of serum albumin measurement must be specified, and the positive interference by globulins in the bye-binding assay should be taken into account in the nutritional assessment of the hip fractured subjects.

Table 1
Comparison of albumin-BCG and albumin-N.

		Albumin-N	
		<3.5 g/dl	≥3.5 g/dl
Albumin-BCG	<3.5 g/dl	13	0
	≥3.5 g/dl	36	37

Albumin-BCG and Albumin-N indicate the serum albumin level determined by BCG assay, and that by nepherometry, respectively. Data were analyzed by chi-square test ($p = 0.002$).

Conflict of interestv statement

None of the authors have any conflicts of interest.

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29 July 2010

0261-5614/$ – see front matter
doi:10.1016/j.clnu.2010.09.005

たんぱく質・アミノ酸の必要量に関する研究

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摂取エネルギーは十分であっても，摂取たんぱく質が不足した時にクワシオコールが発症し，感染症などを併発しやすい。たんぱく質必要量は，身体の構造と機能を正常に維持するために必要な摂取量（代謝要求量）であり，食事たんぱく質必要量は，それらの要求量を満たす量である。たんぱく質必要量は，窒素出納法によって決定されてきた。しかし，窒素出納法にはその方法上様々な問題がある。指標アミノ酸酸化（IAAO）法は，窒素出納法とは原理が大きく異なり，窒素出納法の代替法として動物とヒトにおいて開発された。私たちは，指標アミノ酸酸化法を用いてラットと健康成人男性の食事たんぱく質必要量とたんぱく質の質を再評価した。その結果，指標アミノ酸酸化法は全てのライフステージ（幼児，小児，学童，成人，高齢者）の食事たんぱく質必要量の評価だけでなく，代謝要求量が大きく変化している術後，傷害，感染症などいろいろな病態時の食事たんぱく質必要量の推定，また，たんぱく質の質の評価にも利用できることがわかった。

栄養学雑誌，Vol.69 No.6 285-293（2011）

キーワード： たんぱく質・アミノ酸，たんぱく質必要量，代謝要求量，窒素出納法，指標アミノ酸酸化法

はじめに

たんぱく質必要量に関する議論は1955年に「タンパク質必要量に関するFAO委員会」[1]で行われた。このFAO委員会[1]では，人の不可欠アミノ酸必要量パターンを重視し，これと同じ理想的なアミノ酸組成を持つたんぱく質（比較基準たんぱく質）の必要量が決められた。

1963年の「タンパク質必要量に関するFAO/WHO合同専門グループ」[2]では，たんぱく質必要量は無たんぱく質食摂取時に身体から失われる不可避窒素損失量によって規定されるという新しい概念が導入された。

1971年の「エネルギーとタンパク質の必要量に関するFAO/WHO合同特別専門家委員会」[3]では，エネルギーとたんぱく質が初めて一緒に検討された。この特別専門家委員会では，生物価の高いたんぱく質であっても，窒素平衡維持のための最小必要量は，不可避窒素損失量よりも大きいとした。また，集団に対する必要量を決定する場合，エネルギーとたんぱく質とでは考え方が異なることも明確にした。

1981年の「エネルギーとタンパク質必要量に関する協議会」[4]では，個人のたんぱく質必要量を最適レベルの身体活動を行ってエネルギー平衡を維持している人の，身体から失われる窒素と等しい最小の食事たんぱく質摂取量と定義された。子どもや妊婦，授乳婦では，良好な健康状態を維持しながら，組織の増殖肥大，あるいは乳汁分泌に必要なたんぱく質も含まれる。すべての必要量の算定値は，適当な期間続けて求められた要求量を参考に決められた。このような期間の摂取量は，ある特定の1日の摂取量と区別するために，「習慣的」あるいは「日常の摂取量」といえる。習慣的摂取量を「1日当りの摂取量」で表しているが，これらの量が毎日摂取しなければならない量であることを意味しているわけではない。

2002年の「タンパク質とアミノ酸の必要量に関するWHO/FAO/UNU合同専門家協議会」[5]では，成人のアミノ酸必要量の算定根拠が窒素出納法の成績から^{13}C-指標アミノ酸を用いたトレーサー実験に変わった。たんぱく質必要量については，引き続き窒素出納法の成績が用いられたが，皮膚などからの損失は1981年の8 mgN/kg/日より低い5 mgN/kg/日の値が採用され，安全摂取量が0.75から0.83 g/kg/日に改定された。

このように，たんぱく質とアミノ酸の必要量に関する研究は，確実に進歩してきた。しかし，窒素出納法と^{13}C-指標アミノ酸を用いたトレーサー実験の結果の解釈等，議論を必要とする課題は山積している。本稿では，私たちの最新のデータを示すとともにたんぱく質とアミノ酸の必要量の考え方について概説する。

Ⅰ．たんぱく質欠乏症

たんぱく質欠乏症は，イギリス領黄金海岸（現ガーナ共和国）で1933年にCicely D. Williams[6]によって最初に報告され，クワシオコール（kwashiorkor）と命名された。クワシオコールの主原因は，エネルギーは足りているがたんぱく質が不足することである。浮腫，毛髪の変

本論文は，平成23年度（第58回日本栄養改善学会学術総会）学会賞受賞対象論文である。

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色，ペラグラ様皮疹，下痢，低たんぱく質血症，発育障害などが特徴である。1990年から1年間，国際協力事業団（現国際協力機構）の専門家として，ガーナ共和国ガーナ大学医学部野口記念医学研究所で，現地の乳幼児の栄養調査と栄養改善プログラムの開発に関わることができた。この時はじめて，途上国における栄養問題の重要性を実際に感じることができた。現地では，ガーナ共和国保健省，WHO，UNICEFなど関係組織とともに2回のセミナーを開催した[7~9]。

Ⅱ．たんぱく質必要量の考え方

食事中のたんぱく質必要量とは，生体が必要とする量，すなわち代謝要求量を満たすために必要な摂取量である（図1）。代謝要求量は，アミノ酸を消費する代謝経路を維持するために必要な量と成長，妊娠，授乳など特別な必要量の和として求めることができる。維持必要量とは，アミノ酸を消費し，尿，糞便，皮膚，毛髪，分泌物など生体から排泄される全ての損失を補完できる量をいう。

たんぱく質の必要量がエネルギー摂取量に大きく影響を受けるにもかかわらず，健康づくりのための運動基準相当の運動を行った時のたんぱく質必要量については全く検討されていなかった。そこで，厚生省（現厚生労働省）の健康づくりのための運動所要量（現健康づくりのための運動基準2006）に相当する運動施行時にたんぱく質必要量が変動するかを検討した結果，運動所要量に相当する運動を行ってもたんぱく質必要量を増加させる必要がないことを明らかにした[10,11]。窒素出納法を用いたこの研究では，尿，糞便，だけでなく皮膚など生体から排泄される窒素損失量を測定した。表1に示したように，皮膚などから排泄される窒素を測定していれば，「健康づくりのための運動（200～400 kcal/日のエネルギー消費）をしても摂取するたんぱく質を増やす必要はない」と結論できる。しかし，皮膚などから排泄される窒素を測定しないと，「健康づくりのための運動（200～400 kcal/日のエネルギー消費）を実施すると，摂取するたんぱく質を増やさなくても体たんぱく質の蓄積が増加する」という結論になる。すなわち，窒素出納試験を用いてたんぱく質代謝を評価するためには，尿，糞便，皮膚，毛髪，分泌物など生体から排泄される全ての損失を測定しなければならない。実際に，尿，糞便，皮膚，毛髪，分泌物など生体から排泄される全ての損失を測定することは非常に困難である。

食事たんぱく質必要量とは，代謝要求量を満たし，窒素平衡を維持するために食事として摂取すべきたんぱく質またはその成分であるアミノ酸，またはその両者である。したがって，食事たんぱく質必要量は次式で示すことができる。

食事たんぱく質必要利用＝代謝要求量÷利用効率

食事からの窒素摂取量がゼロで，エネルギーとその他の栄養素が十分量摂取されている場合に，尿中に排泄される窒素量は徐々に減少し，一定の値となる。尿中に排泄される窒素量が一定となるためには5～7日間を要することが報告されている（図2）[12]。すなわち，食事からのたんぱく質摂取量を変化させた時には，少なくとも7日間の適応期間を要し，このようにして得られた窒素平

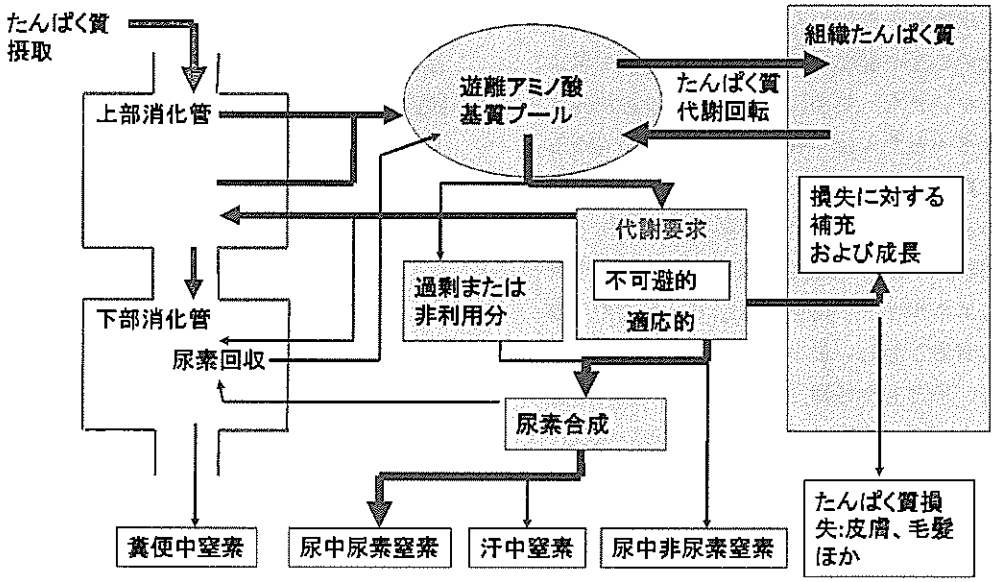


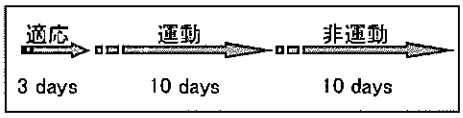
図1　アミノ酸の代謝要求の概略図

成人の不可欠アミノ酸必要量は，窒素平衡をもたらすために必要なアミノ酸摂取量として測定される。

表1　1.08 g/kg/日のたんぱく質摂取量時の窒素出納値に及ぼす運動（400 kcal/日）の影響[10)]

Subjects	Non-exercise period						Exercise period					
	IN	FN	TD	DN	UN	NB	IN	FN	TD	DN	UN	NB
L	181.9	15.5	98.3	7.6	150.6	8.1	181.0	15.5	98.3	17.6	149.3	−1.4
M	177.2	19.4	96.0	3.5	124.8	29.5	177.8	16.1	97.9	9.7	123.5	28.5
N	181.9	12.6	99.9	10.3	145.9	13.1	180.0	17.5	97.2	18.4	134.0	10.0
O	179.0	16.8	97.6	8.2	147.9	6.1	179.2	17.8	97.0	9.8	145.5	6.1
P	177.7	20.7	95.3	8.4	152.0	−3.4	178.3	24.0	93.5	9.5	136.8	8.0
Q	180.5	17.7	97.1	5.6	142.2	15.0	179.2	16.8	97.6	7.4	135.6	19.5
Mean	179.7	17.1	97.4	7.3	143.9	11.4	179.3	18.0	96.9	12.1*	137.5*	11.8
SD	2.0	2.9	1.6	2.4	10.0	11.0	1.2	3.1	1.7	4.7	9.1	10.6

（$p<0.05$ vs non-exercise period）



摂取たんぱく質量：1.08 g/kg/日
エネルギー摂取量：42.8〜43.8 kcal/kg/日
代謝性糞中排泄量：12.4 mgN/kg/日
運動強度：65% VO_2max
運動によるエネルギー消費量：400 kcal/日

略語：IN，摂取窒素：FN，糞中窒素：TD，真の吸収率：DN，経皮窒素：UN，尿中窒素：NB，窒素出納値

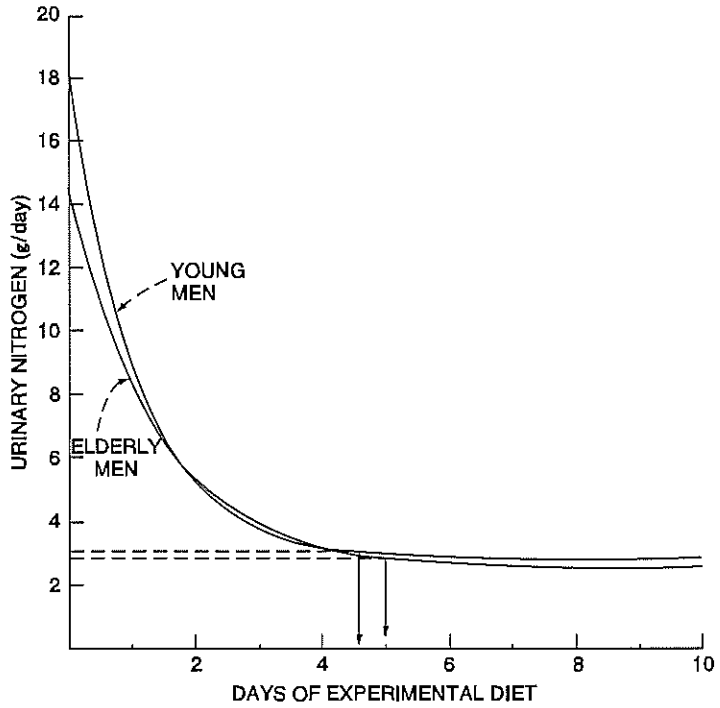


図2　無たんぱく質摂取後の尿中窒素排泄量の変化

（J Nutr; 108, 97（1978）を改変）

衡維持に必要な食事からのたんぱく質摂取量は，最小たんぱく質必要量と定義できる。

私たちは，たんぱく質代謝には，適応現象が存在することに着目した。習慣的なたんぱく質摂取状態に適応しており，低たんぱく質代謝適応が成立していない状態（たんぱく質摂取レベルを変更した実験日）で，たんぱく質代謝を推定できる指標アミノ酸酸化（indicator amino acid oxidation: IAAO）法を用いることにより，習慣的なたんぱく質摂取状態でのたんぱく質代謝要求量の推定を試みた。たんぱく質の摂取量を習慣的な摂取量よりも少ない摂取量に変化させた時に，その少ない摂取たんぱく質量でのたんぱく質代謝状態を反映する期間は，少なくとも7日間を要する（図3）。つまり，一過性のたんぱく質代謝応答は，その時の習慣的なたんぱく質摂取時の代謝を反映していることを意味する。習慣的に十分量のたんぱく質を摂取している時に，生体内で合成されるたん

ぱく質と分解されるたんぱく質はほぼ一定であり，たんぱく質代謝回転が定常状態であると考えられる。この時たんぱく質合成に必要なアミノ酸は，体内の遊離アミノ酸プールから供給される。この遊離アミノ酸プールのアミノ酸の供給源は，食事，体たんぱく質の分解，および体内合成である（図4）。

たんぱく質必要量とたんぱく質代謝要求量は，その意味するところが異なる。窒素出納法で求めるたんぱく質必要量は，低たんぱく質栄養状態に適応した状態での最小たんぱく質摂取量を意味する。この最小たんぱく質必要量を下回る摂取量が続くと，たんぱく質欠乏症が発症すると考えられる。一方，IAAO 法で求めるたんぱく質代謝要求量は，その時の習慣的なたんぱく質摂取時の代謝を維持するために必要なたんぱく質摂取量を意味する。このたんぱく質代謝要求量を下回る摂取量が続いてもたんぱく質欠乏症が発症することはなく，その時のたんぱく質摂取量でのたんぱく質代謝に適応していくと考えられる。

たんぱく質摂取量を窒素出納法で求めたたんぱく質必要量に適応させた時のたんぱく質代謝要求量は，たんぱく質必要量と一致すると考えられる。すなわち，窒素出納法で求めた最小たんぱく質必要量にたんぱく質代謝が適応すると，体内の遊離アミノ酸プールもその時のたんぱく質代謝に見合ったサイズになると推定される。このため，この低たんぱく質状態に適応したたんぱく質代謝を維持するために必要なたんぱく質代謝要求量は，最小たんぱく質必要量と一致すると考えられる。

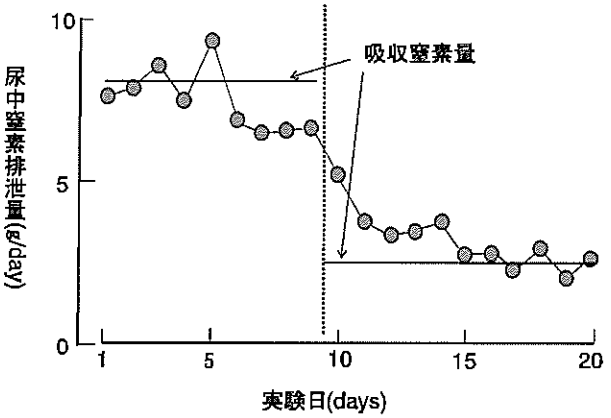


図3　たんぱく質の摂取量を変化させた時の尿中窒素排泄量の変化

Ⅲ．指標アミノ酸酸化（IAAO）法の原理

IAAO 法の理論は，食事に含まれているあるアミノ酸がたんぱく質代謝要求量以下であれば（すなわち，制限アミノ酸），他のすべての不可欠アミノ酸（^{13}C-標識アミノ酸を含む）はたんぱく質合成には利用することができず，この余分の不可欠アミノ酸は酸化されて，不可逆的に重炭酸塩プールに遊離され，呼気中に排泄される，というものである。例えば，図5に示したように，遊離アミノ酸プール中の制限アミノ酸（ここではリシン）がたんぱく質要求量よりも少ないと，たんぱく質合成量は低下し，余った指標アミノ酸（ここでは［1-^{13}C］フェニルアラニン（^{13}C-Phe））の酸化量が増加し，その炭素骨格は $^{13}CO_2$ として排泄される。この $^{13}CO_2$ 排泄量は，摂取するたんぱく質量が増加し，遊離アミノ酸プール中の制限アミノ酸（ここではリシン）がたんぱく質要求量たんぱく質要求量と等しくなるまで減少する。制限アミノ酸（ここではリシン）が合成すべきたんぱく質に必要な量以上に供給されると，たんぱく質をそれ以上合成する必要がないので，指標アミノ酸由来の呼気 $^{13}CO_2$ 排泄量は一定となる（図6）。この屈曲点が食事たんぱく質必要量と考えられる。この条件を満たすためには，指標アミノ酸として ^{13}C-Phe を利用する場合に，組織や血液中の ^{13}C-Phe と ^{12}C-Phe 濃度の割合と量が一定であることが必要である。

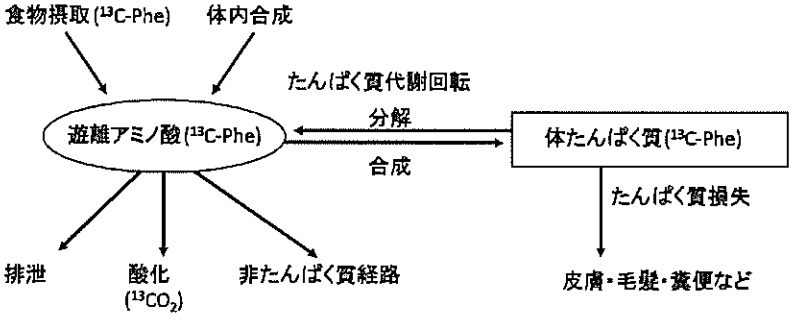


図4　たんぱく質必要量の考え方

成人のたんぱく質必要量は，体外に失われる窒素量を補い，体たんぱく質量を維持するために必要な食事たんぱく質の最小摂取量である。

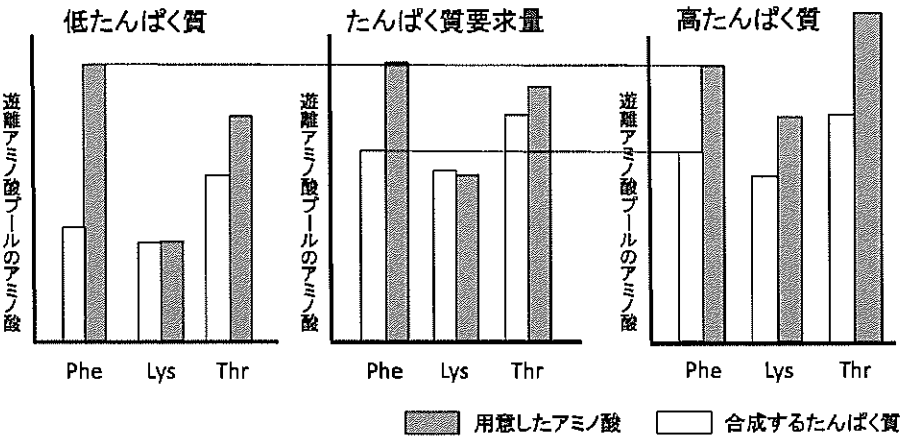


図5　指標アミノ酸酸化法の原理

リシンが第一制限アミノ酸と仮定すると，合成するたんぱく質よりも用意したアミノ酸の量が少ない状態（低たんぱく質）では，合成すべきたんぱく質に必要なリシン量が供給されないので，たんぱく質合成量は低下し，他の余った指標アミノ酸は分解され，その炭素骨格は呼気 CO_2 として排出される。しかし，合成すべきたんぱく質に必要な量以上にリシンが供給される（高たんぱく質）と，たんぱく質はそれ以上合成する必要がないので，指標アミノ酸由来の呼気 CO_2 の排泄量は一定となる。

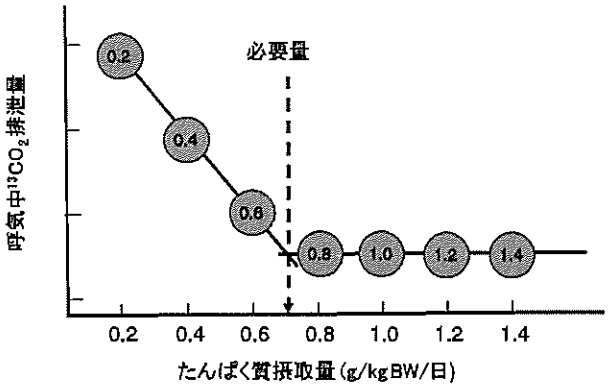


図6　指標アミノ酸酸化法（IAAO 法）

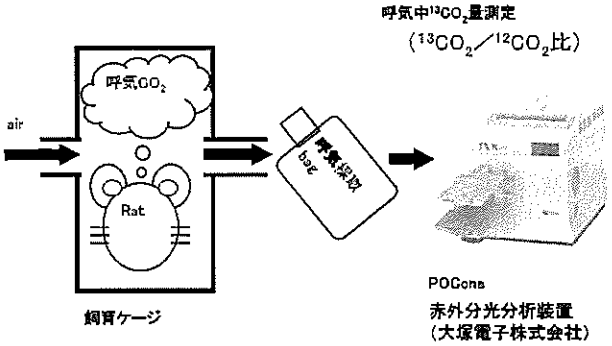


図7　呼気の採取と呼気分析の方法

Ⅳ．たんぱく質代謝研究における IAAO 法の利点

たんぱく質代謝研究における IAAO 法の利点は次の3つが考えられる。

第1に，トレーサーが試験たんぱく質とは別なので，栄養学的にかなりの量のトレーサーを与えても問題がないことである。指標アミノ酸の摂取量は一定に保たれているので，試験たんぱく質よりも指標アミノ酸のほうが濃度の変化が小さい。指標アミノ酸としては，^{13}C-Phe が最も高い頻度で利用されてきた。今後，様々なアミノ酸を指標アミノ酸として利用し，たんぱく質代謝に用いる指標アミノ酸としての評価も必要である。

第2に，出納試験を必要とせず，異なるたんぱく質摂取レベルに対して事前に実験食に適応させる必要がないことである。習慣的な食生活の条件でたんぱく質代謝要求量を求めることが可能である。個々人に見合ったたんぱく質代謝要求量が算出でき，体調や生活スタイルが変化すれば，その都度，最適なたんぱく質代謝要求量を算出することができる。また IAAO 法は，成人だけでなく成長期から高齢者まで同じ方法でたんぱく質代謝要求量を再評価できると考えられる。

第3に，アミノ酸酸化測定の精度や正確さについて高いレベルが要求されないことである。屈曲点は，試験たんぱく質摂取量が十分であることの操作上の指標であり，それは指標物質の酸化率が正確に測定されているか否かに依存しない。私たちは，一定速度で空気を送り込んでいる飼育ケージにラットを入れ，飼育ケージ内の気体を呼気採取バッグに採取し，赤外分光分析装置（大塚電子株式会社）を用いて，呼気 $^{13}CO_2$ 量を ^{12}C との割合として測定している（図7）。

V．ラットにおけるIAAO法によるたんぱく質代謝要求量の測定[13)]

実験食のたんぱく質源としてカゼインと小麦グルテンを用い，IAAO法によるたんぱく質代謝要求量について，実験食のたんぱく質源により違いが見られるかを検討した。ラットは，小麦グルテンを実験食として用いる場合も含めたすべての実験について，実験前24時間以上，20%カゼイン食を自由摂取とした。実験日，ラットは，6段階のカゼインを含む実験食（4.3，8.6，12.9，17.2，21.5，25.8%カゼイン食），または，6段階の小麦グルテンを含む実験食（7.2，10.8，14.4，18.0，21.6，25.2%小麦グルテン食）のうち一つを09：00から18：00まで3時間ごとに4回摂取した。1回の給餌量はラットの1日摂食量の1/8量ずつとした。^{13}C標識物質投与は3回目の給餌時の15：00（$NaH^{13}CO_3$，0.88 mg/kg BW; $NaHCO_3$，7.92 mg/kg BW; ^{13}C-Phe，3.3 mg/kg BW; Phe，29.7 mg/kg BW）に開始し，16：00，17：00，18：00（^{13}C-Phe，6.0 mg/kg BW; Phe，54.0 mg/kg BW）まで続けた。^{13}C標識物質経口投与後ただちにラットをチャンバーに入れた。15：00から19：00まで30分ごとに，チャンバー内の気体を呼気サンプルとして呼気採取バッグに採取し，赤外分光分析装置（POCone；大塚電子株式会社）により呼気中$^{13}CO_2$量を測定した。

たんぱく質含量が6段階のカゼイン食を実験食とする実験（n=8）と小麦グルテン食を実験食とする実験（n=8）それぞれ6回のIAAO法は，実施日は2日間間隔とし，2週間以内に完了した。実験食の組成を表2に示した。

IAAO法においては，低たんぱく質食から十分なたんぱく質食に食事内容を変化させても，食事中の^{13}C-Pheと^{12}C-Pheの量および［1-^{12}C］チロシン（^{12}C-Tyr）の量を一定に保ことが必要である。このように調整された食事を摂取した時に，組織や血漿中の^{13}C-Pheだけでなく，^{13}C-Tyrと^{12}C-Tyrの量も一定であることを確認することが必要である。4.3%カゼイン食と17.2%カゼイン食を摂取した時のラットの血漿PheとTyr濃度を表3に示した。カゼイン食のたんぱく質レベルを4.3%から17.2%に変化させても，血漿^{13}C-Pheと^{12}C-Phe濃度の割合と量が一定であった。また，血漿^{13}C-Tyrと^{12}C-Ty濃度の割合と量も一定であった。さらに，^{12}C-Tyに対する^{13}C-Tyrの割合は，^{12}C-Pheに対する^{13}C-Pheの割合よりも小さく，このことは，PheからTyrへの代謝は亢進していないことを示唆している。また，肝臓および腓腹筋の遊離アミノ酸について測定した結果，血漿と同様にカゼイン食のたんぱく質レベルを4.3%から17.2%に変化させても，血漿^{13}C-Pheと^{12}C-Phe濃度の割合と量および血漿^{13}C-Tyrと^{12}C-Ty濃度の割合と量も一定であった（表3）。

たんぱく質代謝要求量は，18：30の$^{13}CO_2$量を特異的回帰法（2段階線形交差）[14)]により解析し，段階的なたんぱく質摂取量に対する呼気中$^{13}CO_2$が最小値となる屈曲点として算出した。本研究では，ラットにおいてIAAO法により，カゼインをたんぱく質源とした時のたんぱく

表2　実験食の組成

Protein	Casein diet						Wheat gluten diet					
	4.3%	8.6%	12.9%	17.2%	21.5%	25.8%	7.2%	10.8%	14.4%	18.0%	21.6%	25.2%
	g/kg diet						g/kg diet					
Casein	50	100	150	200	250	300	−	−	−	−	−	−
Wheat gluten	−	−	−	−	−	−	100	150	200	250	300	350
Cornstarch	557	523	490	457	423	390	527	498	470	440	411	383
Sucrose	278	262	245	228	212	195	265	250	235	221	206	190
Rapeseed oil	35	35	35	35	35	35	31	27	22	18	14	9
Soy bean oil	15	15	15	15	15	15	12	10	8	6	4	3
Vitamins	10	10	10	10	10	10	10	10	10	10	10	10
Minerals	35	35	35	35	35	35	35	35	35	35	35	35
Cellulose	20	20	20	20	20	20	20	20	20	20	20	20
L-Phenylalanine	11	9	7	5	2	−	9	7	5	3	1	−
L-Tyrosine	13	10	8	5	3	−	13	11	10	9	8	6
Energy（kJ/g）	15.4	15.4	15.5	15.5	15.5	15.6	15.5	15.5	15.5	15.5	15.6	15.6

カゼインのたんぱく質含量は86.2%（N×6.38），小麦グルテンのたんぱく質含量は72.0%（N×5.70）である。食事中のフェニルアラニン含量は，全ての食事で13,500 mg/kg dietとした。ただし，25.2%小麦グルテン食の場合には，14,350 mg/kg dietとした。また，食事中のチロシン含量は，全ての食事で15,000 mg/kg dietとした。

表3　血漿，肝臓，腓腹筋のフェニルアラニンおよびチロシン濃度

Diet	Phenylalanine			Tyrosine		
	^{13}C-Phe	^{12}C-Phe	Total	^{13}C-Tyr	^{12}C-Tyr	Total
			Plasma (nmol/mL)			
4.3% casein	13.2 ± 2.9	47.2 ± 4.3	60.4 ± 7.0	7.5 ± 2.0	113.0 ± 29.4	120.6 ± 30.7
17.2% casein	12.1 ± 2.5	50.8 ± 10.0	62.9 ± 11.8	8.5 ± 1.4	119.8 ± 15.2	128.3 ± 16.2
			Liver (nmol/g)			
4.3% casein	10.6 ± 0.4	40.9 ± 5.1	51.5 ± 4.9	7.4 ± 1.3	99.4 ± 32.0	106.9 ± 33.2
17.2% casein	10.4 ± 2.1	43.1 ± 10.5	53.6 ± 12.3	8.8 ± 2.8	92.5 ± 7.5	101.4 ± 9.2
			Gastrocnemius muscle (nmol/g)			
4.3% casein	13.0 ± 1.7	46.6 ± 4.5	59.6 ± 5.7	8.4 ± 0.8	91.9 ± 8.7	100.3 ± 8.5
17.2% casein	11.6 ± 1.9	48.2 ± 2.5	59.8 ± 3.6	7.0 ± 1.1	84.7 ± 5.8	91.7 ± 5.0

平均値±SE（4.3% casein, $n=5$; 17.2% casein, $n=5$）．全てのデータに4.3% casein 群と17.2% casein 群との間に Student's t-test にて有意差を認めなかった。

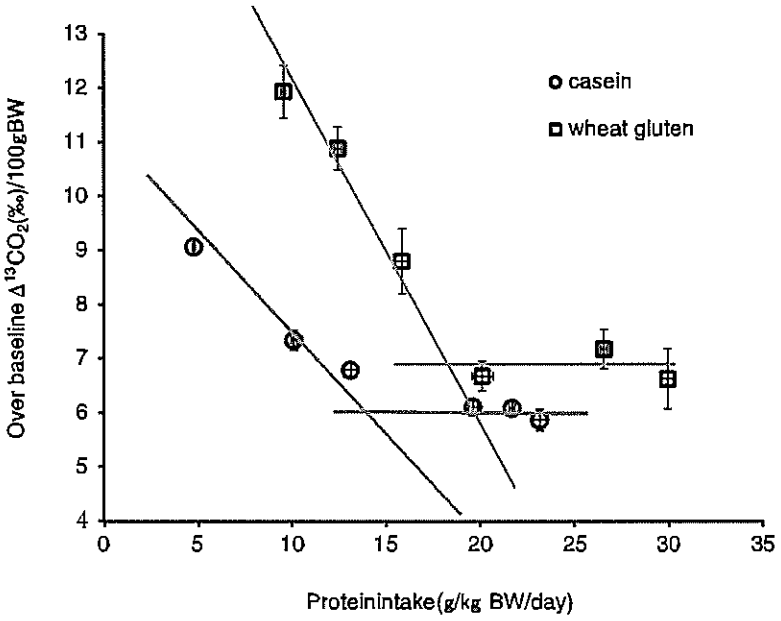


図8　たんぱく質代謝要求量の算出

カゼイン食（$n=8$）と小麦グルテン食（$n=8$）のたんぱく質摂取量を変化させた時の呼気 $^{13}CO_2$ 産生量の変化を平均値±標準偏差で示した。カゼイン食の回帰直線式は，$y=10.73-0.35x$ と $y=6.17$ であり，小麦グルテン食の回帰直線式は，$y=18.87-0.66x$ と $y=6.92$ であった。屈曲点は，カゼイン食が 13.1 g/kg BW/日，小麦グルテン食が 18.1 g/kg BW/日であった。

質代謝要求量は 13.1 g/kg BW/day に相当すると推定された（図8）。

小麦をたんぱく質源とした IAAO 法では，たんぱく質代謝要求量は 18.1 g/kg BW/day と算出され，カゼインをたんぱく質源とした時よりも高い値であった。たんぱく質必要量は良質のたんぱく質摂取で低く，劣質のたんぱく質摂取で高くなったという結果は，我々の仮説に合致し，IAAO 法はたんぱく質の質評価に利用することができると考えられた。

Ⅵ. ヒトにおける IAAO 法によるたんぱく質代謝要求量の測定

1日の総窒素必要量は，不可欠アミノ酸の適切な摂取レベルとバランス，それに α-アミノ窒素源となる十分な可欠アミノ酸を供給することを満たすものである。2007年に Humayun ら[15] は，IAAO 法を用いて成人のたんぱく質必要量を再評価している。彼らによると成人男性のたんぱく質必要量は，0.93 g/kg BW/日であった。我々も，IAAO 法を用いて日本人成人男性のたんぱく質代謝要

求量を評価した。その結果，0.91 g/kg BW/日と推定した（データは未発表）。また，IAAO 法を用いて，成人女性では 0.91 g/kg/日[16]，学童期では 1.3 g/kg/日[17] と報告されている。いずれも，窒素出納法で策定された値よりも大きい。

以上のように，IAAO 法はたんぱく質代謝要求量の評価だけでなく，たんぱく質の質の評価にも利用できることがわかった。さらに，ライフステージ別のたんぱく質代謝要求量やいろいろな病態時のたんぱく質代謝要求の推定にも利用できる方法であると考えられた。

謝　辞

本稿を終えるにあたり，栄誉ある日本栄養改善学会学会賞を授与されたことに対し，選考委員の先生をはじめ，本学会の諸先生に厚く御礼申し上げます。本研究は，大塚製薬株式会社徳島研究所，徳島大学大学院栄養学研究科ならびに京都府立大学大学院生命環境科学研究科で行われたものです。研究を遂行するにあたり終始ご指導いただいた岸恭一徳島大学名誉教授（現名古屋学芸大学教授）に深謝致します。また，大塚製薬株式会社では多大なご指導とご助言をいただきました郡英明博士（元佐賀研究所所長）に心より感謝申し上げます。京都府立大学では常に多くのご助言とご指導をいただきました中坊幸弘京都府立大学名誉教授（現川崎医療福祉大学教授）に厚く御礼申し上げます。最後になりましたが，徳島大学大学大学院栄養学研究科栄養生理学講座および京都府立大学大学院栄養科学研究室での共同研究者ならびに大学院・学部卒業生，在校生の皆さんに深く感謝いたします。

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（受理：平成23年11月28日）

Dietary Requirements of Protein and Amino Acids

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ABSTRACT

Even when energy intake is adequate, the classic protein deficiency disease kwashiorkor increases susceptibility to infection. The protein requirement defines the requirement in terms of the needs to maintain the physical structure and body functions, i.e. metabolic demands, and the dietary protein requirement will satisfy those demands. Protein requirement is generally determined by nitrogen balance studies, but various limitations are associated with this method. The indicator amino acid oxidation (IAAO) method, with a theoretical foundation quite different from that of the nitrogen balance method, was developed as an alternative for studies in animals and humans. We employed the IAAO technique to evaluate dietary protein requirements and protein quality in rats and healthy men. The results indicated that the IAAO method is effective for evaluating the dietary protein requirements for people of all ages and for postoperative patients or those with injuries or infections, all of who represent a wide range of metabolic demand. This method could also be used to evaluate protein quality.

Jpn. J. Nutr. Diet., **69** (6) 285～293 (2011)

Key words: amino acid and protein, protein requirement, metabolic demand, nitrogen balance method, indicator amino acid oxidation method

4. 生化学検査
(3) ビタミンと微量ミネラル

Vitamins and microminerals

柴田克己／福渡　努／吉田宗弘

SUMMARY

ビタミンと微量ミネラルは，食品中にまんべんなくは含まれておらず，加工・調理過程によって損失しやすい。また，ビタミンはきわめて壊れやすい。したがって，食事調査と食品成分表から算定される摂取量だけを用いて栄養管理を行うことはきわめて困難である。多くのビタミンといくつかの微量ミネラルは尿中に排泄されるので，栄養状態良好時の尿中排泄量値と比較することで，栄養管理が可能になる。

KEY WORDS

■ ビタミン
■ 微量ミネラル
■ 尿
■ 目標排泄量
■ 栄養管理

I
はじめに

動物が生きていくためには，とにかく何かを食べなければならない。この能力は本能として獲得している。しかし，健康長寿のためには，すべての栄養素を適量摂取するという，さらなる能力が必要である。われわれは，食事内容が不適であっても，それ自体で痛みや苦しみを伴うことはないので自覚することができない。つまり，すべての栄養素を適量摂取する能力はない。過不足の状態が長期間持続して症状が顕在化し，病的な状態になって，はじめて食事の欠陥に気付くのである。

したがって，健康長寿のためには，後天的に栄養状態の評価方法を学ばなければならない。最も普及している栄養状態の評価方法は，「習慣的な栄養素摂取量」を「必要量」と比較する方法である。「習慣的な栄養素摂取量」は，食べた食品の重量を測定すれば，「食品成分表」（現在は，『日本食品標準成分表 2010』[1]が利用できる）を利用して，摂取した栄養素量を知ることができる。「必要量」は『日本人の食事摂取基準』（2010年版）[2]に記載されている。「習慣的な栄養素摂取量」と「必要量」を比較することで，栄養状態を評価することができる。しかし，この最も多用されている評価方法の限界として以下が挙げられる。

①摂取した食品の原材料名と重量を精度高く記録することができない。
②食品成分表の値は，単なる「化学的」な分析値の代表値であること。
③食事摂取基準の値は，あくまでも基準を示す値であること。

よって，栄養評価方法として多用されている「習慣的な栄養素摂取量」を「必要量」と比較する方法を補完する方法が必要となる。そこでわれわれは，非侵襲性の尿を生体指標とする栄養評価法を確立しつつある。

本稿では，尿中のビタミン量を測定することで栄養状態の何を知ることができるかを紹介する。また，微量ミネラルに関しては，生体試料を指標とした栄養管理において，尿を栄養状態の指標として使用できるものと尿以外の生体試料を用いなければならないものを紹介する。

II
ビタミン

1．理論

13種類のビタミンのなかで，ビタミンA，ビタミンDおよびビタミンKを除く10種類のビタミンあるいはその異化代謝産物が尿中に排泄されることが報告されている[3)4)]。α-トコフェロールの異化代謝産物である2,5,7,8-テトラメチル-2（2'-カルボキシエチル）-6-ヒドロキシクロマン（α-CEHC）は，α-トコフェロールの摂取量がある量以上に達すると摂取量に応じて増大することが報告されている[5)]。9種類の水溶性ビタミンではビタミンB_{12}を除く8種類のビタミンが摂取量に応じて尿中への排泄量が増大することが明らかにされている[6)-9)]。

ビタミン欠乏食を投与した後の血液，尿中のビタミン量の変化の概念図を**図1**に示す。

2．健康長寿を達成するための目標尿中水溶性ビタミン排泄量

われわれが報告した健常人の尿中排泄量を基にして作成した健康長寿を達成するための目標尿中水溶性ビタミン排泄量を**表1**に示す。α-CEHCに関しては，現在検討中であるため記載していない。

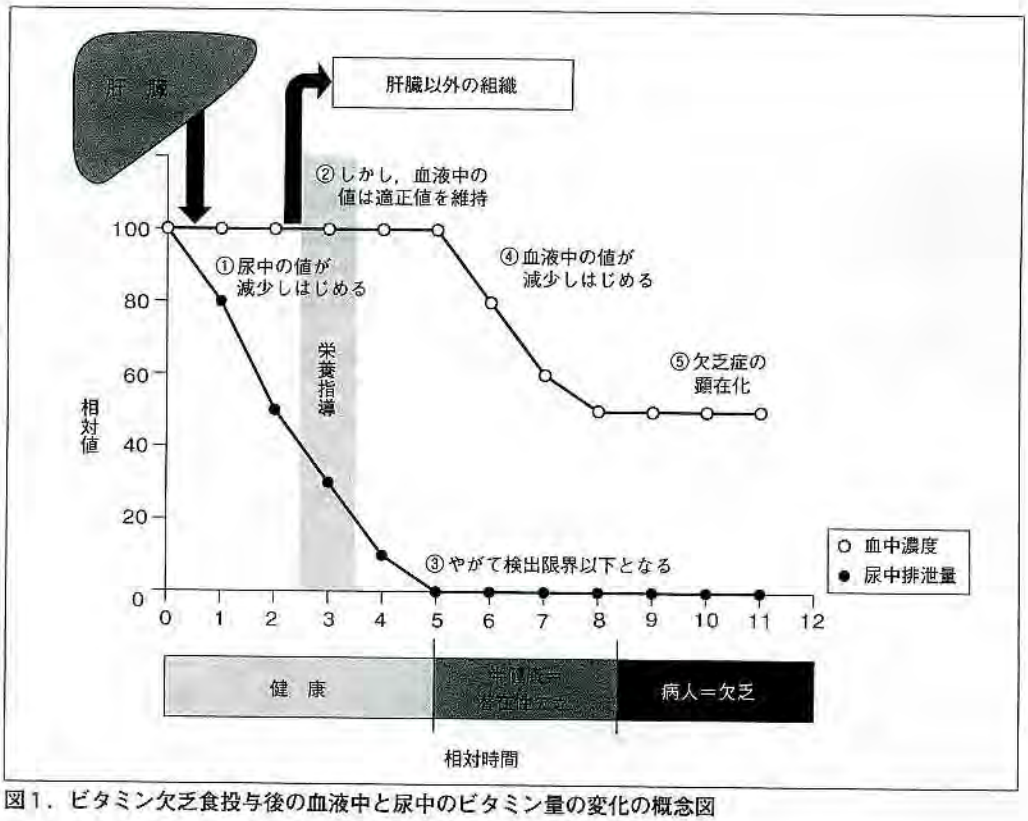

図1．ビタミン欠乏食投与後の血液中と尿中のビタミン量の変化の概念図

表1．健康を維持するための目標尿中水溶性ビタミン排泄量

ビタミン（単位）	2～5歳	10～12歳	18～69歳	70歳以上
ビタミンB_1（μg/日）	30～150	70～300	100～400	100～400
ビタミンB_2（μg/日）	40～150	60～250	70～350	70～350
ビタミンB_6（μg/日）	150～600	300～1,000	500～1,400	500～1,400
ナイアシン（mg/日）	2.5～10	4.5～20	6～25	6～25
パントテン酸（mg/日）	0.9～3	1.5～5.5	2.2～7	2.2～7
葉酸（μg/日）	3～70	4.5～15	7～20	7～20
ビオチン（μg/日）	5～15	8.5～30	10～40	10～40
ビタミンC（mg/日）	9～70	20～90	25～90	25～90

3．目標尿中水溶性ビタミン排泄量を活用した栄養指導例

ある症例の1日尿中の水溶性ビタミン排泄量を表2に示す。各ビタミンのコメントと総合コメントは表2に示した通りである。α-CEHCに関しては記載していない。

Ⅲ 微量ミネラル

1．微量ミネラルの栄養管理における尿試料の位置づけ

ここで述べる微量ミネラルとは『日本人の食事摂取基準』(2010年版)[2]が対象とする，鉄，亜鉛，銅，マンガン，ヨウ素，セレン，クロム，モリブデンの8元素である。これら8元素のうち尿で摂取量を把握し栄養管理が実施できるのは，消化管吸収率が高く，かつ主排泄経路が尿である，ヨウ素，セレン，モリブデンの3元素である。ただし，これら3元素でも計算に基づく摂取量推定値と尿中濃度との関連は変動が大きく，回帰式の精度が低い。したがって，現状では個人の摂取量を尿中濃度から正確に推定するのは難しい。出納試験での摂取量実測値と尿中排泄量との関連は図2のモリブデンのようにきわめて大きい[10]。ゆえに，ヒトを対象とした実験から摂取量と尿中排泄量間の高精度な回帰式の確立が必要である。

残り5元素は消化管吸収率が低く，かつ変動する。さらに，クロムを除く4元素は血中でたんぱく質に結合し，尿は主排泄経路ではない。また，クロムは尿が主排泄経路とされるが，定量が難しく，信頼できる摂取量や尿中排泄量の情報が少ない。ゆえに，これら5元素では尿を用いた栄養管理は難しく，血清中濃度などを用いる必要がある。

2．ヨウ素とセレンの栄養管理

ヨウ素，セレン，モリブデンの3元素は尿中排泄量が摂取量と関連するので，尿を用いた栄養管理がある程度は可能である。ただし，前述のように尿中濃度と摂取量との回帰式の精度が不十分なので，複数回の測定が必要である。ここでは疾病発生との関連が大きいヨウ素とセレンに関して尿を用いた栄養管理を述べ，セレンに関しては血清を用いた栄養管理についても述べる。

（1）ヨウ素

集団では，1日尿のヨウ素濃度とヨウ素摂取量との間に「ヨウ素摂取量（μg/日）＝尿中ヨウ素濃度（μg/ℓ）×0.0235×体重（kg）」の回帰式が成立する[11]。『日本人の食事摂取基準』（2010年版）[2]における成人のヨウ素推奨量130 μg/ℓ/日を回帰式に代入すると，体重60 kgでは尿中濃度92 μg/ℓが得られる。この回帰式は集団を対象としているが，個人でもこの数値が1つの目安となる。なお世界保健機関（WHO）では尿中ヨウ素濃度100 μg/ℓを基準としており，集団では尿中濃度100μg/ℓ未満の人の割合がヨウ素摂取不足の人の割合に相当する[12]。

（2）セレン

尿セレンを扱った総説では摂取セレンの尿中排泄率を50～70%としている[13]。一方，セレンの1日尿中排泄量（μg/日）

表2．ある症例の1日尿中の水溶性ビタミン排泄量

測定ビタミン（単位）	測定結果	参考値（成人）	コメント
ビタミンB_1（μg/日）	1,938	100～400	サプリメントからビタミンB_1を大量に摂取している可能性があります。用量に気をつけてビタミンB_1を摂取してください。必要量は1.2mg/日です。
ビタミンB_2（μg/日）	2,526	70～350	サプリメントからビタミンB_2を大量に摂取している可能性があります。用量に気をつけてビタミンB_2を摂取してください。必要量は1.2mg/日です。
ビタミンB_6（μg/日）	3,978	500～1,400	サプリメントからビタミンB_6を大量に摂取している可能性があります。用量に気をつけてビタミンB_6を摂取してください。必要量は1.1mg/日です。
ナイアシン（mg/日）	10.3	6.0～25	ナイアシンの摂取に問題はありません。必要量は12mg/日です。
パントテン酸（mg/日）	1.3	2.2～7.0	パントテン酸を十分に摂取できていない可能性があります。必要量は5 mg/日です。
葉酸（μg/日）	6.2	7～20	葉酸を十分に摂取できていない可能性があります。必要量は240μg/日です。
ビオチン（μg/日）	35	10～40	ビオチンの摂取に問題はありません。必要量は50μg/日です。
ビタミンC（mg/日）	12	25～90	ビタミンCを十分に摂取できていない可能性があります。必要量は100mg/日です。
総合コメント	一部のビタミンを十分に摂取できていない可能性，一部のビタミンを大量に摂取している可能性があるので，一度，管理栄養士に食事相談をしてください。		

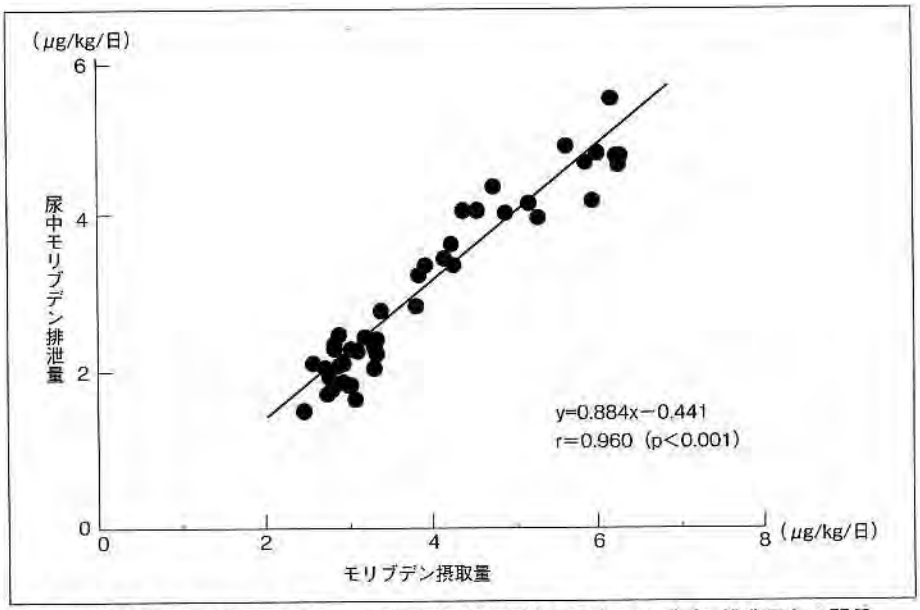

図2．若年日本人女性におけるモリブデン摂取量実測値と尿中モリブデン排泄量との関係

とクレアチニン（Cre）濃度あたりの随時尿のセレン濃度（μg/g Cre）との間には高い相関が観察される[14]。これらからセレン摂取量を随時尿のセレン濃度から推定可能である。日本人の尿中セレン濃度に関する報告は多いが，その値はおおむね25～60 μg/g Creである[15) 16)]。個人単位でのセレンの栄養管理の場合，Creの１日排泄量と成人のセレン推奨量（男性30 μg/日，女性25 μg/日）を考慮すると，随時尿のセレン濃度が常に20 μg/g Creを下回る状態であればセレンの摂取不足といえる。

一般には個人のセレン栄養状態の評価に血清セレン濃度を用いる。日本人の血清セレン濃度の多くは110～130 μg/ℓの範囲にある[16]。世界13地域におけるセレン摂取量と血清セレン濃度からは，両者間に「セレン摂取量（μg/日）＝血清セレン濃度（μg/ℓ）×0.672＋２」という回帰式が得られ[17]，推奨量に対応する血清セレン濃度は30～35 μg/ℓとなる。しかし，このような低血清セレン濃度は臨床症状を引き起こす可能性が高く，栄養管理での基準にはできない。セレン欠乏は長期間の静脈栄養または経腸栄養の施行中に発生しており，欠乏症例の血清セレン濃度は13～80 μg/ℓである[14]。一方，低セレン状態が種々の部位における癌発生の危険因子であることは広く知られており，血清セレン濃度60～70 μg/ℓを下回るとリスクが高まるとする報告が多い[18]。以上より，セレンの栄養管理という観点からは血清セレン濃度70～80 μg/ℓを下限の目安とすべきである。

３．鉄と亜鉛の栄養管理

摂取量と尿中濃度との関連が小さい鉄，亜鉛，銅，マンガン，クロムでは尿を用いた栄養管理は難しい。ここでは鉄と亜鉛に関して，血清データを用いた栄養管理を述べる。

（１）血清データに基づく鉄の栄養管理

わが国の成人女性の約25%は鉄欠乏性貧血である。さらに妊娠期では胎児への鉄供給のため鉄需要が高く，鉄の栄養管理がきわめて重要である。現在，鉄の栄養状態の指標として，血清鉄濃度，血清総鉄結合能（total iron-binding capacity；TIBC），血清不飽和鉄結合能（unsaturated iron-binding capacity；UIBC），および血清フェリチン濃度が利用されている。鉄欠乏では，血清鉄濃度は低下，TIBCは上昇するため，両者の差であるUIBC，および血清鉄濃度とTIBCの比（血清鉄濃度/TIBC）が鉄欠乏のより感度の高い指標となる。貯蔵鉄であるフェリチンは，理論的には鉄欠乏の最も鋭敏な指標であるが，感染など鉄以外の要因によっても変動するため，鉄摂取量との関連を認めないこともある[19]。フェリチンは分析費用が高額なので，血清鉄濃度およびTIBCの測定に基づく栄養管理が現実的である。これらの基準値は検査機関ごとに若干の差異があり，女性の場合では血清鉄の下限が40～50 μg/dℓ，TIBCの上限が400～450 μg/dℓである。ただし栄養管理ではこれらの数値をそのまま流用せず，少し厳しい基準値を設定するのが適切である。

（２）血清亜鉛濃度

亜鉛は体内存在量や１日摂取量が鉄に匹敵し，鉄と同程度に必要量が多いミネラルである。亜鉛摂取不足による健康障害として味覚異常が有名である。また，寝たきりの高齢者では亜鉛栄養状態の低下が褥瘡発生を促進するため，介護・福祉施設では亜鉛の栄養管理が特に重要とされる[20]。現在のところ，亜鉛栄養状態を反映する指標として血清亜鉛のみが有効とされている。多くの検査機関での血清亜鉛の正常下限は60～65 μg/dℓだが，これ以上でも味覚障害や褥瘡の進展などが観察されるので下限を80 μg/dℓにすべきとの提言がある[21]。栄養管理でも後者を目安にするのが適切であろう。血清亜鉛濃度は日内変動するので採血は早朝空腹時に統一するのがよい。

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日本栄養・食糧学会誌　第64巻　第5号　313-321（2011）

報　文

ストレプトゾトシン誘導糖尿病ラットのトリプトファン–ニコチンアミド代謝

—摂取ビタミン量との関係—

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（2011年3月4日受付；2011年6月28日受理）

要旨：トリプトファン（Trp）の異化代謝にはビタミン B_1，B_2，B_6 が関わっている。そこで，低用量，中用量，十分量のビタミン混合を含む飼料を投与した時にTrp異化代謝がどのように変動するのかを，健常ラットと糖尿病ラットを用いて比較した。健常ラットにおいても，糖尿病ラットにおいても，飼料中のビタミン混合含量の差異はTrp異化代謝には全く影響をおよぼさなかった。糖尿病ラットにおけるTrp-ニコチンアミド（Nam）転換率は健常ラットの1/3程度にまで低下していた。N^1-メチルニコチンアミドが体内に蓄積しやすい代謝状態にあった。この現象は体内の遊離状態のNam濃度の上昇を引き起こす可能性がある。遊離型のNamは，poly(ADP-ribose) 合成酵素やヒストンデアセチラーゼなど種々の酵素の阻害剤である。したがって，糖尿病時にはNam代謝変動による影響が表れる可能性があり，さらなる研究が必要である。

キーワード：ストレプトゾトシン，糖尿病，トリプトファン，ニコチンアミド，ビタミン

糖尿病は，一般的に糖質の利用が極度に制限されるため，肝臓における脂質の利用が著しく増大し，かつ糖新生が盛んとなる。すなわち，タンパク質の分解が進み，アミノ酸の異化代謝が亢進する。そのため，脂質代謝に関わるビタミン B_2，ナイアシン，パントテン酸の要求量が増大し，かつアミノ酸の異化代謝に関わるビタミン B_6 の要求量が特に高まることが予想される。本研究では，糖尿病におけるビタミン量の違いがトリプトファン–ニコチンアミド代謝におよぼす影響について，また，トリプトファン異化代謝について明らかにすることを目的とし，以下の二つのことについて調べた。一つ目は，ビタミン摂取量の差異がトリプトファン異化代謝にどのような影響をおよぼすのかである。

二つ目は糖尿病ラットと健常ラットのニコチンアミドそのものの異化代謝の違いについてである。1型糖尿病のモデル動物であるストレプトゾトシンおよびアロキサン誘導糖尿病ラットでは，健常ラットと比べ，トリプトファンの異化代謝がかなり亢進しているものと考えられている。Mehler *et al.*[1]，Ikeda *et al.*[2]，Sanada *et al.*[3] は糖尿病ラットでは肝臓のアミノカルボキシムコン酸セミアルデヒド脱炭酸酵素活性が健常ラットと比べて顕著に高くなることを報告している。この酵素活性の増大はトリプトファン–ニコチンアミド転換経路の鍵中間体となるキノリン酸の生成量の低下を引き起こし，ニコチンアミド経路への流入が低下し，グルタル酸経路への流入量が多くなり，最終産物であるアセチル-CoAの生成量の増大を招くことになる。McDaniel *et al.*[4] は，アロキサン糖尿病SD系ラットではトリプトファンを負荷すると健常ラットと比べてニコチンアミドの異化代謝産物である N^1-メチルニコチンアミド（MNA）の尿中への排泄量が低くなっていたが，この現象はニコチンアミドのメチル化反応の低下によるものではなく，トリプトファンからニコチンアミドへの転換経路が低くなったためであろうと報告している。したがって，MacDaniel *et al.*[4] は，糖尿病はニコチンアミドの異化代謝には影響をおよぼすことはないものと結論した。後に，Shibata *et al.* はアロキサン誘発[5] およびストレプトゾトシン誘発[6] 糖尿病SD系ラットでは，トリプトファンを負荷しない通常食摂取時でも，トリプトファンから生合成されるニコチンアミドの量が低下していることをMNA，N^1-メチル-2-ピリドン-5-カルボキサミド（2-Py）および N^1-メチル-4-ピリドン-3-カルボキサミド（4-Py）を測定することで，直接証明している。さらに，Shibata *et al.* は，アロキサン誘発糖尿病SD系ラット[5] とストレプトゾトシン誘発糖尿病SD系ラット[6] では，ニコチンアミドの異化代謝産物である4-Py/2-Py排泄量比ならびに（2-Py＋4-Py）/MNA

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排泄量比が健常ラットと比較して，差異が認められなかったことを証明し，MacDaniel *et al.*[4] が推測したように糖尿病はニコチンアミドの異化代謝には影響をおよぼすことはないという結論に支持を与えた。

ところが，ラットの系統をSD系ラットからWistar系ラットに変えて同様な実験を行ったところ，Wistar系ラットでは糖尿病になると，上記のニコチンアミド異化代謝産物の排泄量比が顕著に低下することをShibata *et al.*[7] は見いだした。この原因は，SD系ラットでは糖尿病状態でもMNA酸化酵素活性が低下しなかったが[6]，Wistar系ラットでは低下したことであった[7]。トリプトファンの異化代謝は種差や系統差あるいは性差による差異が著しいことが知られている[2)8-13)]。したがって，SD系ラットとWistar系ラット間で見られたニコチンアミドの異化代謝経路の差異はラットの系統に起因する特有のものである可能性が高いが，補酵素として関わっているビタミンの要求量の差異に起因する可能性も否定できない。

ビタミンB_2はキヌレニン3-モノオキシゲナーゼ[14]と2-Py生成MNA酸化酵素，4-Py生成MNA酸化酵素の補酵素として[15]，ビタミンB_6はキヌレニナーゼ[16]，キヌレニンアミノトランスフェラーゼ[17]の補酵素として必要である。間接的ではあるが，PRPPの前駆体のリボース-5-リン酸の生成に関与するトランケトラーゼ反応にビタミンB_1が必要である[18]。このように，トリプトファンの異化代謝は複数のビタミンが関与する。そこで，ニコチンアミドの異化代謝経路がストレプトゾトシンで影響を受けるWistar系ラットを用いて，低用量，中用量，十分量のビタミン混合を含む飼料を投与した時にトリプトファン-ニコチンアミド代謝がどのような変動を示すかを調べた。また，糖尿病ラットと健常ラット間のトリプトファン異化代謝についても比較した。

実 験 方 法

1. 動物飼育

本実験は滋賀県立大学動物実験委員会の承認を受けた。飼育室の温度は22℃前後，明暗サイクルは，午前6時～午後6時を明，午後6時～午前6時を暗とした。

5週齢のWistar系雄ラットを日本クレア(株)より購入した。購入後，新しい環境に順応させるために，直ちに，ラット用代謝ケージ（日本クレア社製，CT-10）に個別に入れ，表1に示した1%AIN-93ビタミン混合[19]を含む20%カゼイン食と水を自由に与え，1週間予備飼育した。そして，6週齢となった時点で，ラットの平均体重がほぼ均等になるように15匹ずつ二群に分けた。一群のラットを糖尿病にするために，0.1 mol/Lのクエン酸でpHを4.4に調整した0.5%食塩水1.0 mLにストレプトゾトシンをラットの体重1 kg当たり70 mgとなるように溶解した液を腹腔内に注射した。ストレプトゾトシン投与3日後，飽食時での血糖値が200 mg/dL以上のものを使用し[20)21)]，ストレプトゾトシンを注射した群を糖尿病ラット群とした。他の群にはストレプトゾトシンを含まない同じ液を腹腔内に注射した。この群を健常ラット群とした。注射をした時間は午前9時から9時30分とした。さらに，糖尿病ラット群と健常ラット群の二つの群は，摂取させた飼料によって三つの群に細分化した。0.3%ビタミン混合食飼料を摂取させた低用量ビタミン混合食群，0.5%ビタミン混合食飼料を摂取させた中用量ビタミン混合食群，1.0%ビタミン混合食飼料を摂取させた十分量ビタミン混合食群の三群である。ストレプトゾトシンを投与したラットの飼料摂取量は，健常ラットよりも徐々に増え始め，糖尿病状態が落ち着いてきた10日後あたりから，約2倍になり飼料摂取量も安定化してくることを予備実験で確認した。既報もこの現象が見られることを報告している[6]。糖尿病ラット群のビタミン摂取量を健常ラットの比較すべき群と揃えるために，実験開始10日間は，予備実験時の糖尿病ラットの飼料摂取量を基にしてビタミン混合量を毎日変えた飼料を投与した。糖尿病ラット群は，実験開始11日からは，表1に示したように，健常ラットに与えた各飼料の1/2量のビタミン混合を含む飼料を与えることで，比較すべき健常ラット群のビタミン摂取量と揃えた。

飼育期間は70日間とした。この間，飼料と水は自由

表1 飼料組成

	健常ラット			糖尿病ラット		
	0.3% VX 飼料群	0.5% VX 飼料群	1.0% VX 飼料群	調整 0.3% VX 飼料群[1]	調整 0.5% VX 飼料群[1]	調整 1.0% VX 飼料群[1]
ビタミンフリーミルクカゼイン	20	20	20	20	20	20
L-メチオニン	0.2	0.2	0.2	0.2	0.2	0.2
α-コーンスターチ	46.8	46.8	46.8	46.8	46.8	46.8
ショ糖	24.2	24	23.5	24.35	24.25	24
コーン油	5	5	5	5	5	5
ミネラル混合（AIN-93-G-MX）	3.5	3.5	3.5	3.5	3.5	3.5
ビタミン混合（ニコチン酸欠 AIN-93-MX）	0.3	0.5	1	0.15	0.25	0.5

各値は%で示した。[1] 健常群ラットと糖尿病群ラットのビタミン混合摂取量のみを各ビタミン混合飼料群間でそろえるために，糖尿病ラットの飼料中のビタミン含量を1/2にした。その理由は，糖尿病ラットは健常ラットの約2倍の飼料摂取量であるためである。

摂取とし，毎日新しいものに交換した。ラットの世話は午前 8 時～10 時の間に行い，体重と飼料摂取量を測定した。実験開始日を Day 1 として，飼育最終日の Day 70 の 1 日尿（Day 70 の午前 9 時～Day 71 午前 9 時：24 時間）を集めた。非絶食時の尿を塩酸酸性下で集め，採尿後，分析に供するまで－20℃で保存した。

2. 化学薬品

ビタミンフリーミルクカゼイン，ショ糖，L-メチオニン，ニコチンアミド，アンスラニル酸は和光純薬工業(株)(大阪) より購入した。コーンオイルは味の素(株)(東京) より購入した。α-コーンスターチ，ミネラル混合 (AIN-93-G-MX)[19]，ビタミン混合 (AIN-93-VX)[19] はオリエンタル酵母(株) より購入した。キヌレン酸，キサンツレン酸，MNA は東京化成工業(株)(東京) より購入した。2-Py および 4-Py は，各々 Pullman & Colowick[22] および Shibata *et al.*[23] の方法により合成した。他の化学薬品は市販品の中で最高純度のものを使用した。

3. 尿中のトリプトファン異化代謝産物の測定

アンスラニル酸[24]，キヌレン酸[25]，キサンツレン酸[26]，MNA[27]，Nam[23]，2-Py[23]，および 4-Py[23] は各々文献に示した方法で測定した。

4. 統計学的解析

すべてのデータは平均値±標準誤差で示した。データの比較には，二元配置分散分析 (2-way ANOVA) を行い，差があると推定されたとき，Tukey 法にて多重比較検定を行い，$p<0.05$ をもって有意とした。計算には日本語 Windows 版 Statistical Package for Social Science (SPSS, Chicago, IL) Ver.14 を用いた。

結　　果

1. 飼料中のニコチン酸欠–ビタミン混合含量の違いが尿量，血液および尿中生化学検査値におよぼす影響

ストレプトゾトシンを投与した糖尿ラットの尿量は，健常ラットに比べておよそ 19～35 倍であった。また，血中グルコースおよび尿中グルコース排泄量，尿中尿素窒素排泄量についても健常ラットに比べ糖尿ラットにおいて有意に高値を示したが，ビタミン混合の摂取量の違いによる影響は受けなかった（表 2）。

2. 飼料中のビタミン混合量の差異が通常ラットのトリプトファンの異化代謝におよぼす影響

トリプトファンの異化代謝産物で，尿中に排泄される主要な化合物の測定を行った。表 3 に示したように，健常な Wistar 系雄ラットを低用量(0.3%VX)，中用量(0.5% VX)，十分量 (1.0%VX) のニコチン酸フリーのビタミン混合を含む飼料を自由に摂取させた時のアンスラニル酸，キヌレン酸，キサンツレン酸排泄量は，ビタミン混合の摂取量の違いによる影響を全く受けなかった。

本実験ではニコチン酸フリー飼料を投与したので，生成したニコチンアミドとその異化代謝産物はすべて飼料

表 2　飼料中のニコチン酸欠–ビタミン混合含量の違いが体重，飼料摂取量，尿量，生化学検査値におよぼす影響

	実験開始時の体重 (g)	実験終了時の体重 (g)	体重増加量 (g/70日)	飼料摂取量 (g/70日)	尿量 (mL)	血漿グルコース (mmol/L)	尿中グルコース (mmol/日)	尿中尿素窒素 (mmol/日)
健常ラット×0.3% VX	154±3	455±10	301±9	1415±24	9±1	5.7±0.3	0.0073±0.0005	12.9±0.9
健常ラット×0.5% VX	154±1	445±12	292±13	1428±26	15±3	5.3±0.3	0.0063±0.0017	13.1±0.9
健常ラット×1.0% VX	154±1	468±12	313±12	1471±22	12±1	6.4±0.2	0.0090±0.0007	13.6±0.5
糖尿ラット×調整 0.3% VX	157±3	198±19*	41±22*	2792±114*	312±16*	61.8±3.8*	193±19*	34.3±4.0*
糖尿ラット×調整 0.5% VX	155±2	219±25*	60±21*	2824±147*	281±45*	66.4±7.3*	173±36*	32.5±5.8*
糖尿ラット×調整 1.0% VX	154±3	205±16*	51±16*	2815±81*	346±19*	60.0±4.9*	193±9*	32.7±2.2*
2-way ANOVA *p*-values								
ストレプトゾトシン	0.493	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ビタミン含量	0.810	0.833	0.795	0.897	0.349	0.777	0.796	0.966
ストレプトゾトシン×ビタミン含量	0.810	0.494	0.592	0.953	0.288	0.627	0.796	0.920

各値は平均値±標準誤差 ($n=5$) で示した。*健常ラットに対して有意差 ($p<0.05$) が認められたことを示す。

表3　飼料中のニコチン酸欠-ビタミン混合含量の違いが尿中へのトリプトファンの異化代謝におよぼす影響

	飼料摂取量[1]（g/日）	Trp 摂取量[1]（μmol/日）	AnA 排泄量（nmol/日）	AnA 排泄率[2]（%）	KA 排泄量（nmol/日）	KA 排泄率[2]（%）	XA 排泄量（nmol/日）	XA 排泄率[2]（%）
健常ラット×0.3% VX	20.4±0.4	232±5	98±4	0.045±0.002	976±66	0.432±0.036	984±76	0.424±0.043
健常ラット×0.5% VX	20.9±0.5	237±5	91±9	0.038±0.003	1017±151	0.426±0.053	886±113	0.373±0.042
健常ラット×1.0% VX	21.0±0.6	239±6	103±9	0.043±0.004	1107±108	0.465±0.045	1003±76	0.420±0.029
糖尿ラット×調整 0.3% VX	42.3±1.4*	462±32*	121±21	0.025±0.004*	1195±288	0.259±0.062*	893±186	0.193±0.040*
糖尿ラット×調整 0.5% VX	44.3±2.1*	502±24*	122±22	0.024±0.006	1207±244	0.237±0.041*	948±425	0.189±0.074*
糖尿ラット×調整 1.0% VX	41.3±1.4*	475±13*	124±35	0.027±0.008	1244±124	0.258±0.029*	848±167	0.179±0.031*
2-way ANOVA *p*-values								
ストレプトゾトシン	<0.001	<0.001	0.1332	<0.001	0.230	<0.001	0.725	<0.001
ビタミン含量	0.455	0.452	0.939	0.648	0.878	0.807	0.995	0.829
ストレプトゾトシン×ビタミン含量	0.464	0.575	0.965	0.826	0.974	0.933	0.871	0.802

各値は平均値±標準誤差（$n=5$）で示した。*健常ラットに対して有意差（$p<0.05$）が認められたことを示す。[1]採尿日の値を示す。[2]Trp 摂取量に対する排泄量の割合を示す。略称：Trp，トリプトファン；AnA，アンスラニル酸；KA，キヌレン酸；XA，キサンツレン酸。

表4　飼料中のニコチン酸欠-ビタミン混合含量の違いがニコチンアミドの異化代謝産物におよぼす影響

	合計量[1]（nmol/日）	Trp-Nam 転換率[2]（%）	Nam（nmol/日）	MNA（nmol/日）	2-Py（nmol/日）	4-Py（nmol/日）	4-Py/2-Py	(2-Py+4-P)/MNA
健常ラット×0.3% VX	3169±269	1.37±0.13	69±4	155±21	136±21	2809±235	21.7±2.6	19.6±2.2
健常ラット×0.5% VX	3072±467	1.13±0.14	73±14	158±37	140±23	2701±400	20.0±2.2	19.6±2.4
健常ラット×1.0% VX	3287±172	1.38±0.06	79±8	150±6	150±17	2908±159	20.2±2.4	20.4±0.9
糖尿ラット×調整 0.3% VX	2088±250	0.43±0.04*	N.D.[3]	1086±82*	114±20	907±242*	9.6±1.7*	0.9±0.3*
糖尿ラット×調整 0.5% VX	1981±362	0.52±0.16*	N.D.	961±94*	115±20	906±279*	7.6±2.4*	1.0±0.3*
糖尿ラット×調整 1.0% VX	2028±539	0.43±0.10*	N.D.	1018±315*	111±17	900±306*	8.1±2.1*	1.4±0.5*
2-way ANOVA *p*-values								
ストレプトゾトシン	<0.001	<0.001	—	<0.001	0.089	<0.001	<0.001	<0.001
ビタミン含量	0.932	0.735	—	0.908	0.962	0.938	0.687	0.877
ストレプトゾトシン×ビタミン含量	0.963	0.254	—	0.900	0.901	0.930	0.997	0.989

各値は平均値±標準誤差（$n=5$）で示した。*健常ラットに対して有意差（$p<0.05$）が認められたことを示す。[1]合計量は Nam，MNA，2-Py，4-Py の合計を示す。[2]Trp-Nam 転換率（%）＝合計量（nmol/日）/トリプトファン摂取量（nmol/日）×100。（例：健常 0.3% VX ラットで，トリプトファン摂取量が 230 μmol/日の時では，3169/230000×100＝1.378≒1.38%となる。[3]検出限界以下であることを示す。

中に含まれるカゼインのトリプトファンから生合成されたものである。ニコチンアミドとその異化代謝産物の尿中への合計排泄量と採尿時のトリプトファン摂取量からトリプトファン–ニコチンアミド転換率がもとめられる。その結果を表3に示した。この転換率も飼料中のビタミン含量の差異によって影響を受けることはなかった。

ニコチンアミドおよびその異化代謝産物であるMNA, 2-Py, 4-Pyの各排泄量を表4に示した。また異化代謝産物排泄量比, 4-Py/2-Pyおよび(2-Py+4-Py)/MNAを表3に示した。これらの値は, 摂取ビタミン量の差異によって影響を受けることはなかった。

3. 飼料中のビタミン混合量の差異が糖尿病ラットのトリプトファンの異化代謝におよぼす影響

ストレプトゾトシンを注射することで作成したI型糖尿病ラットのトリプトファン異化代謝が, 摂取するビタミン量の違いによってどのように変動するのかを調べた。上記の健常ラットと同様に, 低用量, 中用量, 十分量のビタミン混合を含んだ飼料を自由に摂取させた。投与した飼料のビタミン混合量は, 後述の健常ラットと糖尿病ラット間での比較を行うに当たり, ラット1匹当たりの摂取するビタミンの絶対量を比較すべき二つの群間内で等しくするために調整を行った。

トリプトファンの異化代謝産物であるアンスラニル酸, キヌレン酸, キサンツレン酸は表3に示したように, 糖尿病Wistar系雄ラットを低用量, 中用量, 十分量のニコチン酸フリーのビタミン混合を含む飼料を自由に摂取させた時において, 各々の排泄量は, ビタミン混合の摂取量の差異による影響を全く受けなかった。

糖尿病ラットのトリプトファン–ニコチンアミド転換率を求めた。その結果を表4に示した。転換率は摂取するビタミン量の差異による影響を受けなかった。

糖尿病ラットのニコチンアミドおよびその異化代謝産物であるMNA, 2-Py, 4-Pyの各排泄量を表4に示した。また異化代謝産物排泄量比, 4-Py/2-Pyおよび(2-Py+4-Py)/MNAを表4に示した。これらの値は, 摂取ビタミン量の差異によって影響を受けることはなかった。

4. 健常ラットと糖尿病ラットのトリプトファン異化代謝の比較

上述のように, 健常ラットおよび糖尿病ラットに低用量, 中用量, あるいは十分量のビタミン混合を含む飼料を与えて, トリプトファンの異化代謝産物排泄量への影響, トリプトファン–ニコチンアミド転換率への影響, およびニコチンアミド異化代謝産物への影響を調べたが, 調べた範囲内でのビタミン混合量の差異は, これらの指標に全く影響をおよぼすことはなかった(表3, 4)。

実験期間中の飼料摂取量は約2倍であったにもかかわらず, 体重増加量は, 糖尿病ラットの方が健常ラットよりも顕著に低い値であった。アンスラニル酸, キヌレン酸, キサンツレン酸の1日尿中に排泄された絶対量は, 糖尿病ラットと健常ラット間で差異は認められなかったが, 糖尿病ラットのトリプトファン摂取量が2倍であったために, トリプトファン摂取量に対する排泄量比率は糖尿病ラットが健常ラットの約0.4～0.5倍の値となった。糖尿病ラットのトリプトファン–ニコチンアミド転換率は健常ラットの約1/3にまで低下していた。これは, ニコチンアミドとその異化代謝産物量がトリプトファン摂取量に比して, 低い値であったことを意味している。しかしながら, 糖尿病ラットの1日尿中に排泄されたニコチンアミドおよびその異化代謝産物の絶対量が, 健常ラットに比して一様に低いわけではなく, 2-Py排泄量はほぼ等しく, MNAは高い値, 4-Pyは顕著に低い値を示した。糖尿病ラットのニコチンアミド排泄量は検出限界以下であったが, これは, 糖尿病ラットの尿量が300 mL/日と健常ラットの20倍も多く, ニコチンアミドが希釈されすぎて, 検出限界以下になったものと推察された。

考　　察

トリプトファン代謝には, B群ビタミンが必要である。ビタミンB_6の栄養状態を判断する一つの指標として, トリプトファン負荷後の尿中へのキサンツレン酸排泄量を調べる方法[28,29]が知られている。また, 糖尿病患者の尿中には多量の3-ヒドロキシキヌレニンとキサンツレン酸が検出されること[30]や糖尿病ラットにトリプトファンを負荷するとキサンツレン酸の排泄量が顕著に高くなることも古くから明らかにされており[31], 糖尿病状態ではビタミンB_6の栄養状態が悪化していることが推察される。これは, キヌレニンの代謝経路と関係している。キヌレニンはビタミンB_2の補酵素型であるFADを補酵素とするキヌレニン3-モノオキシゲナーゼ[14]によって3-ヒドロキシキヌレニンになるのか, ピリドキサールリン酸を補酵素とするキヌレニナーゼ[16]によってアンスラニル酸となるのか, あるいはピリドキサールリン酸を補酵素とするキヌレニンアミノトランスフェラーゼ[17]によってキヌレン酸になるのか, 三つの可能性がある。キヌレニナーゼのキヌレニンと3-ヒドロキシキヌレニンに対する反応性は, K_m値などのデータから[16], 3-ヒドロキシキヌレニン→3-ヒドロキシアンスラニル酸の方が高いと考えられている。したがって, キヌレニナーゼは本来3-ヒドロキシキヌレニナーゼと呼ばれるべきである。3-ヒドロキシキヌレニンはキヌレニナーゼによって3-ヒドロキシアンスラニル酸になるか, キヌレニンアミノトランスフェラーゼによってキサンツレン酸になるのか, 二つの可能性がある。キヌレニナーゼは細胞質画分[16]に, キヌレニンアミノトランスフェラーゼはミトコンドリアの外膜中に存在する酵素である[32]。ビタミンB_6欠乏になると, 細胞質に存在するキヌレニナーゼはミトコンドリア外膜に存在するキヌレニンアミノトランスフェラーゼよりも早くピリドキサールリン酸を失い, 活性が低下するものと考えられる。その結果, キヌレニ

ンはキヌレニン3-モノオキシゲナーゼによって選択的に水酸化され，3-ヒドロキシキヌレニンが生成される。3-ヒドロキシキヌレニンは，ビタミンB_6不足では，キヌレニナーゼの触媒反応により3-ヒドロキシアンスラニル酸になることができないので，トリプトファン–ニコチンアミド代謝経路から考えれば，側路に位置するキヌレニンアミノトランスフェラーゼによってキサンツレン酸へと代謝される。つまり，深刻なビタミンB_6欠乏状態では，キヌレニンを代謝できる酵素が二つともビタミンB_6酵素であるために，キヌレニンが蓄積するものと考えられる。しかし，穏やかなビタミンB_6欠乏の場合は，ミトコンドリアの外膜に存在するキヌレニンアミノトランスフェラーゼよりも，細胞質画分に存在するキヌレニナーゼの方が，より早くピリドキサールリン酸を失うため，キヌレニンがキサンツレン酸の生成へと偏向するものと考えられている。しかし，どれほどのビタミンB_6欠乏でこのような代謝変動が起きるのかについては未だ十分な解明はなされていなかった。今回の実験結果では，幼若ラットの最大成長を保証する最低量の0.3%ビタミン混合食を投与しても，アンスラニル酸，キヌレン酸，キサンツレン酸の尿中排泄量が十分量のビタミン混合食を投与した時と比較して変動は認められなかった。したがって，トリプトファン–キサンツレン酸経路が主な代謝経路となるようなビタミンB_6栄養状態は，かなり深刻なビタミンB_6欠乏状態であると思われた。Schaeffer *et al.* は[33]，トリプトファン負荷によるキサンツレン酸の測定はビタミンB_6欠乏の鋭敏な判断方法としては適さないことを報告している。また，ビタミンB_6はトリプトファンから生成する神経伝達ホルモンであるセロトニンの合成にも関わっていることから[34]，糖尿病による神経障害を，ビタミンB_6の積極的な投与が緩和させることも報告されている[35]。今回の実験では，セロトニン関連物質の測定は行わなかったが，今後検討してみたい課題である。

ビタミンB_2が欠乏すると，FAD酵素であるキヌレニン3-モノキシゲナーゼ活性が低下し，キヌレニンの代謝はアンスラニル酸へと偏向することが予想される。今回の実験はすべてのビタミンを低用量にした群を設けたが，低用量ビタミン混合飼料を投与してもアンスラニル酸が増大することはなかった。したがって，0.3%ビタミン混合食に含まれるビタミンB_2量で，キヌレニン3-モノオキシゲナーゼは十分に酵素活性を発揮できるものと推察された。

ビタミンB_1は直接トリプトファンの異化代謝には関与していないが，キノリン酸からニコチン酸モノヌクレオチドが生成するときに必要な5-ホスホリボシル-1-ピリロン酸（PRPP）[36]の生成系の一員であるトランスケトラーゼの補酵素として関与している。トリプトファン–ニコチンアミド転換率は飼料中のビタミン混合量の差異によって変動しなかったので，PRPP生合成反応系にも異常がないものと推察された。ビタミンB_1の量もビタミン混合量が0.3%で必要量をまかなうことができたものと考えることができる。

今回の実験結果から，低用量ビタミン混合食として使用した0.3%ビタミン混合食でも十分に幼若ラットの成長を支えることができ，さらにトリプトファンの異化代謝経路も正常に動かすことができることが明らかとなった。

糖尿病ラットは健常ラットの飼料摂取量の2倍の摂食があるにもかかわらず，アンスラニル酸，キヌレン酸，キサンツレン酸の排泄量が健常ラットに比して高い値を示すことはなく，ほぼ健常ラットと等しい値を示した。したがって，トリプトファンからの生成比率では糖尿病ラットは健常ラットの1/2程度であったことになる。このことは，糖尿病ラットではトリプトファンをアセチル-CoAに転換する経路の活性が強くなり，側路への流入量を著しく低下させる代謝変動が起きていることを意味する。これは，3-ヒドロキシアンスラニル酸ジオキシゲナーゼ活性の増大[6)7)]とアミノカルボキシムコン酸セミアルデヒド脱炭酸酵素活性の増大[1-3)6)7)]という *in vitro* 実験の結果から説明が可能である。すなわち，糖尿病によってキサンツレン酸が増えるという現象は，トリプトファンの大量負荷により，トリプトファンの異化代謝の本経路が飽和した結果時にのみ見られる現象で，通常の飼料中から得られるトリプトファン摂取量では，トリプトファンはほとんど本経路で処理されてしまい，側路に流入する量は減少し，糖尿病時ではむしろ側路に位置するアンスラニル酸，キヌレン酸，キサンツレン酸の各生成量比は低下していることが明らかとなった。

ビタミンB_2欠乏ラットではトリプトファンからのニコチンアミドの生合成量が低下することが報告されている[37]。これは，キヌレニン3-モノオキシゲナーゼがFADを補酵素とするからである[14]。さらに，ニコチンアミドの異化代謝酵素である2-Py生成MNA酸化酵素と4-Py生成MNA酸化酵素はFAD酵素であると推測されている[38]。したがって，ビタミンB_2欠乏ラットではこれらの酵素活性が検出限界以下にまで低下することで，MNA → 2-Pyおよび MNA → 4-Pyの反応が抑制され，MNA排泄量が著しく高くなると考えられている。そして，(2-Py + 4-Py)/MNA排泄量比が著しく低下するものと思われる。今回の実験では低用量のビタミン混合食でもこれら二つの比率は変動しなかった。したがって，この結果も0.3%ビタミン混合食に含まれるビタミンB_2含量で必要量をまかなっていることが推測された。

以上のことをまとめてみると，トリプトファン代謝にはB群ビタミンが補酵素として関わっていることから，飼料中のビタミン混合量を十分量，中用量，低用量とし，三つの飼料を健常ラットと糖尿病ラットに投与し，どのような代謝変動が起きるのか調べてみたが，ビタミン摂取量の差異による違いは全く見られなかった。このこと

は，糖尿病ラットにおいては，脂肪酸とアミノ酸の異化代謝が亢進され，これらの代謝に関わるB群ビタミンの必要量に影響を与えると考え，通常食に混入される1.0%ビタミン混合[19]を基本として，1/2量の0.5%ビタミン混合食，1/3量の0.3%ビタミン混合食を投与したが，トリプトファンの異化代謝には変動がなかった。おそらく，他のアミノ酸や脂肪酸の異化代謝においても，0.3%ビタミン混合食で必要量をまかなうことができるものと思われた。さらに，低用量のビタミン混合食で調べてみる必要がある。

健常ラットと糖尿病ラットのトリプトファン異化代謝を比較してみると，多くの差異が観察された。従来から指摘されていたように[1-7]，トリプトファンからのニコチンアミドへの生合成量は約1/3にまで低下していた。今回の実験においてはじめて明らかとなった最も顕著な特徴はMNAから2-Pyおよび4-Pyの生成量が著しく低下していたことであった。このことは，体内へのMNAの蓄積を意味する。MNAの蓄積はニコチンアミドメチルトランスフェラーゼ活性を阻害することで[39]，ニコチンアミドの蓄積を招き，蓄積したニコチンアミドがポリADP-リボース合成酵素[40]やヒストン脱アセチル化酵素活性[41]を阻害することで，細胞機能に悪影響をおよぼす可能性がある。このような推論が正しいとすると，糖尿病でトリプトファン-ニコチンアミド転換率が下がるのは，遊離型のニコチンアミドの蓄積を抑制する防御機構の一つとも考えることができ，したがって，糖尿病患者にニコチンアミドを投与することは好ましくないものと思われる。

本研究は，平成19年度～21年度厚生労働科学研究費補助金循環器疾患等生活習慣病対策総合研究事業「日本人の食事摂取基準を改定するためのエビデンスの構築に関する研究—微量栄養素と多量栄養素摂取量のバランスの解明—」(主任研究者，柴田克己)の成果の一部である。関係各位に謝意を表する。

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J Jpn Soc Nutr Food Sci **64**: 313–321 (2011)

Original Paper

Tryptophan-nicotinamide Metabolism in Rats with Streptozotocin-induced Diabetes: Association of Dietary Vitamin Content

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(Received March 4, 2011; Accepted June 28, 2011)

Summary: The B-group of vitamins, including vitamins B_1, B_2, and B_6, are involved in the catabolic metabolism of tryptophan. We investigated the effects of dietary vitamin content (low, moderate and sufficient) on the catabolism of tryptophan in both diabetic and healthy rats. We found that tryptophan catabolism was not affected by differences in dietary vitamin content in both diabetic and healthy rats. However, the tryptophan-nicotinamide conversion ratio was one-third lower in diabetic rats than in healthy rats. In addition, nicotinamide catabolism differed between diabetic and healthy rats. N^1-methylnicotinamide, a nicotinamide catabolite, accumulated in diabetic rats, but not in normal rats, possibly contributing to the increase in free nicotinamide. It seems likely that this increase in the level of nicotinamide inhibited the activities of poly (ADP-ribose) synthetase and histone deacetylase. These results suggest that administration of nicotinamide might offer some effects, and warrants further investigation.

Key words: streptozotocin, diabetes, tryptophan, nicotinamide, vitamin

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日本栄養・食糧学会誌　第64巻　第5号　329-334（2011）

資　料

ビタミンB_1最小必要量飼料投与ラットあるいは十分量飼料投与ラットを寒冷曝露させた時の肝臓，血液および尿中のビタミンB_1量

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（2011年2月1日受付：2011年6月29日受理）

要旨：低温環境下ではエネルギー代謝の亢進が起こることが知られている。そこで本研究では，低温環境下においてビタミンB_1の要求量がどの程度高まるかを調べた。まず最初にラットを低温環境下で14日間飼育すると，飼料摂取量は1.1倍に増加したが，体重増加量は0.6倍程度であった。したがって，寒冷曝露により1.7倍のエネルギー代謝の亢進が認められた。同時に，14日間の寒冷曝露により，褐色脂肪組織（BAT）が増加し，尿中のビタミンB_1排泄量は約1/2にまで抑制された。これらの事実によって，低温環境がエネルギー代謝の亢進をもたらし，ビタミンB_1必要量が増加したことが示唆された。さらにこのことを確認するために，ビタミンB_1を最小必要量加えた飼料をラットに投与して寒冷曝露し，ビタミンB_1欠乏が引き起こされるか否かを調べたが，ビタミンB_1を十分量投与した群と比べて顕著な体重増加量の低下は認められなかったが，ビタミンB_1の尿中排泄量が低下したことから，寒冷曝露によってビタミンB_1の必要量が高まったと考えられる。

キーワード：ビタミンB_1，寒冷，必要量，チロキシン，エネルギー

何らかの環境因子によってエネルギー消費量が亢進された時に，エネルギー代謝に関わっているB群ビタミン必要量がどのような変動を示すかを明らかにすることは，疲労を軽減させる予防策をたてる上で大切な情報となる。日本人の食事摂取基準において，ビタミンB_1の必要量は，エネルギー消費量が高まった時には増大するという考えに基づいてエネルギー消費量当たりで算定されている[1]。ビタミンB_1欠乏であるベリベリ（日本では脚気）がインドネシアなどの熱帯地方で多発していた時代には，環境温度とビタミンB_1の必要量に関する研究がなされていた。日本においても，脚気には季節的な流行性があり，夏になると脚気に罹るヒトが増え，冬になると自然に治癒してしまったことから，明治時代には，脚気は細菌による伝染病であると多くの著名な脚気学者は考えていた。このことはすなわち，ビタミンB_1の必要量が生活環境温度の影響を受ける可能性を示唆している。

我々は，栄養評価方法として多用されている「習慣的な栄養素摂取量」を「必要量」と比較する方法を補完する一つの方法として，非侵襲性の尿を生体指標とする評価方法を提案しつつある[2-5]。尿中のビタミン排泄量は，摂取量の影響を最も強く受けるが[6]，他の環境因子にも影響を受けることが報告されている。

哺乳動物を低温環境下で飼育すると，体熱維持のためのエネルギー代謝を行わなければならず，エネルギー消費量が増大する。一方，高温環境下では体温のための熱産生を行う必要がないことから，エネルギー消費量は低下する[7]ということが明らかになっている。しかしMills[8-10]はビタミンB_1の必要量は不快な91°F（32.8℃）でラットを飼育すると65°F（18.3℃）で飼育した場合と比較して2倍も高くなったと報告している。このMillsら[8-10]の報告は，ビタミンB_1の必要量はエネルギー消費量が高まった時には増大するという考え方[1]からすると矛盾する報告である。一方で，Edisonら[7]は，ラットを高温環境下（90°F：32.2℃）で飼育すると快適温度環境下（72°F：22.2℃）の時と比較すると飼料摂取量が低下し体重増加量が落ちるが，ビタミンB_1の必要量は高温環境下で高くなることはないとMills[8-10]の説を否定している。むしろ，ラットのビタミンB_1欠乏症である多発性神経炎の発症を指標にすると，高温環境下ではビタミンB_1の必要量は，快適温度環境下よりも低下すると報告している。Kline *et al.*[11]は，ラットの多発性

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神経炎の治療に要するビタミン B_1 量と体重増加量から，高温環境下（90°F：32.2℃）は快適温度環境下（78°F：25.6℃）よりもビタミン B_1 必要量を低下させるということ，そして，この現象は高温環境下ではエネルギーの必要量が少ないためであろうと推察している。1950年代になると Hegsted & McPhee[12] は，55°F（12.8℃）では78°F（25.6℃）よりもビタミン B_1 の必要量が増大することを，体重増加量を指標として明らかにした。すなわち，低温環境ではビタミン B_1 の必要量が増すことを報告した。しかしながら，それ以降，ビタミン B_1 の必要量と飼育温度との関係を調べた実験報告はなく，低温環境下で飼育した時の肝臓や血液中のビタミン B_1 量が通常温度飼育下と比較し，低下しているか否かの情報はない。そこで，本実験では，ビタミン B_1 摂取量が通常飼育温度下で十分量もしくは最小必要量になるように調整した飼料を与えたラットを，4℃と22℃に調整した飼育温度室で飼育した時の飼料摂取量，体重増加量，肝臓，白色脂肪組織（WAT）および褐色脂肪組織（BAT）重量，肝臓および血液中ビタミン B_1 濃度，尿中のビタミン B_1 排泄量の比較を行った。

実験方法

1. 動物飼育

本実験は滋賀県立大学動物実験委員会の承認を受けた。飼育室の温度は通常温度飼育群は22℃前後，寒冷曝露飼育群は4℃前後で，明暗サイクルは，午前6時～午後6時を明，午後6時～午前6時を暗とした。

3週齢の Wistar 系雄ラットは日本クレア(株)より購入した。購入後，新しい環境に順応させるために，直ちに，ラット用代謝ケージに個別に入れ，Table 1 に示した20%カゼイン食（チアミン塩酸塩含量は0.6 mg/100 g 飼料）と水を自由に与えた。2週間予備飼育した後（すなわち5週齢となった時点で）ラットの平均体重がほぼ均等になるように5匹ずつ4群に分け，飼育温度と摂取ビタミン B_1 量によって通常温度飼育-ビタミン B_1 十分量飼料投与群，通常温度飼育-ビタミン B_1 最小必要量飼料投与群，低温度飼育-ビタミン B_1 十分量飼料投与群，低温度飼育-ビタミン B_1 最小必要量飼料投与群として，それぞれの環境温度下でそれぞれの飼料を与えて14日間飼育した。

通常温度飼育群には，Table 1 に示したチアミン塩酸塩含量が0.6 mg/100 g のビタミン B_1 十分量飼料，もしくはチアミン塩酸塩含量が0.1 mg/100 g 飼料のビタミン B_1 最小必要量飼料を投与した。事前の予備実験の結果，ラットを寒冷曝露すると，ラットの飼料摂取量が1.1倍に増加したことを踏まえ，本実験では比較する両温度飼育間のラットのビタミン B_1 摂取量を同じとするために，寒冷曝露群の飼料中のビタミン B_1 含量を調整した。低温度飼育群のビタミン B_1 十分量飼料投与群のチアミン塩酸塩含量を0.54 mg/100 g とし，ビタミン B_1 最小必要量飼料投与群のチアミン塩酸塩含量を0.09 mg/100 g とした（Table 1）。

飼育期間は14日間である。この間，飼料と水は自由摂取とし，1日ないし2日おきに新しいものに交換した。ラットの世話は午前8時～10時の間に行い，体重と飼

Table 1 Compositions of the diets.

	$V.B_1$ sufficient diet for normal temperature (%)	$V.B_1$ minimum requirement diet for normal temperature (%)
Vitamin free milk casein	20.0	20.0
L-Methionine	0.2	0.2
Gelatinized cornstarch	46.9	46.9
Sucrose	23.4	23.4
Corn oil	5.0	5.0
Mineral mixture (AIN-93G-MX)	3.5	3.5
Vitamin mixture (Vitamin B_1 free AIN-93M-VX)	1.0	1.0
Thiamin-HCl	0.0006 (1.78 μmol/100 g diet)	0.0001 (0.30 μmol/100 g diet)
	$V.B_1$ sufficient diet for cold temperature (%)	$V.B_1$ minimum requirement diet for cold temperature (%)
Vitamin free milk casein	20.0	20.0
L-Methionine	0.2	0.2
Gelatinized cornstarch	46.9	46.9
Sucrose	23.4	23.4
Corn oil	5.0	5.0
Mineral mixture (AIN-93G-MX)	3.5	3.5
Vitamin mixture (Vitamin B_1 free AIN-93M-VX)	1.0	1.0
Thiamin-HCl	0.00054 (1.60 μmol/100 g diet)	0.00009 (0.27 μmol/100 g diet)

料摂取量を測定した。実験開始日を Day 1 として，飼育最終日の Day 14 の 1 日尿（Day 14 の午前 9 時～Day 15 の午前 9 時：24 時間）を採取した。尿は塩酸酸性下で集め，分析に供するまで－20℃で保存した。

採尿終了後の Day 15 の午前 9 時～10 時に断頭にて屠殺し，血液を頸動脈から採取した。その後，肝臓，WAT，BAT を摘出し，重量を測定した。血液と肝臓はビタミン B_1 測定用の試料を作成するための調製を直ちに行った（3. チアミンの測定を参照）。

精巣周囲の白色組織量を白色脂肪組織量（WAT）とした。

褐色脂肪量（BAT）は，肩胛骨の下部周辺の褐色がかった組織の量とした。

2. 化学薬品

ビタミンフリーミルクカゼイン，ショ糖，L-メチオニン，チアミン塩酸塩（$C_{12}H_{17}ClN_4OS\cdot HCl = 337.27$）は和光純薬工業(株)(大阪）より購入した。コーンオイルは味の素(株)(東京）より購入した。α-コーンスターチ，ミネラル混合（AIN-93-G-MX)[13]，ビタミン混合（AIN-93-VX)[13] はオリエンタル酵母工業(株)より購入した。他の化学薬品は市販品の中で最高純度のものを使用した。

3. チアミンの測定

3.1 血液　採取した直後の血液 0.1 mL に 0.5 mL の 5% トリクロロ酢酸を加え，均一化したのち，氷水中に 5 分間以上放置後，遠心分離（10,000×g，5 分間，4℃）により得られた上清をミクロフィルター（ポアサイズ 0.45 μm）でろ過した液 50 μL を直接 HPLC に注入した。

3.2 肝臓　摘出した直後の肝臓から約 0.2 g を切り出し，切り出した肝臓重量の 10 倍量の 5% トリクロロ酢酸を加え，テフロン-ガラスホモゲナイザーで均一化したのち，氷水中に 5 分間以上放置後，遠心分離（10,000×g，5 分間，4℃）により得られた上清をミクロフィルター（ポアサイズ 0.45 μm）でろ過した液 50 μL を直接 HPLC に注入した。

3.3 尿　凍結保存した尿を解凍後，ミクロフィルター（ポアサイズ 0.45 μm）でろ過した。そのろ液 20 μL を直接 HPLC に注入した。

HPLC の定量操作は文献 14) に示したポストカラム-HPLC 法にしたがった。分析条件の概要は，カラム：Cosmosil $5C_{18}$-MS-II (ϕ4.6×250 mm)，移動相および流速：5 mmol/L ペンタンスルホン酸ナトリウム塩と 1% アセトニトリルを含む 0.2 mol/L NaH_2PO_4，1.0 mL/min，反応液 1：0.01% $K_3Fe(CN)_6$，0.15 mL/min，反応液 2：15% NaOH，0.15 mL/min，カラム温度：40℃，検出器：分光蛍光光度計，励起波長 365 nm，蛍光波長 435 nm で行った。

4. 有意差検定

すべてのデータは平均値±SEM で示した。有意差検定は two-way ANOVA と Bonferroni ポストテストで行い，交互作用があったものに関しては追加で one-way ANOVA と Tukey の多重比較検定を行った。有意差は，$p<0.05$ で判定した。なお，検定は，統計ソフト GraphPad Prism (version 5.01; GraphPad 社。San Diego, CA, USA) を使用して行った。

結　果

1. 基本的な栄養パラメーター

幼若ラットを，ビタミン B_1 を十分量含む飼料あるいは必要量含む飼料を投与して飼育室の温度が 22℃ の通常温度室あるいは 4℃ の低温室にて，14 日間飼育した。基本的な栄養パラメーターを Table 2 に示した。

飼料摂取量は飼料中のビタミン B_1 含量に関係なく，予

Table 2 Effect of cold environment on the body weight gain, food intake, FER, the weights of liver, WAT and BAT.

	V.B_1 sufficient group		V.B_1 minimum requirement group		2-way ANOVA p-values[4]		
	22℃	4℃	22℃	4℃	V[5]	℃[6]	V×℃
Initial body weight (g)	129.8±2.3	132.1±2.5	129.2±2.9	129.2±3.5	ns	ns	ns
Final body weight (g)	232.1±4.4	190.3±3.5*	231.5±4.2	194.1±6.7*	ns	<0.0001	ns
Body weight gain (g/14 d)	102.3±2.5	58.2±1.9*	102.3±0.7	64.9±4.2*	ns	<0.0001	ns
Food intake (g/14 d)	234.1±2.1	252.4±4.4*	227.7±1.5	253.1±3.9*	ns	<0.0001	ns
FER[1]	0.437±0.005	0.231±0.007*	0.449±0.004	0.256±0.005*	0.0033	<0.0001	ns
VB_1 intake (μg/14 d)	1,405±13	1,363±39	228±5[§]	228±4[§]	<0.0001	ns	ns
VB_1 intake (μmol/14 d)	4.17±0.038	4.04±0.116	0.68±0.015[§]	0.68±0.013[§]	<0.0001	ns	ns
Liver weight (g/rat)	10.84±0.32	8.67±0.39	11.17±0.38	9.92±0.48[§]	ns	0.0005	ns
WAT[2] (g/rat)	2.66±1.0	1.89±0.09	2.07±0.07	1.76±0.13	ns	ns	ns
BAT[3] (g/rat)	0.527±0.040	0.812±0.076*	0.496±0.035	0.704±0.036*	ns	0.0001	ns

Values are expressed as mean±SEM for 5 rats. [1] (body weight gain)/(food intake). [2] White adipose tissues surrounding testes. [3] Brown adipose tissues under scapulae. [4] The p-values, obtained from two-way ANOVA analysis, are given for interaction effects. ns = not significant ($p>0.05$). [5] V: Vitamin. [6] ℃: Temperature. * Statistically significant difference at $p<0.05$ between the two groups (22℃ or 4℃) fed the same diet (V.B_1 sufficient group or V.B_1 minimum requirement group), as determined by two-way ANOVA with Bonferroni's post-hoc test. [§] Statistically significant difference at $p<0.05$ between the two groups (V.B_1 sufficient group or V.B_1 minimum requirement group) of the same temperature (22℃ or 4℃), as determined by two-way ANOVA with Bonferroni's post-hoc test.

Table 3 Effect of cold environment on the vitamin B_1 contents in liver, blood, and urine.

	$V.B_1$ sufficient group		$V.B_1$ minimum requirement group		2-way ANOVA p-values[1]		
	22℃	4℃	22℃	4℃	V[2]	℃[3]	V×℃
Liver (nmol/g)	17.4±1.3	17.3±0.7	4.5±0.3[§]	3.6±0.2[§]	<0.0001	ns	ns
Whole blood (pmol/mL)	421±25	412±25	126±16[§]	116±4[§]	<0.0001	ns	ns
Urine (nmol/d)	67.4±5.4[a]	33.6±7.2[ab]	1.6±0.1[c]	1.2±0.24[d]	<0.0001	0.0016	0.0019

Values are expressed as mean±SEM for 5 rats. [1] The p-values, obtained from two-way ANOVA analysis, are given for interaction effects. ns=not significant ($p>0.05$). [2] V: Vitamin. [3] ℃: Temperature. [§] Statistically significant difference at $p<0.05$ between the two groups ($V.B_1$ sufficient group or $V.B_1$ minimum requirement group) of the same temperature (22℃ or 4℃), as determined by two-way ANOVA with Bonferroni's post-hoc test. Same alphabet denotes statistically insignificant difference between the respective means, while those with different alphabets denote statistically significant difference between such means at $p<0.05$ by one-way ANOVA with Tukey multiple comparison test.

備実験時と同じく寒冷曝露（4℃にて飼育）により，通常温度飼育群（22℃にて飼育）と比較して，1.1倍に増加した。しかしながら，体重増加量は，通常温度飼育群の0.6倍に減少した。したがって，飼料効率比は，寒冷曝露により，約1/2の値となった。ビタミンB_1の摂取量にかかわらず，寒冷曝露により，肝臓重量は約0.9倍に低下し，BAT重量は寒冷曝露により1.5倍に増大した。

2. ビタミンB_1含量

肝臓および血液中ビタミンB_1濃度，尿中のビタミンB_1排泄量をTable 3に示した。肝臓中の濃度（1g当たりの含量）と血液中の濃度は寒冷曝露の影響を受けなかった。逆に，尿中への排泄量は寒冷曝露により，ビタミンB_1摂取量が十分量の場合でも最小必要量摂取量でも，約0.5倍に低下した。

考　　察

ラットを低温環境下で飼育すると，褐色脂肪細胞が多くなり，また，飼料摂取量が1.1倍に増加しても体重の増加量は0.6倍程度と低かったことから，本実験条件下では，低温曝露によって1.7倍のエネルギー代謝の亢進がもたらされたものと考えられた。

低温で飼育されたラット肝臓のビタミンB_1量は，肝臓単位重量当たりでは，飼育温度による差異は認められなかった。血液中の濃度は，飼育温度の影響を受けなかったが，尿中への排泄量は，寒冷曝露により顕著に低下した。これらの結果は，寒冷曝露により，体温を維持するために，炭水化物などからのエネルギー代謝が亢進され，ビタミンB_1の必要量が高くなった結果，尿中への排泄量が抑制されたと考えられる。

ビタミンB_1最小必要量飼料投与群ラットの実験期間中の1日平均ビタミンB_1摂取量は約12.5 nmol（チアミン塩酸塩量として4μg程度），平均体重は約150gであった。1kg体重当たりのチアミン塩酸塩摂取量は25μg程度と計算された。おそらく，この25μg/日/kg体重程度のチアミン塩酸塩を摂取することができれば，ビタミンB_1欠乏に陥ることはないと予想される。過去の報告によると，幼若ラットにビタミンB_1欠乏食を与えると，概ね5日後当たりから飼料摂取量が落ち，10日後当たりから体重が減少しはじめ，25日後あたりでは死亡するラットが出てくることが見いだされている[15)16)]。実際に，ビタミンB_1欠食を与え，すでにビタミンB_1欠乏が顕在化しているラットの肝臓中のビタミンB_1の値は2.75 nmol/g程度であったと報告されている[15)]。本実験の低温度飼育-ビタミンB_1最小必要量投与ラットの肝臓ビタミンB_1濃度は3.6 nmol/g程度であった。この数値は上記の2.75 nmol/gよりも高い値であった。このことからも低温度飼育-ビタミンB_1最小必要量投与ラットはビタミンB_1欠乏状態に陥っていないと考えられる。

血液中の値は，摂取した飼料からの吸収量，貯蔵庫である肝臓からの放出量，非肝臓組織の取り込み量，さらに腎臓を介する尿中への排泄量などによって厳密に調節されているものと推察される。したがって，血液中のビタミンB_1濃度は肝臓中のビタミンB_1の備蓄量が枯渇しない限り，低下しないものと考えられる。本実験においても，肝臓中のビタミンB_1量が寒冷曝露により枯渇しなかったため，血液中のビタミンB_1濃度も変化しなかったものと考えられた。

尿中へのビタミンB_1排泄量は，寒冷曝露により，ビタミンB_1十分量摂取群では約1/2にまで低下した一方で，血液中の値は通常温度飼育群と同じ値が維持されていた。ビタミンB_1十分量飼料投与群と最小必要量飼料投与群を比較すると，ビタミンB_1の摂取量は約1/5であったが，肝臓中も血液中もビタミンB_1含量は約1/3であった。このことは，ラットは低温にさらされると，すべての組織中のビタミンB_1必要量が高まり，その結果引き起こされる欠乏リスクを下げるために，ビタミンB_1の備蓄庫である肝臓からの放出を促進させる一方で，尿中への排泄量を抑制する（言い換えれば，腎臓での再吸収を高める）機構が作動することが示唆された。なお，ビタミンB_1最小必要量飼料投与群では，基本的な排泄量が低く，正確な比較をすることはできなかったが，尿中への排泄量は抑制されていた。これらの事実は，尿を用いるビタミンB_1の栄養評価をより正確にするためには，評価する対象者の主要な生活がどのような環境温度であったのかを調査要因の一つとして加えておく必要性を示している。

寒冷曝露によりエネルギー代謝の亢進が起こることがHegsted & McPhee[12]によって報告されていたが，本実験においてもこのことが確認された。その結果，ビタミン B_1 の要求量が高まり，ビタミン B_1 最小必要量飼料投与ラットでは，ビタミン B_1 十分量飼料投与群と比べて，顕著な悪影響が認められることを期待したが，今回の実験条件下では，肉眼的な外見所見では摂取ビタミン B_1 含量による差異は認められなかった。大きな差異が認められたのは，摂取ビタミン B_1 量に関係なく，通常温度飼育群と寒冷曝露群間の体重増加量の差異であった。寒冷曝露により体重増加量が約半分となった。寒冷曝露-ビタミン B_1 最小必要量飼料投与群の体重増加量と寒冷曝露-ビタミン B_1 十分量飼料投与群の体重増加量には差異は認められなかった。寒冷曝露による体重増加量の低下は，本実験条件下では，少なくとも，摂取するビタミン B_1 量とは関係がないことが明らかとなった。低温度飼育-ビタミン B_1 最小必要量飼料投与群においても，肝臓のビタミン B_1 量は，完全なビタミン B_1 欠乏ラット[15]よりも高い値であった。しかしながら，本実験条件下において，寒冷曝露により，尿中排泄量が顕著に抑制されたことから，低温環境下では，ビタミン B_1 の必要量が高くなることが示唆された。

これまでの報告において，寒冷曝露によって甲状腺の機能亢進が起こり，チロキシンの放出が著しく増加することが明らかにされている[17]。ラットにチロキシンを含む飼料を投与すると，含まない飼料投与群に比して，チロキシン投与7日後より，1.5倍量の飼料を摂取するにもかかわらず，体重増加量が約半分であったことも報告されている[18]。また，副腎髄質からアドレナリンが分泌され，肝臓や筋肉細胞の活動を高めて，熱の発生量を増加させることも知られている[19]。アドレナリンをラットに注射すると，飼料摂取量が落ちて体重増加量が低下することも報告されている[20]。さらに寒冷曝露(5℃)によって褐色脂肪組織でのUCP mRNAの発現量が増加することも報告されている[21]。

チロキシン投与の報告は，今回の実験結果と同じ現象であった。したがって，寒冷曝露による飼料摂取量の増大と体重増加量の遅延という現象は，エネルギー代謝亢進によるビタミン B_1 必要量の増大に起因するビタミン欠乏の結果ではなく，寒冷曝露によりチロキシンの放出が高まったこと，また同時に熱産生のためにUCPの発現量が増加したといった複数の要因による現象ではないかと考えられる。

本研究により，寒冷環境下においてビタミン B_1 の尿中の排泄量が顕著に低下し，ビタミン B_1 の必要量が増大することが明らかになった。しかし，寒冷曝露により尿中へのビタミン B_1 排泄量が低くなるという現象に関する機構の解明については明らかになっておらず，今後の課題としたい。

本研究は，平成19年度～21年度厚生労働科学研究費補助金循環器疾患等生活習慣病対策総合研究事業「日本人の食事摂取基準を改定するためのエビデンスの構築に関する研究―微量栄養素と多量栄養素摂取量のバランスの解明―」(主任研究者，柴田克己)の成果の一部である。関係各位に謝意を表する。

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J Jpn Soc Nutr Food Sci **64**: 329–334 (2011)

Research Data

Liver, Blood, and Urine Vitamin B_1 Content in Cold-exposed Rats Fed a Vitamin B_1-sufficient or Minimum-requirement Diet

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(Received February 1, 2011; Accepted June 29, 2011)

Summary: Energy metabolism is facilitated by exposure to cold because of thermogenesis. However, it is unclear how a low temperature environment affects vitamin B_1 requirements. Therefore, in this study, we examined how the requirement for vitamin B_1 in rats is increased upon exposure to cold. Rats housed for 14 days at 4℃ showed a 1.1-fold increase in food intake and a 0.6-fold decrease of body weight gain. Hence, the cold environment increased energy expenditure 1.7-fold. Furthermore, these rats had larger brown adipose tissue depots, while urinary excretion of vitamin B_1 was decreased 0.5-fold. These results suggest that the requirement for vitamin B_1 was increased by cold exposure. We then examined the effects of feeding cold-exposed rats a diet containing the minimum level of vitamin B_1, in terms of changes in body weight, in comparison with rats fed a diet containing a sufficient level of vitamin B_1. Urinary excretion of vitamin B_1 was lower in rats housed for 14 days at 4℃ than at 22℃. However, no marked changes in body weight were observed. These results indicate that exposure to a low-temperature environment increases energy metabolism, and that the requirement for vitamin B_1 also increases.

Key words: vitamin B_1, cold, requirement, thyroxin, energy

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資　料

ビタミン B_{12} 欠乏ラットの種々の臓器，血清，尿中のB群ビタミン含量

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Vitamins (Japan), **85** (1), 18-22 (2011)

The contents of B-group vitamins in various tissues, serum, and urine of vitamin B_{12}-deficient rats

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We measured the contents of B-group vitamins in the urine, serum, liver, and kidney of vitamin B_{12}-deficient rats. Urine and liver vitamin B_{12} contents in urine and liver were lower in the B_{12}-deficient rats than in the control rats. Contents of vitamin B_2, nicotinamide, and pantothenic acid in the urine, liver and kidney were not different between the B_{12}-deficient and control rats. On the other hand, contents of vitamin B_1, vitamin B_6, folate, and biotin in the urine, liver, and kidney were different between the two groups. Vitamin B_1 and folate contents in the urine, liver, and kidney were lower in the B_{12}-deficient rats than in the control rats. Vitamin B_6 content in the liver was almost equal between both groups, but, the content in urine was lower and that of kidney was higher in the B_{12}-deficinet rats than in the control rats. Biotin content in the urine and kidney was higher in the B_{12}-deficient rats, but the content in the liver was lower in the B_{12}-deficient rats.

Key words : B-group vitamin, vitamin B_{12} deficiency, urine, liver, kidney

(*Received November 11, 2009*)

緒　言

B群ビタミン8種類すべては，酵素の補酵素として機能しているため，体内動態が相互に影響を及ぼしているものと考えられる．相互関係がよく知られているビタミンとして，複合酵素系として研究されてきたα-ケト酸脱水素酵素系のナイアシン，ビタミン B_1，ビタミン B_2，パントテン酸がある[1]．さらに，ホモシステイン代謝に関わる葉酸，ビタミン B_6，ビタミン B_{12} がある[2]．そこで，一つのB群ビタミンを欠乏させた時に，他のB群ビタミンの体内動態がどのような影響を受けるのか否かを調べる研究を開始した．

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ビタミンの体内動態を研究するためには濃度を測定するだけでは極めて不十分であることは言うまでもないが，研究の第一段階としてそのビタミンの臓器中の濃度を知ることは何らかの手がかりをもたらすと考えられる．8種類のB群ビタミン欠乏動物のすべての結果を得るまでには長期間を要するので，今回はビタミンB_{12}欠乏動物を作成し，尿，肝臓，腎臓，脳，精巣，血清中のB群ビタミン濃度を測定した結果を報告する．なお，ビタミンの種類によって一部の臓器に関する結果が欠落しているが，これは試料量が不十分で分析ができなかったためである．

実験方法

1．動物の飼育方法

10週齢のWistar系雌ラットを交配，受精確認後，直ちに妊娠期間，授乳期間を通しビタミンB_{12}欠乏飼料(表1)を給与した親ラットから出生，離乳した雌雄ラットを実験に用いた．

離乳したラット(3週齢)は，雌雄別々の群とし，さらにビタミンB_{12}欠乏ラット作成群と対照ラット作成群とに分けた．ビタミンB_{12}欠乏ラット作成群はビタミンB_{12}欠乏飼料のみを与えた．

一方，対照ラット作成群はビタミンB_{12}欠乏ラット作

表1．ビタミンB_{12}欠乏食の栄養素組成

栄養成分	ビタミンB_{12}欠乏食(g/kg飼料)
分離大豆タンパク質[1]	180
グルコース[2]	673.5
脂溶性ビタミン[3]混合大豆油[4]	100
ミネラル混合[5]	35
水溶性ビタミン混合(ビタミンB_{12}欠)[6]	10
塩化コリン[7]	1.5

[1] アジプロンSU，粗タンパク質含量は85.4%，味の素株式会社(東京都)より購入．
[2] サンエイ糖化株式会社(愛媛県)より購入．
[3] 飼料1kg当たりの脂溶性ビタミン含量：dl-α-トコフェリルアセテート，35 mg；レチニルアセテート，4,000IU；コレカルシフェロール1,000 IU．これらのビタミンは和光純薬工業株式会社(東京都)より購入．
[4] 林化学工業株式会社(東京都)より購入．
[5] ミネラル混合1kg当たりのミネラル含量(g/kgミネラル混合)，AIN-76ミネラル混合：$CaHPO_4$, 500；NaCl, 74；$K_3C_6H_5O_7$-H_2O, 220；K_2SO_4, 52；MgO, 24；$MnCO_3$, 3.5；Fe-citrate (Fe 17%), 6；$ZnCO_3$, 1.6；$CuCO_3$-Cu $(OH)_2$-H_2O, 0.3；KIO_3, 0.01；Na_2SeO_3-$5H_2O$, 0.01；and CrK $(SO_4)_2$-$12H_2O$, 0.55．ショ糖で1kgに調製した．これらのミネラルは和光純薬工業株式会社(東京都)より購入．
[6] 水溶性ビタミン混合1kg当たりの水溶性ビタミン含量(mg/kg水溶性ビタミン混合)，AIN-76ビタミン混合：塩酸チアミン，600；リボフラビン，600；塩酸ピリドキシン，700；ニコチン酸，3,000；パントテン酸カルシウム，1,600；プテロイルモノグルタミン酸，200；ビオチン，20；メナジオン，5．ショ糖で1kgに調製した．これらのビタミンは和光純薬工業株式会社(東京都)より購入．

表2．ビタミンB_{12}欠乏食の投与が体重と組織重量におよぼす影響

	雄ラット		雌ラット	
	Pair-fed対照群	V.B_{12}欠乏群	Pair-fed対照群	V.B_{12}欠乏群
体重増加量(g)	178 ± 11 (n = 10)	131 ± 19* (n = 10)	162 ± 7 (n = 10)	129 ± 22* (n = 7)
肝臓(g)	5.78 ± 0.43 (n = 10)	7.16 ± 1.00* (n = 10)	4.55 ± 0.34 (n = 10)	7.10 ± 0.89* (n = 7)
脳(g)	1.72 ± 0.08 (n = 10)	1.56 ± 0.09 (n = 10)	1.66 ± 0.06 (n = 10)	1.60 ± 0.07 (n = 7)
腎臓(g)	1.36 ± 0.14 (n = 10)	1.95 ± 0.35* (n = 10)	1.18 ± 0.13 (n = 10)	1.83 ± 0.18* (n = 7)
精巣(g)	2.57 ± 0.11 (n = 10)	0.71 ± 0.18* (n = 10)	-	-

約3週齢の離乳したての雌雄ラットをpair-fed対照群とビタミンB_{12}欠乏群の二群に分けた．二群ともビタミンB_{12}欠乏食を投与したが，pair-fed対照群のラットには毎日1μgのシアノコバラミンを経口投与した．シアノコバラミンを投与したラットの食欲はビタミンB_{12}欠乏食のみを投与したラットよりも高いので，摂食量をビタミンB_{12}欠乏食群のラットと同じ摂取量になるようにpair-fedした．雄ラットは98－108日間，雌ラットは150－159日間飼育したのち，賭殺した．
数値は平均値±SEMで示した．
*Pair-fed対照群とビタミンB_{12}欠乏群との間でStudentのt検定を行った結果，有意差($p<0.05$)が認められたことを示す．

成群と同じ飼料を与え，かつ pair-fed としたが，実験期間中シアノコバラミン水溶液を 1 μg/rat/day となるように 50 μL 経口投与し，ビタミン B_{12} 欠乏を回避させた．なお，飼料の交換，シアノコバラミン投与は飼育期間中毎日 9 時から 12 時に行った．

なお，飼育期間は，雄ラットは 98 ～ 108 日間，雌ラットは 150 ～ 159 日である．表 2 に解剖時の体重増加量，臓器重量を記載した．

飼育最終日直前の 24 時間尿を採取した後，心臓採血により血液を採取，肝臓，腎臓，脳，精巣を摘出，分析まで -80℃で保存した．なお，本研究は動物の飼養及び保管等に関する基準（昭和 55 年 3 月，総理府告示，第 6 号），岡山大学動物実験指針，教育学部動物実験指針に従った．

2．ビタミンの測定方法

採尿方法，血液の採集，並びに各々の B 群ビタミンの測定方法は文献 3 に記載した方法を用いた．

3．統計処理

値は平均値 ± SEM で示した．二群間の値の比較は，GraphPad Software 社（Sandiego, CA, USA）の GraphPad Prism 5 を使用し，un-paired Student's t-test を行い，$p<0.05$ を有意差があるとした．

測定結果

対照ラットとビタミン B_{12} 欠乏ラットの B 群ビタミン含量を，雄ラットについては表 3 に，雌ラットについて

表 3．ビタミン B_{12} 欠乏食投与が雄ラットの尿中および肝臓，腎臓，脳中の B 群ビタミン含量におよぼす影響

	尿（nmol/day）	肝臓（nmol/g）	腎臓（nmol/g）	脳（nmol/g）
ビタミン B_1				
Pair-fed 対照群	20.6 ± 2.6 (n = 10)	19.6 ± 1.2 (n = 8)	55.6 ± 6.0 (n = 8)	6.35 ± 0.37 (n = 8)
V.B_{12} 欠乏群	5.2 ± 0.4* (n = 10)	14.8 ± 1.5* (n = 5)	26.5 ± 2.3* (n = 5)	4.70 ± 0.61 (n = 5)
ビタミン B_2				
Pair-fed 対照群	69.0 ± 7.9 (n = 10)	77.8 ± 6.2 (n = 8)	75.4 ± 6.4 (n = 8)	3.93 ± 0.21 (n = 8)
V.B_{12} 欠乏群	60.4 ± 8.8 (n = 10)	72.8 ± 4.2 (n = 5)	71.7 ± 4.0 (n = 5)	3.99 ± 0.34 (n = 5)
ビタミン B_6[1]				
Pair-fed 対照群	85.5 ± 4.3 (n = 10)	27.1 ± 0.9 (n = 10)	21.5 ± 2.4 (n = 6)	8.4 ± 0.3 (n = 8)
V.B_{12} 欠乏群	34.2 ± 2.0* (n = 10)	28.6 ± 1.2 (n = 5)	40.5 ± 2.1* (n = 4)	8.7 ± 0.2 (n = 8)
ビタミン B_{12}				
Pair-fed 対照群	13.7 ± 3.7 (n = 10)	47.4 ± 4.4 (n = 8)	no data	no data
V.B_{12} 欠乏群	0.3 ± 0.1* (n = 10)	13.0 ± 1.2* (n = 5)	no data	no data
ニコチンアミド[2]				
Pair-fed 対照群	2241 ± 164 (n = 10)	1379 ± 86 (n = 8)	1255 ± 81 (n = 8)	546 ± 20 (n = 8)
V.B_{12} 欠乏群	2918 ± 259 (n = 10)	1224 ± 132 (n = 5)	1251 ± 113 (n = 5)	517 ± 8 (n = 6)
パントテン酸				
Pair-fed 対照群	381 ± 46 (n = 10)	501 ± 5 (n = 8)	314 ± 50 (n = 5)	77 ± 12 (n = 6)
V.B_{12} 欠乏群	322 ± 32 (n = 10)	557 ± 15 (n = 5)	343 ± 28 (n = 5)	48 ± 9 (n = 4)
葉酸				
Pair-fed 対照群	2.78 ± 0.31 (n = 10)	10.0 ± 1.0 (n = 8)	4.71 ± 0.19 (n = 8)	no data
V.B_{12} 欠乏群	1.30 ± 0.08* (n = 10)	4.7 ± 0.9* (n = 5)	1.67 ± 0.33* (n = 5)	no data
ビオチン				
Pair-fed 対照群	0.97 ± 0.09 (n = 10)	1.29 ± 0.13 (n = 8)	1.08 ± 0.06 (n = 8)	no data
V.B_{12} 欠乏群	2.18 ± 0.30* (n = 10)	0.80 ± 0.21* (n = 5)	1.57 ± 0.08* (n = 5)	no data

実験条件は表 2 の下の説明に記載した．
数値は平均値 ± SEM で示した．
*Pair-fed 対照群とビタミン B_{12} 欠乏群との間で Student の t 検定を行った結果，有意差（$p<0.05$）が認められたことを示す．
[1] この表ではビタミン B_6 と表記したが，4-ピリドキシン酸の量である．4-ピリドキシン酸は尿中に排泄される主要なビタミン B_6 の異化代謝産物である．
[2] この表ではニコチンアミドと表記したが，ニコチンアミドとその異化代謝産物である N^1-メチルニコチンアミド，N^1-メチル-2-ピリドン-5-カルボキサミド，N^1-メチル-4-ピリドン-3-カルボキサミドの合計量である．

表4. ビタミン B_{12} 欠乏食投与が雌ラットの尿中および血清，肝臓，腎臓，脳中のB群ビタミン含量におよぼす影響

	尿 (nmol/day)	血清 (nmol/ml)	肝臓 (nmol/g)	腎臓 (nmol/g)	脳 (nmol/g)
ビタミン B_1					
Pair-fed 対照群	52.4 ± 4.1 (n = 10)	no data	18.0 ± 1.0 (n = 9)	45.6 ± 1.4 (n = 6)	4.98 ± 0.32 (n = 8)
V.B_{12} 欠乏群	7.0 ± 1.0* (n = 6)	no data	11.2 ± 0.8* (n = 7)	17.2 ± 1.2* (n = 7)	4.61 ± 0.61 (n = 4)
ビタミン B_2					
Pair-fed 対照群	65.0 ± 2.8 (n = 10)	no data	65.1 ± 5.5 (n = 10)	68.5 ± 1.9 (n = 6)	3.57 ± 0.04 (n = 10)
V.B_{12} 欠乏群	64.4 ± 7.1 (n = 6)	no data	67.3 ± 5.6 (n = 7)	67.9 ± 1.5 (n = 7)	3.66 ± 0.18 (n = 6)
ビタミン B_6[1]					
Pair-fed 対照群	80.5 ± 3.7 (n = 10)	1.27 ± 0.08 (n = 10)	21.4 ± 0.6 (n = 10)	20.8 ± 1.2 (n = 10)	9.1 ± 0.4 (n = 8)
V.B_{12} 欠乏群	56.1 ± 3.7* (n = 6)	1.17 ± 0.11 (n = 7)	24.1 ± 1.9 (n = 6)	40.1 ± 3.6* (n = 7)	9.3 ± 0.4 (n = 4)
ビタミン B_{12}					
Pair-fed 対照群	27.0 ± 6.1 (n = 10)	0.73 ± 0.06 (n = 10)	46.2 ± 3.7 (n = 10)	no data	no data
V.B_{12} 欠乏群	3.3 ± 1.6* (n = 6)	0.29 ± 0.01* (n = 6)	15.3 ± 0.9* (n = 6)	no data	no data
ニコチンアミド[2]					
Pair-fed 対照群	2688 ± 228 (n = 10)	no data	1284 ± 78 (n = 10)	1267 ± 20 (n = 6)	585 ± 28 (n = 8)
V.B_{12} 欠乏群	2596 ± 443 (n = 6)	no data	1286 ± 93 (n = 6)	1302 ± 48 (n = 5)	599 ± 22 (n = 6)
パントテン酸					
Pair-fed 対照群	379 ± 18 (n = 10)	no data	560 ± 25 (n = 10)	no data	no data
V.B_{12} 欠乏群	336 ± 30 (n = 6)	no data	565 ± 74 (n = 7)	no data	no data
葉酸					
Pair-fed 対照群	3.54 ± 0.24 (n = 6)	no data	12.5 ± 0.8 (n = 10)	5.23 ± 0.30 (n = 6)	no data
V.B_{12} 欠乏群	1.40 ± 0.11* (n = 13)	no data	4.3 ± 0.5* (n = 7)	2.11 ± 0.43* (n = 7)	no data
ビオチン					
Pair-fed 対照群	1.21 ± 0.07 (n = 10)	no data	1.09 ± 0.07 (n = 10)	1.32 ± 0.18 (n = 3)	no data
V.B_{12} 欠乏群	2.49 ± 0.21* (n = 6)	no data	0.64 ± 0.02* (n = 7)	1.87 ± 0.06* (n = 3)	no data

実験条件は表2の下の説明に記載した.
数値は平均値±SEMで示した.
*Pair-fed 対照群とビタミン B_{12} 欠乏群との間で Student の t 検定を行った結果，有意差 ($p<0.05$) が認められたことを示す.
[1] この表ではビタミン B_6 と表記したが，4-ピリドキシン酸の量である．4-ピリドキシン酸は尿中に排泄される主要なビタミン B_6 の異化代謝産物である.
[2] この表ではニコチンアミドと表記したが，ニコチンアミドとその異化代謝産物である N^1-メチルニコチンアミド，N^1-メチル-2-ピリドン-5-カルボキサミド，N^1-メチル-4-ピリドン-3-カルボキサミドの合計量である.

は表4に示した.

1．ビタミン B_{12} 含量

予測されたように，ビタミン B_{12} 欠乏ラットの方が対照ラットよりも有意に低い値を示した．肝臓中のビタミン B_{12} 含量の低下は1/3程度であったが，尿中の排泄量の低下はより顕著であり，特に，雄ラットでは1/45にまで低下していた.

2．ビタミン B_1 含量

雄ラットにおいても，雌ラットにおいても，ビタミン B_{12} 欠乏により，尿中，肝臓中，腎臓中のビタミン B_1 含量が有意に低値を示した．この結果は，Nishino と Itokawa[4] の報告と一致する.

3．ビタミン B_2 含量

ビタミン B_2 含量は，全くビタミン B_{12} 欠乏の影響を受けなかった.

4．ビタミン B_6 含量

ビタミン B_6 含量に関して，肝臓における含量は両群間でほとんど同じであったが，ビタミン B_{12} 欠乏ラットでは，尿における排泄量は対照ラットよりも低かったが，腎臓においては高い値を示した.

5．ニコチンアミド含量

ニコチンアミド含量は，全くビタミン B_{12} 欠乏の影響を受けなかった.

6．パントテン酸含量

パントテン酸含量は，全くビタミン B_{12} 欠乏の影響を受けなかった．

7．葉酸含量

ビタミン B_{12} 欠乏により，尿中，肝臓中，腎臓中の葉酸含量が有意に低値を示した．

8．ビオチン含量

ビタミン B_{12} 欠乏がビオチン含量におよぼす影響は複雑であった．尿中への排泄量はビタミン B_{12} 欠乏により有意に増大し，腎臓中の含量も有意に高い値を示した．ところが，肝臓中のビオチン含量はビタミン B_{12} 欠乏により低値を示した．

謝 辞

本資料は，平成 19 年度～21 年度厚生労働科学研究費補助金循環器疾患等生活習慣病対策総合研究事業「日本人の食事摂取基準を改定するためのエビデンスの構築に関する研究―微量栄養素と多量栄養素摂取量のバランスの解明―」（主任研究者，柴田克己）の成果の一部である．関係各位に謝意を表する．

（平成 21.11.13 受付）

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Osteoporosis Japan

地域在住の女性後期高齢者における血中ビタミンD濃度と転倒発生に関する縦断研究

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はじめに

一般に，加齢とともに皮膚でのビタミンD産生能は低下し[1)]，血中ビタミンD濃度は低下する。われわれの先行研究[2)]でも，65歳以上の地域在宅女性で年齢とともに血清25(OH)D濃度が低下していた。血中ビタミンDの不足は，骨量減少を助長し，骨粗鬆症の進行およびそれに伴う大腿骨頸部骨折の受傷可能性を増大させる重要な原因と考えられる。同時にわれわれの先に行った65歳以上の地域在宅高齢者2,957名の横断研究では，血清25(OH)D濃度が低い群で運動機能，特に筋力，バランス能力，および歩行速度が有意に低く，転倒と有意に関連していたと報告した[2)]。

本研究では，女性の地域在宅高齢者，中でも虚弱あるいは要介護状態に至る割合の高い75歳以上の後期高齢者を対象として，血中ビタミンD濃度として血清25(OH)D濃度を測定し，血清25(OH)D濃度と転倒発生との関連性について縦断的追跡研究を行い，両者の関連性を明らかにすることを目的とした。

1　対象と方法

1)研究対象者の選定

本研究の対象者は，東京都板橋区に在住する75歳以上の高齢女性である。

初回調査として，2008年10月～11月に実施された健診に対し，合計1,399名が受診した。さらに2009年11月に追跡郵送調査を行い，過去1年間の転倒経験を含む健康状況が把握できた1,393名を本研究の追跡対象とした。健診については高齢者に特有の老年症候群のリスク把握と予防のための健診(「お達者健診」)であり，このようなモデル的検診に関する詳細は他の多くの論文に記載されている[3)]。転倒の把握については，初回調査の健診時の聞き取り，および追跡調査時の郵送調査共に，「ここおよそ1年間に転んだことはありますか(転びそうになった，転びかけた，交通事故などはのぞきます)」という設問に対して「ある」と回答した場合を「転倒あり」とした。

なお本研究は，東京都健康長寿医療センターの倫理委員会の審査を得て実施した。2008年のベースライン健診時に，受診者に健康情報の研究への使用に関して説明し，書面にて同意署名を得た。

Serum 25-Hydroxyvitamin D Levels and Fall Risk among Japanese Community-Dwelling Elderly Women Aged 75 Years or Older

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Key words：Serum 25-hydroxyvitamin D, Falls, Elderly women

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表1　対象者の特性の推移(女性, $n=1285$)

項目	2008年健診	2009年追跡調査	p値
年齢(歳)　平均値±標準偏差(範囲)	78.6±2.8(75～90)($n=1,285$)		
年齢階級(歳)			
75～79	809名(63.0%)		
80～84	456名(35.5%)		
85～90	20名(1.6%)		
健康度自己評価(健康である)	1,074名(83.6%)($n=1,285$)	1,005名(78.5%)($n=1,280$)	$p=0.001$[1]
過去1年間の転倒あり	241名(18.8%)($n=1,285$)	312名(24.4%)($n=1,277$)	$p<0.001$[1]
転倒回数			
1回	181名(75.1%)	153名(49.0%)	$p<0.001$[1]
2回以上	60名(24.9%)	146名(46.8%)	
不明	0名(0%)	13名(4.2%)	

データ数(n)は項目により異なる。[1]：χ^2検定

表2　ベースライン健診項目からみた追跡1年間の転倒発生のリスク

ベースライン健診項目	追跡1年間の転倒者数(割合)		オッズ比[1]	95%信頼区間	p値
年齢	75～79歳($n=804$)	177(22.0%)	1.42	1.09～1.84	0.009
	80歳≦($n=473$)	135(28.5%)			
アルブミン	4.3g/dL≦($n=657$)	143(21.8%)	1.32	1.02～1.70	0.037
	4.3g/dL＞($n=614$)	168(27.4%)			
握力	19kg≦($n=626$)	123(19.6%)	1.60	1.23～2.10	0.001
	19kg＞($n=586$)	169(28.8%)			
5m通常歩行時間	3.9秒＞($n=593$)	123(20.7%)	1.37	1.05～1.79	0.019
	3.9秒≦($n=682$)	188(27.6%)			
開眼片足立ち時間	16秒≦($n=652$)	128(19.6%)	1.61	1.24～2.10	＜0.001
	16秒＞($n=621$)	182(29.3%)			
健康度自己評価	健康である($n=1,067$)	237(22.2%)	1.91	1.39～2.62	＜0.001
	健康でない($n=210$)	75(35.7%)			
過去1年間の転倒経験	なし($n=1,039$)	196(18.9%)	4.11	3.04～5.54	＜0.001
	あり($n=238$)	116(48.7%)			

[1]：年齢以外のベースライン健診項目については，Mantel-Haenszel検定により年齢(2層化)調整したオッズ比を算出。

2) 解析対象者の選定

追跡郵送調査で最終的に回答のあった1,285名(回収率92.2%)を，本研究の解析対象者とした。統計解析は，統計解析用ソフトウェアSPSS15.0を用いた。統計学的有意水準は5%($p=0.05$)とした。

2　結　　果

1) 対象者の特性の推移(表1)

対象者の年齢は，75歳以上90歳以下，平均78.6±2.8歳(平均値±標準偏差)であった。ベースライン健診(2008年)と1年後の追跡調査における対象者の特性の推移をみた結果，健康度

表3　25(OH)Dの分布とintact PTHとの関係

	年齢階級(歳)		全体	intact PTH
	75～79	80～90	(n＝	(pg/mL)[3]
	(n＝808)	(n＝471)	1,279)	(n＝1,279)
25(OH)D[1](ng/mL)	22.3±6.7	21.8±6.8	22.1±6.7	
平均値±標準偏差(範囲)	(6～81)	(6～48)	(6～81)	
三分位				
≦19(低値群)[2]：ビタミンD不足(20ng/mL未満)	271名 (33.5%)	179名 (38.0%)	450名 (35.2%)	45.5±0.8
20～24(中間値群)	264名 (32.7%)	140名 (29.7%)	404名 (31.6%)	42.0±0.8
25≦(高値群)	273名 (33.8%)	152名 (32.3%)	425名 (33.2%)	38.3±0.8*

[1]：年齢階級間のt検定にて有意な差なし($p=0.05$)。[2]：年齢階級間のχ^2検定にて有意な差なし($p=0.05$)。[3]：分散分析(年齢調整)後の平均値±標準誤差。*：p for trend＜0.001

自己評価で「健康である」と回答した割合は有意に低下していた。過去1年間の転倒経験は，18.8%から24.4%と有意に増加していた。転倒経験者のうち複数回転倒する割合は，24.9%から46.8%と有意に増加していた。

2) ベースライン健診項目からみた追跡1年間の転倒発生のリスク(表2)

ベースライン健診項目ごとに対象者を2群に分割し，追跡1年間の転倒発生のオッズ比を年齢調整して算出した。アルブミンと運動機能検査項目については，中央値で2群に分割した。追跡1年間の転倒発生リスクは，年齢80歳以上では80歳未満に比較して1.42倍，有意に高かった。アルブミン4.3g/dL未満では4.3g/dL以上に比較して1.32倍，握力19kg未満では19kg以上に比較して1.60倍，5m通常歩行時間3.9秒以上では3.9秒未満に比較して1.37倍，開眼片足立ち時間16秒未満では16秒以上に比較して1.61倍，健康度自己評価で「健康でない」と回答した群は「健康である」群に比較して1.91倍，有意に転倒発生リスクが高かった。ベースラインから過去1年間に転倒経験のある群は，ない群に比較して4.11倍，有意に転倒発生リスクが高かった。

3) 25(OH)Dの分布とintact PTHとの関係(表3)

25(OH)Dの平均値は22.1±6.7ng/mLで，二つの年齢階級間で平均値に有意な差を認めなかった。25(OH)Dの分布を三分位に分割すると，19ng/mL以下の低値群は35.2%であり，20ng/mL未満(19ng/mL以下)をビタミンD不足とすると，ビタミンD不足者の割合は35.2%であった。ビタミンD不足の割合は年齢階級別では33.5%と38.0%と高年齢層で高かったが，有意な差ではなかった。三分割した各群のintact PTHの平均値(年齢調整)は，25(OH)Dが高くなるほど有意に低くなった(p for trend＜0.001)。

4) 25(OH)D濃度の追跡1年間の転倒発生へのリスク

25(OH)Dの分布で三分位に分割し，高値群(25ng/mL以上)に対する中間値群(20～24ng/mL)，低値群(19ng/mL以下：ビタミンD不足群)の追跡1年間の転倒リスクを，多重ロジスティックモデル(年齢調整)で解析した。転倒を1回以上発生するリスクは，25(OH)Dが低くなるほど有意に高くなり，低値群(ビタミンD不足群)は高値群に対して1.56倍と有意にリスクが高かった。また転倒を2回以上発生するリス

クは，25(OH)Dが低くなるほど有意に高くなっていた。また，追跡1年間の転倒発生に関する関連要因のリスクについては多重ロジスティックモデルを用いた回帰分析を行った。その結果，血中25(OH)D濃度は他の要因を調整してもなお有意で独立した転倒の予防因子であることが明らかにされた。

考　　察

わが国では，65歳以上の在宅高齢者の1年間の転倒率は，おおむね15～20%であると言われており[4]，また女性後期高齢者の介護に至った主な原因の中で骨折・転倒によるものが約12%を占めていることからも，女性後期高齢者において転倒を予防することは極めて重要である。

最近，高齢者の転倒に関連する要因として血中ビタミンD濃度(25(OH)D)が注目されている。本研究においても，25(OH)Dの分布で三分位に分割し，高値群(25ng/mL以上)に対する中間値群(20～24ng/mL)，低値群(19ng/mL以下：ビタミンD不足群)の追跡1年間の転倒リスクを，多重ロジスティックモデル(年齢調整)で解析したところ，転倒を発生するリスクは，25(OH)Dが低くなるほどオッズ比が有意に高くなり，低値群は高値群に対して有意にリスクが高くなっていた。

高齢者の転倒の血中ビタミンD濃度との横断的ならびに縦断的研究のいずれもから，血中ビタミンD濃度が転倒の発生に関連していることは確実である。わが国でも最近，活性型ビタミンD_3製剤の投与により転倒に関連する運動能力の維持向上が認められることが(萌芽的研究ではあるが)報告されている[5]。今後さらなる規模の大きな臨床的研究が望まれる。

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Lecture

解　説

欧米の循環器疾患予防のための食事ガイドラインの現状*

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Key Words : dietary guideline, diet, lifestyle, cardiovascular disease

はじめに

昨今，さまざまな媒体を通じ，健康と栄養に関する情報が身近なものとなった結果，国民の健康意識，“健康的な食事”，“バランスのとれた食事”の重要性が認識されてきている．

生活習慣病の予防，健康の維持・増進のためにエネルギー・栄養素の摂取基準値を示したものとしては，日本では厚生労働省が5年おきに公表している「日本人の食事摂取基準2010年版(平成22年から26年まで使用)」があげられるが，諸外国においても同様なものが作成されている．しかし，通常食品を購入し食事という形で栄養を摂取する多くの消費者にとっては，エネルギー・栄養素個々の値から全体の食事バランスを考え，健康な食生活を図ることは，非常に困難である．それぞれの栄養素の働きについての十分な知識なしに，エネルギー・栄養素の基準である「日本人の食事摂取基準」を日常の食事で活用することには限界がある．

食事ガイドラインとは

食事ガイドラインは，政府，もしくは政府関連機関が，循環器疾患および特定のがんなどの生活習慣病を予防し，健康増進を図ることを目的に，理想的な栄養素組成や食品構成，食習慣のあり方を平易な文章で示したものである．対象は主として一般住民であるが，種々の疾病予防ガイドライン策定にかかわる政府関係者，ならびに健康教育を進める医療従事者にも用いられる．

なお，食事ガイドラインの多くは，生活習慣病全般を予防することを目的に，一般住民を対象としたものであり，疾患を持つ特定集団を対象とした治療ガイドライン(高血圧ガイドライン，糖尿病ガイドライン，心疾患関連ガイドライン，虚血性心疾患の一次予防ガイドラインなど)とは区別されたい．

ところで，食事ガイドラインを視覚的にわかりやすくしたものが，日本における食事バランスガイドや，欧米諸国における食品ピラミッド，食事プレートである．欧米諸国の中には，視覚媒体とした食事プレートと食事ガイドラインが一体になっているものがあるが，本稿では，食事ガイドラインを中心に概要を記述する．

欧米の食事ガイドライン

表1に，日本と欧米の食事ガイドラインを示した[1)~9)]．

疾病予防・健康増進のための実践活動では，文化，環境，政治，経済，行動学的背景の影響を多分に受けるため，その国独自の方法が求められる．しかし，生活習慣病予防および健康増進を目的とした食事ガイドラインの内容は，日

* Dietary guidelines for the prevention of cardiovascular disease in United States and Europe.

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表1　日本および欧米諸国の食事ガイドライン

		日本[1]	アメリカ[2]	イギリス[3]
機関		厚生労働省	Department of Health and Human Services, and the U.S. Department of Agriculture	Food Standard Agency
年		2000	2010	2006
項目数		10	11	5
類似点	果物	↑	↑	↑ } 少なくとも
	野菜	↑	↑	↑ } 5ポーション/日
	豆類	↑	（ナッツ，種実含）↑	○肉・魚に代わる蛋白質源として
	穀類	↑	↑全粒粉，↓精製粉	↑全粒粉
	油脂類	↓	○種類を変える	−
	飽和脂肪酸	−	↓10%	↓
	トランス脂肪酸	−	↓	−
	コレステロール	−	↓300mg/day未満	−
	魚介類	↑小魚	↑量・種類	○ } ＋卵
	肉類	−	○赤身	○ } 適正に
	乳・乳製品	↑	↑無脂肪，1％脂肪	↑低脂肪
	食塩	↓	↓	↓
	砂糖	−	↓	↓
	飲酒	−	男性2杯/日，女性1杯/日まで	
	適正体重	○	○	−
相違点		食文化 食品安全 無駄の軽減 食事のリズム 団欒，コミュニケーション 食事のバランス （主食，主菜，副菜）	食品安全 食事バランス（食事パターン，カロリーバランス） 運動	
特記事項				視覚媒体：食事プレートと一体

↑：摂取増加，↓：摂取減少，○：表示有

本および欧米諸国でそれほど大きな差がないのが特徴である．

〔類似している推奨項目〕

1．野菜，果物摂取を増加させる

野菜，果物摂取を推奨する理由として，アメリカのガイドライン[2]では3つあげている．

①葉酸，マグネシウム，カリウム，食物繊維，ビタミンA・C・Kの主要な供給源である．

②野菜・果物摂取は，種々の生活習慣病リスクの減少と関連している．特に，1日少なくとも2.5カップ以上の野菜・果物摂取をしている人々では，循環器疾患のリスクを下げることが報告されている．

③多くの野菜・果物は，低カロリーである．高カロリーの食品を食べる代わりに野菜・果物をとることで，適正体重の維持につながる．

同様の記述は，ほかの国でも認められる[4)8)]．

表記については，a)イギリス，フランス，ドイツ，デンマークなどのように，具体的な摂取量を示している国，b)調理方法を示している国[5)9)]，とそれぞれ自国の食文化や摂取量に応じた推奨を行っている．

2．穀類摂取の増加

米を主食とする日本と異なり，欧米諸国はパン，パスタなど小麦粉を主食とすることから，精製された粉ではなく，栄養価の高い全粒粉を

表1

フランス[4]	ドイツ[5]	イタリア[6]	デンマーク[7][8]	スウェーデン[9]
Agence française de sécurité sanitaire des aliments	German Nutrition Society	Instituto Nazionale di Ricerca per gli Alimentie la Nutrizione	The Danish Veterinary and Food Administration	LIVSMEDELS VERKET
2001	2008	2003	2008	1991
7	10	10	8	7
↑ 5ポーション/日以上	↑ 5ポーション/日以上，できるだけ生で，調理は短く，ジュースは1杯まで	↑	↑ 6ポーション/日以上	↑ 季節の物を，できるだけ生で
↑ ＋芋類	−	−	−	↑肉・魚の代わりに
↑ 全粒粉	↑全粒粉＋芋類	↑	↑＋芋類，毎日	↑全粒粉
↓全体的に	↓60～80g/日	○量・質を気をつける	↓	↓低脂肪のものを
−	↓（↑植物由来）	−	−	−
−（↑植物由来 ↓動物由来）	−	−	−	−
−	−	−	−	−
↑ 少なくとも2回/週	○ 1～2回/週	−	○ 数回/週	↑
○ ＋卵類	○ 赤身，300～600g/週	−	○ 赤身	○ 赤身
○3杯/日	○ 毎日	−	○ 低脂肪	○ 低脂肪
↓ヨウ素入り食塩の使用	↓	↓	−	−
−	↓種類を変える	○ 適正に	↓	−
○ワイン：男性3グラス/日，女性2グラス/日	○ ときどき	○ 適正に	−	−
−	○	○	○	−
菓子・嗜好飲料↓ 運動：少なくとも30分/日 水分↑（嗜好飲料は除く）	食事を楽しむ 食事バランス（高栄養，低エネルギー） 調理方法 食品安全 水分↑1.5*l*/日	菓子・嗜好飲料：適正に 食品安全 種類を多く 運動↑	菓子↓ 運動：30分/日 水分↑	
				視覚媒体：食事サークル，および食事プレートと一体

摂取することが勧められている[2)～5)9)]．

全粒粉とは，小麦の外皮に含まれるすべて―ふすま，胚芽，胚乳―を含んだもので，鉄，マグネシウム，セレン，ビタミンB群，食物繊維を豊富に含んでいる．全粒粉摂取を勧める根拠として，アメリカのガイドラインでは，全粒粉を摂取している人々では循環器疾患のリスクが減ること，適正な体重の維持につながっていることをあげている[2)]．そのため，全穀類摂取の少なくとも半分以上を全粒粉にするよう推奨している．

3．油脂類摂取を減らす

全体としては摂取を控えることが勧められている．なかでも，「動物由来の油脂に代えて植物由来の油脂摂取を勧める」など，具体的にどの種類の油脂を摂取すべきかを示している国も散見される[2)5)6)]．

油脂類は，エネルギー，必須脂肪酸を供給するほか，脂溶性ビタミンの吸収を助ける働きがある．脂肪酸は，飽和脂肪酸，一価不飽和脂肪酸，多価不飽和脂肪酸に分類される．油脂はこれら異なった脂肪酸が混ざったものである．アメリカのガイドラインでは，飽和脂肪酸をエネルギーの10％未満に抑え，代わりに循環器疾患リスクを下げる一価不飽和脂肪酸，多価不飽和脂肪酸を使用するよう推奨している[2)]．

ところで，近年問題となっているトランス脂肪酸は不飽和脂肪酸であるが，自然食品に含まれる通常の不飽和脂肪酸と構造的に異なり，人工的なトランス脂肪酸は循環器疾患のリスクを増加させることが知られている．

4．蛋白質源

蛋白質は，魚介類，肉類，卵類，豆類，種実類などに含まれる．これら食品群は蛋白質のほか，ビタミンB群(例；ナイアシン，ビタミンB_1，ビタミンB_2，ビタミンB_6)の供給源としても重要である．その他，蛋白質は乳・乳製品などにも含まれる．

全体として，魚介類，豆類からの摂取を増加させ，肉類から摂取する際は脂肪の少ない赤身の肉や鶏肉などの食品を選択することを勧めている．特に魚介類の摂取に関しては，週あたりの推奨量を示している国もある[2)4)5)8)]．具体例として，アメリカのガイドラインでは週あたり8オンス以上，蛋白質全摂取量では20％以上を魚介から摂取することを勧めている[2)]．

5．食塩摂取を減らす

食塩，ナトリウムは人体に不可欠な栄養素であるが，その必要量は非常に少量であり，平均的には多く取りすぎていることは知られている．適切な食塩量の摂取は血圧を適正に保ち，循環器疾患のリスクを下げる．

6．乳・乳製品の摂取を増加させる

乳・乳製品はカルシウム，ビタミンD，カリウムの供給源として重要である．特に小児期においては，乳・乳製品の摂取が骨と関連することがいわれている．また，成人においては，乳・乳製品の摂取が循環器疾患，糖尿病のリスクを下げることが報告されている．

世界的に日常的に摂取されている豆乳を含む乳・乳製品は，推奨される量より少ないことがいわれており，摂取の増加が求められる．しかしその一方で，乳・乳製品は高脂肪でもあるため，低脂肪や無脂肪の乳・乳製品を摂取することが推奨される[2)3)7)9)]．

7．適正な飲酒

また，食事以外の項目として，体重を定期的に測定し，適正体重を維持することの重要性が述べられている．

異なる推奨項目としては，日本やドイツなどのように，食事バランスや調理方法，食事を楽しむ団欒の重要性といった食事にかかわる文化的背景について言及している国がある一方[1)5)]，健康の維持に重きを置き，水摂取の増加や運動を推奨している国が認められた．具体的にどのような表記がなされているかについては，それぞれの出典を参照されたい．

参考までに，2011年1月31日アメリカが最新のDietary Guidelines for Americans, 2010を公表したので紹介する(**表2**)．

Dietary Guidelines for Americansは，Department of Health and Human Services(HHS)ならびにthe U.S. Department of Agriculture(USDA)より1980年に初版が発表されて以降，5年ごとに改定されている．

Dietary Guidelines for Americans, 2010では，国民がトータルで健康的な食事を選択することができるよう3つの目標を軸に，一般住民に対しては23の提言，妊産婦や50歳以上の高齢者など特段の注意が必要な対象については6つの追加提言がなされている．

循環器疾患に特化した食事ガイドライン

ところで，諸外国の中には，一般住民を対象とした食事ガイドラインとは別に，循環器疾患ガイドラインの一部として，食事に関する特記があるものが認められる．

アメリカ心臓協会の食事，生活習慣のためのガイドライン〔The American Heart Association (AHA) 2006 Diet and Lifestyle Recommendation〕

2006年，AHAは，アメリカにおいて死亡，障害の主要な要因である循環器疾患を予防するためのAHA 2006 Diet and Lifestyle Recommendationを報告した(**表3**)[10)]．

本策定の初回は2000年であるが，2006年ではいくつかの改訂が行われた：

1)食事は健康的な生活習慣の一部という認識から，生活習慣をタイトルに加えた

2)2000年のガイドライン策定以降，明らかとなった新たな科学的エビデンスを追加した

表2 Dietary Guidelines for Americans, 2010[2)]

＜目標＞
1. 体重を維持するために食事と運動のバランスをとる
2. 果物，野菜，全粒穀物，無脂肪・低脂肪の乳製品，および魚介類の積極的摂取に努める
3. ナトリウム(食塩)，飽和脂肪酸，トランス脂肪酸，コレステロール，砂糖追加，精製された穀物の摂取を控える

＜具体的な推奨項目＞
(体重維持)
・食習慣と運動習慣の改善により過体重および肥満を防ぐ/減らす
・体重を維持するために総カロリー摂取量を調整する．特に過体重・肥満の人々については，食事，飲料からのカロリー摂取を控える
・運動量を増加し，デスクワーク中心の活動を減らす
・それぞれのライフステージにおける適切なカロリーバランスを維持する—小児期，青年期，成人期，妊娠中および授乳中，高齢期
(摂取量の増加が望まれる食品・栄養素)
それぞれのカロリー摂取量に応じた健康的な食事パターンの一部として以下の推奨を実施することが望ましい
・野菜，果物摂取の増加
・さまざまな種類の野菜，特に葉物，赤色やオレンジ色の野菜，豆類，種実類の摂取
・総穀物摂取のうち，少なくとも半分は全粒穀物を摂取する．精製された穀物の代わりに全粒穀物の摂取を増加させる
・無脂肪・低脂肪牛乳および乳製品(牛乳，ヨーグルト，チーズ)や大豆強化製品の摂取増加
・さまざまな食品から蛋白質源を摂取—魚介類，脂肪の少ない肉および鶏肉，卵，豆・種実類，大豆製品，無塩の豆・種類
・肉，鶏肉の代わりに，魚介類を選択する．魚介類摂取の量と種類を増加させる
・飽和脂肪やカロリーの低い食品，油の種類を変えることで蛋白質源となる食品の質を変える
・可能な限り飽和脂肪の代わりに油を使う
・カリウム，食物繊維，カルシウム，ビタミンDを供給してくれる食品—野菜，果物，全粒穀物，牛乳および乳製品—を選択する
(摂取量の減少が望まれる食品・栄養素)
・ナトリウムの摂取を2,300mg未満，51歳以上の高齢者やアフリカ系アメリカ人もしくは高血圧・糖尿病，腎臓病を持つ全年齢のものについては1,500mg未満に努める．ナトリウム摂取量1,500mgは子どもや多くの成人を含むアメリカ国民の半分に推奨される
・一価不飽和脂肪酸や多価不飽和脂肪酸を使用することにより，飽和脂肪酸の摂取を10％減少させる
・食事由来コレステロールを300mg/day未満に抑える
・トランス脂肪酸の摂取を，可能な限り低くする
・固形油脂，砂糖の追加によるカロリー消費を抑える
・精製された穀物，特に固形油脂，加糖，ナトリウムの含まれた精製穀物の摂取を制限する
・もし飲酒をする場合は女性なら1杯/日，男性なら2杯/日までとし，適度な摂取に努める
(健康的な食事パターンの形成)
・長期にわたって適切なカロリーレベルを維持できる食事パターンを選択する
・あらゆる飲食の摂取を考慮に入れ，総合的に健康的な食事パターンを満たすための方法を検討する
・料理および食事の際には食品安全の勧告に従う

3)2006年の策定では，よりわかりやすい情報提供を心がけた

4)ガイドラインでは，循環器疾患を予防するための環境に関する項目を加えた

5)積極的に食事や生活習慣の変容を行うための実践的な手引きとした

6)自宅および外出先で食事する際，ガイドラインに従う重要性を強調した

7)人々がガイドラインを順守するために，医療従事者，レストランや，食品業界，学校，地域の果たす役割の重要性を強調した．

2006年版食事・生活習慣のためのガイドラインは，2歳以上の一般住民が対象である．なお，成長期の子供については，別項のAHA食事ガイドラインにも記載しているので参照されたい．

方向性としては先述の欧米の食事ガイドラインと類似しているが，食事だけでなく生活習慣も含めた包括的な取り組みが必要なことを示し

表3　AHA 2006 Diet and Lifestyle Recommendation[10)]

＜目標＞
1. 健康的な食事
2. 健康的な体重を目標とする
3. 推奨されるレベルの，LDLコレステロール，HDLコレステロール，中性脂肪の維持
4. 適切な血圧の維持
5. 適切な血糖値の維持
6. 積極的な運動
7. 能動・受動喫煙を避ける

＜具体的な推奨項目＞
- 健康で適正な体重を維持するために，カロリー摂取と運動のバランスをとる
- 野菜・果物の積極的摂取
- 全粒粉，高食物繊維の食品を選択する
- 魚介類の摂取―特に油の多い魚介類を少なくとも週に2回以上
- 飽和脂肪酸を，全摂取カロリーの7％未満，トランス脂肪酸は1％未満，コレステロールは300mg未満に抑える
  ―脂肪の少ない肉の摂取や，代わりに野菜を選択する
  ―無脂肪(スキムミルク)，1％，低脂肪の乳・乳製品を選択する
  ―一部水素化された油脂類の摂取を最小限にする
- 砂糖の添加された嗜好飲料，食品の摂取を最小限にする
- きわめて少ないか，無塩の食品(できあいの食品)を選択する
- もし飲酒をするなら適量に
- 外食するときには，本AHA Diet and Lifestyle Recommendationに従う

＜推奨項目を実施するための具体的なヒント＞
(生活習慣)
- 健康的な体重を達成・維持するために，必要なカロリーを知ろう
- あなたが普段食べている食べ物，飲み物に含まれるカロリーを知ろう
- 体重，運動量，カロリー摂取量を記録しよう
- 少なめのポーション(食事量)で準備し，食べよう
- 可能なら，スクリーンを見る時間を記録し，減らそう(例；テレビを見る，ネットサーフィンをする，コンピューターゲームをする)
- 普段の生活の中に身体的動きのあることを取り入れよう
- 喫煙や，たばこ製品の使用をやめよう
- 飲酒するなら適量にしよう(男性なら1日あたり2杯，女性なら1杯にとどめる)

(食品選択および準備)
- 食品を購入する際は，食品表示や原材料名の欄を活用しよう
- 高カロリーのソース，塩や砂糖の添加されていない，新鮮，冷凍，缶詰の野菜や果物を食べよう
- 高カロリーの食品の代わりに野菜と果物を食べよう
- 豆類，全粒穀物，果物，野菜などから，食物繊維摂取を増加させよう
- 固形油脂の代わりに液体の植物由来の油脂を使用しよう
- 砂糖の添加された嗜好飲料や食品摂取を制限しよう．通常の，砂糖添加の形態としては，ショ糖，ブドウ糖，果糖，麦芽糖，デキストリン，コーンシロップ，濃縮果汁，はちみつがある
- 練り菓子や，高カロリーの菓子パンを減らそう(例；マフィン，ドーナッツ)
- 無脂肪や低脂肪の乳・乳製品を選択しよう
- 以下の方法で食塩の摂取を減らそう
  ―類似品の食塩量を比較し，食塩の少ない方を選択しよう(例；異なったブランドのトマトソース)
  ―食塩量の少ないシリアルやパンといった加工品を選ぼう
  ―調味料の使用を制限
- 赤身の肉を使用したり，食べるときに皮を除こう
- 飽和脂肪やナトリウムの多い加工肉は食べる量を制限しよう
- 魚，肉を食べるときは，(鉄板で)焼く，(オーブンで)焼く，ゆでるといった調理をしよう
- 自分のレシピに植物ベースの肉(大豆など)を代用しよう
- ジュースの代わりに野菜，果物そのものを利用しよう

た点，栄養・食品摂取と循環器疾患リスクに関する先行研究から，エビデンスがあり，特に重視してほしい項目を示した点，具体的な行動としてどのようなことを実践・選択したらいいのかを示した点が特徴的である．

〔具体的な推奨項目〕

1．健康で適正な体重を維持するために，カロリー摂取と運動のバランスをとる

体重増加を避けるために，カロリーコントロール，すなわちエネルギー摂取量と消費量が一致することが求められる．このコントロールのためには，自身が自分の普段摂っている食べ物，飲み物に含まれるカロリーに関心を持つことが肝要である．

一方，運動習慣に関しては，体重にかかわらずすべての人々において，循環器疾患のリスクを下げる点で勧められる．

AHAでは，すべての成人に対しほぼ毎日合計して30分以上の運動を推奨している．また，体重を減らしたい人々に関しては，少なくとも60分以上の運動が勧められる．

これらを達成するためには，テレビやパソコンなどのスクリーン画面を長時間見るといった座位の活動を減らす，日々の生活の中でできるだけ動く(例；エレベーターの代わりに階段を利用)，といった生活習慣の変容が求められる．

2．野菜・果物の積極的摂取

さまざまな種類の野菜・果物摂取が勧められる．特に，色の濃い野菜・果物(例；ホウレンソウ，ニンジン，モモ，ベリー)は，ほかの野菜・果物よりビタミン，ミネラルなどの栄養素が多く含まれていることから，より摂取を勧める．

フルーツジュースは食物繊維が豊富な果物の代わりにはならず，勧められない．

3．全粒粉，高食物繊維の食品を選択する

先行研究から，全粒粉や食物繊維の多い食事パターンは食事の質を上げ，循環器疾患のリスクを下げることが明らかとなっている．食物繊維は満腹中枢を刺激し，結果としてカロリー摂取を抑えることにつながる．

AHAでは，少なくとも穀類摂取の半分を全粒粉からの摂取にするよう勧める．

4．魚介類の摂取―特に油の多い魚介類を，少なくとも週に2回以上

魚，特に油の多い魚は，オメガ3を多く含む多価不飽和脂肪酸，なかでもeicosapentaenoic acid(EPA)，docosahaxaenoic acid(DHA)を多く含む．EPA，DHA含有量の高い魚類を週2回以上食べる人々は，突然死，ならびに冠状動脈疾患による死亡リスクが減少することが報告されている．

5．飽和脂肪酸を全摂取カロリーの7％未満，トランス脂肪酸は1％未満，コレステロールは300mg未満に抑える

飽和脂肪酸，トランス脂肪酸，コレステロールの低い食事はLDLコレステロール値を下げることから，結果として循環器疾患リスクを下げることが知られている．

飽和脂肪酸，コレステロールを下げるためには，同じ動物由来油脂でも，不飽和脂肪酸の多いものに代える，低脂肪の食品にするといった方法がある．なかでも，肉の代わりに代替品(大豆)や，魚類を使用することは，一つの戦略と考えられる．

6．砂糖の添加された嗜好飲料，食品の摂取を最小限にする

砂糖添加を減らすことの主要な理由としては，砂糖添加を減らすことでカロリー摂取が抑えられ，栄養の適正化を促進することにつながることがあげられる．

7．きわめて少ないか，無塩の食品(できあいの食品)を選択する

食塩はさまざまな食品に含まれるため，ナトリウム1.5g/日未満とすることはかなり困難である．暫定的な目標として，2.3g/日未満とすることを推奨する．

8．もし飲酒をするなら適量に

適量の飲酒が循環器疾患のリスクを下げることが種々の対象集団において検討されている．

9．外食するときには本AHA Diet and Lifestyle Recommendationに従う

健康的な食事を達成するためには，外食時においても賢い選択をすることが求められる．

結　語

以上，欧米における食事ガイドラインをみてきた．食文化，食習慣が異なるにもかかわらず，生活習慣病予防，健康増進のために推奨される項目は種々の国で類似していた．近年では科学的エビデンスの増加に伴い，具体的な数値や種類まで言及している国もみられた．“健康的な食事”，“バランスのとれた食事”といった人々の行動変容を促す一助として，食事ガイドラインの使用は有用であると考えられる．

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研究・事例報告

諸外国における栄養士養成のための臨地・校外実習の現状に関する調査研究

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Study on Supervised Professional Practice for Training of Dietitians in Foreign Countries

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要旨：国際栄養士連盟（International Confederation of Dietetic Associations, ICDA）が示す栄養士養成教育の最低必須条件は、①学士号（大学卒業）と②監督下での専門的な実習500時間である。本研究では、この国際的スタンダードの達成状況を明らかにすることを目的として、諸外国の栄養士養成における臨地・校外実習制度を調査した。

各国を代表する栄養士にかかわる職能団体および関連の政府機関から出されている、通知文書、報告書、ホームページ等から、栄養士の免許取得前に実施する臨地・校外実習に関する情報を収集した。詳細に関しては、各国の担当者に直接連絡することにより具体的な内容を把握した。日本は管理栄養士の臨地・校外実習を調査対象とした。

調査した21カ国では、日本とノルウェーを除くほとんどの国で国際的スタンダードの500時間を超える臨地・校外実習を実施していた。実施時期は国によって異なり、日本と同じく養成課程在学中に実施するタイプと、養成課程卒業後にインターンシップとして実施するタイプが混在していた。臨地・校外実習の科目は、臨床栄養、食事療法を中心に学ぶ国が多かった。一方、人口10万人対の栄養士数は日本が最も多く、活躍する職域の数も最も多かった。

本研究の結果から、わが国の管理栄養士養成制度の弱点および利点が浮き彫りとなった。わが国は、管理栄養士の人数および職域の数が多く、国民の身近に活躍しているという利点を生かしながら、臨地・校外実習の時間数を拡大し、専門家としての資質を向上させる独自の対策が求められる。

キーワード：栄養士、制度、臨地・校外実習、教育

I 緒言

わが国の制度は、栄養士と管理栄養士の2種類の資格が存在する点、栄養士から管理栄養士に比較的容易に移行できる点において他国に例がなく、世界的に見てもユニークである。しかしながら、わが国

平成20年度政策課題の報告
受理日：平成22年4月1日、採択日：平成23年3月11日
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の管理栄養士・栄養士を取り巻く国内外の状況は変化しており、特定保健指導等の新たなニーズの増大や、国際的な考え方との整合性への対応がますます必要となってきている。国際栄養士連盟（International Confederation of Dietetic Associations, ICDA）では、国際的な栄養士の定義、および栄養士教育の最低必須条件を国際的スタンダードとして示している[a)]。栄養士の定義として、「栄養士とは、栄養と食事療法の分野で、国が承認した資格を有するものをいう。栄養士は、栄養に関する科学的知識を、集団および個人を対象とした、健康と疾病に関する教育や食事療法に応用するものである」が示され、栄養士は栄養科学のトランスレーターであるとされている[a,1)]。また、栄養士養成の最低必須条件として、①学士号（大学卒業）、②監督下での専門的な実習500時間を示している。この内容は、2008年にわが国で初めて開催された第15回国際栄養士会議（International Congress of Dietetics, ICD2008）においても確認されている[1)]。

本研究では、諸外国の栄養士養成制度における国際的スタンダードの達成状況について、現状を調査した。諸外国においては、栄養士制度のみを有する国が一般的であるため、栄養士制度を調査対象とした。わが国においては管理栄養士と栄養士の2種類の制度が存在するが、管理栄養士が国際的スタンダードの役割を担うべきであることから、わが国の調査対象は管理栄養士制度とし、諸外国の臨地・校外実習制度との比較検討を行った。ICDAの国際的スタンダードでは、実習をsupervised professional practiceとしており、必ずしも臨地・校外実習に限定して明言しているわけではない。しかしながら、国際的一般的認識として、supervised professional practiceは栄養士免許を取得する前に実施する臨地・校外実習と解釈されている国が多いことから、本研究においては栄養士免許（日本においては管理栄養士）を取得する前に実施する臨地・校外実習に焦点を当て、調査研究を行った。

須永らは、厚生労働科学研究費研究班として諸外国の栄養士養成制度に関して報告[2,3)]しているが、わが国におけるこの分野での研究的な側面からの系統的な検討はわずかであり[2-4)]、諸外国の臨地・校外実習に関する情報は少ないのが現状である。また、諸外国においても、さまざまな検討がなされていると思われるが、政策的・制度的背景が大きく異なり、教育制度も各国独自の制度を有するため、その結果をそのままわが国に適用することはできない。

したがって、本研究では、わが国の管理栄養士制度を考える上できわめて重要な課題として、諸外国における栄養士養成のための臨地・校外実習の時間数、具体的な制度やその内容、育成された人材の就職先を対象に調査研究を行った。

Ⅱ 方 法

ICDAの報告書[5)]より、栄養士制度を有する国を抽出した。2002年にICDAは、37カ国の栄養士会を対象に国際的な栄養士養成制度に関するアンケート調査を実施している。回答の得られた31カ国（オーストラリア、オーストリア、ベルギー、カナダ、チリ、キプロス、デンマーク、フィンランド、フランス、ドイツ、ギリシャ、ハンガリー、アイスランド、インド、アイルランド、イスラエル、イタリア、日本、ルクセンブルク、オランダ、ニュージーランド、ノルウェー、南アフリカ、スペイン、スーダン、スウェーデン、スイス、トリニダード・トバゴ、トルコ、イギリス、アメリカ）のうち、栄養士養成にかかわる独自の教育制度を持っていない国は3カ国で、キプロス、アイスランド、ルクセンブルクであった[5)]。独自の栄養士養成制度を有する国々の中から、栄養士免許を取得する前に実施する臨地・校外実習の時間数に関して論文、公の報告書などのデータが公表されている国を21カ国抽出し、養成課程の年数、臨地・校外実習の時間数を調査した。人口10万人対の栄養士数については、ICDAが2008年に実施した調査報告書も参照した[6)]。さらに、比較的信頼度の高いデータを有する11カ国については、臨地・校外実習の科目、方法、職域などに関するより詳細な内容を調査した。

日本では、栄養士と管理栄養士の2種類の資格が存在するが、本研究では国際的スタンダードとの比較という観点から、その責務を果たすべき管理栄養士を調査対象とした。また、アメリカには登録栄養士（registered dietitian, RD）と、RDの補助的な

仕事を行うテクニシャンである dietetic technician, registered（DTR）が存在するが、栄養士として認知されている RD を調査対象とした。また、栄養士免許を取得する前に実施する臨地・校外実習の範囲は、栄養士養成課程において実施される supervised professional practice、practical training、supervised practice、professional practice、practicum program、practice placement 等と表記されている実習とした。また、養成課程卒業後に実施される上記トレーニングおよびインターンシップであっても、栄養士免許取得前に実施する場合には調査対象とし、臨地・校外実習として取り扱った。一方、栄養士免許取得後に栄養士に対して実施する実習や教育トレーニング、卒後研修は調査対象から除外した。臨地・校外実習の最低必須時間数に関する情報が得られない国に関しては、養成課程において一般的に実施されている臨地・校外実習の時間数やカリキュラムの単位から換算した臨地・校外実習に相当する時間数を示した。

調査方法は、栄養士にかかわる代表的な職能団体、政府機関の栄養に関連する部署および栄養・食事に関連する学術団体等から出されている、論文、通知文書、各種報告書、およびインターネットのホームページ等を対象とし、当該各国において栄養士免許を取得する前に実施する臨地・校外実習制度の情報を収集した。詳細が不明な国に関しては、各国の栄養士にかかわる代表的な職能団体の担当者、政府機関の関連部署の担当官に電子メールおよび国際電話等により直接連絡することにより詳細かつ具体的な実態を把握し、2名以上から同様の情報が得られた場合のみ結果として採用した。また、事例を収集する目的で、栄養士を養成する施設（大学、大学院など）の教員、担当者に電子メールおよび国際電話等により直接連絡をし、各校での取り組み、プログラム内容に関する実態調査を行った。収集したデータについてわが国の管理栄養士養成制度と比較検討を行った。

Ⅲ 結 果

◆◆1 諸外国の栄養士制度の現状

調査した21カ国のうち、日本のように栄養士と管理栄養士という2種類の制度で栄養士を分類している国はなかった。アメリカでは、アメリカ栄養士会（American Dietetic Association, ADA）が認定する RD を栄養士として位置づけ、RD の補助的な仕事を行うテクニシャンとして DTR を認定し、それぞれの仕事内容を明確にしていた。また、欧州を中心とした数カ国においては、専門性により栄養士を分類していた。卒業後に専門性を分けるのではなく、栄養士養成課程の段階から専門的な教育を受ける制度であり、卒業した栄養士には特有の名称を与えていた。administrative dietitian は、給食管理を専門として学んだ学生に与えられ、clinical dietitian は、治療や疾病予防のための臨床栄養や食事療法に関して重点的に学んだ学生に与えられていた。これらの国々においては、日本のように栄養学全般を広く学ぶプログラムもあり、このような一般的な課程を卒業した栄養士を general dietitian としていた。

◆◆2 諸外国の栄養士数

人口10万人対の栄養士数を表1に示した。日本の管理栄養士数は、世界で最も多かった[6]。さらに、栄養士の数を加えれば日本の管理栄養士・栄養士総数は世界でも類を見ない最高レベルであった。

◆◆3 諸外国の栄養士養成課程年数、必須学位

調査対象とした21カ国における栄養士養成課程の年数、必須学位を表1に示した。養成課程の年数は、教育制度を背景に各国異なっていた。学位に関しては、国際的スタンダードである学士号を必須学位としている国が多かったが、日本をはじめフランス、ドイツ、スペイン、スイスでは、学士号が必須ではなかった。ノルウェーには、主に臨床に携わる clinical dietitian と給食業務に携わる administrative dietitian の2種類が存在し、clinical dietitian は5年間の教育を受けた学士であるのに対し、administrative dietitian は2年間で取得でき、学士号も必須ではなかった。アメリカ、カナダ、オーストラリア、イギリスでは大学院においても栄養士の養成を実施していた。当該大学院は、研究活動ではなく専門家養成のための大学院であり、専門教育とインターンシップを学び、修士号を取得できる。大学で栄養学以外の学問を学んだ幅広い能力を持つ栄養

表1　各国栄養士の養成課程年数、必須学位、臨地・校外実習時間数、人口10万人対の栄養士数

	日本(管理栄養士)	アメリカ	カナダ	オーストラリア	イギリス	フランス	ドイツ
養成課程の年数	4年間*2	4年間[b]（大学院でも取得可）	4年間[d]（大学院でも取得可）	4年間[7, c]（大学院でも取得可）	4年間[9, 10, e]（大学院でも取得可）	2年間（BTSおよびDUT）[10]	3年間[10, g]
必須学位	学士以外	学士(大学院でも取得可)	学士(大学院でも取得可)	学士(大学院でも取得可)	学士(大学院でも取得可)	学士以外	学士以外
臨地・校外実習の時間数*1	4週間程度（最低4単位）*2 最低180時間	約24-96週間[b] 最低1,200時間*3	約40週間[d] 約1,600時間[d]	最低20週間[7, c] 約800時間	28週間[8, 9, e] 約1,040時間[10]	BTS：20週間[17, f] DUT：15週間[16, f] 約1,015-1,305時間[10]	約39週間[10] 1,400時間[g]
人口10万人対の栄養士数	56人[6]	16-20人[6]	21-25人[6]	16-20人[6]	6-10人[6, 10]	6-10人[6, 10]	—

	イタリア	アイルランド	スウェーデン	オーストリア	ベルギー	デンマーク	フィンランド
養成課程の年数	3年間[10]	4.5年間[10]	3-4年間[10, i]	3年間[10]	3年間[10]	3.5年間[10]	5年間[10]
必須学位	学士	学士	学士	学士	学士	学士	学士
臨地・校外実習の時間数*1	50週間[10]	約34週間[10] 1,210-1,430時間[10]	約10-約13週間[10] 約440-520時間[10]	約67週間[10] >3,315時間[10]	約18週間[10] 約990-1,210時間[10]	約17週間[10] 660-780時間[10]	約24週間[10] >845時間[10]
人口10万人対の栄養士数	2-5人[5]	16-20人[6]	clin.：6-10人 adm.：21-25人[5, 10]	11-15人[6, 10]	—	>25人[6] clin.：6-10人[5, 10] adm.：>25人[5, 10]	>1-<2人[5]

	ギリシャ	ハンガリー	オランダ	ノルウェー	スペイン	トルコ	スイス
養成課程の年数	4年間[10]	4年間[10]	4年間[10]	clin.(学士)：5年間 adm.(学士以外)：2年間[10]	学士：3年間 学士以外：2年間[10]	4年間[10]	3年間[10]
必須学位	学士	学士	学士	学士：clin. 学士以外：adm.	学士以外	学士	学士以外
臨地・校外実習の時間数*1	約38週間[10] >910時間[10]	約25週間[10] 約665-855時間[10]	約30週間[10] 約1,045-1,235時間[10]	clin.：約3週間[10] adm.：0週間[10] clin.：約83-98時間[10] adm.：0時間[10]	学士：約12週間[10] 学士以外：約10週間[10] 学士：約455-585時間[10] 学士以外：<490時間[10]	約34週間[10] <1,015時間[10]	約69週間[10] 約2,750-3,250時間[10]
人口10万人対の栄養士数	2-5人[5]	6-10人[6]	16-20人[6, 10]	clin.：2-5人 adm.：11-15人[5]	2-5人[6]	2-5人[6]	11-15人[6, 10]

BTS：中級技術者養成課程食事療法学[2]。DUT：技術短期大学部生物工学食事療法学選択課程[2]。clin.：clinical dietitian。adm.：administrative dietitian

*1 必須時間数または各種調査データ等からの概算値。*2 栄養士法施行規則（昭和23年1月16日厚生省令第2号、最終改正：平成19年12月25日厚生労働省令第152号）。*3 2009年3月より900時間から引き上げ[b]、調査時（2010年3月）は移行期間。

士を育成でき、より高い専門性を学べる意味で栄養士の資質向上に寄与していると思われた[3]。

◆◆4　諸外国の臨地・校外実習の時間数

21カ国中、多くの国はICDAによる国際的スタンダードの500時間を超える臨地・校外実習を実施していた（表1）。アメリカは臨地・校外実習の必須時間として900時間を設定していたが、2009年3月より新制度となり1,200時間に引き上げ、調査時（2010年3月）は移行期間であった。しかしながら、新制度に移行する以前から、アメリカでは実際には1,000時間を超えるプログラムが大部分であった[b]。また、オーストラリアは最低20週間を必須条件としており、これを1日8時間、5日/週で計算すると800時間に相当した[7, c]。アメリカ、オーストラリア以外の国において最低必須時間に関する情報は得られなかった。そのため、一般的に実施されている臨地・校外実習時間や養成課程カリキュラムから換算した臨地・校外実習時間数など公表されている時間数を示した。カナダ[d]では、約40週間の臨地・校外実習を実施しており、時間数に換算すると1,600時間に相当した。また、イギリスは、28週間の臨地・校外実習を実施しており[8, 9, e]、時間数に換算すると1,120時間であった。ヨーロッパにおける代表的な栄養士団体であるEuropean Federation of

the Associations of Dietitians（EFAD）では、2002年に欧州各国の栄養士制度に関する調査を実施し、栄養士課程の年数、時間数、カリキュラム全体に占める臨地・校外実習の割合などを報告している[10]。このEFAD報告書によると、イギリスの臨地・校外実習時間は1,040時間とされていた。また、ドイツ[11]は1,400時間以上の臨地・校外実習を実施していた。

一方、調査対象とした21カ国のうち、国際的スタンダードである500時間を明確に下回っていたのは日本とノルウェーの2カ国だけであった。日本では、栄養士法施行規則で管理栄養士の臨地・校外実習時間を4単位以上と規定しており、厚生労働省では1単位を45時間と定めていることから、最低必須時間は180時間となった。わが国においても、臨地・校外実習を重視した教育を実施している施設も存在するが、平均的な臨地・校外実習の時間数に関するデータは不明である。ノルウェーでは、臨床に携わるclinical dietitianでも臨地・校外実習時間は約3週間（時間数にして約83～98時間）であり、給食管理に携わるadministrative dietitianでは臨地・校外実習は必須ではなかった[10]。

◆◆5　諸外国の臨地・校外実習の内容

各国の臨地・校外実習の内容を表2に示した。科目・分野に関しては、「臨床栄養」、「公衆栄養」、「給食(経営)管理」を中心にプログラムが組まれている国が多かった。その中でも、多くの国で臨床栄養を重視しており、病態栄養や食事療法を中心とした医療スタッフとしての臨地・校外実習に多くの時間が費やされていた。イギリスは臨地・校外実習の内容に特徴があり、臨地・校外実習を段階的に設定し、Placement A、B、Cと呼ばれる3つに分類されていた[8,9]。Placement Aは、仕事内容や役割、環境を知る、Placement Bは、Placement Aで得た経験をもとに、特定の患者、集団を対象とした場合の理論、知識、スキルを獲得する、Placement Cは、臨床的な理論を実践に応用するステージで、より複雑な臨床ケースに介入する機会を与えられ、多くの専門家領域で臨地・校外実習を行うとされていた。学士号を必須としていないフランス、ドイツにおいても、医療機関における十分な臨地・校外実習を実施し、臨床スタッフとしての実習が実施されていた。

また、指導方法については、日本では少数グループ単位で指導を受けることが一般的であるが、マンツーマンでの徹底した指導を重視したアメリカをはじめ、マンツーマン指導を取り入れている国も存在した。

臨地・校外実習の時期は、多くの国で養成課程在学中に実施していた。アメリカおよびカナダでは、2種類の養成制度が存在し、①養成課程を卒業した後にインターンシッププログラムを修了する、②養成課程とインターンシップが組み合わされたプログラム（コーディネートプログラム、インテグレーテッドプログラム）を卒業する[b,d]であった。しかし、アメリカおよびカナダ共に養成課程を卒業した後にインターンシップの形で臨地・校外実習を実施するのが一般的であった[b,d]。インターンシップは有料であることが多く、インターンになるための選抜試験等が存在した。アメリカ、カナダ共にインターンシップの受け入れ数に制限があるため、養成課程卒業生の約半数しかインターンシップへ進むことができない[12,d]。一方、在学中にインターンシップを実施するコースでは、全員がインターンシップを受けられるが、週末や夏期休暇などの長期休暇中にもインターンシップを組み込んでいる場合もあった[13]。

◆◆6　諸外国の栄養士の職域

栄養士免許取得後の進路は、卒業生の大多数が栄養士として勤務する国が多数を占めた（表2）。分野は臨床栄養分野が多く、医療スタッフとして医療現場で働く国が多いことが明らかとなった。アメリカでは約55%[14]、カナダでは約61%[d]、イギリスでは約60%[9]、ドイツは約90%[11]が医療分野で医療スタッフとして勤務していた。一方、日本では卒業生が栄養士として就業する率が低く、平成19年度の調査では管理栄養士課程卒業生のうち栄養士として就職した者の割合は約55%であった[15]。栄養士として就職した者のうち、病院勤務者の割合は約27%[15]であった。わが国では、病院勤務の管理栄養士であっても、給食管理(フードサービス)スタッフとしての位置づけがある場合もあり、他国とは事

表2　各国栄養士の臨地・校外実習内容および職域

	日本（管理栄養士）	アメリカ	カナダ	オーストラリア
臨地・校外実習の時期	在学中[*1]	卒業後が一般的[b]（コーディネートプログラム[*3]は在学中）	卒業後が一般的[d]（integrated undergrad programは在学中）	在学中[7, c]
実習科目および各時間数	「臨床栄養学」、「公衆栄養学」、「給食経営管理論」合計で4単位以上（ただし「給食の運営」に係る校外実習の1単位を含む）[*1]	一般型：「臨床栄養」、「コミュニティー栄養」、「フードサービス」などを含む 専門型：「栄養療法」、「栄養教育」、「母子栄養」など専門性を重視[b] プログラムは一般型から専門性重視型まで多様[b] 科目時間は選択プログラムにより異なる[b]	「病院」、「長期療養施設」、「健康管理施設」、「コミュニティー施設」、「食品業界」など特定機関で35-40週	「個別ケアマネジメント」最低10週間、「地域・集団の健康問題」最低4週間、「食品・栄養システムマネジメント」最低4週間、「選択実習」最低2週間[7, c]
臨地・校外実習の方法	少数グループが一般的[*2]	マンツーマンが一般的（1-2週間以上）[*2] 現役のRDプリセプターから直接指導[b] ローテーションにより複数のRDから指導[13]	現役のRDプリセプターの監督下でマンツーマンおよびグループ実習[d, *2]	個別ケアマネジメント実習のうち4週間は常勤栄養士が2名以上の病院での実習が必須[2, 7] 個人の経験、雇用による経験が実習期間に認められる場合も有[7]
臨地・校外実習を受ける学生の割合	100%（養成課程の学生全員が実習）[*1]	約50%（希望者は60%以上）[12]	約50%	100%[*2]
臨地・校外実習の出願の有無および選抜方法	出願：無 選抜方法：大学から派遣。養成課程の学生は全員実習可能	出願：有[b] 選抜方法：CADE認定施設に出願[b]（コーディネートプログラムは養成課程に実習を含む） コンピューターマッチングシステムにより選抜（学業成績、ボランティア/職務経験、小論文など）[b]	出願：有[d] 選抜方法：コンピュータ選択プロセス（integrated undergrad programは養成課程に実習を含む）	出願：無 選抜方法：大学から派遣
臨地・校外実習の費用	無料（学費に含まれる）	有料が一般的[b]（無料および有給のインターンも一部有）	有料が一般的[d]	無料（学費に含まれる）が多数[*2]
就職状況および職域	栄養士業務への就職率は約55%（卒業時点）[15] 新卒の就職状況（栄養士業務の内訳）[15]：工場・事業所38.2%、病院27.2%、福祉施設15.3%、学校4.5%、官公署4.5%、養成施設0.7%	多くはRDとして就職[*2] 職域[14]：臨床栄養55%、食品・栄養管理12%、地域栄養11%、コンサルタント・ビジネス11%、教育・研究6%	大多数が栄養士の職に就く[d, *2] 新卒の就職状況[d]：臨床栄養士61%、コミュニティー栄養士24% 職域[d]：一般および急性期病院37%、コミュニティー、クリニック29%、長期療養施設、リハビリ23%、開業、食品産業8%	大多数が栄養士の職に就く[*2]

	イギリス	フランス	ドイツ	イタリア	アイルランド	スウェーデン	ノルウェー
臨地・校外実習の時期	在学中[10, e]	在学中[2]	在学中[2]	在学中[*2]	在学中[h]	通常在学中[*2]	—
実習科目および各時間数	「臨床栄養」（一部「コミュニティー栄養」含む）[e] 3段階のプレイスメントにより実施 Placement A 4週間、Placement B 12週間、Placement C 12週間[8, 9 e]	BTS：「食堂」実習6週間、「臨床医学治療法」実習10週間、選択実習4週間[17, f] DUT：治療学分野最低8週間[16, f]	「栄養学」（食事療法が中心、一部厨房作業含む）700時間、「調理・厨房管理」衛生含む200時間、「食・栄養カウンセリング」150時間、ディストリビューション120時間、その他230時間[11, g] 実習は病院にて実施[11, g]	—	—	「臨床栄養学」[i]	—
臨地・校外実習の方法	資格を持つ栄養士の監督下にてその他の医療関係者と共に実習[9]	マンツーマンもしくは最大2-3人の少数グループ（病院の場合）	—	—	—	—	—

	イギリス	フランス	ドイツ	イタリア	アイルランド	スウェーデン	ノルウェー
臨地・校外実習を受ける学生の割合	—	—	—	—	—	—	—
臨地・校外実習の出願の有無および選抜方法	出題：無 選抜方法：実習は養成課程に含まれる。health professional council によって承認された病院やコミュニティにて実習	—	—	—	—	—	—
臨地・校外実習の費用	無料(学費に含まれる)[*2]	無料(学費に含まれる)[*2)]	—	無料[*2]	—	—	—
就職状況および職域	職域[10)]：病院 40%、ファミリードクター 20%、健康教育 10%	ほとんどが栄養士として就職[*2] 職域[10)]：病院・ファミリードクター・健康関連施設・看護施設・健康教育・地域アドバイザー 65%、コンサルタント(開業)20%、その他 15%	ほとんどが栄養士として医療専門職に就職(病院、リハビリテーションクリニック勤務が大多数)[g)] 職域[10)]：病院 90%、健康教育 5%、コンサルタント(開業)5%	職域[10)]：病院 80%	職域[10)]：病院 60%、ファミリードクター 19%、製薬会社 10%	職域[10)]：clin.；病院 50%、ヘルスセンター・ファミリードクター 14%、製薬会社 2% adm.；病院 25%、コミュニティーアドバイザー 15%、給食会社 10%	職域[10)]：病院 40%、看護施設 40%、社員食堂 5%、軍隊食堂 5%

BTS：中級技術者養成課程食事療法学[2)]。DUT：技術短期大学部生物工学食事療法学選択課程[2)]。clin.：clinical dietitian。adm.：administrative dietitian。RD：registered dietitian。CADE：Commission on Accreditation for Dietetics Education（栄養士教育公認委員会）

[*1] 栄養士法施行規則（昭和 23 年 1 月 16 日厚生省令第 2 号、最終改正：平成 19 年 12 月 25 日厚生労働省令第 152 号）。[*2] 各国の栄養士関連職能団体の担当者、政府機関担当官等からの直接回答。[*3] アメリカのコーディネートプログラムおよびカナダの integrated undergrad program は、養成課程とインターンシップが統合されたコース。

情が異なる。しかし、それであっても管理栄養士課程の卒業生が病院で勤務する割合は、諸外国と比較すると少ないことが明らかとなった。

一方、職域の幅では、多くの国の栄養士が医療職に特化しているのに対し、日本の管理栄養士は臨床分野だけでなく、給食管理、福祉施設、学校、官公庁等に勤務し、諸外国に比較して多くの分野で活躍していることが明らかとなった。

Ⅳ 考 察

本研究により、日本の管理栄養士養成課程における臨地・校外実習は、諸外国と比べ全体の時間数が非常に少なく、マンツーマンでのトレーニングが実施されていないことから、「人を診る専門職」という観点から考えると実習内容が不十分であることが明確となった。管理栄養士の資質向上のためにも、臨地・校外実習の時間数の拡大が望まれる。ICDA が提唱する国際的スタンダードである「監督下での専門的な実習 500 時間」をわが国において実施するためには、以下のような方法が考えられる。

①在学中の週末、夏期休暇など長期休暇期間中に臨地・校外実習を実施する。

②在学中の臨地・校外実習時間数、比率を増加させる（それに伴う授業時間数の減少の可能性あり）。

③臨地・校外実習終了後、選択実習を設ける。

④在学中のボランティア実習、施設研修などを単位化する。

⑤大学院において臨地・校外実習を重視した養成課程を設ける。

⑥アメリカ・カナダで実施している養成課程終了後のインターンシップ制度を導入する。

しかしながら、日本の管理栄養士の養成数が世界でも最多であることから、すべての学生に対して臨地・校外実習を必須とする現行の制度では、臨地・校外実習の時間数増大に受け入れ施設が対応できずに破綻する可能性が考えられる。アメリカおよびカナダにおいても、インターンシップの枠は限られており、養成課程卒業生の約半数しかインターンシップに進学することができない[12, d)]。インターンシップへ進めなかった卒業生は、次期にインターンシップへ進む場合、栄養士免許は取得せずに栄養の専門家として活躍する場合などがある。また、インターンシップへ進めた場合でも、受け入れ施設のインターンシップだけでは修了できないことが多い。アメリ

カの例では、臨床栄養を学ぶため病院でのインターンに進めた場合でも、フードサービスやコミュニティー等の他分野のインターンシップ経験も必要であることから、自ら他分野のインターンシップ先を探さなければならない場合もある。日本においても、すべての養成課程の学生に臨地・校外実習を必須として、実習先を与える制度を再考する必要もある。管理栄養士として勤務を希望している学生のみを対象とした強化型臨地・校外実習や専門型の養成課程を設けるなど、わが国独自の対応が必要である。また、日本では在学中に種々の学内実習や実験を実施しているが、諸外国では学内の実験実習を実施していない例も存在する。例えば、アメリカでは養成課程において授業実習や学内実習はほとんど実施しておらず、実務レベルでの経験はインターンシップに集中させている。ICDAが示している「監督下での専門的な実習」の範囲や定義をわが国として再考する必要もある。

また、オーストラリアの卒後教育制度も参考になる。オーストラリアでは新卒の栄養士は条件付APD（provisional accredited practising dietitian）とされ、Full APDの指導のもとで勤務する[7]。Full APDに昇格するためには、卒後2年以内に指導者の承認と共に申請しなければならない。この制度は、免許を持っているだけの実務経験のない栄養士と、実務を行っている栄養士を明確にすることができる点で、わが国のように就業率が低い場合には有効である。さらに、専門職意識を高める可能性もある。

ICDAが提唱する国際的スタンダードである「監督下での専門的な実習500時間」を実施するためには、上述した種々の方法が考えられるが、本研究の結果から、わが国の管理栄養士養成制度の利点も浮き彫りとなった。第一に、管理栄養士数が世界でも最多であること、第二に、管理栄養士の職域が広く、国民の身近で活躍していることである。管理栄養士数が最多であることは、国際的スタンダードである500時間の臨地・校外実習の実現を困難にしている一因でもある。しかし、わが国が諸外国に例を見ない管理栄養士数を有することは、国民全体の健康に寄与している可能性も否定できない。わが国の管理栄養士制度は、健康を支える社会的基盤となり、世界をリードするための根拠となり得る可能性も十分考えられる。ICDAの掲げる栄養士の国際的スタンダードを諸外国と単純に比較するのではなく、専門職としての社会における役割や影響力の大きさ等も考慮し、わが国の利点を生かした現状に合った独自の対策が求められる。

V 結　論

諸外国において栄養士免許取得前に実施されている臨地・校外実習制度を調査し、日本の管理栄養士養成制度の現状と比較した。その結果、ほとんどの国でICDAが提唱する国際的スタンダードである「監督下での専門的な実習500時間」を実施していることが明らかとなった。さらに、500時間にとどまらず時間数のさらなる拡大の動きがアメリカの制度改定等にも見られることから、日本の管理栄養士養成においても、人を診る専門職として臨地・校外実習の時間数の増加が望まれる。一方、本研究の結果から、わが国の管理栄養士数が世界でも最多であること、管理栄養士の職域が広く、国民の身近で活躍していることが明らかとなった。多数の専門職の存在はわが国の健康を支える基盤とも考えられることから、わが国の利点を生かした今後の対策が望まれる。

謝　辞

本研究は、平成20年度(社)日本栄養士会政策経費事業の助成を受け、全国研究教育栄養士協議会の政策事業「管理栄養士養成のための臨床研修制度についての検討」の一環として行った。

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Abstract：The International Confederation of Dietetic Associations (ICDA) stipulates the minimum level of education of a dietitian is "1: a bachelor's degree, and 2: a period of supervised professional practice of at least 500 hours." In this study, we conducted a survey of practical training systems for dietetic students in the world, and compared the result with the current educational system of registered dietitians in Japan. We collected information from notification documents and reports published by government agencies and national professional dietetic organizations and web pages. To collect more detailed information, we contacted the representatives of countries. Over 500 hours of practical training in accordance with ICDA educational standards were conducted in 21 countries investigated, but not in Japan and Norway. Timing of practical training differs depending on the country; in some countries as Japan students receive practical training during the dietetic course; whereas in the U. S. and Canada, students generally receive such training after completing the dietetic course. The contents of training in many countries were mainly clinical nutrition and nutritional therapy. Of the countries investigated, Japan has the largest number of dietitians per 100,000 of the population and the largest number of occupational areas. This study brought out weak points and advantages of the registered dietitian training system in Japan. Japan should adopt unique countermeasures for improving the expertise of dietitians by increasing the hours of practice training of dietetic students by taking advantage of a great number of dietitians and their occupational areas and their proximity to people's lives.

Key words：dietitian, system, practice, education

Trace Nutrients Research 28 : 27 - 31 (2011)

原　著

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

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Comparison of Calculated Values with Analyzed Values in Intake of Microminerals from Diets in Hospital and Nursing Home

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Summary

Intake of microminerals (iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), iodine, selenium (Se), chromium (Cr) and molybdenum (Mo)) from diets in hospitals and nursing home was determined by atomic absorption spectrometry or inductively coupled plasma mass spectrometry, and compared with those estimated by a calculation using the Standard Tables of Food Composition in Japan, 5th edition (Fe, Zn, Cu and Mn) or 2010 (iodine, Se, Cr and Mo). In the intake of Fe, Zn, Cu and Mn, correlation coefficients between the analyzed values and calculated values ranged from 0.6 to 0.9 and Y-intercepts of the regression equations were near zero; however, slopes of the equation ranged from 0.50 to 0.69. In the daily intake of Se and Mo, the estimated values using the Standard Tables, 2010 were almost equal to the analyzed values. On the other hand, the calculated iodine intake in some diets was markedly lower than the analyzed values. In addition, the calculated Cr intake was less than one-quarter of the analyzed values. These results indicate that considerable loss of Fe, Zn, Cu and Mn occurs in cooking and the estimated Se and Mo intake calculated using the Standard Tables of Food Composition in Japan, 2010 is reliable.

日常の食事からのエネルギーと栄養素の摂取量を知ることは，個人や集団の食生活を評価する上で重要である。食事からの栄養素摂取量を推定する場合，献立に用いられている個々の食材の量と食品成分表に記載されている各食材中の栄養素含有量にもとづく計算値を用いるのが一般的である。また，給食施設などにおいて，管理栄養士が献立を設計する場合も，食品成分表からエネルギーと栄養素の摂取量を計算している。

このようにして算定されたエネルギーと栄養素摂取量が真の摂取量を反映しているかの検討はしばしば行われており，少なくともタンパク質のような主要栄養素では計算値と化学分析にもとづく実測値との間に高い一致性が認められている[1)]。しかし，一部のビタミンやミネラルについては，献立によって一致しない事例も認められている[2,3)]。また水溶性ビタミンやミネラルでは調理中の損失も無視できないといわれる[4)]。このような成分表と実測値との間の一致性が低い場合には，食事摂取基準における推奨量を満足させる献立を設計する場合に一定の配慮が必要となる。本研究では，病院および介護施設で提供されている献立からの鉄，亜鉛，銅，マンガンの摂取量について，五訂食品成分表にもとづく計算値と化学分析にもとづく実測値との差異を検討した。一方，2010年秋に公表された「日本食品標準成分表2010」においては，収載食品の3分の1に相当する約500食品に対して，ヨウ素，セレン，クロム，モリブデンの含有量が示された[5)]。そこで，これら4元素の摂取量についても，計算値と化学分析にもとづく実測値との差異を検討した。

実験方法

1. 食事試料

大阪府下の2つの病院（AおよびB）において，通常食として提供されていた食事を，それぞれ3日間（病院食A1～3，B1～3），朝，昼，夕食別に収集した（合計で，2×3×3＝18試料）。収集時期は病院Aが2010年7月，病院Bが2010年11月である。また神奈川県下の介護施

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設において，通常食1日分と介護食1日分を，朝，昼，夕食別に収集した。収集時期は2010年1月である。介護食については別に昼食1回分を2009年11月にも収集した（合計で，1＋2＋3＋1＝7試料）。これらのうち，介護食とは，咀嚼または嚥下が困難な要介護者に対するもので，加水もしくはとろみづけを行い，ミキサーなどによって均一後，裏ごしなどによって堅い固形物を除去した半流動食である。各食事は，朝，昼，夕食別に凍結乾燥した後，細粉化し，分析用試料とした。朝，昼，夕食に分けたため，食事試料は総計で25種類となった。

2. 微量ミネラルの分析

1）鉄，亜鉛，銅，マンガン，セレン，モリブデンの定量：粉末試料1 gをケルダールフラスコに入れ，硝酸10 mLと沸騰石を加えて約30分加熱後，過塩素酸2 mLを加え，過塩素酸の白煙が生じるまで加熱して完全に灰化した。そして灰化液に標準ロジウムとテルルを終濃度20 ng/mLとなるように加え，純水で10 mLにメスアップしたものを測定用試料とした。この試料中の鉄，亜鉛，銅，マンガンをフレーム式原子吸光光度計，セレンとモリブデンを誘導結合プラズマ質量分析器（ICPMS），を用いて定量した。ICPMSの測定において，セレンに対してはテルル，モリブデンに対してはロジウムを内部標準とした。

2）ヨウ素の定量：粉末試料200 mgに20 ng/mLのテルルを含む0.5％テトラメチルアンモニウムヒドロキシド溶液40 mLを加え，室温で一夜放置した。さらに，60℃で4時間加熱後，遠心および濾過を行い，抽出液を得た。抽出液中のヨウ素を，テルルを内部標準として，ICPMSを用いて定量した。

3）クロムの定量：粉末試料500 mgを磁製るつぼに入れ，550℃で16時間加熱した。灰化後の残渣を20 ng/mLのロジウムを含む0.1 M硝酸に溶解し，含有されるクロムを，ロジウムを内部標準として，ICPMSを用いて定量した。

3. 成分表からの微量ミネラル摂取量の算定

1）鉄，亜鉛，銅，マンガンの摂取量の算定：鉄，亜鉛，銅，マンガン摂取量の成分表からの計算値は各施設において五訂食品成分表にもとづき算定された数値を使用した。

2）「日本食品標準成分表2010」を用いたヨウ素，セレン，クロム，モリブデン摂取量の算定：「日本食品標準成分表2010」においてヨウ素，セレン，クロム，モリブデン含有量の記載があるのは全体の約3分の1である。実際，今回の収集献立で使用されていた138食品中で，成分表にこれら4元素の含有量記載があったのは49％だった。記載のない食品に関しては，①近縁のもので代用（ロース肉→牛肉の平均値，ロールパン→パンの平均値，魚種の細かな違いを同属のもので代用（例，サワラ→同じアジ科のサバで代用）など），②ミックスベジタブルなどのように加工食品において，複数の食品を組み合わせていることが明らかな場合は，構成食品の平均値を使用，③その食品が属する食品群ごとの平均値で代用，のいずれかの方式で数値をあて，摂取量の算定を行った。結果として，①は138食品中の17％，②は7％，③は27％だった。

結　果

Fig. 1に，朝，昼，夕食別の25試料に関して，鉄，亜鉛，銅，マンガンの摂取量の計算値を独立変数（X），分析値を従属変数（Y）とした回帰分析の結果をまとめた。なお，鉄に関しては，計算値（7.1 mg）と実測値（0.65 mg）との乖離が著しい1試料を除外して関連を検討した。4種の微量ミネラル摂取量の計算値と実測値との相関は強く（$r = 0.6 \sim 0.9$），その回帰式は，Y切片の95％信頼区間（鉄，$-0.53 \sim 1.12$；亜鉛，$-0.16 \sim 0.79$；銅，$-0.08 \sim 0.03$；マンガン，$-0.01 \sim 0.25$）がゼロを含み，傾き（回帰係数）が0.5～0.7の範囲だった。

Table 1は，病院と介護施設の食事8日分について，ヨウ素，セレン，クロム，モリブデンの1日摂取量の計算値と分析値を一覧にしたものである。なお，ヨウ素とクロムに関しては，介護施設の食事が分析できなかったため，病院食6日分のみの比較である。対応のあるt検定では，クロムのみ計算値と実測値に有意な差があり，他の3元素は有意差なしだった。

ヨウ素摂取量において，分析値／計算値の比は0.85～5.85だった。すなわち，計算値は6日中2日が100 μg/d未満，残り4日が250～420 μg/dだったのに対して，分析値は6日すべてが290～420 μg/dであり，3分の1において計算値と分析値との乖離が認められた。一方，クロムの摂取量においては，計算値と分析値の相関は大きかったが，分析値／計算値の比は4.64～6.85であり，いずれの日も分析値が計算値を大きく上回っていた。

これに対して，セレンとモリブデンの摂取量では，分析値／計算値の比がそれぞれ0.78～1.84と0.98～1.80であり，計算値と分析値がおおむね一致する傾向にあった。そこでこの2元素については，朝，昼，夕食別に分けた24試料（別途収集した介護施設の介護食昼食は，献立についての詳細な情報が得られず，摂取量の計算値がもとめられなかったので検討から除外した）を対象として，計算値と分析値との関連を検討し，Fig. 2に示した。相関係数は，セレンが0.67，モリブデンが0.78であり，比較的大きかった。回帰式を求めたところ，セレンにおいてはY切片が比較的大きく（95％信頼区間11.4～32.0），傾きは0.74となった。これに対してモリブデンの回帰式では，Y切片の95％信頼区間（$-43.7 \sim 30.7$）がゼロを含み，傾きは1.39だった。

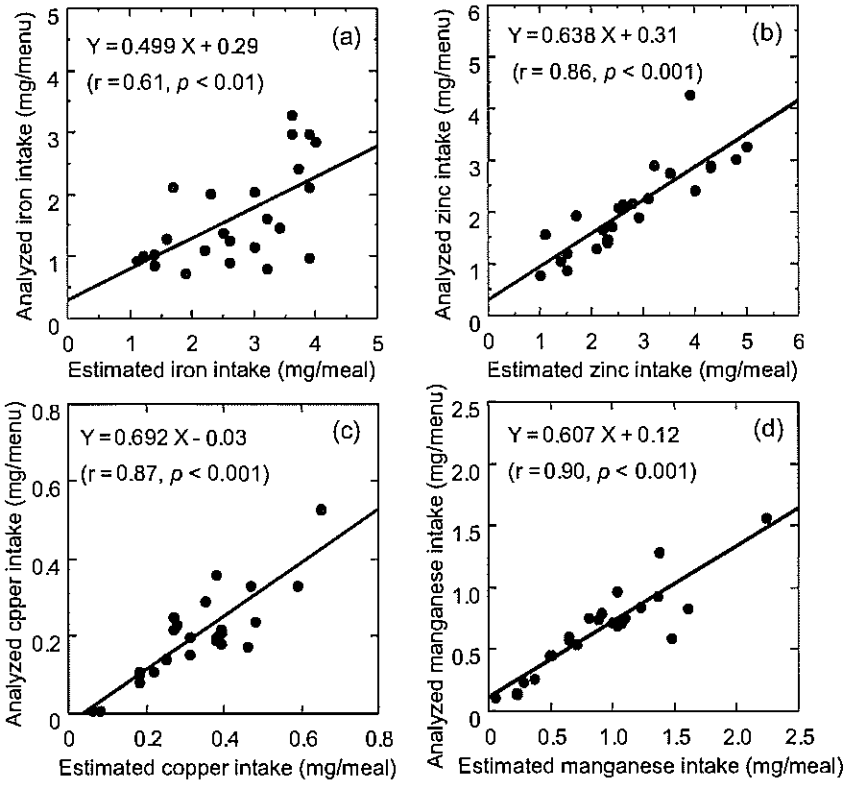

Fig. 1 Relation between estimated and analyzed values in intakes of iron (a), zinc (b), copper (c) and manganese (d)

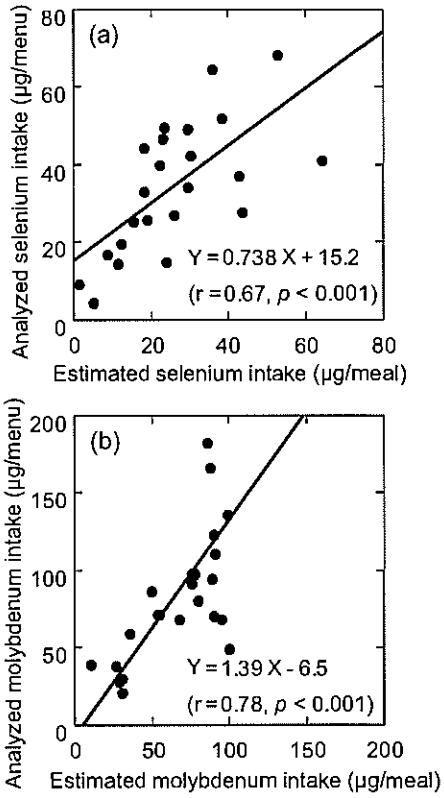

Fig. 2 Relation between estimated and analyzed values in intakes of selenium (a) and molybdenum (b)

Table 1 Comparison of estimated values with analyzed values in intakes of iodine, selenium, chromium and molybdenum from diets in hospital and nursing home

Diets	Iodine (μg/d)		Selenium (μg/d)		Chromium (μg/d)		Molybdenum (μg/d)	
	Estimated	Analyzed	Estimated	Analyzed	Estimated	Analyzed	Estimated	Analyzed
Hospital								
Usual diet A1	347	411	108	101	7	48	242	302
Usual diet A2	80	340	146	114	5	22	269	289
Usual diet A3	52	304	73	90	5	25	253	247
Usual diet B1	246	332	120	125	9	28	223	177
Usual diet B2	345	292	82	151	13	67	218	333
Usual diet B3	419	415	86	146	14	65	267	480
Nursing home								
Usual diet	–	–	58	59	–	–	157	230
Soft semi-liquid diet	–	–	17	24	–	–	65	106
Mean	248	349	86	101	9	43	212	271
Paired t-test	NS		NS		p = 0.005		NS	
Correlation coefficient	0.56 (NS)		0.67 (NS)		0.88 (p = 0.017)		0.71 (p = 0.049)	

考　察

鉄において，計算値と分析値との間で著しい乖離を示したのは，水戻しされた状態で販売されているヒジキを用いた献立だった。このようなヒジキでは，水戻し操作の過程において大部分の鉄が流出していると思われる。

鉄，亜鉛，銅，マンガン摂取量において，五訂成分表にもとづく計算値と実測値との間の関連を検討したところ，相関係数は比較的大きく，回帰式のY切片はいずれもゼロを含み，その傾き（回帰係数）は0.5～0.7の範囲だった。すべての食品に関して成分表記載の数値が実際のものよりも小さいということは考えにくい。したがって，高い相関係数と0.5～0.7の傾きは，これらの微量ミネラルでは調理中の損失が30～50%であることを示している。調理によるミネラルの損失はミネラルの種類に関わらず25%程度といわれているが[4]，今回の結果はこれよりも明らかに大きい。今回の病院食では煮物のように損失が大きい調理法のものが多かったことが関係しているのかもしれない。なお介護食では，通常の調理に裏ごしなどの操作が加わっており，栄養素の損失はさらに大きくなる可能性が

高いが，今回は確認できなかった。

2010年秋に公表された「日本食品標準成分表2010」においては，ヨウ素，セレン，クロム，モリブデンの含有量が初めて示されたが，含有量記載の対象となった食品は全体の約3分の1に過ぎなかった[5]。このため，これら4元素の摂取量計算においては，使用食品の約半分に関して数値のあてはめ作業を行う必要があった。収載されていない食品に関して，その食品が含まれる食品群の平均値を使用することは，あてはめのルールとして単純で好ましいといえる。しかし，現実には，全体の約4分の1近くが，パンの種類や肉の部位，魚種の細かな違いなど，明らかに近縁の食品が利用可能なケース，または調理済み食品などで食材が特定できるケースであったため，食品群の平均値使用のケースは全体の27％にとどまった。あてはめ作業は恣意的に陥る危険性もあるので，今後，4元素含有量を記載した食品数の増加が期待される。

「日本食品標準成分表2010」を利用したヨウ素，セレン，クロム，モリブデン摂取量の計算値と実測値との関連を検討したところ，ヨウ素では両者の乖離が著しいケースが認められた。ただし，ヨウ素摂取量の把握では，まず低ヨウ素の献立と摂取量が数mgにも達する高ヨウ素の献立の区別が期待されるが，今回の献立はいずれも1日500 μgまでの低ヨウ素の献立であったため，今回の結果からは成分表を用いた計算値によってこの区別を行えるかという判断はできない。ヨウ素濃度の高い食品は水産物，とくに海藻類であり，なかでも昆布製品のヨウ素摂取への寄与は著しいことが知られている[6]。しかし，昆布製品，とくに「だし」として使用される場合，これを定量的に把握することは難しい。すなわち一般論としては，ヨウ素摂取量を計算によって把握する場合，布施らが提案しているように特別な調査票を使用するか[7]，あるいは食事記録をとるさいに昆布製品の使用量をできるかぎり詳細に記載することを心がけることが必要であろう。

クロムでは計算値と分析値との相関係数は高かったが，分析値は計算値の数倍となった。分析値が高くなった理由としては，調理加工段階でのクロムの汚染，および分析段階でのクロムの汚染が考えられる。今回の分析ではステンレス製の実験器具を100％回避することができていないので，分析中の汚染は否定できない。ただし，今回の実測によるクロム摂取量の数値は，国内外における陰膳収集献立のクロム分析値とほぼ等しい[8-10]。したがって，分析での汚染よりも調理加工の段階でのクロム汚染が寄与している可能性が高い。かりに調理加工における汚染の寄与が大きいとすれば，実測値の意義が大きく，成分表からの計算値は実態を反映していないことになる。この点を解明するには，厳格に汚染をコントロールした分析を前提として，調理加工に伴うクロム含有量の変化を追跡する必要がある。ただし，成分表においても高クロム濃度の食品は，各食品群に散見され，一定の傾向が認められない。このことは調理前の食品においても，高クロムが汚染によって引き起こされていることを意味している。以上より，現状では，成分表からのクロム摂取量の算定は困難であり，かつその意義も小さいといわざるを得ない。

ヨウ素，クロムとは異なり，セレンとモリブデンでは，計算値と分析値がおおむね一致しており，「日本食品標準成分表2010」を用いた計算による摂取量把握は可能と判断できる。これは，セレンが魚介類や小麦製品，モリブデンが穀類と豆類といった主要食品が供給源であり[11,12]，これらの食品は食事記録において定量的把握が容易であるためと思われる。セレンに関しては，両者の回帰式が原点からやや離れ，傾きが0.74となった。しかし，献立の数値自体は近似しているケースが多かった。したがって，傾きの数値から，ただちにセレンに関して25％程度の調理損失があると結論することはできない。検討例が増加すれば，回帰式が原点に近づき，傾きも大きくなる可能性も考えられる。一方，モリブデンでは，回帰式が原点付近を通過するものの，傾きが1.4となっており，分析値が計算値よりもやや高い値となる傾向にあった。しかし，これも検討例を増加することにより，1に近い傾きとなる可能性も考えられる。今後より多くの献立について検討し，計算値と実測値との正確な関連を明らかにする必要があるだろう。

本研究は，平成23年度厚生労働科学研究費補助金（循環器疾患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関する総合的研究）により行われたものである。

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Trace Nutrients Research 28：79－83（2011）

原　著

市販離乳食からのヨウ素とクロムの摂取量の推定

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Estimation of Iodine and Chromium Intakes from Commercial Baby Foods

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Summary

To estimate iodine and chromium intake in Japanese infants dependent on commercial baby food and human milk, 53 commercial baby food samples (24 samples for ≥7-month-old baby and 29 samples for ≥9-month-old baby) were collected and their iodine and chromium concentrations were determined by inductively coupled plasma mass spectrometry. The iodine concentrations were markedly elevated by the use of *kombu* or *hijiki* and their medians (25-75 percentile) in baby food for a ≥7-month-old baby and a ≥9-month-old baby were 30 (21-103) and 42 (27-1045) ng/g wet weight, respectively. Mean iodine intake by 6 to 8-month-old babies and 9 to 11-month-babies was estimated to be 144 and 691 μg/d, respectively although their medians were estimated to be 89 and 84 μg/d, respectively. On the other hand, chromium concentrations (median (25-75 percentile)) in baby foods for a ≥7-month-old baby and a ≥9-month-old baby were 12 (7-12) and 10 (7-16) ng/g wet weight, respectively. Mean chromium intake by 6 to 8-month-old babies and 9 to 11-month-babies was estimated to be 8 and 10 μg/d, which were about 10 times higher than the value shown as the Adequate Intake in the Dietary Reference Intakes for Japanese. These results indicate that intermittent high-iodine baby food ensures sufficient iodine intake, and chromium intake is increased in Japanese infants after beginning to eat baby food.

わが国の食事摂取基準では，現在の日本の乳児において，栄養素の不足・過剰に起因する顕著な健康障害が認められないという事実をふまえ，各栄養素の母乳中濃度にもとづいて，生後6か月未満乳児の栄養素摂取の目安量を設定している。一方，生後5か月を経過すると，多くの乳児は母乳とともに離乳食を摂取し始める。したがって，生後6か月以降に関しては，母乳と離乳食からの栄養素摂取量にもとづいた目安量の策定，もしくは1歳以降と同様に，推定平均必要量にもとづいて摂取の推奨量を設定するのが妥当である。しかし，微量ミネラルの場合，2010年版食事摂取基準では，6か月以降乳児に対して，鉄が推定平均必要量と推奨量，銅とマンガンが離乳食摂取を考慮した目安量が設定され，その他に関しては生後6か月未満乳児に対する目安量を体重にもとづいて外挿した値を目安量としている[1]。これは，6か月以降乳児を対象とした，栄養素必要量に関する研究や栄養素摂取量調査が不十分であることに起因している。

われわれは，市販離乳食の利用頻度が増加していることから，市販離乳食を収集して，その鉄，亜鉛，銅，マンガン，セレン，モリブデン濃度を測定し，市販離乳食と母乳に依存した場合の6か月以降乳児におけるそれらの摂取量を推定し，報告した[2]。今回，同じ収集試料について，ヨウ素とクロム濃度を測定し，前報と同様に，市販離乳食と母乳に依存した場合の6か月以降乳児におけるこれらの摂取量を推定したので報告する。

実験方法

1．試料の収集

2008年の6月から10月にかけて，5つの国内メーカーより，レトルト，もしくは瓶詰めの状態で販売されていた離乳食53食を購入し，微量ミネラル測定用の試料とした。収集した離乳食は「7か月頃より」の表示のあるのが24食，「9か月頃より」の表示のあるのが29食であった。

2．分　析

収集した離乳食は，1食ごとにすべてを凍結乾燥後，ミル（Retsch GM200）で均一・細粉化した。細粉化した試料

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200 mg に 20 ng/mL のテルルを含む 0.5 %テトラメチルアンモニウムヒドロキシド溶液 40 mL を加え，室温で一夜放置した。さらに，60℃で 4 時間加熱後，遠心および濾過を行い，抽出液を得た。抽出液中のヨウ素を，テルルを内部標準として，誘導結合プラズマ質量分析器（島津 ICPM-8500）を用いて定量した。一方，これとは別に，細粉化試料 500 mg を磁製るつぼに入れ，550℃で 16 時間加熱した。灰化後の残渣を 20 ng/mL のロジウムを含む 0.1 M 硝酸に溶解し，含有されるクロムを，ロジウムを内部標準として，誘導結合プラズマ質量分析器を用いて定量した。分析に用いた質量数は以下のとおりである。クロム，52；ロジウム，103；ヨウ素，127；テルル，126，128，130。

3. 市販離乳食からのヨウ素とクロムの摂取量の推定

分析結果をもとに，各離乳食について，離乳食湿重量（g）あたりとエネルギー（kcal）あたりのヨウ素とクロムの濃度を求めた。本研究で収集した離乳食は「7 か月頃より」，および「9 か月頃より」の表示があったことから，1 日の離乳食をすべて単一の製品に依存したと仮定し，6～8 か月児が前者，9～11 か月児が後者を摂取した場合のヨウ素とクロムの摂取量を以下の式にもとづいて算定した。

［ヨウ素またはクロム摂取量］
＝［各離乳食のエネルギーあたりのヨウ素またはクロム濃度］×［6～8 か月児，または 9～11 か月児の離乳食からのエネルギー摂取量の報告値の平均値（6～8 か月児 171 kcal/d，9～11 か月児 452 kcal/d）[3]］。

さらに，この算定した市販離乳食からのヨウ素またはクロム摂取量に，日本人の食事摂取基準 2010 年版で採用されている母乳中ヨウ素またはクロム濃度（ヨウ素，133 ng/mL；クロム，1.0 ng/mL）[1] と乳児の母乳摂取量（6～8 か月児，600 mL/d；9～11 か月児，450 mL/d）[4, 5] から算定される母乳由来のヨウ素またはクロム摂取量を加え，市販離乳食と母乳を摂取した場合のヨウ素またはクロム摂取量をもとめた。

結　果

離乳食のヨウ素濃度の測定結果を Fig. 1 と Table 1 にまとめた。離乳食のヨウ素濃度は試料ごとの変動が大きく，最小値と最大値との間に約 100 倍の差を認めた。すなわち，約半数は湿重量あたり 50 ng/g 未満であり，日本人の母乳中ヨウ素濃度（133 ng/mL）よりも低かったが，湿重量あたり 1 μg/g を超える離乳食も 10 試料近くあった。7 か月以降用と 9 か月以降用を比較した場合，中央値には大きな差がなかったが，湿重量あたり 1 μg/g を超えるものがいずれも 9 か月以降用であったため，75 パーセンタイル値においては 9 か月以降用が大きく上回っていた。

今回収集した 53 種の市販離乳食中，15 の試料については，「昆布だし」，「昆布エキス」などのように，食材に昆布の使用が明記されていた。そこで，市販離乳食の湿重量あたりヨウ素濃度を，昆布使用表示の有無別に比較し，Table 2 にまとめた。両者のヨウ素濃度には極端な違いが

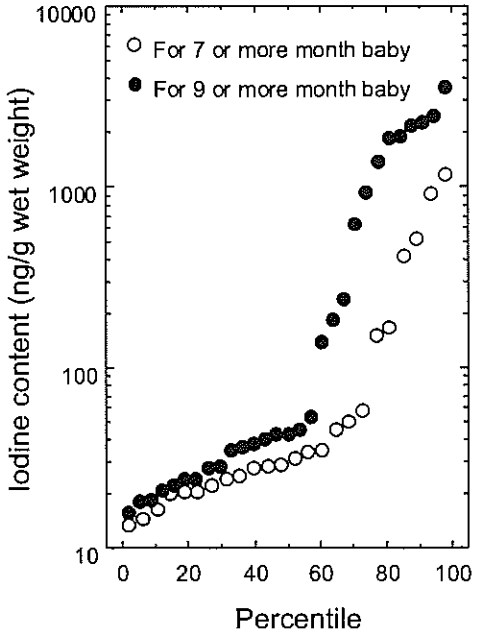

Fig. 1 Percentile curve of iodine content of commercial baby foods.

Table 1 Iodine concentrations in commercial baby foods

	For 7 or more month baby (n=24)		For 9 or more month baby (n=29)	
	ng/g wet weight	ng/kcal	ng/g wet weight	ng/kcal
Minimum	13	29	16	28
25 percentile	21	41	27	41
Median	30	51	42	54
75 percentile	103	174	1045	432
Maximum	1152	2818	3558	9489
Mean	159	378	629	1396
Geometrical mean	53	99	125	205

Table 2 Effect of use of *kombu* on iodine concentration of commercial baby foods

Use of *kombu*	Iodine concentration (ng/g wet weight)				
	Mean	Geometric mean	Median	Minimum	Maximum
+ (n=15)	1340	920	1151	149	3558
− (n=38)	51	33	37	13	614

The Mann-Whitney test showed that difference between "+" and "−" in iodine concentration was significant ($p < 0.05$).

認められ，昆布使用明記の離乳食はいずれも明らかに高いヨウ素濃度を示した。昆布使用が明記されていない離乳食の中で，一部の昆布使用離乳食よりも高いヨウ素濃度示したのが3試料あったが，これらは，「鰹とヒジキの煮つけ」，「とりヒジキごはん」などであり，相当量のヒジキが使用されたものだった。

離乳食のクロム濃度の測定結果をFig. 2とTable 3にまとめた。離乳食のクロム濃度は試料ごとの変動が小さく，そのほとんどが湿重量あたり20 ng/g未満であり，100 ng/gを超えたのは53試料中1試料のみだった。また，7か月以降用と9か月以降用との間に差は認められなかった。

1日の離乳食をすべて単一の製品に依存すると仮定した上で，6～8か月児または9～11か月児が，今回測定対象とした離乳食と母乳を摂取した場合のヨウ素またはクロムの摂取量を算定し，Table 3とFig. 3にまとめた。ヨウ素摂取量の中央値は，6～8か月児が89 μg/d，9～11か月児が84 μg/dであり，母乳のみの摂取を想定して策定されている食事摂取基準 2010年版における6か月以降乳児のヨウ素の目安量（130 μg/d）を下回った。しかし，平均値は，高ヨウ素濃度の試料が散見されたことを反映し，

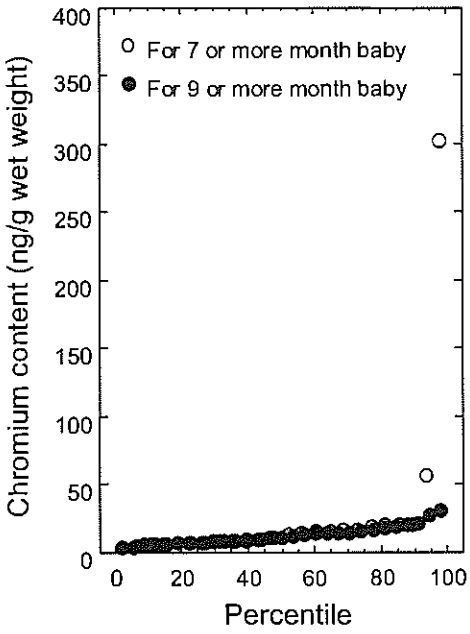

Fig. 2 Percentile curve of chromium content of commercial baby foods.

Table 3 Chromium concentrations in commercial baby foods

	For 7 or more month baby (n=24)		For 9 or more month baby (n=29)	
	ng/g wet weight	ng/kcal	ng/g wet weight	ng/kcal
Minimum	4	7	4	6
25 percentile	7	15	7	11
Median	12	20	10	18
75 percentile	18	29	16	25
Maximum	303	397	31	63
Mean	26	45	12	21
Geometrical mean	13	24	11	18

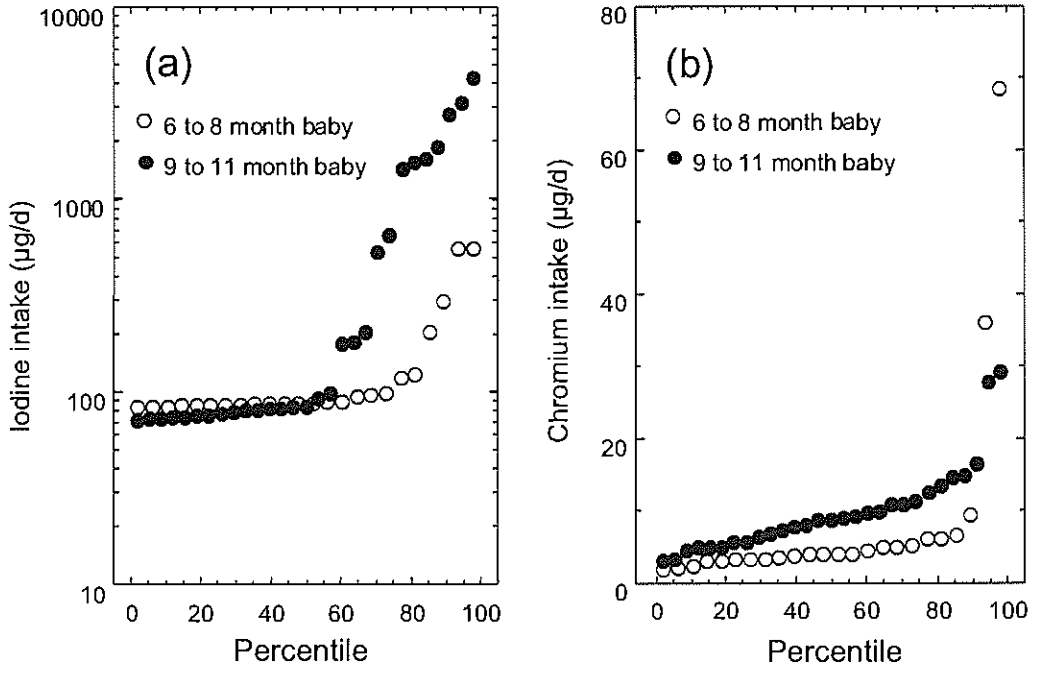

Fig. 3 Percentile curve of estimated iodine (a) or chromium (b) intake from breast milk and commercial baby foods.

6～8か月児が144 μg/d，9～11か月児が691 μg/dであった。とくに9～11か月児では，9か月児以降用離乳食のいくつかが湿重量あたり1 μg/g以上であったことを反映し，食事摂取基準2010年版の耐容上限量である250 μg/dを上回っていた。

一方，クロムの摂取量は，試料間の変動の小ささを反映し，6～8か月児，9～11か月児にかかわりなく，平均値，幾何平均値，中央値のいずれもが4～10 μg/dの範囲であった。

考　察

Table 1に示したように，9か月以降用市販離乳食の湿重量あたりヨウ素濃度の75パーセンタイル値は1 μg/gを超えていた。このことは9か月以降用市販離乳食の約4分の1が高ヨウ素濃度であることを意味している。昆布をはじめとする海藻類，および昆布を原料とした調味料には高濃度のヨウ素が含まれることがよく知られている[6,7]。今回，Table 2に示したように，高ヨウ素濃度の離乳食がいずれも昆布だしまたはヒジキを使用したものであったことは，昆布だしやヒジキの摂取が多量のヨウ素摂取につながることを明確に示すものといえる。

今回測定した市販離乳食の中で，レトルトタイプのものは，ほとんどが1袋あたりの湿重量が80 g（エネルギー量が30～80 kcal），瓶詰めタイプのものは，ほとんどが1瓶あたりの湿重量が130 g（エネルギー量が約100 kcal）だった。この量は1日の離乳食全体の数分の1未満に過ぎない。それでも湿重量当たりで1 μg/gを超える高ヨウ素濃度の製品の場合，1袋または1瓶を食べきった場合のヨウ素摂取量は100～350 μgとなる。1日の離乳食をすべてこのような高ヨウ素濃度の製品に依存することは考えにくいが，1回利用すれば母乳や他の離乳食からの摂取分が加わることで容易に500 μg/d近い摂取量となる。したがって，4分の1の確率で高ヨウ素の離乳食を利用することを考えれば，Table 3に示した離乳食と母乳を摂取した場合の9～11か月児のヨウ素摂取量の平均値691 μg/dは，間欠的に生じる高ヨウ素摂取を含めた平均的な値に位置づけられるだろう。

このように市販離乳食を利用する乳児においては，間欠的に高ヨウ素濃度の製品を摂取する機会があるため，平均的に食事摂取基準におけるヨウ素の耐容上限量を超える摂取が生じている可能性が高い。乳児のヨウ素摂取量の耐容上限量は，高ヨウ素摂取の母親から出生し，甲状腺機能低下を示した母乳哺育児のヨウ素摂取量にもとづき策定されている[1]。したがって，間欠的と考えられる離乳食からの高ヨウ素摂取が，母乳からの連続的摂取と同様に，ただちに甲状腺機能低下を起こすとは考えにくい。しかし，離乳食開始月齢が以前よりも相当早期となっていること，および以前の離乳食は重湯などのように味付けをほとんど行っていなかったことを考慮すると，このような高ヨウ素離乳食の1歳未満乳児への投与の歴史は新しいと考えられる。体質的にヨウ素の影響を受けやすい乳児が存在するという指摘もあることから[5]，とくに昆布だしを使用した離乳食の摂取がもたらす高ヨウ素摂取の影響については注視することが必要だろう。

一方，ヨウ素摂取量推定値の中央値は，6～8か月児が89 μg/d，9～11か月児が84 μg/dであった。平均的なヨウ素濃度の母乳からのヨウ素摂取を6～8か月児で約80 μg/d（133 ng/mL × 600 mL/d），9～11か月児で約60 μg/d（133 ng/mL × 450 mL/d）と見積もっていることから，この中央値は，離乳食の半数以上が20 μg/d未満のヨウ素しか供給できないことを意味している。つまり，母乳と合わせて目安量相当のヨウ素摂取を実現できる離乳食はきわめて少なく，現実には間欠的に摂取する高ヨウ素濃度の離乳食によって必要なヨウ素量を確保しているともいえる。さらに，乳幼児期に昆布だしを摂取させることは，日本人としての健全な味覚の発達にとっては必要と思われる。したがって，市販離乳食においては，昆布だしの使用を控えるのではなく，昆布だしやヒジキなど海藻類を使用している場合は，ヨウ素濃度を明記し，適切な利用回数を示すな

Table 4 Estimation of iodine and chromium intake from commercial baby foods and breast milk in 6 to 8 or 9 to 11 month infants

	Iodine (μg/d)		Chromium (μg/d)	
	6 to 8 months (n=24)	9 to 11 months (n=29)	6 to 8 months (n=24)	9 to 11 months (n=29)
Minimum	85	72	2	3
25 percentile	87	77	3	6
Median	89	84	4	9
75 percentile	110	858	6	12
Maximum	562	4349	68	29
Mean	144	691	8	10
Geometrical mean	116	224	5	9
Criteria in DRI-J 2010*				
Adequate intake	130		1	
Tolerable upper intake level	250		-	

* Dietary Reference Intakes for Japanese, 2010.

どの対応を行うべきだと考える。同様に，家庭における離乳食に対しても，昆布だしの適切な使用頻度について一定の目安を示すことが必要であろう。

なお，繰り返しになるが，乳幼児も含めて日本人は昆布を利用することによって適切なヨウ素摂取を確保している。前段とはやや矛盾した表現にはなるが，現実にヨウ素過剰障害の報告がほとんどない事実をふまえれば，おそらく現在の日本人の昆布の利用と摂取頻度に大きな問題はないものと判断できる。ただし，このような経験的事実を数値によって裏付け，そして高摂取にも関わらず過剰障害が生じない理由を解明することが研究者に課せられた使命であると思う。

他方，市販離乳食のクロム濃度は試料間の差が小さく，大半が湿重量あたり 20 ng/g 未満だった。唯一 100 ng/g を超えたのは「豆腐と野菜とひき肉のあんかけ」だったが，他の豆腐や肉類を使用した離乳食のクロム濃度が低値であることを考慮すると，この高クロム濃度は離乳食製造過程，もしくは分析中の汚染である可能性が高い。母乳と市販離乳食を摂取した場合のクロム摂取量推定値は，平均で約 10 μg/d であり，75 パーセンタイル値もこれに近い値だった。病院普通食の分析結果[9]や一般家庭食事の分析例[10]からは，成人のクロム摂取量が 20～70 μg/d と考えられることから，今回の市販離乳食を利用する乳児のクロム摂取量の推定値（約 10 μg/d）はほぼ妥当なものと判断できる。この値は，母乳のみの摂取を想定して策定されている食事摂取基準における 6 か月以降乳児に対するクロムの目安量（1 μg/d）の約 10 倍である。したがって，マンガンやモリブデンと同様に[2]，クロムは離乳食を開始することによってその摂取量が急激に増加するといえるだろう。ただしクロムに関しては，必須元素ではないという報告も提出されている[11]。したがって，離乳食からのクロム摂取量については，極端な高摂取が生じる可能性がないことを確認するだけで十分かもしれない。

本研究は，平成 23 年度厚生労働科学研究費補助金（循環器疾患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関する総合的研究）により行われたものである。

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日本人の食事摂取基準における目安量は健康人の摂取の中央値でよいのか？

Adequate intake (AI) for vitamin D; how is it to be determined?

本稿はトピックスの原稿として書いたものであるが，最近の話題を取り上げて概説するという本来のトピックスではない．著者らがふだん疑問に感じている点を述べて，ビタミン学会会員諸先生のご意見を承りたいという意図で書いたものであり，いわば著者らの個人的見解を述べたものであることを，最初におことわりしておく．

ビタミンの欠乏により，古典的欠乏症が起こる．ビタミンB_1欠乏による脚気・ウェルニッケ脳症，ナイアシン欠乏によるペラグラ，ビタミンC欠乏による壊血病，ビタミンK欠乏による出血傾向など，多数の有名な例がある．少なくともわが国においては，これらの多くはほぼ克服されたといってよいが，近年ビタミンの新たな意義が注目されている．

古典的な欠乏症を起こすほどのビタミン欠乏（deficiency）より軽度のビタミン不足（insufficiency）であっても，疾患のリスクが増加する．

ビタミンDを例に述べると，骨はタンパク質（主にコラーゲン）の枠組みの上に，リン酸カルシウムが沈着して（石灰化）形成される．ビタミンDの最も基本的な作用は，腸管からのカルシウム・リン吸収促進なので，ビタミンD欠乏の結果，石灰化障害が起こるのがクル病・骨軟化症である．

一方，より軽度の不足の場合，石灰化障害は起こらないが，骨粗鬆症・骨折のリスク増加が起こる．副甲状腺ホルモン（PTH）とビタミンDは血清カルシウム濃度を維持するのが重要な役割であり，PTHはビタミンDの活性化を促進し，活性型ビタミンDはPTH分泌を抑制するという形で，両者は協調して，血清カルシウム濃度を維持するのに作用するが，作用が過剰となって，高カルシウム血症を起こさないように，フィードバック調節を受けている．したがって，ビタミンD不足状態においては，PTH分泌が亢進し，それによって骨吸収が亢進して，骨粗鬆症をきたす．なお，ビタミンD栄養状態の最もよい指標は，血中25-ヒドロキシビタミンD（25OHD）濃度である．

わが国において，残念ながらまだまだ，ビタミン不足の重要性が十分認識されているとは言いがたい状況であるが，その重要性がわかりにくいのも事実である．脚気・クル病・壊血病と列挙すれば明らかなように，古典的欠乏症は外見上の異常を伴うので，個々の人ごとにその有無が判定できる．しかし，ビタミンD不足の場合，外見上は何の異常もなく，疫学調査により初めて疾患リスクが増加していることが明らかになる．

食事摂取基準においても，改訂の度に徐々にビタミンD不足の意義が考慮されるようになってきたと思われる．例えば，第六次改訂日本人の栄養所要量においては，「20～46歳の人で，1.7μg（68 IU）/日以下のビタミンD摂取を数年間続けると骨軟化症が認められるようになり，2.5μg（100 IU）/日では発生はみられなかったとの報告があるので，2.5μg（100 IU）/日とした」と述べられており，この記述はクル病・骨軟化症防止を念頭においた，欠乏症対策と理解される．しかし，現行の2010年版においては，「成人，とくに高齢者において，ビタミンD欠乏とはいえないもののビタミンD不足の状態が長期にわたって続くと，血中副甲状腺ホルモン濃度が上昇し，骨密度が低下する．したがって，正常なカルシウム利用能が保持され，血中副甲状腺ホルモン濃度が上昇しない血中25-ヒドロキシビタミンD濃度を維持するのに必要な量のビタミンDを摂取することが，骨折や骨粗鬆症などの予防の観点から重要と考えられる．しかし，その血中濃度を与えるビタミンD摂取量に関する根拠は乏しいため，その血中濃度を維持していると考えられる集団のビタミンD摂取量の中央値を目安量とした」との記述がみられ，これは明らかに，ビタミンD不足による骨折リスク増加対策をも意識したものである．このように，不足をも考慮する時代になると，目安量の策定において，欠乏対策だけを考えていた時代にはなかった，新たな問題点を生じてきたのではないかというのが，著者らが最近考えていることである．

目安量の策定理論に関して，2010年版の記述を引用すると，「特定の集団において，生体指標等を用いた健康状態の確認と当該栄養素摂取量の調査を同時に行い，その結果から不足状態を示す者がほとんど存在しない摂取量を推測し，その値を用いる．対象集団で不足状態を示す者がほとんど存在しない場合には栄養素摂取量の中央値を用いる．」とされている．

ビタミンDに関する具体的な数字として，「成人において血中副甲状腺ホルモン濃度の上昇を抑制し，骨密度の低下を予防するのに最低限必要な血中25-ヒドロキシビタミンD濃度は50 nmol/L前後であると考えられる」と書かれている．

表1は，食事摂取基準2010年版のビタミンDの項に示されている表を改変引用したものである．確かにここで引用されている論文において，50～69歳の集団における平均25OHD濃度は50 nmol/L（20 ng/mL）を越えているが，平均値が50 nmol/L（20 ng/mL）を越えているからといって，「特定の集団において不足状態を示す人がほとん

表1　日本人女性を対象として血清25-ヒドロキシビタミンD濃度を測定した報告

文献	人数	調査地域	血清25ヒドロキシビタミンD濃度 (nmol/L)	50 nmol/L 未満者の割合	対応する年齢階級のビタミンD摂取量中央値(μg/日)
49	24	新潟(9月)	83±22	6.7%	5.7
48	7	新潟(2月)	54.7±9.4	30.9%	
54	244	長野(限定せず)	50.1±13.6	49.7%	
49	70	新潟(9月)	80±16	3.0%	
50	122	新潟(9月)	78.6±18.2	5.8%	
50	122	新潟(2月)	59.7±17.1	28.5%	
51	151	新潟(2月)	59.9±17.0	28.0%	
52	117	新潟(2月)	59.1±16.1	28.6%	
53	600	新潟(11月)	55.6±14.6	35.1%	

日本人の食事摂取基準2010年版[1]より改変引用した.「50 nmol/L未満者の割合」は,正規分布するものと仮定して,平均値・標準偏差に基づき,著者らが計算したものである.

ど観察されない」と言えるのであろうか.仮に25OHD濃度が正規分布するものとして,摂取基準の表に示されている平均±標準偏差の値に基づき,50 nmol/Lを下回る対象者の割合を概算した結果を,「50 nmol/L未満者の割合」として加筆した.一見して明らかに,多くの文献において,50 nmol/L未満者の割合はかなり高い値であった.するとこれらの集団はビタミンDが充足している集団であるから,それに対応する性・年齢階級における摂取の中央値をもって目安量としてもよいのだろうかという疑問が生じてくる.

そうすると,ビタミンDの摂取基準策定にどういう方法論を用いることができるのであろうか.2010年カルシウム・ビタミンDについて,アメリカ・カナダの食事摂取基準が全面改訂された.方法論からみると,目安量ではなく,推定平均必要量(EAR)・推奨量(RDA)に変わったのが大きな変化である.これらは欠乏・充足実験によって定められるのが原則であり,EARは当該集団において50%の人が必要量を満たす量,RDAはほとんどの人(97～98%)が満たす量として定められる.このアメリカ・カナダの食事摂取基準においては,骨の健康を維持するための血中25OHD濃度(50 nmol/L)を基に,ビタミンDの摂取量が算定されており,RDAは,血中25OHD濃度と摂取量の容量依存性試験の結果から定められている.アメリカ・カナダの食事摂取基準におけるRDAは,1歳から70歳まで600 IU/日となっている[2].一方わが国の平成19年国民健康栄養調査の結果を見ると,成人で600 IU/日摂取できているのは,集団の上位10%に限られているのが実際であり[3],この数字を日本に適用できるのかどうかについては,多くの議論が必要であるが,ビタミン不足をも考慮した場合,この策定の方法論は注目すべきものと思われる.

誤解のないように一言述べておくと,著者らは2010年版に定められた目安量の値が不適切であると述べているのではなく,従来の目安量の概念に従って策定するならば,現行の摂取基準のような定め方にならざるを得ないであろう.しかし,栄養素の不足をも考慮した場合の摂取基準策定において,その摂取の中央値をもって目安量とすることができるような健常人というものはそもそも存在するのであろうか.一見して外見上の異常を伴わない対象者であっても,不足のリスクの低い人から高い人まで連続的に分布すると考える方がより摂取基準の精神に合致するように思われてならない.

ビタミン不足をも考慮しなければならない時代においては,目安量策定の方法論に関して,再検討の時期が来ているのではないだろうか.

Key Words：Dietary reference intakes, adequate intake, vitamin D, median, osteoporosis

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Nutrients **2010**, *2*, 752-761; doi:10.3390/nu2070752

OPEN ACCESS

nutrients

ISSN 2072-6643

www.mdpi.com/journal/nutrients

Article

Fractional Absorption of Active Absorbable Algal Calcium (AAACa) and Calcium Carbonate Measured by a Dual Stable-Isotope Method

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Received: 10 June 2010; in revised form: 2 July 2010 / Accepted: 8 July 2010 / Published: 12 July 2010

Abstract: With the use of stable isotopes, this study aimed to compare the bioavailability of active absorbable algal calcium (AAACa), obtained from oyster shell powder heated to a high temperature, with an additional heated seaweed component (Heated Algal Ingredient,

HAI), with that of calcium carbonate. In 10 postmenopausal women volunteers aged 59 to 77 years (mean ± S.D., 67 ± 5.3), the fractional calcium absorption of AAACa and $CaCO_3$ was measured by a dual stable isotope method. ^{44}Ca-enriched $CaCO_3$ and AAACa were administered in all subjects one month apart. After a fixed-menu breakfast and pre-test urine collection (Urine 0), ^{42}Ca-enriched $CaCl_2$ was intravenously injected, followed by oral administration of ^{44}Ca-enriched $CaCO_3$ without carrier 15 minutes later, and complete urine collection for the next 24 hours (Urine 24). The fractional calcium absorption was calculated as the ratio of Augmentation of ^{44}Ca from Urine 0 to Urine 24/ augmentation of ^{42}Ca from Urine 0 to Urine 24. Differences and changes of ^{44}Ca and ^{42}Ca were corrected by comparing each with ^{43}Ca. Fractional absorption of AAACa (mean ± S.D., 23.1 ± 6.4), was distinctly and significantly higher than that of $CaCO_3$ (14.7 ± 6.4; $p = 0.0060$ by paired t-test). The mean fractional absorption was approximately 1.57-times higher for AAACa than for $CaCO_3$. The serum 25(OH) vitamin D level was low (mean ± S.D., 14.2 ± 4.95 ng/ml), as is common in this age group in Japan. Among the parameters of the bone and mineral metabolism measured, none displayed a significant correlation with the fractional absorption of $CaCO_3$ and AAACa. Higher fractional absorption of AAACa compared with $CaCO_3$ supports previous reports on the more beneficial effect of AAACa than $CaCO_3$ for osteoporosis.

Keywords: active absorbable algal calcium (AAACa); calcium carbonate; dual stable Ca isotope method; fractional absorption (FA); parathyroid hormone (PTH)

1. Introduction

Active absorbable algal calcium (AAACa) prepared from heated oyster shell and seaweed is a unique calcium supplement with high bioavailability, with a characteristic lamellar crystalline structure quite unlike that of calcium oxide and calcium carbonate ($CaCO_3$) [1]. In the Katsuragi Calcium study, a prospective, randomized, double blind and placebo-controlled study compared the effect of AAACa on osteoporosis with that of $CaCO_3$ in hospitalized women with a mean age of 80 years. It was found that AAACa alone increased spinal bone mineral density significantly over the level in subjects given a placebo, whereas $CaCO_3$ did not [2,3]. Fracture occurrence over the two year test period from among 58 subjects was 0 of 5 in the AAACa Group, 2 of 7 in the $CaCO_3$ Group and 3 of 5 in the Placebo Group, on evaluation of all X-rays available at the beginning and end of the test period. The AAACa Group exhibited a significantly lower rate of fracture occurrence than the placebo group, but the $CaCO_3$ Group showed no significant difference from placebo group. Serum parathyroid hormone (PTH) was also suppressed more efficiently by AAACa than $CaCO_3$.

Despite all these indirect lines of evidence indicating a high bioavailability of AAACa, a direct absorption test by a dual isotope method has not been conducted to date. We have therefore attempted to measure the fractional absorption of AAACa by using the dual stable-isotope method [4,5] to

compare it with $CaCO_3$ in subjects in the age group most likely to need effective calcium supplementation to maintain their bone health: postmenopausal women.

2. Experimental Section

2.1. Subjects

Ten postmenopausal women between 59 and 77 years of age (mean ± SD, 67 ± 5.3 years) leading a normal healthy daily life without any known disease possibly affecting bone and mineral metabolism volunteered to participate as test subjects in the present study by providing written consent (Table 1). One subject, shown in parenthesis in Tables 1 and 2, was dropped from analysis because of a measured fractional absorption (FA) value of 0% on giving $CaCO_3$. The Institutional Review Board of the Fujii Medical Clinic approved the study.

Table 1. Background of the test subjects.

No.	Age	Years after menopause	Height (cm)	Weight (kg)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
1	68	19	154	54	138	80
2	72	23	147	50	142	62
3	65	15	157	63	148	70
4	65	13	148	43	125	70
(5) *	(59)	(9)	(153)	(60)	(133)	(88)
6	59	8	152	58	152	85
7	65	13	151	56	150	85
8	77	28	150	48	142	80
9	64	15	145	50	140	90
10	71	19	148	48	122	70
Mean	67	17	150	52	139	76
SD	5.3	6.0	3.7	6.1	10.4	9.3

* Case No. 5 was not included in the statistical analysis.

2.2. Background Data of the Test Subjects

In order to assess the metabolic background of the test subjects, serum Ca, P, albumin, creatinine, BUN, 25(OH)vitamin D, intact parathyroid hormone (PTH), bone specific alkaline phosphatase (BAP), urinary N-terminal type I collagen fragments (NTx) and urinary calcium/ creatinine ratio (UCa/ Cr) were measured prior to the test. The laboratory tests related to bone and calcium metabolism gave results approximately within the normal range, as shown in Table 2, except for one subject, who had a serum 25(OH) vitamin D level in the insufficiency range (7.6 ng/mL). This subject was without symptoms and signs of vitamin D insufficiency such as hypocalcemia, hypophosphatemia, high alkaline phosphatase, muscle weakness and bone pain.

Table 2. Parameters of mineral and bone metabolism of the test subjects.

No.	Serum Ca	Serum P	Serum albumin	Serum creatinine	BUN	25(OH) vitamin D	Intact PTH	BAP	Urine NTx/Cr	Urine Ca/Cr
	mg/dL	mg/dL	g/dL	mg/dL	mg/dL	ng/dL	pg/dL	U/L	nMBCE/ mMCr	mg/mg
1	9.7	3.9	4.4	0.83	11.0	16.8	48	15.2	32.2	0.03
2	9.5	4.5	4.0	0.80	21.9	16.9	31	15.1	16.0	0.06
3	9.7	3.1	4.6	0.71	12.1	11.6	40	35.8	31.1	0.45
4	10.3	3.5	5.1	0.49	12.5	11.7	44	32.4	35.9	0.36
5 *	(9.5)	(3.4)	(4.5)	(0.74)	(14.2)	(21.8)	(61)	(21.6)	(29.0)	(0.08)
6	9.8	3.5	4.7	0.74	17.8	15.4	44	19.1	23.0	0.12
7	9.3	4.4	4.4	0.60	17.8	24.7	42	17.0	42.2	0.22
8	9.9	2.9	4.6	0.59	13.5	7.6	50	34.9	34.5	0.17
9	9.8	3.7	4.4	0.74	13.7	12.7	34	27.5	16.3	0.20
10	9.5	3.3	4.2	0.54	13.6	10.8	34	45.9	21.9	0.30
Mean	9.7	3.6	4.5	0.67	14.9	14.2	41	27.0	28.1	0.21
SD	0.29	0.55	0.31	0.119	3.53	4.95	6.59	11.02	9.20	0.138

Ca: calcium; P: phosphorus; BUN: Blood urea nitrogen; PTH: parathyroid hormone; BAP: Bone specific alkaline phosphatase; BCE: Bone collagen equivalent.

* Case No. 5 was not included in the statistical analysis

2.3. Materials

The first part of the test was performed on March 9, 2009, using ^{44}Ca-enriched $CaCO_3$ for oral load and ^{42}Ca in the form of $CaCl_2$, for intravenous injection (Table 3). On April 13, 2009, after one month, exactly the same procedure was repeated on the same test subjects, except for the use of ^{44}Ca-enriched AAACa in the place of $CaCO_3$ to ensure the stable isotope constituent of the body reached equilibrium. Intrinsic labeling is no doubt ideal, but it is impossible to label the shell of oysters abiding in the ocean, so an extrinsic labeling was adopted as the best substitute for it. The material for AAACa was obtained by heating oyster shell to 1,000 ^{o}C, resulting mostly in CaO powder after losing much of the organic components. To 5,082 mg of this CaO powder, 450.4 mg CaO Ca fraction was added that consisted of 95.9 ± 0.3% ^{44}Ca supplied by TRACE SCIENCES INTERNATIONAL (Ontario, Canada), and was thoroughly mixed in a melting pot. Aqueous solution of a small amount of algal component was pre-heated at a high temperature in a manner similar to the oyster shell to start a chemical reaction lasting for about 10 minutes. After sufficient stirring, it was divided into small portions for actual use and preserved in vacuum. The final product mostly consisted of $Ca(OH)_2$.

$CaCO_3$ labeled with ^{44}Ca was also obtained from the same source (the Ca fraction consisting of 95.9 ± 0.3% ^{44}Ca). To 781.6 mg of this material, 9,075 mg $CaCO_3$ (Japanese Pharmacopeia) was added, thoroughly mixed, and divided into small proportions and stored.

AAACa particle mean size was 5.8 microns; maximum size was 75 microns and $CaCO_3$ particle size ranged from 10 to 20 microns. As these values are based on different occasions of measurements they may not be directly comparable, but appears to lie over a similar range. If anything, a larger size is compatible with slower absorption.

For two subjects, part of the first urine sample was lost; in these cases, both parts of the test were repeated on July 30 and August 27, and the data from the uneventfully performed second set of tests were used to replace those of the first set.

The safety of the intravenous injection of $CaCl_2$ was verified before the study by the absence of any signs of toxicity such as chills, fever, neuromuscular irritability, skin eruptions, disturbance of consciousness, *etc.*

Table 3. Amount of isotope Ca (mg) per subject in 1 study.

Isotope	Oral						IV
	CaX			CaY			Total
	Supplied	Added	Total	Supplied	Added	Total	
^{42}Ca	0.01	1.79	1.80	0.00	1.79	1.79	3.192
^{43}Ca	0.01	0.39	0.40	0.00	0.39	0.39	0.0037
^{44}Ca	25.38	5.52	30.90	25.38	5.53	30.91	0.0334

The contents of Ca isotopes in the material used for the preparation of CaX and CaY on arrival from the supplier (Supplied), their contents in the material added to prepare samples for administration (Added) and the final total (Total) are indicated in Table 3.

A total of approximately 300 mg of Ca containing approximately 30 mg ^{44}Ca isotope (25 + 5) was orally administered to each subject and about 3 mg ^{42}Ca isotope was injected before the study and no symptoms and signs of toxicity were reported.

2.4. Test Procedure

After taking a fixed menu breakfast consisting of fruit juice, toast, eggs and coffee, a pre-test urine sample was collected (Urine 0) and ^{44}Ca-enriched $CaCO_3$ was orally administered followed by the intravenous injection of ^{42}Ca-enriched $CaCl_2$ 15 minutes later. A complete collection of 24 h urine followed (Urine 24). After one month to ensure clearance of the enriched isotope, exactly the same procedure, except for the use of ^{44}Ca-enriched AAACa instead of ^{44}Ca-enriched $CaCO_3$, was repeated.

2.4.1. Measurement of the Stable Isotope

Sample preparation for isotope enrichment measurement was conducted according to the method of Patterson *et al*. [6]. By using the inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500 cs, Agilent Technologies, Inc., Tokyo), ^{42}Ca, ^{43}Ca, ^{43}Ca and other measurable stable Ca isotopes were measured in both Urine 0 and Urine 24. Utilizing ^{43}Ca as an internal standard of the stable Ca

isotopes, the ratio of each stable Ca isotope to ^{43}Ca was calculated. The increase of the $^{42}Ca/^{43}Ca$ and $^{44}Ca/^{43}Ca$ in Urine 24 above the pretest natural abundance level for each test subject over the corresponding value in Urine 0 was then obtained. By dividing the ratio of the actual amount of the enrichment of ^{44}Ca by the corresponding amount of the enrichment of ^{42}Ca from Urine 0 to Urine 24, the FA of the ^{44}Ca-enriched material was obtained; for $CaCO_3$ in the first part of the test and AAACa in the second part (Table 3).

2.5. Statistical Analysis

The Excel Statistical Package was used to compare the FA of $CaCO_3$ and AAACa by paired t-test. A correlation matrix among the FA data, age and parameters of bone and mineral metabolism was constructed and evaluated by the Spearman method in view of the inclusion of variables with uncertain distribution. The p values < 0.05 were considered significant.

3. Results and Discussion

As shown in Table 4, the mean Fractional absorption (FA) of AAACa, 23.1 ± 6.4%, was 1.57-times higher than the corresponding value of $CaCO_3$, 14.7 ± 6.4%, with a significant difference at p = 0.0060 determined using paired t-test.

Table 4. Fractional absorption (FA) of CaCO3 and AAACa by dual stable isotope method.

Subject	FA CaCO3	FA AAACa
1	7.5	21.1
2	20.0	29.7
3	21.9	34.7
4	19.6	20.4
(5) *	(0.0)	(18.8)
6	6.1	22.7
7	14.3	24.9
8	11.7	11.9
9	8.7	22.1
10	22.2	20.4
Mean	14.7	23.1
SD	6.4	6.4

Paired comparison between FA CaCO3 and FA AAACa

p = 0.0060, t = 3.708 (paired t-Test)

* Subject 5 was not included in the statistical analysis.

According to the evaluation by means of the correlation coefficient matrix (Spearman) (Table 5) among the parameters of bone and mineral metabolism summarized in Table 2, no significant correlation was found between the FA of either calcium carbonate or AAACa and each parameter. In the subject with the lowest serum 25(OH) vitamin D of 7.6 ng/mL, the FA of $CaCO_3$ value was medium in the group, *i.e.*, 11.7%, fifth from the lowest, and the FA of AAACa, 11.9%, was the lowest in the group.

Until the advent of the dual isotope method, the true FA of calcium was extremely difficult to measure due to the complex behavior of calcium in living organisms, such as the rapid exchange through multiple Ca pools and various pathways of exit and reentrance [7,8]. Utilizing the presence of multiple stable isotopes in nature, the dual stable isotope method was developed to circumvent this complexity, and it is the only method of directly measuring the fractional intestinal Ca absorption.

Abrams and coworkers as well as other investigators [12-23] have used this method extensively to estimate calcium absorption, establishing it as the gold standard for calcium absorption. Since calcium absorption is influenced by age and the state of bone, as well as mineral metabolism, a correlation matrix was constructed and evaluated by Spearman's method (Table 5). None of the metabolic parameters tested exhibited significant correlation with FA of the calcium compounds. Absence of significant correlation between FA of calcium compound and age was expected because of the narrow age range of this group.

The FA of Ca compounds obtained in this study of postmenopausal women, with a mean age of 66 years and with a tendency of low 25(OH) vitamin D, appears to be much lower than those observed in children and younger subjects: FA; 54.8–63.1% [21], 58.2–64.3% [22], and also younger postmenopausal women with mean age of 56: FA; 34.6–39.1% [23]. In healthy volunteers between 25 to 45 years much lower values, yet still higher than the results in the present study, were reported: FA; 26–31% [24]. The reduced FA in the current study subjects could also be due to reduced estrogen level after menopause. FA is, thus, markedly influenced by age. The age range of the test subjects was quite narrow in this group of subjects, unsuitable for the assessment of the age-FA correlation. Statistically, the tendency of age-FA correlation was non-significant.

Table 5. Spearman's correlation matrix and correlation coefficients among fractional absorption (FA) and parameters of bone and mineral metabolism.

	FA CaCO3	FA AAACa	Age	SCa	SP	Salb	Cre	BUN	25D	PTH	BAP	UNTx	UCa/Cr
FA CaCO3	1.0000	0.1423	0.4238	−0.3882	−0.1925	−0.3405	−0.4854	−0.0335	−0.3167	−0.5630	−0.4833	−0.1333	0.6333
FA AAACa		1.0000	−0.3490	−0.5297	0.4328	−0.2137	0.5042	0.3445	0.5690	−0.5232	−0.3766	−0.2762	0.0418

SCa: serum calcium; SP: serum phosphate; Salb: serum albumin; Cre: creatinine; BUN: blood urea nitrogen; 25D: 25(OH)vitaminD; PTH: parathyroid hormone; BAP: bone specific alkaline phosphatase; UNtx: urine N-terminal type I collagen fragments; UCa/Cr: urinary Ca/creatinine ratio

Although these subjects are reasonably homogeneous and apparently free of any comorbidity, which could potentially influence the test results, the present study is limited by the small number of test subjects. Unlike similar studies conducted in this field in the past, post-menopausal women – who need calcium supplementation most because of high risk of osteoporosis – were asked to participate. A rather low intra-group variation was encouraging, and a clear-cut difference in FA between the two test materials may also add to the credibility of the conclusion.

It is possible that the difference in molecular weight and physicochemical properties of the ^{44}Ca-enriched $CaCO_3$ and AAACa, mostly consisting of $Ca(OH)_2$ as the result of oxidation of $CaCO_3$

obtained from oyster shell, cannot be completely ruled out. The similar molecular size and comparable particle size actually measured as 5.8 to 75 for AAACa and 10 to 20 for $CaCO_3$ and this is a limitation of the result but should not have affected the primary outcome. In view of the similar molecular size and physicochemical properties between $CaCO_3$ and AAACa, both much smaller than organic Ca salts, however, confounding effect exerted on the calculation of the absorptive rate is rather unlikely and the conclusion of difference in the absorption rate between the two compounds should be reasonably supported.

4. Conclusions

This study aimed to compare the bioavailability of active absorbable algal calcium (AAACa), oyster shell powder heated to a high temperature, with an additional heated seaweed component (Heated Algal Ingredient, HAI), with that of calcium carbonate. The Fractional absorption of AAACa, (mean ± S.D.; 23.1 ± 6.4) was distinctly and significantly higher than that of $CaCO_3$ (14.7 ± 6.4; $p = 0.0060$ by paired t-test). The mean was approximately 1.57-times higher for AAACa than $CaCO_3$. Higher fractional absorption of AAACa compared with $CaCO_3$ supports previous reports on the more beneficial effect of AAACa than $CaCO_3$ on osteoporosis.

Acknowledgements

Kazuo Chihara MD, former Dean, Kobe University School of Medicine, encouraged and supported the study. Fujix Corporation provided ^{42}Ca and ^{44}Ca enriched compounds, imported by Taiyo Nippon Sanso Corporation and Kawaguchi Liquefaction Chemical Corporation, clearing up all the difficult official procedures.

The authors have no conflict of interest.

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Original Article

Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly

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There have been methodological problems for studying hypovitaminosis D and K in the elderly. First, studies were done either by evaluating food intake or measuring their circulating levels, but rarely by both in Japan. In this paper, vitamin D and K intakes and their circulating levels were simultaneously determined. Second issue is whether hypovitaminosis D and K are independent of general malnutrition, prevalent in the elderly. We tried to statistically discriminate them by principal component analysis (PCA). Fifty institutionalized elderly were evaluated for their circulating 25 hydroxy-vitamin D (25OH-D), intact parathyroid hormone (PTH), phylloquinone (PK), menaquinone-7 (MK-7) levels, and their food intake. Although average vitamin D intake (7.0 μg/day) exceeded the Japanese Adequate Intake (AI) of 5.0 μg/day, average serum 25OH-D concentration was in the hypovitaminosis D range (11.1 ng/mL). Median vitamin K intake was 168 μg/day, approximately 2.5 times as high as AI for vitamin K. Nevertheless, plasma PK and MK-7 concentrations were far lower than those of healthy Japanese elderly over 70 years old. PCA yielded four components; each representing overall nutritional, vitamin K_2, vitamin D, and vitamin K_1 status, respectively. Since these components are independent of each other, vitamin D- and K-deficiency in these subjects could not be explained by overall malnutrition alone. In summary, institutionalized elderly had a high prevalence of hypovitaminosis D and K, and the simultaneous determination of their circulating level and dietary intake is mandatory in such studies. PCA would yield fruitful results for eliminating the interference by confounders in a cross-sectional study.

Key Words: hypovitaminosis D, hypovitaminosis K, principal component analysis, adequate intake, institutionalized elderly

INTRODUCTION

Vitamin D is of utmost importance in enhancing the intestinal absorption of calcium and phosphorus,[1,2] with its deficiency causing skeletal mineralization defect; rickets and osteomalacia. Recently, it has come to the general attention that inadequate supply of vitamin D, even in its milder form (vitamin D insufficiency), is associated with increased risk of fracture through negative calcium balance, hence secondary hyperparathyroidism.[1,2] Vitamin D insufficiency is also reported to be associated with muscle weakness. Recent clinical studies have indicated that intervention with vitamin D supplementation reduced the incidence of falling in elderly subjects.[3] Clinically important non-vertebral fractures, such as hip and wrist fractures are triggered by falling. Thus, vitamin D insufficiency would render the elderly subjects more prone to fracture through its effects both on the skeleton and muscle. Recently, lower serum level of 25 hydroxy-vitamin D (25OH-D) was reported to be a significant risk factor even for mortality.[4]

Vitamin D insufficiency is quite common in the elderly population,[5,6] and institutionalized elderly are at even higher risk for vitamin D insufficiency.[7-10] Factors hitherto postulated to be responsible include low dietary vitamin D intake,[7,9] reduced dermal capacity to produce vitamin D with aging and minimal sun exposure.[11,12]

In contrast to vitamin D, the skeletal action of vitamin K has called our attention only quite recently. The only biological action of vitamin K has been considered to be its role as the coenzyme of γ-glutamyl carboxylase (GGCX) in the liver, by which additional carboxyl group is introduced into the glutamic acid residue in four of the

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Manuscript received 8 July 2009. Initial review completed 13 October 2009. Revision accepted 23 November 2009.

Table 1. Background profiles and results from blood tests of the study subjects

	Total	Male	Female	*p* value
n	50	15	35	-
Age (y)	87.6±8.0 (88.5)	84.9±7.9 (83.0)	88.7±7.8 (90.0)	0.133
Level of care needed	3.6±1.1 (4.0)	3.3±1.0 (3.0)	3.7±1.2 (4.0)	0.228
Body height (cm)	144.0±11.6 (142.0)	157.0±7.8 (159.0)	138.4±7.8 (139.0)	<0.01
Body weight (kg)	43.6±9.3 (43.2)	50.3±7.9 (49.9)	40.7±8.3 (38.1)	0.001
Body mass index (kg/m2)	21.0±3.8 (20.1)	20.5±3.4 (19.6)	21.3±4.0 (20.2)	0.476
Serum albumin (g/dL)	3.7±0.4 (3.7)	3.8±0.4 (3.9)	3.6±0.4 (3.6)	0.136
Serum total cholesterol (mg/dL)	184±37 (184)	186±26 (195)	183±41 (183)	0.828
Serum triglyceride (mg/dL)	98±41 (92)	96±47 (75)	98±39 (93)	0.403
Serum aminotransferase (U/L)	22±11 (19)	20±7 (17)	22±12 (19)	0.603
Serum alanine aminotransferase (U/L)	16±10 (13)	16±7 (13)	16±12 (12)	0.235
eGFR (mL/min./1.73m2)	61±20 (60)	67±19 (67)	59±21 (57)	0.208
Serum 25-hydroxyvitamin D (ng/mL)	11.1±3.1 (11.2)	10.3±3.5 (9.3)	11.5±3.0 (11.6)	0.274
Serum parathyroid hormone (pg/mL)	30.8±11.8 (30.0)	29.9±11.1 (31.0)	31.3±12.2 (30.0)	0.736
Plasma phylloquinone (ng/mL)	0.73±0.70 (0.58)	0.62±0.29 (0.60)	0.77±0.82 (0.53)	0.992
Plasma menaquinone-7 (ng/mL)	0.53±0.37 (0.45)	0.59±0.47 (0.47)	0.51±0.32 (0.44)	0.849

Data are expressed as mean±SD with the values in parentheses showing the median.
Comparison of indices between males and females were done by unpaired t test or Mann-Whitney test depending on normality.
eGFR; estimated Glomerular Filtration Rate.

Table 2. Daily dietary intakes of the study subjects

	Total	Male	Female	*p* value
Energy (kcal)	1322±159 (1387)	1374±96 (1416)	1300±175 (1386)	0.160
Protein (g)	51.0±5.8 (53.3)	53.1±3.6 (54.6)	50.2±6.3 (53.5)	0.091
Fat (g)	32.8±3.9 (34.6)	34.2±2.4 (35.3)	32.2±4.3 (34.5)	0.095
Carbohydrates (g)	178±20 (186)	185±12 (189.7)	175±21 (186)	0.093
Calcium (mg)	494±53 (504)	503±50 (506)	490±54 (502)	0.157
Vitamin D (μg)	7.0±1.4 (7.7)	7.4±0.9 (7.8)	6.9±1.5 (7.6)	0.107
Vitamin K (μg)	155±30 (168)	164±19 (172)	151±33 (168)	0.107

Data are expressed as mean±SD with the values in parentheses showing the median. Comparison of indices between male and women were done by unpaired t test or Mann-Whitney test depending on normality.

blood coagulation factors (II, VII, IX, X) to yield γ-glutamic carboxyl (Gla) residue.[13] Other extrahepatic proteins are also γ-carboxylated by GGCX, such as osteocalcin (bone Gla protein; BGP) and matrix gla protein (MGP).[14] Recent evidences suggest that vitamin K deficiency is associated with increased risk of fracture. When subjects were categorized into quartiles according to their vitamin K intake, fracture risk in the lowest quartile was twice as high as that in the highest quartile.[15] The age-adjusted incidence of vertebral fracture was significantly higher in subjects with low plasma phylloquinone levels than those with high plasma levels in Japanese women.[16] In addition, the association of circulating vitamin K level and bone mineral density (BMD) has also been reported. For example, low plasma phylloquinone concentration was associated with low BMD at the femoral neck in men, and lower spine BMD in postmenopausal women without estrogen replacements.[17] High serum concentration of undercarboxyled osteocalcin (ucOC), which is a sensitive indicator of skeletal vitamin K insufficiency, was a significant risk factor of hip fracture independent of BMD.[18,19]

Plasma phylloquinone level is subject to alteration by aging,[20,21] and elderly subjects have been reported to have low plasma phylloquinone concentrations.[22] Of note is the report that elderly nursing home residents generally had a poor dietary vitamin K intake compared to the ambulatory elderly.[23]

Studies on the role of hypovitaminosis D and K in the elderly, especially the institutionalized ones are greatly hampered by the fact that they are also generally malnourished. Arguments against the significance of these vitamins have been made that decreased serum concentrations of these vitamins is merely a reflection of overall malnutrition. In this paper, we have tried to statistically discriminate hypovitaminosis D and K from general malnutrition by using principal component analysis (PCA), which has been employed in clinical nutrition for the analyses of dietary pattern.[24,25]

MATERIALS AND METHODS

Subjects

The study subjects were 50 institutionalized elderly (male 15, female 35) in a nursing home, Kayu-Shirakawa. Exclusion criteria were routine medication that has potential interference with vitamin D or vitamin K status. Detailed information about this study was given and written consent was obtained from the subject or the proxy. The study protocol was approved by the ethical committee in Kyoto Women's University.

Laboratory data

Blood was obtained after overnight fasting. After centrifugation, serum was kept frozen at –30°C until analysis. Serum concentration of 25OH-D was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA).

Circulating level of intact parathyroid hormone (PTH) was measured by electro chemiluminescent immunoassay (ECLIA) (Roche Diagnostics, Mannheim, Germany). Plasma vitamin K_1 (phylloquinone; PK), and menaquinone-7 (MK-7) levels were determined by high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS) using a HPLC system (Shimadzu, Kyoto, Japan) and API3000 LC-MS/MS System (Applied Biosystems, Foster City, CA) with ^{18}O-labeled vitamin K as the internal standard.[26]

Nutrition intake study

Since the subjects were institutionalized and their diet was supplied from the institution, their nutrients and energy intake were calculated by multiplying the supplied nutrients on the basis of the Standard Tables of Food Composition in Japan, 5th ed. with the average percentage intake in a preceding month by the staff.[27] Percentage intake was assessed for each subject at every meal, and the monthly average percentage intake was calculated. Based on these records, their intake of energy and nutrients was calculated using software (Healthy Maker Pro 501, Mushroom Software Corp, Okayama, Japan).

Statistical analyses

Statistical analyses were performed with SPSS 15.0J (SPSS Japan Inc., Tokyo, Japan). Comparison of two independent groups was made with Student's t-test or Mann-Whitney test depending on normality. Multiple regression analyses by stepwise method were performed to determine independent factors for circulating levels of vitamin D and K levels. The relationship between various nutritional indices and circulating vitamin D- and K- levels was analyzed with principal component analysis (PCA), which is a statistical method to summarize the various parameters into a small number of summary factors (components). These components are obtained in such a way that the first component is extracted from the initial raw data with the maximal amount of information (eigenvalue), and the second one is extracted from the remaining information. Therefore, each component is mutually independent. Components with the eigenvalue greater than 1 were adopted, as in usual practice.

RESULTS

Biochemical markers and circulating concentrations of vitamin D and K

Baseline characteristics and data from blood examination are shown in Table 1. There was no gender difference in the age and level of care needed, which is a 5-grade score in the long-term care insurance in Japan with a higher number indicating the need for more intensive care. The level of care needed was higher than grade 3 in 78% of subjects. Most of the present subjects required wheelchair for transportation. Body height and body weight were significantly higher in males than in females. Body mass index (BMI), or serum albumin, total cholesterol and triglyceride concentrations did not significantly differ between the two groups. Generally, serum albumin level less than 3.5 g/dL is considered to indicate malnutrition. Serum albumin level was below this value in 26% of subjects. Inasmuch as the advanced age and high level of care needed, nutritional parameters remained within the reference range in most of the subjects. None of the study subjects had severe hepatic or renal dysfunction. There is a general consensus that a serum 25OH-D concentration less than 20 ng/mL indicates hypovitaminosis D.[2] Serum 25OH-D concentration was <10 ng/mL in 40% of subjects, 10-20 ng/mL in 58%, and ≥20 ng/mL in only one subject. None of the subjects had a serum PTH level above the cut-off value (65 pg/mL). Plasma PK and MK-7 concentrations in all of the subjects were 0.73±0.70 ng/mL and 0.53±0.37 ng/mL, respectively. In the present study, serum PK was less than 1 ng/ml and serum MK-7 was less than 1 ng/ml, in 85% and 90% of the subjects, respectively. The interpretation for these values will be given in the "Discussion" section. There were no gender differences in plasma vitamin K levels, serum 25OH-D or PTH.

Nutritional intake in the study subjects

The nutrients intake in the males and females were not statistically different as shown in Table 2. During the preparation of this paper, Dietary Reference Intake (DRI) for Japanese 2010 (DRI 2010) was released on May 29, 2009.[28] Since this work was done in 2006, however, consideration is made basically according to DRI 2005.[29] The intake of macronutrients such as protein, fat and carbohydrates appeared appropriate for their age and sex. The adequate intakes (AI) for calcium in Japan are 750 mg for men and 650 mg for women over 70 years. The AI for vitamin D is 5 μg/day, and that for vitamin K is 75 μg/day for men and 65 μg/day for women respectively. Although average calcium intakes in both groups were lower than the AI in DRI 2005, the average daily vitamin D intake was 7.0 μg, which is 140% of the AI in DRI 2005. The average daily intake of vitamin K in whole subjects was 155 μg, which is more than twice the AI for each gender. Thus, apparently these subjects had sufficient intakes of vitamin D and K based on AI in DRI 2005.

Multiple regression analyses for the determination of independent factor for circulating vitamin D, K concentrations.

In multiple regression analyses, vitamin D intake was a significant determinant of serum 25OH-D level, although the R^2 was low. Serum triglyceride level was the only significant predictor for plasma MK-7 concentration, and vitamin K intake and serum triglyceride concentrations significantly contributed to plasma PK level (Table 3).

Principal component analysis (PCA)

Since institutionalized elderly are generally malnourished, it is quite important to determine whether the low vitamin D - and K -status is independent of overall malnutrition or not. Then PCA was performed with the parameters included for analysis being serum albumin, triglyceride, cholesterol, 25OH-D, PTH levels and plasma PK, MK-7 concentrations. Four components were obtained and explained 82% of the variance. The first component was composite of high albumin, total cholesterol and 25OH-D, and second component consisted of high triglyceride, low

Table 3. Multiple regression analyses for the determination of independent factors for circulating vitamin D, K concentrations

	R^2	*p* value	Variable	β	*p* value
Serum 25OH-D	0.095	0.033	Vitamin D intake	0.309	0.033
Plasma PK	0.181	0.011	Vitamin K intake	0.290	0.042
			Triglyceride	0.380	0.009
Plasma MK-7	0.255	<0.001	Triglyceride	0.505	<0.001

Only significant predictors are shown. The abbreviations are β for β coefficient, and *p* for *p* value. Independent predictor for serum 25OH-D or plasma PK, MK-7 concentrations was analyzed by multivariate analysis with stepwise regression. Age, level of care needed and serum triglyceride and total cholesterol concentrations were included in all analyses. Vitamin D intake was additionally included in the analysis for plasma 25OH-D concentration. For plasma PK and MK-7, vitamin K intake was additionally included.

Table 4. Principal component analysis of nutrition indices

	Component 1	Component 2	Component 3	Component 4
Serum Albumin	0.880	0.004	0.047	0.059
Serum triglyceride	0.229	0.734	0.119	0.380
Serum total cholesterol	0.800	0.320	-0.046	-0.060
Serum 25OH-D	0.434	-0.457	-0.658	-0.033
Serum PTH	0.156	-0.273	0.877	-0.090
Plasma PK	-0.014	0.030	-0.071	0.986
Plasma MK-7	0.117	0.832	-0.238	-0.152

Factor loadings to four components after varimax rotation are shown. Loadings greater than 0.35 are shown in bold
Four components thus obtained were considered to represent the following nutritional status; component 1: overall nutritional status, component 2: vitamin K_2 status, component 3: vitamin D status, and component 4: vitamin K_1 status.

25OH-D, and high MK-7. The third component was composite of low 25OH-D and high PTH, and the fourth component was composed of high triglyceride and high PK. The interpretation of each component was made as follows; the first component representing overall nutritional status, the second component, vitamin K_2 status, the third component, vitamin D status, and the fourth component representing vitamin K_1 status (Table 4).

DISCUSSION

Nutritional status would be adequately assessed by both evaluating the subjects' food intake and measuring their circulating or urinary markers. This principle would hold true especially in the elderly, since they are at high risk for malabsorption or utilization defects of nutrients. Unfortunately in Japan, vitamin D and K status in the elderly has been studied either by evaluating their food intake, as in the annual National Nutrition Survey Japan (NNS-J) or by measuring circulating level of these vitamins,[21,30-33] but rarely by both.[12,34]

Institutionalized elderly have been our special concern, since they are much more susceptible to hypovitaminosis D and K deficiency than the healthy elderly. The NNS-J in 2006 showed that subjects over 70 years of age, including both genders, had the following daily nutrients intakes: energy 1761 kcal, calcium 551 mg, vitamin D 9.0 μg, vitamin K 273 μg,[35] which were higher than those of the subjects in the present study. Gastrointestinal absorption of nutrients in the present study subjects would be impaired also. These considerations led us to simultaneously evaluate both vitamin D and K intakes and its circulating levels in the present study.

Before the interpretation of our data, determination procedure for vitamin K deserves some discussion. There have been discrepancies on the plasma concentration of vitamin K in the previous literature, which is at least partly due to the different determination procedure employed. Recently we have developed a novel procedure for the determination of vitamin K analogs with high sensitivity and specificity, based on high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS).[26] With this procedure, plasma concentrations of PK and MK-7 were 0.73±0.70 ng/mL (median 0.58 ng/mL) and 0.53±0.37 ng/mL (median 0.45 ng/mL), respectively in the current study. In our recent study, plasma concentrations for PK and MK-7 were 1.29±1.09 ng/mL (median 0.94 ng/mL) and 4.21±6.81 ng/mL (median 2.14ng/mL), respectively in the healthy Japanese elderly over 70 years old using the same assay procedure.[21] In the same study, lowest concentration of plasma vitamin K level to avoid the elevation of serum ucOC concentration was 2.5 ng/ml for PK and 6.4 ng/ml for MK-7.[21] Since serum ucOC level is a sensitive indicator of skeletal vitamin K insufficiency, these figures can yield a rough estimate of circulating vitamin K levels needed by the skeleton.

The median intake of vitamin K in the current subjects was 168 μg, which was more than twice the AI in DRI 2005. The AI for vitamin K was not altered in DRI 2010. Dietary vitamin K intake has been identified as an important determinant of plasma phylloquinone concentration in previous studies.[36,37] In the present study, vitamin K intake was also significantly associated with plasma PK, but not with plasma MK-7. Since they were not supplied

with fermented soybean; natto, which contains exraordinary amount of MK-7,[38] phylloquinone from green vegetables is likely to be the major contributors to the total vitamin K intake in our subjects. Thus plasma PK alone correlated with total vitamin K intake, adjusted by serum triglyceride. These data strongly suggest that these subjects are vitamin K-deficient in spite of the fact that their dietary intake is far above the AI in according to DRI 2005, and increased vitamin K intake would be effective in improving plasma PK levels in institutionalized elderly in present study.

As in the case of vitamin K, average dietary intake of vitamin D was around 7 μg/day, which is approximately 140% of the AI in subjects in the present study. Nevertheless, the average serum 25OH-D concentration was only 11.1 ng/mL. Thus, most subjects in the present study had hypovitaminosis D in spite of apparently sufficient vitamin D intake.

Although the multiple regression analysis has identified vitamin D intake as the significant contributor to serum 25OH-D concentration, the R^2 value was low, which indicates that the current model could explain only a small portion of variation. Several factors could be responsible for the above results. First, because of walking disability and other physical dysfunction, the chance of sun exposure was minimal in most of the current study subjects, but it was not null. Thus, sun exposure may also partly explain the above results. Unfortunately, however, detailed information about sun exposure was unavailable. Furthermore, ADL itself has been reported to be related to serum 25OH-D levels,[39] on which detailed information is not available in the current study. Secondly, the intestinal absorption of vitamin D is likely to decrease due to factors such as compromised intestinal ability for nutrients absorption and limited fat intake.[40] Nevertheless, oral vitamin D intake seems to be of value in the institutionalized elderly for improving their vitamin D status. Cashman *et al.* reported dose-dependent increase in serum 25OH-D concentration after incremental supplementation with vitamin D_3 in free-living adults over 64 years of age.[41] Although AI for vitamin D slightly increased to 5.5 μg/day in recently issued DRI 2010, the elderly subjects are likely to require much more vitamin D intake to avoid hypovitaminosis D considering the various problems to interfere with absorption and utilization as discussed above. A second issue with regard to the above discussion; disturbed intestinal absorption and limited fat intake, will also apply to the discrepant intake and circulating level of vitamin K.

Although serum 25OH-D level was extremely low, average serum PTH level was within the reference range. Circulating 25OH-D concentrations showed significant negative correlation with serum PTH levels ($r= -0.293$, $p= 0.041$; data not shown), which suggests that the negative feedback regulation of PTH secretion by vitamin D is not impaired in the current population. Kuchuk *et al*. reported that the elevation of serum PTH concentration by vitamin D deficiency is moderate in its magnitude, and usually fell into the reference range.[42] Thus they stressed the importance of serum 25OH-D level, and argued that for bone health maintenance and physical performance in the elderly, serum 25OH-D concentration above 50-60 nmol/L (20-24 ng/mL) was required.

Although the institutionalized elderly are considered to be generally malnourished,[43-45] nutritional status appeared rather satisfactory in the present study subjects in face of hypovitaminosis D and K. Then we analyzed the relationship between the overall nutrition and circulating levels of vitamin D and K by PCA. The PCA have yielded four components representing: overall nutritional status, vitamin D status, vitamin K_2 status, and vitamin K_1 status respectively. Serum 25OH-D also exhibited some association with the first component, representing the overall nutritional status. One of the reasons for the above results would be that 25OH-D is bound to vitamin D-binding protein (DBP) and albumin during its transport in circulation.[46] Since these components are independent of each other by their definition, these results suggest that hypovitaminosis D and K in the institutionalized elderly do not merely reflect general malnutrition, and have their own role. Confounders are serious challenge in the clinical studies. In the intervention studies, randomization would eliminate the interference by the confounders. It would be less problematic in the case of cohort studies. Adjustment for confounders is quite difficult in the cross-sectional studies like the current one. Multivariate analyses such as PCA would be of help in eliminating the interference by confounders in this type of studies.

In conclusion, institutionalized elderly had high prevalence of hypovitaminosis D and K in spite of their dietary intake exceeding the AI in DRI 2005 in Japan, which suggests that the requirement for these vitamins would be higher in these subjects. Additionally, hypovitaminosis D and K were shown to be independent of general malnutrition by PCA, which would be a useful analytical procedure for eliminating the interference by confounders in cross sectional studies.

ACKNOWLEDGMENTS

This study was supported by Health and Labor Science Research Grant entitled “Studies on the Dietary Reference Intakes (Recommended Dietary Allowance) for Japanese” from the Ministry of Health, Labor and Welfare, Japan.

AUTHOR DISCLOSURES

None of the authors have any conflicts of interest.

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Original Article

Hypovitaminosis D and K are highly prevalent and independent of overall malnutrition in the institutionalized elderly

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居住機構中的老年人有高盛行率的維生素 D 及維生素 K 缺乏症且與整體的營養不良無相關

研究老年人的維生素 D 及維生素 K 缺乏症有許多方法學上的問題。首先，大多研究是藉由評估食物的攝取或是測量血中的濃度來進行的，但在日本很少同時利用這兩種方法。在本篇文章中，維生素 D 及維生素 K 的攝取以及老年人的血中濃度是同步測量的。第二個議題是維生素 D 及維生素 K 缺乏症是否與盛行於老年人的一般營養不良情形相關。我們試著藉由統計的主成份分析方法去分辨。評估 50 位機構中的老年人血中的 25-羥化維生素 D、副甲狀腺素、維生素 K_1、維生素 K_2 濃度，以及食物攝取。雖然平均維生素 D 攝取量(每天 7 克)超過日本所訂定的足夠攝取量(每天 5 克)，但平均血清中 25-羥化維生素 D 濃度(11.1 ng/mL)卻屬維生素 D 缺乏的範圍。維生素 K 攝取量的中位數為每天 168 克，這幾乎是維生素 K 的足夠攝取量的 2.5 倍。但是，血漿中維生素 K_1 及維生素 K_2 濃度是遠低於 70 歲以上健康的日本老人。應用主成份分析法，結果產生 4 個成份，分別代表整體營養狀況、維生素 K_2、維生素 D 及維生素 K_1 的營養狀況。既然每個成份都各自獨立，則這些老人的維生素 D 及維生素 K 缺乏不能用整體營養不良加以解釋。總之，在這些機構中的老年人具有高盛行率的維生素 D 及維生素 K 缺乏；爾後這類研究應該同時測量血中濃度及飲食攝取。主成份分析法，可排除橫斷性研究中其他干擾因子的作用，而得到有效的結果。

關鍵字：維生素 D 缺乏、維生素 K 缺乏、主成份分析、足夠攝取量、機構中的老年人

特集「日本人の食事摂取基準(2010 年版)」

日本人の微量ミネラルの食事摂取基準 (2010)

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Vitamins (Japan), **84** (5・6), 240-245 (2010)

Dietary Reference Intakes of Trace Minerals for Japanese (2010)

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Key words: Japanese, dietary reference intake, iron, zinc, copper, manganese, iodine, selenium, chromium, molybdenum

はじめに

日本人の食事摂取基準 2010 年版におけるミネラルの区分は,「多量ミネラル(ナトリウム, カリウム, カルシウム, マグネシウム, リン)」と「微量ミネラル(鉄, 亜鉛, 銅, マンガン, ヨウ素, セレン, クロム, モリブデン)」である. 本稿では「微量ミネラル」に関して, その呼称, 範囲, 順序を決めた経緯を概説し, 次いで各微量ミネラルの食事摂取基準策定の根拠と今後の課題について述べる. なお, 各微量ミネラルの食事摂取基準の詳細は, 厚生労働省「日本人の食事摂取基準」策定検討会報告書[1]もしくは他誌に記載されている解説[2]を参照していただきたい.

1. 呼称, 範囲, 順序

食事摂取基準 2005 年版では鉄以下の 8 元素を「微量元素」と呼んでいた. これは trace elements を邦訳したものであり, 学術的には誤りでない. しかし, 医学・生物学分野における微量元素には, 水銀やカドミウムなど, ヒトにおける非必須元素も含まれている. 食事摂取基準で扱う無機元素は栄養上必須のものに限定していることから, 栄養学分野で馴染み深い「ミネラル」という用語を「元素」と置き換え,「微量ミネラル」という呼称を採用した. また, これに合わせて, ナトリウム, カリウム, カルシウム, マグネシウム, リンについても,「多量ミネラル」としてまとめることとした.

摂取基準で扱う微量ミネラルの範囲についても食事摂取基準策定検討会ミネラルワーキンググループにおいて検討した. とくにフッ素について, う歯予防の観点から食事摂取基準を策定することの是非を検討した. しかし, フッ素はヒトにおいて欠乏症は認められておらず栄養学上の必須元素ではない. しかも, う歯予防の効果を得るに必要と推定される摂取量は日常の食事から摂取できる量を大幅に超えている. これらの理由により, フッ素は食事摂取基準が扱う微量ミネラルの範囲には含めないこととした.

報告書におけるミネラルの掲載順序についても検討した. 当初は原子番号, アルファベット, アイウエオなど, 機械的に順序を決める案が有力であったが, 現実の献立作成での優先度, および食品成分表や国民健康・栄養調査における掲載順序を考慮し, 微量ミネラルに関しては, 鉄, 亜鉛, 銅, マンガン, ヨウ素, セレン, クロム, モリブデンの順にすることとした. 今後, 栄養学のテキストにおいて, 微量ミネラルという呼称とこの順序が定着することを期待している.

2. 鉄の食事摂取基準

鉄の食事摂取基準を付録 (p249 参照) に示した. 鉄の食事摂取基準は, 2005 年版と同様に, 米国/カナダの食事摂取基準(以下, 米国と略記)[3]でも採用されている要因加算法にもとづいて策定した. 別の方法, とくに集団データ解析法についても検討したが, 日本人では, 鉄欠乏の指標とされる血清フェリチン濃度が食事からの鉄摂取量

以外の要因に依存して変動することが報告されていることから[4]，今回の策定においても要因加算法を採用することとした．

要因加算法における要因とは，基本的損失(大半は消化管排泄)，月経血への損失，成長に伴う蓄積(大半は血液量増加によるヘモグロビンの増加)，妊娠・授乳に伴う需要増加，および消化管吸収率である．すなわち，鉄の推定平均必要量は，「(基本的損失＋経血損失(思春期以降の閉経前女性のみ)＋妊娠・授乳に伴う需要増加(妊娠・授乳期のみ)＋成長に伴う蓄積(成長期のみ))÷消化管吸収率」の式を用いて算定し，推奨量は推定平均必要量に1.2(成長期は1.4)を乗じて策定した．いずれの要因についても，鉄栄養が健全と推定されるヒトを対象とした内外の研究から妥当と思われる数値を採用した．2005年版と比較した場合，妊婦付加量以外は，基準体位の変化に伴う軽微な変更が生じたのみである．ただし，経血損失量と消化管吸収率に関しては，以下に述べるように，日本人を対象にした詳細な研究が今後必要と思われる．

今回の策定では，18歳以上の経血量として37 mL/回，月経周期31日を採用した[5]．しかし，これらの数値の根拠となる日本人の経血量についての研究は，きわめて例数が少なく，かつ対象としているのはいずれも小地域の特定集団である[6][7]．しかも中には1960年代に実施されたものもある．半世紀前と現在とでは女性の体位も変化し，かつ生活環境が大きく異なっていることから，現代女性を対象とした大規模な経血に関する調査が行われることを期待する．

同様に，鉄の消化管吸収率についても日本人を対象にした研究が見当たらないため，欧米の研究にもとづきFAO/WHOが指示している15%を用いた[8]．しかし，食事中の鉄の吸収率は，鉄の形態，共存成分，鉄摂取量に伴い変化することから，日本人が日常的な献立を摂取した場合の数値を求める必要がある．

妊娠中の鉄付加量は，妊娠に伴う鉄需要増加(胎児・胎盤・臍帯への鉄蓄積と妊娠に伴う循環血液量増加)をもとに算定できる．2005年版においてはWHOの需要量増加推定値[9]を採用したが，2010年版では日本人の体格をもとに循環血液量増加を少なく見積もったので付加量は小さな数値になった．それでも妊娠中期以降(18～29歳)の付加量を加えた推定平均必要量と推奨量は，それぞれ17.5 mg/日と21.0 mg/日となり，一般的な献立からは達成が困難な数値となる．しかし，現実には，妊娠女性の貧血有病率は一般女性よりもわずかに高い程度である[10]．この矛盾は，おそらく妊娠中の鉄需要増加に伴う鉄吸収率の増加がきわめて大きいことに起因していると推定できる．そこで，日本人の妊娠女性の鉄吸収率を40%[11]と高めに見積もった場合の妊娠中期以降の付加量を推定平均必要量8.0 mg/日，推奨量9.5 mg/日と試算した．この試算値にもとづけば，妊娠中期以降(18～29歳)の鉄摂取の付加量を加えた推定平均必要量と推奨量は，それぞれ13.0 mg/日と15.5 mg/日となる．吸収率40%の科学的根拠が低いため，これらの試算値は，食事摂取基準としては採用できなかったが，妊娠女性の鉄摂取の現実的な目標といえるだろう．

なお耐容上限量に関しては，新規な報告が見当たらないため2005年版同様に，FAO/WHOの暫定耐容最大1日摂取量0.8 mg/kg[12]にもとづき策定した．ヒトを対象にして上限量策定に有用な実験を行うことは倫理的に難しい．しかし，多種多様な鉄サプリメントや鉄強化食品が市販されていることから，鉄を大量摂取している症例が存在している可能性は高い．このような症例は，健康障害が認められなくても，上限量策定にとっては有用であることから，積極的に報告されることを希望するものである．

3. 亜鉛の食事摂取基準

亜鉛の食事摂取基準を付録(p249参照)に示した．亜鉛の食事摂取基準も2005年版と同様に，米国[13]でも採用されている要因加算法で求めた．すなわち，「総排泄量＝腸管内因性排泄量＋尿中排泄量＋体表消失量＋精液または月経血への損失量」と考え，総排泄量に見合う真の吸収量を与える摂取量を「真の吸収量＝1.113×摂取量$^{0.5462}$」の式より算定し，男性11.18 mg/日，女性10.03 mg/日という数値を得た．要因中でもっとも寄与の大きい腸管内因性排泄量を求める関係式は男性を対象とした英米の複数の研究から得られるが，これらの研究における対象者の体重は特定できなかった．そこで，この腸管内因性排泄量は米国人男性の基準体位である76 kgの人に対するものであると考え，最終的に得られた上記摂取量を76 kgの人に対する推定平均必要量とした．そして18歳以上は体重比の0.75乗，12～17歳は体重比の0.75乗と成長因子を用いて外挿し，性・年齢階級別の推定平均必要量を策定した．1～11歳に関しては，日本人小児を対象とした出納試験における平衡維持量[14]と米国人の値から推定した小児の体表損失量にもとづいて推定平均必要量を策定した．推奨量は推定平均必要量に1.2を乗じて求めた．2005年版では総排泄量を求めるための要因の一部に体重の小さい日本人の数値を用いていた．このため2010年版において，とくに成人の数値は男女ともに2005年版に比較して大きな数値になった．

以上から明らかなように，亜鉛の食事摂取基準策定の根拠となっている研究は，そのほとんどが欧米のものである．最大の寄与要因である腸管内因性排泄量に関して，日本人を対象にして安定同位体を用いた研究が待たれる．

亜鉛の耐容上限量は，鉄と同様に新規な報告が見当たらないため，2005年版において採用した米国の成人女性を対象とした研究から得られる最低健康障害発現量60 mg/日[15]と不確実性因子1.5にもとづいて策定した．ただし各年齢階級への外挿においては，2005年版とは異な

り，男女ともに年齢階級別基準体重の 61 kg（米国成人女性の基準体重）に対する比を用いた．このため 2010 年版の耐容上限量は，男性において 2005 年版よりも大きな数値になった．亜鉛サプリメントや亜鉛強化食品が市販されていることから，今後は亜鉛大量摂取の症例を積極的に報告することが必要といえる．

4. 銅の食事摂取基準

銅の食事摂取基準を付録（p249 参照）に示した．銅の摂取基準は，銅の栄養状態を示すバイオマーカー（血漿銅濃度，血漿スーパーオキシドジスムターゼ活性など）の値が低下しない最小の摂取量（0.72 mg/ 日）[16)17)] にもとづき策定した．この方法は 2005 年版と同じであり，米国 [18)] も採用している．したがって 2010 年版の数値は，2005 年版に比較して基準体位の変化に伴う軽微な変更のみである．2005 年版発表直後は，推奨量が 6 次改定栄養所要量の約半分の数値になったため，相当な論議を呼んだが，この 5 年の間に上記 0.72 mg/ 日を推定平均必要量策定の基準値にすることを支持する報告 [19)] が提出されており，今後もよほどのことがない限り，大きな変更はないと思われる．耐容上限量に関しても，新規な報告が見当たらないため，2005 年版をそのまま踏襲した．

5. マンガンの食事摂取基準

マンガンの食事摂取基準を付録（p249 参照）に示した．マンガンの食事摂取基準に関しては，2005 年版と同様に，日本人のマンガン摂取量にもとづいて目安量を設定するにとどめた．これは，短期間の出納実験から求められるマンガンの平衡維持量の信頼性を米国同様に低いと判断しているためである．日本人のマンガン摂取量に関する報告も増えていないので，2005 年版と同様に，複数の報告値に基づいて成人日本人の平均的なマンガン摂取量を 3.7 mg/ 日と見積もった [20)]．そして，エネルギー摂取量の性差を考慮し，男性 4.0 mg/ 日，女性 3.5 mg/ 日を 18 歳以上の目安量とした．なお，国民健康・栄養調査の元データと食品成分表に付記されている食品中マンガン濃度にもとづいて成人の性・年齢階級別マンガン摂取量を試算したところ，上記目安量は，日本人のマンガン摂取量の中央値にほぼ一致していた．マンガンは穀物をはじめとする植物性食品に多く含有されている．このため日本人のマンガン摂取量は米国人の約 1.5 倍である．したがって日本人のマンガン摂取の目安量も米国の目安量 [21)] の約 1.5 倍となっている．

以上のように，マンガンは，微量ミネラル中で唯一，推定平均必要量と推奨量が策定できなかった．マンガンは，欠乏症もほとんど認められず，研究者の関心を引きにくい微量栄養素かもしれない．しかし，推定平均必要量策定に根拠を与える質の高い研究が国内の研究者によって行われることを期待している．

6. ヨウ素の食事摂取基準

ヨウ素の食事摂取基準を付録（p249 参照）に示した．ヨウ素の食事摂取基準は，2005 年版に比較して，推定平均必要量と推奨量は数値の丸め方に伴う軽微な変更であったのに対して，耐容上限量は大きな変更が加わった．ここでは耐容上限量について述べる．

米国 [22)] では，健常人（ヨウ素摂取量約 300 μg/ 日）へ 1500 μg/ 日のヨウ素を負荷した場合に甲状腺機能低下が起こること [23)] から，上限量を 1100 μg/ 日としている．2005 年版では，一般的な日本人のヨウ素摂取は最大で 3000 μg/ 日と推定できるが [24)25)]，ヨウ素の過剰摂取に起因する甲状腺機能低下が認められないこと，および北海道住民を対象にした研究 [26)] から甲状腺機能低下が生じるヨウ素摂取量は 10 mg/ 日であると判断できることから，上限量を 3000 μg/ 日としていた．しかし，最近に行われた，中国 [27)]，およびアフリカ [28)] における研究は，継続的な 1500 μg/ 日程度のヨウ素摂取が甲状腺腫の有病率を上昇させることを示しており，日本人のヨウ素摂取と甲状腺機能の関連について再検討する必要があると判断された．

まず，日本人のヨウ素摂取量を尿中ヨウ素排泄量 [29)]，および昆布の消費量 [30)] の両面から検討し，日本人のヨウ素摂取量は，日常的には 500 μg/ 日未満であるが，間欠的に海藻類を大量に摂取するために平均的には約 1500 μg/ 日になると推定した．次に，2005 年版において上限量策定の根拠とした北海道住民を対象とした疫学研究 [26)31)] を再検討し，成人日本人におけるヨウ素の健康障害非発現量を 3300 μg/ 日と推定した．そして，諸外国で行われているヨウ素過剰障害に関する研究に配慮し，安全性を高める観点から不確実性因子を 1.5 と見積もり，18 歳以上の耐容上限量を 2005 年版よりも 800 μg/ 日小さい 2200 μg/ 日とした．

一方，ヨウ素摂取が約 750 μg/ 日である北海道の小学生において甲状腺容積の有意な増大が認められており，小児ではヨウ素摂取が 500 μg/ 日を超えると有害な影響が生じると考えられている [32)]．これにもとづき，6 ～ 11 歳の耐容上限量を 500 μg/ 日とし，他の性・年齢階級にはこの値と成人の値から体重比で外挿した数値を適用した．また，ヨウ素過剰摂取と推定される乳児のヨウ素摂取量 [33)] にもとづき，乳児の耐容上限量は 250 μg/ 日とした．なお，これらの 18 歳未満に対するヨウ素の耐容上限量は 2010 年版において初めて策定したものである．

ヨウ素の耐容上限量の策定において痛感したことは，日本人のヨウ素摂取量を推定している論文が意外に少ないということであった．日本人が本当に米国の耐容上限量を上回るヨウ素を摂取しているのであれば，なぜ過剰障害が起こらないのかを真剣に検討すべきである．その場合，ゴイトロゲン（造甲状腺腫物質）として知られるイソフラボンを含有する大豆製品がヨウ素の影響をどの程

度修飾しているかも検討課題と思う．国内におけるヨウ素の医学・生物学的研究の多くは，内分泌学や小児科学の臨床系研究者に委ねられている．これらの研究者と分析学者，および栄養学者が一同に会して，ヨウ素の耐容上限量について議論することが必要と感じている．

7. セレンの食事摂取基準

セレンの食事摂取基準を付録(p249参照)に示した．セレンの推定平均必要量は，2005年版と同様に，克山病のようなセレン欠乏症を予防するのに必要なセレン摂取量という観点から，血漿の含セレン酵素であるグルタチオンペルオキシダーゼ(GPX)の活性が飽和値の3分の2の値を示すときのセレン摂取量にもとづき策定した．この考え方は，セレン欠乏症の予防には血漿GPX活性が飽和値の3分の2の値で十分とするWHOの報告[34]にもとづくものである．米国[35]などでは，推定平均必要量を血漿GPX活性をちょうど飽和させるセレン摂取量としているため，わが国の策定値は，欧米に比べると低い水準にある．

一方，セレンの耐容上限量は，2005年版に大きな変更を加えた．2005年版では，米国[35]と同様に，慢性セレン中毒症状を指標にしたセレンの健康障害非発現量(13.3 μg/kg/日)[36]と不確実性因子2を用いて上限量を設定していた．しかし，2010年版では，米国において，血清セレン濃度が121.6 μg/L(セレン摂取量84 μg/日に相当)以上の集団に200 μg/日のセレンをセレン酵母サプリメントとして投与すると2型糖尿病の発生率が有意に上昇したと報告されたことから[37]，セレン摂取量が100 μg/日に近い人が200 μg/日のセレンをサプリメントから付加的に摂取し続けることは健康に対して好ましくない影響を与える可能性があると判断した．そこで，性・年齢階級別体重が最大である30～49歳男性(基準体重68.5 kg)の耐容上限量を300 μg/日とし，他の年齢階級には300 μg/68.5 kg/日＝4.4 μg/kg/日を適用した．なお，この4.4 μg/kg/日という数値は，慢性セレン中毒を指標にした場合の健康障害非発現量に不確実性因子3を適用したのと結果的に同じであるので，2010年版の耐容上限量は2005年版のほぼ3分の2の値となった．

低セレン摂取がいくつかのがんの発生リスクを高めるという報告は多い．これらの研究の多くは，対象者を血清セレン濃度などを指標に複数の集団に分割し，がんの発生リスクを集団間で比較している．しかし，セレン摂取とがん発生率に有意な関連が認められるのは，対象者全体のセレン栄養状態が低いときのみである[38]．たとえば，有意な関連ありとする一報告[39]では，もっとも高いセレン栄養状態である集団の血清セレン濃度は78 μg/L(セレン摂取量54 μg/日に相当)以上であるのに対して，関連なしとする別の報告[40]でのもっとも低いセレン栄養状態である集団の血清セレン濃度は99 μg/L(セレン摂取量69 μg/日に相当)未満である．これらの研究結果にもとづき，がん予防に適切なセレン摂取量を目標量として定めることも検討したが，科学的証拠が小さく，時期尚早と判断した．

8. クロムの食事摂取基準

クロムの食事摂取基準を付録(p249参照)に示した．米国ではクロムの食事摂取基準として，クロム摂取量にもとづいて目安量を策定している[41]．しかし，わが国では，食品中クロムに関して信頼性の高い分析値の報告が少なく，クロム摂取量を正確に見積もることが不可能である．このため，2010年版においても，2005年版と同様に，外国の高齢者を対象とした出納実験[42][43]の結果にもとづき，推定平均必要量を策定した．したがって，2010年版の推定平均必要量と推奨量は，2005年版に基準体位の変化に伴う軽微な変更が加わっただけである．

糖尿病患者に200～1000 μg/日のクロムサプリメントを投与すると症状の改善が認められる．しかし，健康な人へのクロムサプリメント投与が健康にとって好ましい影響を与えることは認められていない[44]．一方，200～1000 μg/日のクロムサプリメント継続摂取による副作用の報告が散発的に認められるが[45]，いずれも同時に服用していた医薬品やサプリメント類の影響を否定できない．以上より，クロム摂取と健康障害との量・反応関係に関する研究は不十分と判断し，2005年版と同様にクロムの耐容上限量設定は見合わせた．しかし，このことが200～1000 μg/日のクロム摂取が無害であることを保証するものではないことを強調したい．

なお，乳児の目安量に関して，日本人の母乳中濃度に関する報告[46]が新たに提出されたため，2010年版において初めてこれを策定した．

9. モリブデンの食事摂取基準

モリブデンの食事摂取基準を付録(p249参照)に示した．モリブデンの食事摂取基準は，2005年版に比較して，推定平均必要量と推奨量は基準値から各性・年齢層への外挿法の統一に伴う軽微な変更であったのに対して，耐容上限量は大きな変更が加わった．ここでは耐容上限量について述べる．

2005年版ではアルメニアで発生した事例[47]にもとづいて，モリブデンの耐容上限量を5 μg/kg/日(体重60 kgのヒトで300 μg/日)としていた．しかし，この事例報告には多くの問題点があり，記載されている所見にモリブデンが関わることは疑わしいと判断した．一方，日本人のモリブデン摂取量を150～350 μg/日と見積もる報告[48][49]が提出され，日本人にモリブデン過剰摂取に伴う健康障害の報告がないことから，2005年版の上限量は厳し過ぎるという指摘もなされていた．欧米の食事摂取基準[50][51]では，ラットにおけるモリブデンの健康障害非発現量(900 μg/kg/日[52])にもとづいて上限量を策定している．そこで

2010 年版では，ラットの健康障害非発現量にヨーロッパ食品科学委員会[51]が用いている不確実性因子 100 を適用し，9 μg/kg/ 日（体重 60 kg のヒトで 540 μg/ 日）をモリブデンの耐容上限量の基準値とした．米国における出納実験[53]からは，ヒトにおけるモリブデンの健康障害非発現量を 18 μg/kg/ 日と解釈できるため，上記の 9 μg/kg/ 日はヒトの健康障害非発現量に不確実性因子 2 を適用したことにもなる．

なお，乳児の目安量に関して，クロムと同様に日本人の母乳中濃度に関する報告[46)54)]が新たに提出されたため，2010 年版において初めてこれを策定した．

おわりに

以上，微量ミネラルの食事摂取基準策定の根拠と今後の課題について，成人の平均推定必要量と耐容上限量を中心に解説した．いずれのミネラルに関しても，2005 年版に比較して策定根拠はより明快になったものと判断している．2015 年版に向けては，本文でふれたことに加えて，離乳食からの微量ミネラル摂取量，サプリメントやミネラル強化食品摂取による大量摂取者の把握なども必要と思われる．食事摂取基準策定に必要な情報を得るための研究は地味であり，脚光を浴びることは少ない．しかし，栄養学が定量的な学問であり，その目的が人々に「何を」「どれだけ」「どのようにして」食べるのがよいかを示すことにあると信じている立場からは，より多くの栄養学研究者が食事摂取基準に注目し，その策定に活用できる研究に取り組まれることを期待している．

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ビタミン D による骨折予防効果の意義：医療経済の視点から

Fracture Prevention by Vitamin D: from the Perspectives of Health Economy

ビタミン研究において，基礎研究，疫学・臨床研究は，お互いに合い補いあう関係であろうが，もう一つの研究分野は，社会におけるビタミンの役割である．しかし，このような研究はわが国では従来ほぼ皆無なので紹介したい．ほとんどの読者にとって，ふだん聞きなれない用語・概念がでてくることや一部に私見が入っている点はご容赦願いたい．なお，筆者は骨粗鬆症を主な研究分野としており，以下ビタミン D による骨折抑制を例として述べる．

本稿における骨折の記述は，大腿骨近位部骨折が主となるので，最初に簡単に説明しておく．この骨折はいわゆる「足の付け根」が折れるもので，高齢者に多い．受傷後 1 年以内の死亡率が高いだけではなく，死亡をまぬがれても，生活レベルが低下するため，要介護の原因となる例も少なくない．このことは患者本人にとって，大きな不幸であることは言うまでもないが，医療費・介護に要する費用も莫大であり，社会に及ぼす影響も大きい[1]．従って，大腿骨近位部骨折の予防は医学的にも，社会的にも緊急の課題となっている．

近年種々の骨粗鬆症治療薬が臨床現場で用いられているが，主に投与されているのは骨吸収抑制薬である．その代表である bisphosphonate 系薬剤は，強力な骨吸収抑制薬であり，骨折の発生率を半減させるほどの効果を示す．最近一部の国と地域では，人口の高齢化にも関わらず，大腿骨近位部骨折の発生率が減少しており，その減少は骨密度測定の普及や bisphosphonate のような新薬の処方との関連が考えられている[2]．これに勝るとも劣らない新薬が近年さらに次々と開発されている．このような画期的新薬と比べると，ビタミンをはじめとする栄養療法の効果は小さい．

最近欧米においては，医療行為は有効であることを証明しただけでは不十分で，かけた費用に見合うだけの効果を挙げることを示す必要があると考えられている[3]．この費用に対する効果（費用対効果）の視点から見ると，ビタミンを含む栄養療法には，新たな側面が見えてくる．

疾患の予防は，一次予防，二次予防，三次予防に分類される．三次予防はリハビリテーションのように，疾患に罹った後の対策，二次予防は疾患の早期診断と早期治療，一次予防は生活習慣の改善などによって，疾患を未然に防ぐことを目指すものである．また，予防に関するアプローチの方法から，high risk approach と population approach に分けられる．前者はその名前の通り，疾患発生のリスクの高い群に対して集中的に介入を行うものである．疾患発生リスクが高く，その薬剤の予防効果が大きいほど費用対効果はよい値となる．従って，骨折リスクの非常に高い群に対して bisphosphonate などを用い，薬剤治療介入を行うことは費用対効果に優れた医療行為である．

しかし，中～低リスク集団に対してもこのような治療介入を行うことは，要する費用や発生しうる副作用の可能性を考えると，とうてい実行不可能である．例えば，50 歳以上の全女性に，bisphosphonate を服用させるというのは，あり得ない選択である．このような場合には，集団のリスクを低い方に平行移動させる population approach が行われる．その場合，多数かつ多様な対象者に対して行いうることが必須であるから，安全かつ安価なものでなければならず，栄養，運動などの生活習慣改善が中心となる．

言い換えると，ビタミンなどの栄養療法は絶対的な効果は新薬より小さいが，かかる費用は圧倒的に低いので，費用対効果には優れており，中～低リスク集団に対する一次予防，population approach には非常に適しているということである．わが国ではこのような研究は皆無だが，海外では医療行為に対する経済評価は盛んに行われており，ビタミン D の骨折予防効果を扱ったものもいくつかある[4)-8)]．

一例を示すと，Decalyos Study は，フランスで行われた臨床研究であり，3,270 名の高齢女性を 2 群に分け，一方にはビタミン D_3（800 IU/ 日）＋カルシウム（1,200 mg/ 日）の投与が，他方にはカルシウム（1,200 mg/ 日）のみの投与が，36 ヶ月行われた．大腿骨近位部骨折は，Ca のみ群では 1,127 名中 184 名に起こったのに対し，D 群では 1,176 名中 138 名であった．すなわち，1,000 名あたり 46 件の大腿骨近位部骨折が予防できたことになる．この骨折は非常に医療費のかかる骨折であるので，もし施設入居高齢者全員にビタミン D ＋カルシウムの補充を行うならば，それにより，1 億 5 千万フランの医療費が削減できるものと推測されている[4]．

骨折の危険因子として低骨密度はもちろん重要だが，大腿骨近位部骨折など非椎体骨折の場合，転倒がきっかけになる例が非常に多い．最近ビタミン D の転倒防止効果も注目されていることから，ビタミン D は骨密度低下を防ぐ作用に加えて，複数の作用点から骨折予防に役立つ可能性がある．なお上記の研究は，大腿骨近位部骨折のみに注目したものであるが，実際にはビタミン D 欠乏（不足）の改善によって，それ以外の骨折についても予防

効果が見込まれるので，実際の経済効果はさらに大きく，上記の推定はおそらく過小評価である．

最近海外では，400IU/ 日のビタミン D では骨折を防ぐことができず，最低 800IU/ 日程度を要するという大規模研究やメタアナリシスがあいついで発表され[9)10)]，ビタミン D による骨折予防の社会的側面にも言及されている．予防効果を評価するのに，NNT（number needed to treat）という指標がよく用いられる．これはその疾患（ここでは骨折）の発生を 1 件防ぐためには，何人に対して介入する必要があるかという数字であり，NNT が小さい方が効率のよい予防介入である．当然，疾患発生リスクの高い集団に対して介入した方が NNT はよい値となる．Tang らは，29 の RCT（randomized controlled trial），63,897 例のデータのメタアナリシス結果から，ビタミン D の有効性を報告しているが，NNT＝63 であったと述べている[9)]．この研究は 50 歳以上の女性が対象であり，決して骨折リスクが非常に高い集団だけを選んだものではないことを考えると，NNT＝63 は非常によい数字であり，循環器領域での NNT と比べても遜色ない[11)]．

ビタミン D の骨折予防効果に関しては，近年の海外での進歩に比べて，日本での臨床研究が非常に遅れている．多数の介入試験が海外で発表されてきたが，これまでわが国ではほぼ皆無であった．最近，我々は施設入所高齢者に対する，ビタミン D_3 による介入試験を行った[12)13)]．血液中の 25-hydroxyvitamin D 濃度が最もよいビタミン D 栄養状態の指標だが，日本人の食事摂取基準 2005 年版の目安量である，200 IU/ 日では極めて不十分な効果しか得られず，少なくとも 800 IU/ 日を要することを報告した．しかし，これはまだ血液中のビタミン D 濃度を指標としたのにとどまっており，骨折を指標したものではない．このようなわが国の現状では，本稿に述べたような医療経済からみたビタミンの評価というような話題は時期尚早なのかもしれない．しかし，このようなビタミンの評価は社会と行政に対してビタミンの重要性を訴えていく，一つの有力な視点となるものであり，今後わが国でも研究が行われるべき分野であると考え，あえて紹介した．

Key Words：vitamin D, fracture prevention, health economics, primary prevention, population approach

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CHAPTER 14

Molybdenum in Biological Samples and Clinical Significance of Serum Molybdenum

MUNEHIRO YOSHIDA

SUMMARY

Molybdenum is an essential trace element in human nutrition. The determination of Mo in biological materials can be performed using inductively coupled plasma mass spectrometry (ICP-MS). The Mo status of the Japanese population is assessed on the basis of the Mo content in foods and human milk. A balance study of Mo is reported. Finally, the meaning of serum Mo is discussed.

14.1. INTRODUCTION

Molybdenum is used in the production of steels and alloys and as a chemical catalyst in the manufacture of plastics, lubricants, and pigments. Because of the lower toxicity of Mo as compared with B, Cr, Mn, and Ni, the industrial use of Mo is steadily increasing [1]. On the other hand, several cases of occupational Mo exposure with elevation of serum uric acid and ceruloplasmin levels have been observed [2, 3]. In these cases, serum/plasma and urinary Mo concentrations were monitored as an index of Mo exposure.

Molybdenum is also used as a material for several prostheses in joint-replacement surgery performed on young patients [4]. Due to the possibility that Mo is released from the prosthesis into blood, it has been proposed that monitoring blood Mo is important to detect the long-term effects of Mo exposure [5]. This metal is also important as an essential micronutrient for both plants and animals. In animals, Mo functions as a cofactor for a limited number of enzymes, including xanthine oxidase,

Analytical Techniques for Clinical Chemistry: Methods and Applications, First Edition.
Edited by Sergio Caroli and Gyula Záray.

aldehyde oxidase, and sulfite oxidase [6]. Human nutritional deficiency of Mo was observed in a patient subjected to prolonged total parenteral nutrition [7]. In this case, clinical symptoms suggestive of a deficiency of sulfite oxidase and those including irritability leading to coma, tachycardia, tachypnea, and night blindness were reported; however, it is believed that dietary Mo deficiency is not likely to occur due to the high concentration of Mo in cereals and pulses. Since plasma and urinary Mo concentrations are correlated to dietary Mo intake, these levels may be used as an index of Mo nutritional status [8, 9].

In this chapter, Mo concentrations in biological samples (foods, animal tissues, human milk, blood, and urine) are surveyed and the clinical significance of serum Mo is discussed.

14.2. ANALYSIS OF MOLYBDENUM IN BIOLOGICAL SAMPLES BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

14.2.1. General

Several analytical methods can be used for Mo quantification including electrothermal atomization atomic absorption spectrometry (ETA-AAS), ICP-MS, and neutron activation analysis (NAA). Among these, ETA-AAS and ICP-MS have the lion's share to determine Mo in biological samples. In the following sections the applicability of ICP-MS methods to Mo quantification as developed in the author's laboratory is described [9–12].

14.2.2. Sample Preparation

14.2.2.1. Cereal, Pulse, and Liver Samples Since these samples contain Mo at a high level (≥100 ng/g), a simple short wet digestion is applicable. Up to 1 g of dry samples are digested with 10 mL metal-free grade HNO_3 in a 30 mL micro Kijeldahl flask until the disappearance of insoluble components and then the digestion mixture is diluted to 50 mL with double-distilled water.

14.2.2.2. Food Samples and Animal Tissue Since a large part of these samples contain Mo at a low level (<100 ng/g), long thorough wet digestion is required. Approximately 1 g of sample is carefully heated with 10 mL metal-free grade HNO_3 for 30 min in a 30 mL micro Kjeldahl flask. The mixture is further heated with 2 mL metal-free grade 60% $HClO_4$ until the appearance of white fumes of $HCLO_4$ and the volume of the digest is made up to 10 mL with water.

14.2.2.3. Human Milk and Serum Since Mo concentrations in these samples are very low (<10 ng/g), minimum dilution of Mo concentration is required during preparation. Approximately 2 to 5 mL of milk or serum sample are transferred to a ceramic melting pot (32 ø × 24 mm) and heated in an electric furnace at 550 °C for 16 h. After dry incineration, the remaining ash is dissolved in 5 mL of 0.1 M HNO_3. This dry digestion technique is also applicable to most food samples.

14.2.2.4. Urine Urine specimens are directly diluted with nine volumes of 0.1 M HNO_3.

14.2.3. Determinations by Inductively Coupled Plasma Mass Spectrometry

Molybdenum concentrations in sample solutions thus prepared are determined by ICP-MS with direct nebulization. ICP-MS operating conditions are as follows: instrument, ICPM-8500 (Shimadzu, Kyoto, Japan); forward power, 1200 W; coolant gas flow rate, 7.0 L/min; auxiliary gas flow rate, 1.5 L/min; nebulizer gas flow rate, 0.58 L/min; sampling depth, 5.0 mm; integration time, 2.0 s; number of run, 20; mode of analysis, pulse; isotopes monitored, ^{95}Mo, ^{97}Mo, and ^{98}Mo. An Rh isotope (^{103}Rh) was used as an internal standard. Because three Mo isotopes, ^{95}Mo, ^{97}Mo, and ^{98}Mo, were monitored, three analytical values of Mo were obtained for each sample. Since the analytical values obtained were similar, the mean of three analytical values was used for quantification. As an internal standard of Mo determination using ICP-MS, the isotopes of ^{45}Sc, ^{89}Y, or ^{115}In are also applicable.

Table 14.1 shows analytical results for Mo in several Certified Reference Materials (CRMs) and in milk samples with or without standard addition of Mo. When CRMs

TABLE 14.1 Validation with CRMs and Recovery for Mo Determination [10, 11]

CRMs or Samples	Method of Preparation[a]	Certified Value	Observed Value[b]
		(μg/g)	(μg/g)
Rice flour (SRM 1568a)	S-wet	1.46 ± 0.08	1.40 ± 0.07
Wheat flour (SRM 1567a)	S-wet	0.48 ± 0.03	0.50 ± 0.02
Bovine liver (SRM 1577a)	S-wet	3.5 ± 0.5	3.69 ± 0.30
Nonfat milk power (SRM 1549)	S-wet	0.34[c]	0.34 ± 0.06
Tomato leaves (SRM 1573a)	S-wet	0.46[c]	0.39 ± 0.11
Bovine muscle powder (RM 8414)	L-wet	0.08 ± 0.06	0.095 ± 0.032
Apple leaves (SRM 1515)	L-wet	0.094 ± 0.013	0.087 ± 0.020
Wheat flour (SRM 1567a)	Dry	0.48 ± 0.03	0.51 ± 0.04
Nonfat milk power (SRM 1549)	Dry	0.34	0.35 ± 0.04
			μg/kg or μg/L
Cow's milk	Dry	–	45.5 ± 3.9
Cow's milk + Mo (40 ng/g)	Dry	–	85.7 ± 1.8
Formula milk powder	Dry	–	16.6 ± 0.1
Formula milk powder + Mo (10 ng/g)	Dry	–	26.9 ± 0.3
Pooled human milk	Dry	–	6.3 ± 0.2
Pooled human milk + Mo (5 ng/g)	Dry	–	11.8 ± 0.4
Pooled serum	Dry	–	0.66 ± 0.07
Pooled serum + Mo (1 ng/mL)	Dry	–	1.66 ± 0.18

[a] S-wet, short simple wet digestion; L-wet, long wet digestion; Dry, dry incineration at 550 °C.
[b] Values are the means ± SD for quadruplicate sample preparation and analyses.
[c] Noncertified value.

were digested with acids either for a short or long time, or incinerated at 550 °C, their analytical values for Mo were coincident with the certified values. In addition, recoveries of the amount of Mo added to milk and serum samples were almost 100% with dry incineration. The limit of detection (LOD) was 0.1 μg/L when nebulizing to ICP-MS.

14.3. MOLYBDENUM IN FOOD

14.3.1. Molybdenum Concentration in Food

Molybdenum concentrations in various food groups are summarized in Table 14.2 [10, 13, 14]. The Mo concentration in food is highly different for each food group. Food groups with high Mo concentration are plant foods, such as cereals, pulses, nuts, and their products, whereas the Mo concentration in animal foods, such as meats, fish, and dairy products, is extremely low. The highest Mo concentration is observed in pulses, nuts, and their products.

Molybdenum concentrations in plants change not only as a function of the Mo concentration in soil, but also with soil pH [15]. Therefore, even in the same food commodity, a remarkable difference in Mo concentration can occur due to differences in the area where the plants grew, for example, a value of almost 10 μg/g was observed for the Mo concentration in US soybeans while a value of less than 1 μg/g was observed in Chinese soybeans [16].

14.3.2. Speciation of Molybdenum in Food

There is a little information of the chemical species of Mo in food. Since Mo enzymes exist in animal tissues, it is thought that animal foods include Mo bound to protein; however, no research has been devoted so far to the amount of Mo existing as a protein-bound form in animal food. Such enzymes also exist in plants; however, it is assumed that most Mo in soybeans is the inorganic low molecular weight compound molybdophosphate [16].

TABLE 14.2 Molybdenum Concentration (μg/g Fresh Weight) in Foods Consumed in USA, France, and Japan

Food Group	USA [13]	France [14]	Japan [10]
Wheat and wheat products	0.32	0.29	0.23
Rice and rice products	0.29	0.11	0.63
Pulse, nuts, and pulse products	1.55	1.06	1.32
Potatoes (starchy vegetables)	0.07	0.42	0.08
Vegetables	0.05	0.17	0.12
Fruits	0.03	0.01	0.04
Meat and meat products	0.03	0.02	0.05
Poultry	0.05	0.14	0.03
Fish and fish products	0.01	0.08	0.02
Eggs	0.09	0.03	0.40
Milk	0.05	0.04	0.04
Cheese	0.10	0.07	0.08

TABLE 14.3 Estimated Mo Intake (μg/day per person) in Populations of Several Countries

Countries	Method of Estimation	
	Calculation	Analysis of Diet
USA	120–240 [13]	
Mexico		185 [17]
France	275 [14]	
	114 [18]	
Germany		95 [17]
		175[a] [17]
Japan	225 [10]	217 [9]
		147[b] [9]
		318[c] [9]
Korea	11 [19]	

[a] Vegetarian diets.
[b] Diets without soybean products.
[c] Diets rich in soybean products.

14.3.3. Molybdenum Intake in Human Population

Table 14.3 shows the daily Mo intake from food in several human populations. The Mo intake in most countries ranges from 100 to 300 μg/day. In the Dietary Reference Intake (DRI) of the US/Canada, the Recommended Dietary Allowance (RDA) and the tolerable upper limit (UL) of Mo intake have been set at 45 μg/day and 2 mg/day, respectively [20]. Accordingly, Mo intake in these countries is thought to be adequate. The Mo intake of Korean people was estimated to be only 11 μg/day [19]; however, this figure is doubtful because the analytical values adopted in this report were one order of magnitude lower than those of other reports. Since cereals and pulses contain Mo at a very high level, the Mo intake of vegetarians [17] and people who eat a large amount of soybeans [9] is higher than that of the general population.

14.4. MOLYBDENUM IN HUMAN SAMPLES

14.4.1. Molybdenum in Urine

Table 14.4 shows the urinary Mo concentration for two healthy populations in Europe [21, 22]; these two studies give similar analytical values. The urinary concentration in the two European populations showed a logarithmical normal distribution rather than a normal distribution. In a Mo balance study of healthy Japanese young women, urinary Mo excretion was highly correlated to Mo intake, as

TABLE 14.4 Molybdenum Concentration in Spot Urine Samples from Healthy Italian and Danish Adults

	Italy [21]	Denmark [22]
Number of subjects	51M, 49F	71M, 57F
Age	M 44.1 ± 12.7 F 41.6 ± 13.3	40–70 years
Analytical method	ICP-MS	ICP-MS
Analytical values (μg/L)		
Range	11.1–155.8	3.7–180.7
Mean	54.1	42.0
SD	33.9	26.8
Geometrical mean	44.0	34.8
Median	46.2	36.2
10th percentile to 90th percentile	16.5–101.8	–
95% reference interval[a]	–	10.2–106.1
Analytical values (μg/g creatinine)		
Range	7.4–137.0	3.9–127.8
Mean	44.8	34.1
SD	23.8	22.6
Geometrical mean	39.0	27.8
Median	39.3	27.3
10th percentile to 90th percentile	23.0–72.3	–
95% reference interval[a]	–	7.2–102.8

[a] Nonparametric 95% reference value.

shown in Figure 14.1 [9]; therefore, the urinary Mo concentration of these populations in the two European regions where the dietary pattern is similar may show a common distribution. Urinary Mo concentration in vegetarians or Asian people who eat more cereals and pulses than general Europeans may be somewhat higher than in these two populations.

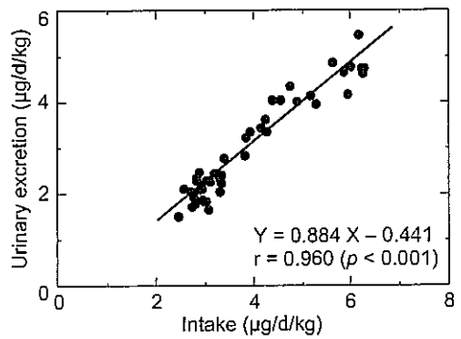

FIGURE 14.1 Relationship between Mo intake and urinary Mo excretion in healthy young Japanese women in balance study [9].

TABLE 14.5 Molybdenum Concentration in Healthy Human Blood

	Number of Subjects	Age	Concentration (μg/L)[a]	Analytical Method	Reference
Serum/plasma					
Japan	22M, 33F	20–59	0.70 (0.27–1.81)[b]	ICP-MS	[11]
Belgium	27M, 23F	18–75	0.55 ± 0.21	NAA	[23]
USA	2M	Unknown	0.5 ± 0.1	ICP-MS	[24]
Whole blood					
UK	44	Unknown	0.62 ± 0.29	ICP-MS	[5]
Germany	50M, 80F	18–70	0.43 (0.14–1.1)[c]	ICP-MS	[25]
Venezuela	244M, 174F	18–27	2.66 ± 0.66	AAS	[26]

[a] Values are arithmetic means ± SD unless otherwise noted.
[b] Geometric mean with SD range in parentheses.
[c] Arithmetic mean with 5th percentile to 95th percentile in parentheses.

14.4.2. Molybdenum in Blood

Table 14.5 shows tbe Mo concentration in healthy human blood measured in various countries. Serum Mo concentration in Japanese subjects showed a logarithmic normal distribution [11], while the variation coefficient of serum Mo in Belgian subjects was comparatively low [23]. The average values of plasma or serum Mo concentration were similar among Japanese, Belgian, and American [24] subjects although several Japanese subjects showed slightly higher values. Because the plasma Mo concentration was correlated to the Mo intake [8], this higher serum Mo concentration in Japanese is probably derived from their high consumption of soybean products rich in Mo.

The values of whole blood Mo in German [25] and British [5] subjects were similar and also similar to the values of serum Mo; however, the Mo concentration of whole blood from Venezuelan subjects [26] was obviously higher than those of European subjects. The Venezuelan study used the AAS method while the two European studies used the ICP-MS technique for Mo determination; however, since the dietary pattern and Mo intake of Venezuelan people are unclear, it cannot be concluded that differences in the analytical method influence the analytical values of whole blood Mo concentration.

14.4.3. Molybdenum in Milk

The Mo concentration of human milk decreased rapidly from 15 μg/L on day 1 to 4.8 μg/L at 7 to 10 days postpartum and 2.6 μg/L by 1 month [27]. Table 14.6 shows the Mo concentration in matured human milk samples from various countries. The Mo concentration in human milk from mothers in European countries was lower than in other areas. The US/Canadian DRI has adopted a value of 2.00 μg/L as the average human milk Mo concentration [20]. The buman milk Mo of mothers in Japan, Guatemala, and Zaire are similar to this value. On the other hand, the human milk Mo level in the Philippines is markedly high compared to that in other areas.

TABLE 14.6 Molybdenum Concentration in Matured Human Milk

Country	Number of Samples	Median (μg/L)	Range (μg/L)	Analytical Method	Reference
Germany[a]	19	0.53	0.18–0.81	ICP-MS	[28]
Guatemala	14	2.12	<0.3–9.00	NAA	[29]
Hungary	13	<0.3	<0.3–3.88	NAA	[29]
Japan	79	3.18	<0.1–25.91	ICP-MS	[12]
Nigeria	9	2.65	0.34–9.71	NAA	[29]
Philippines	15	10.37	6.75–35.41	NAA	[29]
Sweden	10	0.40	<0.3–5.87	NAA	[29]
Zaire	15	1.39	<0.3–5.81	NAA	[29]
USA/Canada	–	2.00[b]	–	–	[20]

[a] Include samples from mothers who live in the Czech Republic and Poland.
[b] Values adopted in DRI to calculate the adequate intake (AI) of Mo for US/Canadian infants.

14.5. CLINICAL SIGNIFICANCE OF SERUM AND PLASMA MO

14.5.1. Index of Dietary Molybdenum Intake

Turnlund and Keyes examined variations of the plasma Mo concentration in four young American subjects who consumed five levels of dietary Mo for 24 days each [8]. As shown in Figure 14.2, which is based on this study, the plasma Mo strongly correlated to the dietary Mo intake. This indicates that the serum/plasma Mo concentration is an index of dietary Mo intake. As shown in Table 14.3, a higher dietary Mo intake (more than 300 μg/day) occurs in vegetarians or Japanese people with a high consumption of soybean products, but dietary Mo intake more than

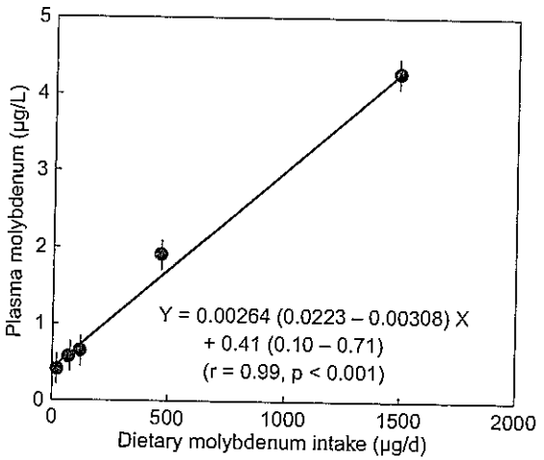

FIGURE 14.2 Relationship between Mo intake and serum Mo concentration of four American male adults in a balance study performed by Turnlund and Keyes [8]. Values within parentheses indicate 95% confidence intervals.

500 μg/day cannot occur. When the highest dietary Mo intake is estimated to be 500 μg/day, the highest serum/plasma Mo concentration (95% confidence interval) is in the range 1.22–2.25 μg/L. Accordingly, when the serum/plasma Mo concentration is more than 2.25 μg/L, a metabolic abnormality or abnormal exposure must be considered. This criterion is reasonable when considering the serum Mo concentration of Japanese healthy adults [11].

14.5.2. Index of Molybdenum Exposure

14.5.2.1. Occupational Exposure There are several reports on occupational exposure to Mo. Twenty-five workers employed at a molybdenite roasting plant in Denver in the US were exposed to Mo dust at an 8 h time-weighted average value of 9.47 mg/m^3 [2]. This Mo exposure caused a large elevation of serum ceruloplasmin and smaller increases in serum uric acid levels. Joint pains, backaches, headaches, and nonspecific hair and skin changes were the most frequent complaints of the workers. Plasma Mo concentration of the workers ranged from 9 to 330 μg/L. This plasma Mo concentration is markedly higher than the upper limit of serum/plasma Mo in healthy adults established above. Accordingly, measurement of serum/plasma Mo can demonstrate occupational exposure to Mo.

14.5.2.2. Artificial Joint Joint-replacement surgery has revolutionized the treatment of osteoarthritis as the most effective therapy. Due to the possibility that several trace elements are released from the prosthesis into blood, it is necessary to monitor the trace element levels in blood and to examine the long-term effects of trace element exposure. Since a metal-on-metal prosthesis is frequently used in current joint-replacement surgery, the chance of exposure to several trace elements is deemed to increase. This metal is used as a material for several prostheses, as well as Al, Co, Cr, Ni, Ti, and V. In fact, there are several reports on the monitoring of blood Mo in patients with artificial joints [30, 31]. Luetzner et al. determined changes in serum levels of Co, Cr, and Mo in 41 patients after cemented unconstrained total knee arthroplasty without patellar resurfacing, 18 with unilateral total knee arthroplasty, and 23 patients with bilateral total knee arthroplasty surgeries. While serum Co and Cr increased in patients compared to the controls without implants, the serum Mo in patients (median, 2.55 μg/L) was not significantly higher than in controls (median, 2.11 μg/L). Since the control values in this study are close to the upper limit of serum/plasma Mo in healthy adults (2.25 μg/L), a problem in the analysis of serum Mo may exists in this study. Thus, no evidence of Mo exposure by artificial joints using blood Mo has been found; however, it is thought that measuring blood (serum/plasma or whole blood) Mo can monitor exposure to this metal from artificial joints.

14.5.3. Index of Various Diseases

14.5.3.1. Liver Dysfunction In a Belgian study, serum Mo concentration was significantly increased in subjects with various types of liver disease and significant correlations between serum Mo and activities of serum aspartate aminotransferase

(AST) and alanine aminotransferase (ALT) were observed [23]. Significant correlations between serum Mo and the activities of AST and ALT were also found in the Japanese study [11]. Since Mo accumulates in the liver at a level of more than 300 μg/kg [32], high serum Mo in subjects with liver dysfunction may be derived from a liver leakage. Similarly to serum Mo, serum levels of xanthine oxidase, a molybdoenzyme, were significantly higher in patients with liver disease than in healthy controls [33]. Thus, serum Mo is increased by liver disease and is a suitable index for liver function.

14.5.3.2. Renal Failure Serum Mo levels of 60 patients with chronic renal failure were examined before and after hemodialysis [34]. The serum Mo significantly decreased from 27 ± 19 μg/L before hemodialysis to 14 ± 7 μg/L after hemodialysis. The correlations between serum Mo and serum β2-microglobulin, serum parathyroid hormone, serum calcium, and duration of hemodialysis were significant. Serum Mo in patients markedly exceeded the upper limit of healthy adults even after hemodialysis. Since the main excretion route of Mo is urine, subjects with renal failure show remarkably high serum Mo values [9, 35]. However, subjects with high serum urea in a medical examination did not show high serum Mo (Yoshida, M. et al., unpublished data). Therefore, serum Mo is not an index of early-stage renal dysfunction, but an index of renal failure.

14.6. CONCLUSIONS

The determination of Mo in biological materials can be performed using ICP-MS. Food groups with high Mo concentration are plant foods, such as cereals, pulses, nuts, and their products. The Mo intake in most countries ranges from 100 to 300 μg/day and is thought to be adequate. A large part of Mo in diet is readily absorbed in intestine and then excreted to urine. Serum Mo and urinary Mo are strongly associated with Mo intake. When the Mo concentration in serum is more than 2.25 μg/L, a metabolic abnormality or abnormal exposure should be assumed.

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In: Vitamin D: Nutrition, Side Effects and Symptoms ISBN: 978-1-61728-601-8
Editor: Stephanie R. Malone

Chapter 8

PATIENTS WITH CROHN'S DISEASE HAVE HYPOVITAMINOSIS D AND K, WHICH IS INDEPENDENT OF GENERAL MALNUTRITION

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INTRODUCTION

Inflammatory bowel disease (IBD) consisting of Crohn's disease (CD) and ulcerative colitis (UC) is an inflammatory disease affecting the gastrointestinal tract. As its name implies, UC mainly affects large intestine. In contrast, CD can affect all part of the gastrointestinal tract from mouth to anus, but in most cases with CD, small intestine is involved. Nutritional complications are common in patients with IBD, especially those with CD[1,2]. Deficiencies of proteins, calories, and vitamins are quite prevalent in subjects with CD, which may be caused by such factors as inadequate dietary intake, intestinal loss of protein, or malabsorption[3-6]. Previous studies have reported the high prevalence of hypovitaminosis D or K in patients with IBD [7-11].

Vitamin D, amongst its diversity of actions, enhances intestinal absorption of calcium and phosphorus as its fundamental action [12]. Its deficiency causes mineralization defect; rickets and osteomalacia[13]. Its inadequacy, even in its milder form (insufficiency) increases the risk of fracture through negative calcium balance and secondary hyperparathyroidism[14]. Recently, the skeletal action of vitamin K has come to our attention, although its sole action of clinical significance has long been considered to be the one as the co-factor for γ-

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carboxylation of four of the clotting factors in the liver [15]. Skeletal vitamin K insufficiency has been demonstrated to be a risk factor for fracture [16,17].

Osteoporosis is a common complication of IBD [18-22]. It has also been reported that the incidence of fracture among patients with IBD was greater than that in general population [23]. Thus, hypovitaminosis D and K is likely to be of great importance in the evaluation and possibly the treatment of osteoporosis associated with IBD.

In our recent report [24], CD patients had significantly lower plasma vitamin K and D concentrations, and significantly higher serum levels of markers for the inadequacy of these vitamins; parathyroid hormone (PTH), protein induced by vitamin K absence (PIVKA)-Ⅱ and undercarboxylated osteocalcin (ucOC), which are indicators for vitamin D insufficiency, hepatic vitamin K insufficiency, and skeletal vitamin K insufficiency, respectively. Subjects with CD had significantly lower BMD scores at almost all measurement sites than those with UC. Plasma levels of vitamin D and K correlated with BMD and the patients' fat intake, but not with their intake of these vitamins. Multiple regression analysis revealed that low plasma concentrations of vitamin D and K were independent risk factors for low BMD at radius. These results suggested that maintaining vitamin D- and K-adequacy is necessary for the bone health. The insufficiency of these vitamins is likely to arise from their malabsorption probably due to restricted fat intake as well as the compromised intestinal ability to absorb the nutrients.

However, nutritional indices such as serum albumin and total cholesterol levels were also lower in patients with CD than those with UC. Thus one could argue against the role of these vitamins by stating that decreased blood concentrations of these vitamins merely reflect the overall malnutrition.

In this paper, we have attempted to demonstrate that decreased blood concentrations of vitamin D and K in IBD patients are independent of general malnutrition by use of one of the multivariate analyses; principal component analysis (PCA).

SUBJECTS AND METHODS

Study Subjects

The study subjects were 128 patients with IBD (male:78, female:50) consisting of 60 patients with CD (male:36, female:24) and 68 patients with UC (male:42, female:26) visiting the Gastroenterology Clinic of Kyoto University Hospital. Consent to participate in this study was obtained after explanation of the objective and protocol of this study.

Bone Mineral Density (BMD) Measurement

BMD was measured at various skeletal sites; lumbar spine, femoral neck, total hip, distal one-third of radius, ultradistal radius with dual energy X-ray absorptiometry (DXA) using Hologic QDR-2000 (Bedford, MA). Data were expressed as z-value, which shows a standard deviation from age- and sex-adjusted average based on the Japanese reference database.

Blood Examination

Blood was obtained after overnight fasting. After centrifugation, plasma or serum samples were stored at –30℃ with protection from light until analysis. Serum concentration of 25-hydroxyvitamin D (25OH-D) was measured by radioimmunoassay (RIA) (DiaSorin, Stillwater, MN, USA). Plasma vitamin K_1 (phylloquinone; PK), and menaquinone-7 (MK-7) levels were determined by high-performance liquid chromatography-tandem mass-mass spectrometry with atmospheric pressure chemical ionization (LC-APCI-MS/MS), HPLC analyses were conducted with a HPLC system (Shimadzu, Kyoto, Japan), Mass spectrometry was performed with API3000 LC-MS/MS System (Applied Biosystems, Foster City, CA). ^{18}O-labeled vitamin K was the internal standard in the analysis of plasma vitamin K [25]. Serum intact-PTH was measured by an electro immunoradiometric assay (IRMA) (Scantibodies Laboratory, Santee, CA, USA), with 14-66 pg/ml as the reference range.

Other biochemical markers were measured by auto-analyzer. Approximately half of serum calcium is bound to albumin, and its serum concentration is affected by alteration in serum albumin level. Therefore, serum calcium concentration was corrected as follows; corrected calcium=calcium – (albumin – 4).

Statistical Analysis

Statistical analyses were done with SPSS 17.0J (SPSS Japan, Tokyo). Comparison of independent two groups was done with Student's t-test or Mann-Whitney test depending on normality. Multiple regression analyses were performed to investigate the determinants for BMD at each site. Data on various nutritional indices and circulating vitamin D- and K-levels were analyzed with principal component analysis (PCA), which is a statistical method to summarize the various parameters into small number of summary factors (components). These components are obtained in such a way that first component is extracted from the initial raw data with the maximal amount of information (eigenvalue), and the second one is extracted from the remaining information. Thus, they are independent of each other. Components with the eigenvalue greater than 1 were adopted as is used in the usual practice.

RESULTS

Biochemical Markers

The baseline characteristics of the study subjects are shown in Table 1. Patients with CD were significantly younger than those with UC. CD patients had higher CRP level, and lower nutritional indices such as BMI, hemoglobin, serum albumin and cholesterol concentrations than those with UC. Other indices were not significantly different between two groups, and largely fell into the reference ranges.

Table 1. Baseline characteristics of the study subjects

	IBD (n=128)	CD (n=60)	UC (n=68)	p value
Age (years)		34.4+/-7.5	41.7+/-16.4	<0.01
Sex (Male/Female)		36/24	42/26	-
Body mass index (kg/m^2)		19.2+/-2.7	21.1+/-3.0	<0.01
C-reactive protein (mg/dl)		0.7+/-1.0	0.3+/-0.6	0.01[b]
White blood cell (x10^3)		6.1+/-1.9	5.9+/-1.9	NS
Red blood cell (		4.3+/-0.5	4.5+/-0.6	NS
Hemoglobin (g/dL)		11.8+/-1.9	13.1+/-2.0	<0.01
Total protein (g/dL)		6.8+/-0.7	7.1+/-0.4	0.01
Albumin (g/dl)		3.9+/-0.4	4.3+/-0.4	<0.01
Triglyceride (mg/dL)		95.4+/-40.7	101.3+/-77.6	NS
Total cholesterol (mg/dl)		131.7+/-25.2	176.5+/-36.6	<0.01
Alkaline phosphatase		228.9+/-72.3	227.5+/-95.8	NS
Corrected calcium (mg/dl)		8.8+/-0.3	8.8+/-0.3	NS
Phosphorus		3.3+/-0.7	3.4+/-0.4	NS

Data are expressed as mean+/-SD.

The p value indicates the statistical difference between CD and UC patients based on Student's t-test or Mann-whitney test depending on normality.

Table 2. Serum concentrations of 25(OH)D, PTH, and vitamin K in IBD patients

	IBD (n=128)	CD (n=60)	UC (n=68)	p value
25OH-D (ng/ml)		14.1+/-8.5 (12.6)	18.5+/-7.2 (18.2)	<0.01
PTH (pg/ml)		30.6+/-12.4 (28.0)	26.0+/-9.7 (25.5)	0.04
PK (ng/ml)		0.60+/-0.72 (0.45)	0.86+/-0.54 (0.78)	<0.01
MK-7 (ng/ml)		2.70+/-7.49 (0.45)	6.43+/-10.1 (1.67)	<0.01

Data are expressed as mean+/-SD with the values in parentheses showing the median.

The p value indicates the statistical difference between CD and UC patients based on Student's t-test or Mann-whitney test depending on normality.

Circulating Levels of Vitamins D and K

Serum 25OH-D concentration was significantly lower and PTH level was significantly higher in CD patients than UC subjects. There is a general consensus that serum 25OH-D concentration less than 20 ng/mL indicates hypovitaminosis D. Serum 25OH-D level was below 20 ng/ml in 82% of patients with CD and 57% of patients with UC. Most of the subjects had serum PTH level within the reference range (≤66 pg/mL). Serum concentrations of PK and MK-7 were significantly lower in patients with CD than those with UC (Table 2). In our recent report using the same assay procedure, mean plasma PK level was 1.74 ng/ml, and plasma MK-7 level was 4.96 ng/ml in healthy Japanese women aged 30-49 [26]. Therefore, circulating levels of vitamin K were considered to be extremely lower in IBD patients than that in the healthy subjects.

Table 3. BMD in patients with CD and UC

	CD (n=27)	UC (n=31)	p value
Lumbar spine (L1-4)	-0.52+/-0.64**	-0.45+/-1.15*	NS
Femoral neck	-1.02+/-0.85**	-0.45+/-1.12	NS
Total hip	-0.87+/-0.81**	-0.27+/-1.08	0.03
Distal one-third of radius	-1.85+/-1.15**	-1.79+/-1.51**	NS
Ultra distal radius	-1.87+/-0.86**	-1.20+/-1.36**	NS

Data are shown as the z-score normalized by gender and age and expressed as mean +/- SD. The p value denotes the statistical significance in BMD in CD and UC subjects. The asterisks show the statistically significant difference from zero (*: $p<0.05$, **<0.01).

BMD Measurement

In Table 3 are shown the data on BMD. Although patients with CD had z-score significantly lower than zero at all measurement sites, UC subjects had z-score below zero at lumbar spine, distal one-third and ultradistal radius. BMD in CD patients was significantly lower at total hip

Principal Component Analysis (PCA)

PCA was performed with the parameters included for analysis being hemoglobin serum albumin, total cholesterol, 25OH-D level and plasma PK, MK-7 concentrations (Table 4). Two components were obtained and explained 56.7% of the variance. The first component was composite of high albumin, total cholesterol and hemoglobin, and second component consisted of high 25OH-D, PK and MK-7. The interpretation of each component was made as follows; the first component representing overall nutritional status, the second component, vitamin D and K status. When CD and UC subjects were analyzed separately, vitamin D and K status was also independent of overall nutritional status (data not shown).

Table 4. Principal component analysis of nutrition indices

	Component 1	Component 2
Serum Albumin	**0.828**	0.040
Serum total cholesterol	**0.675**	0.056
Hemoglobin	**0.786**	0.241
Serum 25OH-D	0.309	**0.730**
Plasma PK	-0.125	**0.820**
Plasma MK-7	0.130	**0.498**

Factor loadings to four components after varimax rotation are shown. Loadings greater than 0.35 are shown in bold

Two components thus obtained were considered to represent the following nutritional status; component 1 overall nutritional status, component 2 vitamin status

Table 5. Comparison of two parameters obtained from PCA.

	CD (n=60)	UC (n=68)	P value
First Component (Overall Nutritional Status)	-0.54+/-0.87	0.47+/-0.86	<0.001
Second Component (Vitamin D and K Status)	-0.25+/-1.08	0.22+/-0.87	0.006

The p value indicates the statistical difference between CD and UC patients based on Student's t-test.

Next, these two summary scores were compared between CD and UC subjects. Both of them were significantly lower in CD patients than UC subjects, which suggests that patients with CD are both under-nourished and in vitamin D- and K-insufficiency (Table 5).

Multiple Regression Analyses for Factors Associated with BMD Z Scores At Various Site

Multiple regression analyses were done to study to what extent the BMD (z-score) could be explained by these summary scores (Table 6). BMD at all measurement sites except lumbar spine and distal one-third radius were significantly contributed by these two summary scores, with first component (overall nutritional status) contributing to BMD at ultradistal radius, and second component (vitamin status) significantly contributed to BMD at femoral neck, total hip, and ultradistal radius.

Table 6. Multiple Regression Analyses for BMD.

Sites	R^2	p value	Variable	β	p value
Lumbar spine	0.06	0.07	Overall Nutritional Status	0.10	0.44
			Vitamin D and K Status	0.27	0.04
Femoral neck	0.21	<0.01	Overall Nutritional Status	0.11	0.45
			Vitamin D and K Status	0.47	<0.01
Total hip	0.18	<0.01	Overall Nutritional Status	0.22	0.09
			Vitamin D and K Status	0.36	<0.01
Distal one-third of radius	0.02	0.65	Overall Nutritional Status	0.10	0.51
			Vitamin D and K Status	0.07	0.63
Ultradistal radius	0.14	<0.01	Overall Nutritional Status	0.28	0.04
			Vitamin D and K Status	0.26	0.05

Abbreviations are as follow; β for β coefficient and p for p value. Determinants of independent predictors for BMD at each site were analyzed by multivariate analysis with stepwise method. Variables included were two parameters obtained from PCA.

DISCUSSION

The insufficiency of vitamin D and K are associated with increased risk of fracture[14,27-29]. However, the importance of vitamin insufficiency is often overlooked. Classical vitamin deficiency diseases are easily diagnosed, since they are accompanied by phenotypic abnormalities such as rickets or osteomalacia in vitamin D deficiency, and bleeding tendency in vitamin K deficiency. In contrast, vitamin insufficiency could not be diagnosed for the individual subjects phenotypically, and the increased risk of chronic diseases due to vitamin insufficiency becomes apparent only by the epidemiological studies.

Thus each subject could only be evaluated by the surrogate markers. Serum concentration of 25OH-D best reflects the vitamin D status. It is a general consensus that its concentration below 20 ng/mL is associated with increased fracture risk, although recent evidences indicate that higher concentration around 30 ng/mL is necessary for fracture prevention[30,31]. In the case of vitamin K, there are some methodological problems. First, vitamin K consists of phylloquinone (vitamin K_1) and menaquinones (vitamin K_2), the latter further composed of several analogs with different length of side chain. Therefore, vitamin K status cannot be evaluated by a single measurement of blood level of one of the vitamin K analogs. In addition, although circulating vitamin K levels have been measured with various methods, results are different between assay procedures. The present data were obtained with our newly developed LC-APCI-MS/MS procedure with stable isotope-labeled internal standard yielding high sensitivity and specificity [25]. Thus, measurement of circulating levels of these vitamins aids us to evaluate the subjects' vitamin status, especially vitamin insufficiency.

In the present study, patients with IBD had hypovitaminosis D and K, especially those with CD. Patients with CD had also lower overall nutritional status than those with UC. We have recently reported that hypovitaminosis D and K were associated with decreased BMD in IBD subjects[24]. However, osteoporosis is a common complication of IBD, for which many factors have been reported to be responsible; low body weight, inflammatory process, and therapeutic glucocorticoid use[18-22].

Thus, it must be decided whether the decreased concentrations of vitamin D and K are merely the reflection of overall malnutrition or not. Confounders are serious challenges in the clinical and epidemiological studies. In the intervention studies, randomized controlled trial (RCT) would largely eliminate the interference by confounders. Of the observational studies, cohort study would be less sensitive to the confounding variables. In the cross-sectional studies, eliminating the interference by confounders is a tough methodological challenge.

The main strategies to control for confounding in observational epidemiological investigations and, in particular, in case-control studies, are restriction, matching, stratification and fitting of regression models. In the present study, we have attempted to discriminate hypovitaminosis D and K from general malnutrition by use of another statistical method; principal component analysis (PCA). It is a useful statistical procedure to summarize the complex and diverse data into the small number of independent components (summary score). In the epidemiological or clinical studies, PCA has been employed in the dietary pattern analysis [32,33].

Two components or the summary scores obtained in the current study were considered to represent the overall nutritional status, and vitamin D- and K-status, respectively. Both of them were lower in patients with CD than those with UC. Since they are independent of each

other, these results suggested that subjects with CD had both hypovitaminosis D and K, and general malnutrition, but the former is not merely a reflection of the latter. The results by multiple regression analyses indicated that insufficiency of these vitamins would negatively affect BMD at various skeletal sites.

We believe that our current report carries significance both clinically and methodologically, it is not free from limitation. Although the multiple regression analysis has identified vitamin D and K status as the significant contributor to BMD at femur and radius, the R^2 value was low, which indicates that the current model could explain only a small portion of variation. Further studies are required for the additional determinants for BMD in IBD.

In conclusion, patients with CD have hypovitaminosis D and K independent of general malnutrition, and analyzing data from cross-sectional studies with PCA would be of help in eliminating the interference by confounding variables.

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