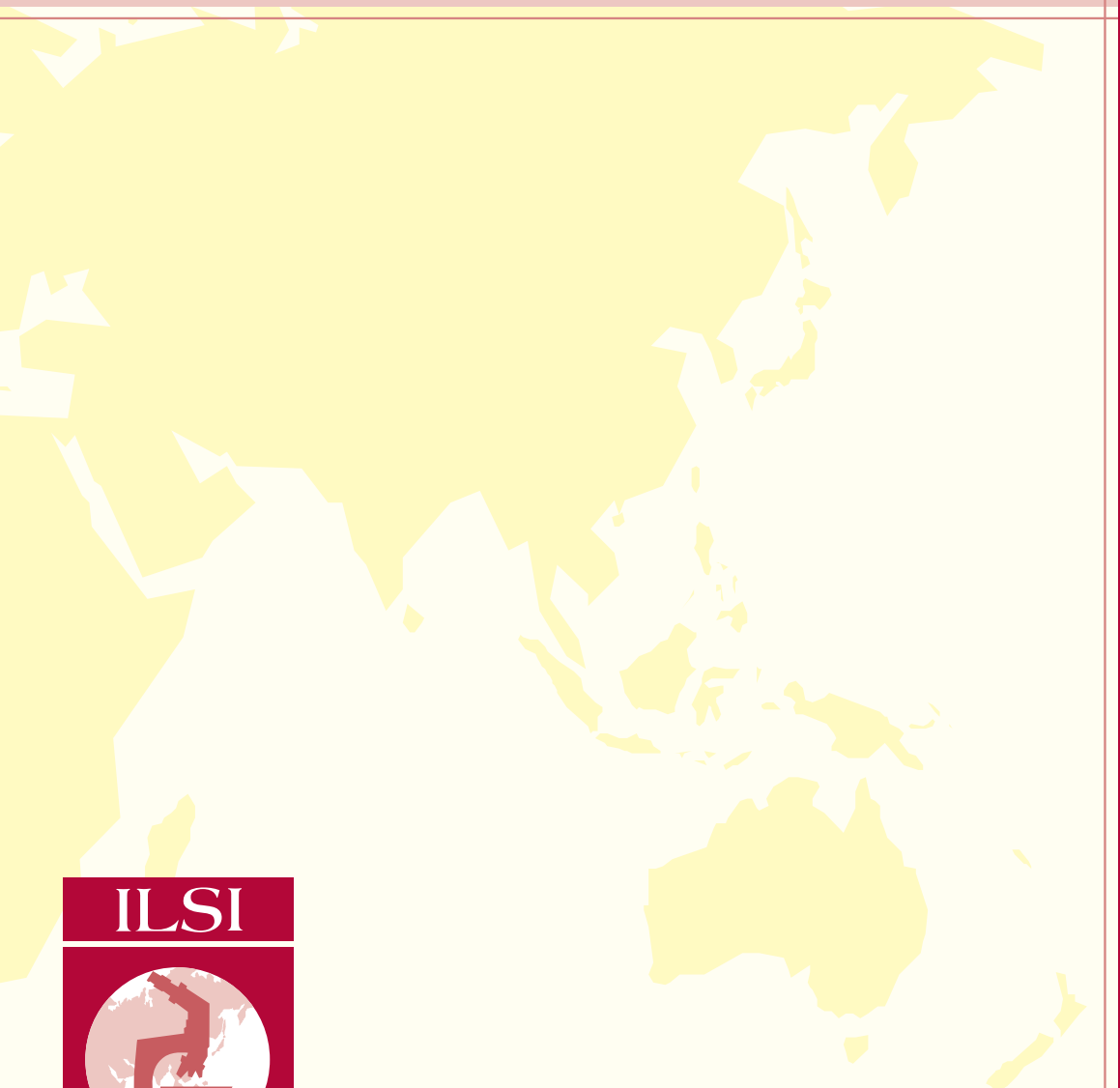
RECOMMENDED DIETARY ALLOWANCES:

HARMONIZATION IN SOUTHEAST ASIA



ILSI

International Life Sciences INSTITUTE

ASEAN • Australasia & Pacific

INTERNATIONAL LIFE SCIENCES INSTITUTE
Southeast Asia Region

MONOGRAPH SERIES

The International Life Sciences Institute (ILSI) is a nonprofit, worldwide foundation based in Washington, DC established in 1978 to advance the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. By bringing together scientists from academia, government, industry and the public sector, ILSI seeks a balanced approach to solving problems of common concern for the well-being of the general public. ILSI receives financial support from industry, government, and foundations. ILSI branches include Argentina, Brazil, Europe, India, Japan, Korea, Mexico, North Africa and the Gulf Region, North America, North Andean, South Africa, South Andean, Southeast Asia Region, the Focal Point in China, and the ILSI Health and Environmental Sciences Institute. ILSI also accomplishes its work through the ILSI Research Foundation, comprising the ILSI Human Nutrition Institute, the ILSI Risk Science Institute and the ILSI Center for Health Promotion. ILSI is affiliated with the World Health Organization as a non-governmental organization (NGO) and has specialized consultative status with the Food and Agriculture Organization of the United Nations.

ILSI Southeast Asia Region (ILSI SEA Region), located in Singapore, serves as the regional office for the administration, coordination of scientific programs, research and information dissemination in Southeast Asia, Australia, New Zealand and the Pacific.

This publication is made possible with the support of the ILSI SEA Region Micronutrients and Food Fortification Task Force and its members. The Task Force has worked closely with scientific institutions and regulatory agencies in the region to review current national recommended dietary allowances (RDAs) and to develop a set of harmonized RDAs for Southeast Asia. The Task Force will continue to develop and promote appropriate technologies, standards and regulatory guidelines for the fortification of selected staples, complementary foods and condiments to reduce micronutrient deficiencies in the region.

RECOMMENDED DIETARY ALLOWANCES: HARMONIZATION IN SOUTHEAST ASIA

Editors

E-Siong Tee

Rodolfo F. Florentino

International Life Sciences Institute (ILSI)
Southeast Asia Region

Monograph Series

Singapore

Southeast Asia Recommended Dietary Allowances Harmonization
Steering Committee

Consultants

Dr E-Siong Tee

Dr Rodolfo F. Florentino

Chairperson

Dr Corazon VC Barba

Secretariat

Ms Ma. Isabel Z. Cabrera

Members

Dr Ouk Poly (*Cambodia*)

Dr Muhilal (*Indonesia*)

Dr Somphou Outhensackda (*Laos*)

Dr Mohd Ismail Noor (*Malaysia*)

Dr May Khin Than (*Myanmar*)

Ms Ma. Isabel Z. Cabrera (*Philippines*)

Ms Low Yen Ling (*Singapore*)

Dr Prapaisri Sirichakwal (*Thailand*)

Dr Sangsom Sinawat (*Thailand*)

Dr Do Thi Kim Lien (*Vietnam*)

FOREWORD

Recommended Dietary Allowances (RDAs) are the most common and well-accepted standards among nutrition experts for nutrient intake. RDAs reflect current scientific judgment on nutrient allowances for growth of children and maintenance of good health for all. New scientific knowledge on nutrient needs to prevent chronic diseases and promote optimal health, and recent developments in national food labeling regulations have spurred the review of current RDAs in the Southeast Asian region.

Recognizing the need to harmonize RDAs in Southeast Asia, the International Life Sciences Institute (ILSI) Southeast Asia Region (ILSI SEA Region) has, with support from the Food and Agriculture Organization (FAO) of the United Nations, organized a total of 6 workshops and 1 working group meeting between 1997 and 2003. The meetings aimed to help countries and government agencies to update national RDAs based on the latest science and the utilization of national data. The objective was to facilitate consumer education, public health improvement and trade. The participants included country representatives from Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam, as well as other international and regional nutrition experts and regulators. The meetings provided a platform for the development of a harmonized set of RDAs which can be used as references by the Southeast Asian countries.

This Monograph documents the process of developing and establishing the harmonized RDAs. It also comprises state-of-the-art papers on 14 selected core nutrients prepared by several country representatives. This Monograph will serve as a working document and reference for countries in the region that are formulating or revising their national RDAs.

Many have contributed to the successful completion of RDA harmonization, as well as the publication of this Monograph. Firstly, we would like to express our sincere appreciation to all the Authors of the state-of-art papers. The Editors of this Monograph, Dr E-Siong Tee and Dr Rodolfo F. Florentino, worked tirelessly to fine tune and perfect all the papers. ILSI SEA Region is indebted to the Editors for their guidance and excellent work. We also thank the Chairperson of the Southeast Asia Recommended Dietary Allowances Harmonization Steering Committee (SEA-RDA Committee), Dr Corazon VC Barba and the Head of the Secretariat, Ms Ma. Isabel Z. Cabrera, both from the Food and Nutrition Research Institute of the Philippines, for their leadership in facilitating the discussion and preparation of the state-of-art papers.

Several international and regional nutrition experts have assisted and offered their expertise to the SEA-RDA Committee. Special thanks are due to Dr Biplab Nandi of FAO, Dr Paul Lachance of Rutgers University, USA, Dr Allison Yates of the University of Southern Mississipi, USA, Dr Geoffrey Marks of the University of Queensland, Australia, Dr Normasa Hosaya of University of Tokyo, Japan, Dr Denise Bienz and Dr B.H. Lim of Roche Vitamin Ltd and the late Dr Vernon Young of the Massachusetts Institute of Technology, USA. ILSI SEA Region also gratefully acknowledges the valuable contributions made by all workshop participants. Last but not least, special thanks to Ms Pauline Chan, Senior Program Manager and Task Force Coordinator of ILSI SEA Region, who has worked closely with all parties concerned to bring this Monograph to fruition.

We are confident that this Monograph will provide useful and up-to-date information on nutrient intakes to the regional and global scientific community, health professionals and industry.

Yeong Boon Yee
Executive Director, ILSI SEA Region
April 2005

CONTENTS

6. IRON

Dr Pattaneee Winichagoon
Institute of Nutrition, Mahidol University, Thailand

7. IODINE

Dr Hardinsyah
Bogor Agricultural University, Indonesia
Dr Djoko Kartono
Ministry of Health, Indonesia

8. ZINC

Dr Khor Geok Lin
Universiti Putra Malaysia, Malaysia
Dr Zalilah Mohd Shariff
Universiti Putra Malaysia, Malaysia

9. SELENIUM

Ms Ma. Isabel Z. Cabrera
Food and Nutrition Research Institute, the Philippines
Dr Corazon VC Barba
Food and Nutrition Research Institute, the Philippines

10. VITAMIN A

Dr Muhilal
Nutrition Research & Development Centre, Indonesia
Ms Ma. Isabel Z. Cabrera
Food and Nutrition Research Institute, the Philippines

11. VITAMIN D

Dr Do Thi Kim Lien
National Institute of Nutrition, Vietnam

1. INTRODUCTION

1.1 Background

In March 1997, the International Life Sciences Institute Southeast Asia Region (ILSI SEA Region) held the first Regional Forum and Workshop on Recommended Dietary Allowances (RDAs) in Singapore (1st RDA Workshop) to exchange information on the status of RDAs in Southeast Asia. Attended by food and nutrition scientists and other participants from countries in the region such as Indonesia, Malaysia, Philippines, Thailand and Vietnam, discussions focused on the scientific basis for RDAs, identifying research needs and setting future directions. It was also recognized that there was a need for regional collaboration and harmonization of RDAs. Such collaboration and harmonization would provide a common framework that can help countries and governments in formulating their own standards. A consensus was then reached for a follow-up workshop to be organized, and participants would work towards achieving agreement on areas such as common approaches in the development of RDA; concepts and terminologies; application and uses; framework (age groupings, reference body weights, core nutrients etc) and research agenda applicable throughout the region.

During the 2nd RDA Workshop held in July 1998 in Kuala Lumpur, Malaysia, a framework for RDA harmonization was agreed upon. The initial agreements and recommendations that were reached included the purpose and definition of harmonization as *"the consensus on certain elements of the RDA for the purpose of obtaining a better understanding not only of the RDAs in various countries but also of their application and use as practiced there at"*. The sharing of resources (manpower and materials) through training visits and analysis of food and biological samples was identified as one strategy to facilitate the setting up of a regional RDA database. To this end, a Southeast Asia Recommended Dietary Allowances Harmonization Steering Committee (the SEA-RDA Committee) was formed, consisting of representatives from local/national RDA Committees of the participating Southeast Asian countries, namely, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. It was agreed that an inventory survey of the countries' expertise and resources for RDA formulation would be conducted to serve as a basis for identifying Country Focal Points and for drawing up a Plan of Action for regional collaboration towards RDA harmonization. Results of the survey showed that there were similarities in certain areas that could forge immediate harmonization or even alignment. There were, however, also differences that may be resolved in due time with proper research and development.

In the course of a series of 6 RDA Workshops and 1 working group meeting, held between 1997 and 2003, organized by ILSI SEA Region and supported by the Food and Agriculture Organization of the United Nations (FAO), consensus has been reached in several key areas, including:

1. Definition of RDAs for the Southeast Asia region
2. Minimum list of core nutrients to be included
3. Population groupings
4. Reference body weights

It was further agreed that state-of-the-art papers will be prepared to serve as working documents for formulating the RDAs for the Southeast Asian population.

1.2 Development of Recommended RDAs for Southeast Asia

The formulation of the recommended RDAs for Southeast Asia (SEA-RDAs) was undertaken by the SEA-RDA Committee, comprised of country representatives who are either members of their national RDA committees or are involved in the review and revision of their respective country's RDAs as data generators or users. Initial agreements were reached on the purpose of harmonization and the goal of the harmonized RDAs. "RDA" was taken to mean the levels of intake of energy and nutrients which are considered adequate for the maintenance of health and well-being for nearly all healthy persons in the population.

Most participating countries felt that they were not ready for multiple reference values such as adequate intake (AI) and tolerable upper intake level (UL). There is insufficient data in the region on health outcomes arising from intakes greater than the RDAs. The move to incorporate the concept of risk reduction of chronic degenerative diseases, such as cardiovascular disease, would mean doing an extensive review of scientific literature in and outside the region, and may also require primary research on health outcomes at various levels of intakes among the Asian population. The SEA-RDA Committee therefore agreed to expand its goals to include reducing the risk of chronic diseases and the attainment of optimum health in the future. In the meantime, it was agreed that a single level be used and that the term "RDA" be retained. However, it was also agreed that national committees reviewing their RDAs could start working towards establishing AIs and ULs for their respective countries.

The SEA-RDA Committee agreed on the framework of the harmonized SEA-RDAs, as well as their applications and uses. It was also agreed that the population groupings would be consistent with, where possible, the groupings used in the 2002 FAO/WHO Expert Consultation Report on Recommended Nutrient Intakes (RNIs) for Vitamins and Minerals (2002 FAO/WHO Report). The SEA-RDA Committee also set sub-categories for adolescent and adult groupings in consideration of energy and protein requirements. The SEA-RDA Committee agreed on the list of nutrients, known as "core nutrients", to be included in the SEA-RDAs, and which could serve as the SEA-RDA Committee's immediate target. The nutrients to be included are those considered to be of nutritional importance to the populations in the region. Other nutrients may be added to national RDA tables.

Reference body weights for each population group were agreed upon. However, each country may also use, if available, national data derived from national surveys to incorporate trends in body weight within the country. Thus, for adults, the use of local national averages was recommended, but for children, "ideal" body weights using reliable and more recent local data was agreed upon. In the absence of national data on reference body weights, the US National Center for Health Statistics (NCHS) references may be used.

After agreements were reached on the framework of the harmonized SEA-RDAs, the SEA-RDA Committee members were each assigned to prepare state-of-the-art papers on specific core nutrients. Each of the papers outlined the characteristics and functions of the specific core nutrient; absorption, utilization and excretion; effects of deficiency and excess; food sources and usual intakes; factors affecting requirements; estimating requirements and recommended intakes; current RDAs for the nutrient in Southeast Asia; the SEA-RDA Committee's proposed SEA-RDAs; and research recommendations. The preparation of the papers was viewed by the SEA-RDA Committe Members as an excellent collaborative activity to jointly review the current concepts of RDA development; to share knowledge on the use of newer concepts; to initiate the practice of sharing databases, building upon what was available at the national/regional level and to discuss possible future cooperative efforts.

In view of the limited studies done in most Southeast Asian countries, the SEA-RDA Committee members drew heavily on the following sources in preparing their papers: the report of the 2002 FAO/WHO Expert Consultation on RNIs for Vitamins and Minerals; the 1985 FAO/WHO/UNU report on Energy and Protein Requirements; the 2004 FAO/WHO/UNU report on Energy Requirements; and the reports of the US Institute of Medicine (IOM), Food and Nutrition Board (IOM, 1997, 1998, 2000, 2001 and 2002). Databases used by these organizations together with other relevant studies; meta-analyses published in refereed and non-refereed journals; and unpublished data, particularly on nutrient requirements and bioavailability, nutritional status assessment and supplementation studies in the region were also examined. Findings of studies done in the Southeast Asian countries or in the region, which demonstrated commonalities in many aspects, were given precedence, followed by the recommendations of FAO/WHO and other foreign organizations.

The papers were then circulated to the other members of the SEA-RDA Committee and then presented and discussed during the SEA-RDA Committee's follow-up meetings, where consensus was reached on the SEA-RDAs for the various nutrients.

The finalized papers and the SEA-RDAs recommended by the SEA-RDA Committee are published as this Monograph. This Monograph begins with an introduction on the need for harmonization of RDAs within the Southeast Asian region and the efforts of the SEA-RDA Committee to achieve this objective. Next, the concepts and general principles employed by the SEA-RDA Committee in arriving at the SEA-RDAs are set out. This is followed by chapters on each of the specific core nutrients included in the framework of the SEA-RDAs. This Monograph ends with a conclusion on the recommendations of the SEA-RDA Committee.

Bibliography

FAO/WHO (2002). *Human Vitamin and Mineral Requirements.* Report of a Joint FAO/WHO Expert Consultation. FAO, Rome.

FAO/WHO/UNU (1985). *Energy and Protein Requirements.* Report of a Joint Expert Consultation. WHO Technical Report Series 724. World Health Organization, Geneva.

FAO/WHO/UNU (2004). *Human Energy Requirements.* Report of a Joint FAO/WHO/UNU Expert Consultation. Food and Nutrition Technical Report Series, Food and Agriculture Organization, Rome.

IOM (1997). *Dietary references for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC.

IOM (1998). *Dietary References Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.

IOM (2000). *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.

IOM (2001). Vitamin A. In: *Dietary References Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; pp 82-161.

IOM (2002). *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients).* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC.

2. CONCEPTS AND GENERAL PRINCIPLES

2.1 Terminology, Definition and Goal

The harmonized SEA-RDAs set by the SEA-RDA Committee are defined as *"the levels of intake of energy and dietary components which, on the basis of current scientific knowledge, are considered adequate for the maintenance of health and well-being of nearly all healthy persons in the population."* Operationally, it is equivalent to the mean requirement plus 2 standard deviations (SD), or also coefficient of variation (CV), to meet the needs of almost all (97.5%) individuals in a population group.

2.2 Uses and Applications

The SEA-RDAs have the following uses and applications:

- Assessment of food and nutrient intake of populations and individuals
- Planning of nutrient intakes, such as in menu planning, planning for therapeutic diets, food and nutrient supplementation, catering guidelines etc
- Risk assessment of adequacy of nutrient intake among individuals and populations
- As a tool for nutrition education and in defining food and nutrition policy (e.g., in the planning and assessment of food production)
- As a basis for food product formulation (e.g., in food fortification) and nutrition information. The guidelines of the Codex Alimentarius system will be used for labeling purposes.

2.3 Nutrients Included

The list of core nutrients included in the SEA-RDA framework are:

- Energy
- Iron
- Zinc
- Vitamin D
- Niacin
- Protein
- Iodine
- Vitamin A
- Thiamin
- Folate
- Calcium
- Selenium
- Vitamin C
- Riboflavin

Unfortunately, national and regional data on food composition, deficiency prevalence, the roles of nutrients in the prevention of chronic degenerative diseases, direct studies on requirements and nutrient-nutrient interrelationships are limited if not unavailable for most of these nutrients. Nevertheless, the importance of these nutrients are well-recognized, and foreign literature is replete with information on requirements and metabolism.

2.4 Age/Physiological Group Categories and Reference Body Weights

Currently, there is divergence among the RDAs of the Southeast Asian countries in terms of the categorization of populations by age, sex and physiologic states. To achieve consistency, age categories within the SEA-RDA framework essentially follow those of the 2002 FAO/WHO Expert Consultation on RNIs for Vitamins and Minerals (FAO/WHO, 2002). In relation to the energy requirements, the SEA-RDAs also have sub-categories for adolescent and adult groupings (Table 2.1).

The reference weights for specific age categories are also presented in Table 2.1. The reference weights for adults are 60 kg for males, and 50 kg for females. These are rounded up figures of the mean of average weights for male and female adults respectively, as reported by 4 countries which conducted national surveys between 1998 and 2002 (Indonesia, Philippines, Thailand and Vietnam), of 58 kg and 51 kg. The reference body weights for infants and children up to 9 years old are based on the NCHS P50 weights for males. The weights for older children lie between the body weights of children aged 7 to 9 years and young adults, taking into consideration the fact that weights at the end of adolescence approximate those of young adults (Figure 1).

Table 2.1 Population groupings and reference body weights within the SEA-RDA framework

Population Groups	Reference Body Weight (kg)	
Infants (months)		
0 – 5	6	
6 – 11	9	
Children (years)		
1 – 3	14	
4 – 6	20	
7 – 9	27	
Adolescents (years)	Male	Female
10 – 12	34	36
13 – 15	47	45
16 – 18	56	49
Adults (years)	Male	Female
19 – ≥ 65	60	50

2.5 Basis and Criteria of Adequacy

As mentioned above, the SEA-RDA Committee drew heavily on recent reports of the FAO and WHO, in particular, the 2002 FAO/WHO report on RNIs for Vitamins and Minerals; the 1985 FAO/WHO/UNU report on Energy and Protein Requirements; the 2004 FAO/WHO/UNU report on Energy Requirements; and the reports of the US Institute of Medicine (IOM), Food and Nutrition Board (IOM, 1997, 1998, 2000, 2001 and 2002). The SEA-RDA Committee was guided by the concepts and definitions adopted by these organizations. The estimated requirements in all of these reports were based on thoroughly-

examined evidences derived from both experimental and epidemiological/population studies. The conceptual framework of the SEA-RDAs is presented below in Figure 2.

Figure 1 Reference Body Weighs: SEA RDAs vs FAO/WHO

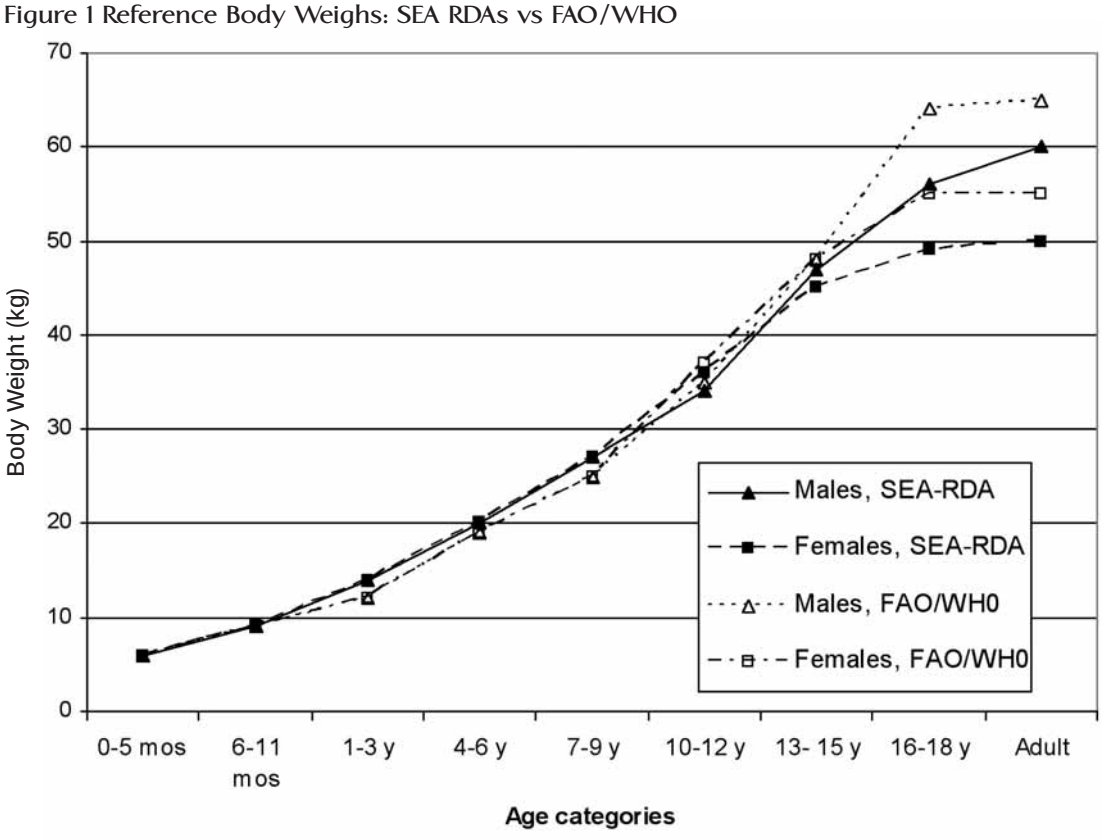
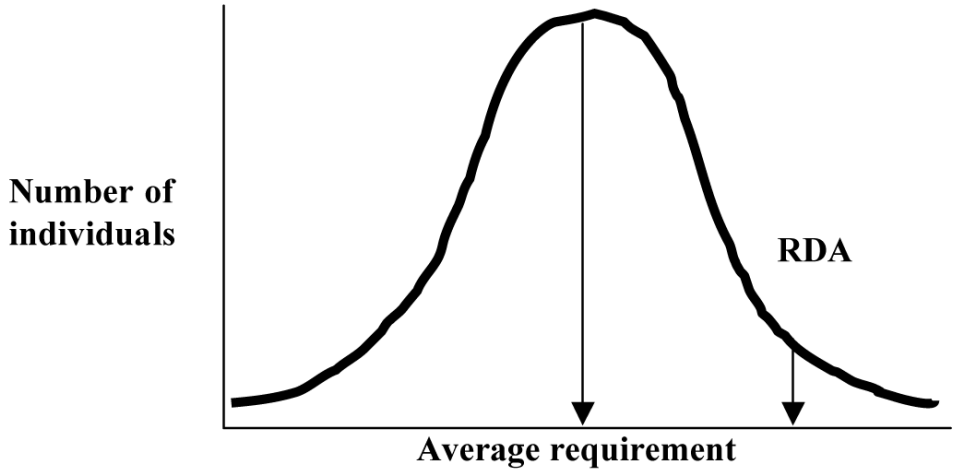


Figure 2 Conceptual framework of the SEA-RDAs



"Average requirement" refers to the lowest mean intake that, for a selected criterion, will prevent deficiency in a group of individuals with specific characteristics (e.g., age, gender, physiologic status).

The chosen criterion may be different depending on life stage or gender groups or both. The estimated nutrient needs of half of the individuals in a group is met at this level of intake (the risk of inadequacy is 50%). This intake level includes an adjustment for an assumed bioavailability of the respective nutrient.

"RDA" refers to the daily intake which meets the nutrient requirements of almost all apparently healthy individuals in an age- and sex-specific population group. It is based on an estimated average requirement (EAR) plus 2 SD above the mean:

$$\text{RDA} = \text{EAR} + 2\ \text{SD}^{\text{AR}}$$

This intake level meets the nutrient needs of almost all (97% to 98%) individuals in a specific group.

However, the above operational definition of RDA for nutrients does not apply to energy requirements. Unlike the RDAs for nutrients, which are set at the top of the distribution of requirements to meet the needs of nearly all (97% to 98%) individuals in a group, the RDA for energy is set at the computed average requirement of individuals in that group because intakes consistently above the individual's requirements can lead to overweight or obesity.

For some nutrients, a "recommended safe or adequate intake" (AI) rather than RDA is provided because of insufficient scientific evidence to derive an EAR. There may also be uncertainties in deriving recommended intake levels. This intake level corresponds to the observed average or experimentally set intake by a defined population or subgroup that appears to sustain a defined nutritional status, such as growth rate, normal circulating nutrient values, or other functional indicators of health.

An additional level, the "upper tolerable intake level" (UL), has also been introduced by FAO/WHO and IOM. The need for this new level arose from the growing practice of fortifying foods with nutrients, as well as the increasing use of large doses of dietary supplements. The UL is the maximum intake from food that is unlikely to pose risk of adverse effects from excessive intake. If an individual's usual nutrient intake remains below the UL, it is unlikely that there would be an increased risk of adverse effects from excessive intake, given current knowledge. There is no established benefit for healthy individuals from consuming amounts of nutrients that exceed the recommended intakes. For most nutrients, no adverse effects are anticipated when they are consumed as foods because their absorption and/or excretion are regulated. The SEA-RDAs have adopted the ULs recommended by FAO/WHO and IOM.

2.6 Basis of Population Groups

Most requirement estimates are based on studies on adults. The basis and criteria of adequacy for energy and nutrients are described in detail in the following chapters on specific nutrients. In general, the requirements for children were extrapolated from those of adults.

For most nutrients, SEA-RDAs for infants from birth to less than 6 months were derived from adequate intakes of fully breast-fed infants, based on an average milk volume of 750 mL for the first 6 months multiplied by the nutrient concentration in breast milk.

For older infants (6 to 12 months), the SEA-RDA includes the amount of nutrients provided in both breast milk (based on average breast milk volume of 600 mL) and complementary foods. As with adults, values derived by extrapolation from EARs are increased by 2 SD or 2 CV to derive the RDA that would cover the needs of 97.5% of the individuals in the group.

Additional requirements during pregnancy are based on estimates of amounts laid down in fetal and maternal tissues. For lactating women, the requirements are based on amounts secreted in breast milk (750 mL and 600 mL for the 1st and 2nd 6 months respectively, multiplied by nutrient concentration). Adjustments for efficiency of conversion to tissue/milk are incorporated and these amounts are then added to the requirements of non-pregnant, non-lactating women.

2.7 Application of the SEA-RDAs to Individuals and Populations

The SEA-RDAs are intended to cover the requirements of most individuals to ensure that the needs of nearly all apparently healthy (not ill based on clinical signs and symptoms and function, normally assessed by routine laboratory methods and physical evaluation) individuals in the population are met. The SEA-RDAs are expressed in terms of usual intakes of nutrients that population groups should consume over a period of time.

The comparison of intakes with RDA is a statement of risk of inadequacy; that is, the chance that the intake is inadequate to meet the actual requirement. It is a probability statement and is not a measure of severity of inadequacy. It is not possible to judge nutritional status of individuals on the basis of RDA as this can be done only through clinical, biochemical and anthropometric means. Thus, if the intake of an individual is grossly below the RDA, it does not necessarily mean that the individual is inadequately nourished, but that the probability that he or she may be malnourished is high. As such, RDA functions as a tool in assessing the dietary nutrient intake of individuals. For example, assuming a CV of 15% and recommended allowance of 2 SD above the mean, an intake of 88.5% of the RDA has about 7% probability that the individual's intake is below requirement.

The SEA-RDAs relate to individual nutrients. The recommended intake levels refer to specific nutrients and not certain foods or diets. These nutrient intake levels have to be translated to food-based dietary guidelines for populations and individuals to be applicable to food and agriculture planning and nutrition education. The food pyramid, for example, explains how persons can consume adequate nutrients in terms of foods that consumers buy, cook and eat. Essentially, RDAs for energy and nutrients translate into a recommendation to eat a variety of foods, which in turn translates into a recommendation to follow the food guide pyramid.

As a nutrient-based dietary standard, RDA acts a basis for formulating dietary guidelines and food guides, and gives direction for change in dietary patterns of the populations and nutrition education programs. The following are some examples of the functional roles of RDAs:

Example 1

The consumption of green leafy vegetables, yellow fruits and vegetables, milk and eggs can materially increase vitamin A and riboflavin intakes. To meet the higher RDA for folate, higher intakes of vegetables and fruits, which are among the best sources of folate, should be promoted.

Example 2

To achieve sufficient calcium intakes among the population, food fortification and enrichment programs and the consumption of milk and other foods rich in calcium should be promoted. Efforts to promote consumption of milk and milk products must also be matched by efforts to increase available milk supply and to improve access to milk by a significant segment of the population.

Example 3

The SEA-RDA for iron is higher than for most Western population groups due to the low bioavailability of iron in the average regional diet. The recommended intake levels for iron may be lowered by an overall improvement in dietary quality, such as inclusion of sources of vitamin C and heme iron (e.g. animal products) at every meal. There is a need for a vigorous and rigorous implementation of food fortification and supplementation initiatives for iodine since the only reliable sources of this nutrient are fortified foods (iodized salt) and supplements.

Example 4

The higher RDAs for pregnant and lactating women, particularly for iron and folate, may be difficult to meet by dietary sources alone. Supplementation can help meet the increased requirements of these physiologic groups.

Bibliography

FAO/WHO (2002). *Human Vitamin and Mineral Requirements.* Report of a Joint FAO/WHO Expert Consultation. FAO, Rome.

FAO/WHO/UNU (1985). *Energy and Protein Requirements.* Report of a Joint Expert Consultation. WHO Technical Report Series 724. World Health Organization, Geneva.

FAO/WHO/UNU (2004). *Human Energy Requirements.* Report of a Joint FAO/WHO/UNU Expert Consultation. Food and Nutrition Technical Report Series, Food and Agriculture Organization, Rome.

IOM (1997). *Dietary references for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC.

IOM (1998). *Dietary References Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.

IOM (2000). *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.

IOM (2001). Vitamin A. In: *Dietary References Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; pp 82-161.

IOM (2002). *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids* (*Macronutrients*). Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC.

3. ENERGY

3.1 Introduction

The first attempt to establish human energy requirements at population level was initiated by FAO in 1950. At its first Expert Consultation on energy requirements, FAO proposed an estimate of requirements based largely on energy intakes of different population groups. The estimated requirements were applicable only to groups and not to individuals. A "reference" man, woman and child were each created, with defined patterns of physical activity, body size and age, enabling a mean yearly average energy requirement to be calculated. In 1957, the second FAO Expert Consultation on energy requirements was convened and the earlier approach was maintained. However, some of the underlying concepts and definitions were refined.

In 1973, FAO/WHO jointly convened an ad hoc committee of experts (the FAO/WHO Expert Consultation) to revise the energy requirements and also to address the issue of protein requirements. The FAO/WHO Expert Consultation retained the concept of a "reference man" and its application to groups rather than individuals. However, it also redefined the "reference individuals" and amplified the specifications. Categories of energy requirements were refined by introducing several occupational activities applicable to diverse populations, taking into account lifestyles and living conditions in developing countries. The FAO/WHO Expert Consultation stated that estimates of energy requirements should be based, as far as possible, on estimates of energy expenditure. Nevertheless, it also acknowledged that most of its recommendations on energy requirements were based on measurements of food intake. The factorial approach (see below) was endorsed and adjustments made to the reference requirements on the basis of age, body size of specific populations, and the climate conditions they face.

Following the FAO/WHO Expert Consultation's 1973 Report, an Expert Consultation jointly organized by FAO, WHO, and the United Nations University (UNU) met in 1980 to discuss and update energy and protein requirements. FAO/WHO/UNU published its report in 1985 (the 1985 FAO/WHO/UNU Report), wherein energy requirement was defined as *"the level of energy intake from food that will balance energy expenditure when the individual has a body size and composition, and level of physical activity, consistent with long-term good health; and that will allow for maintenance of economically necessary and socially desirable physical activity"*. It then specified the extra needs of children and pregnant and lactating women. The FAO/WHO/UNU Expert Consultation retained most of the general approaches adopted at the previous Expert Consultations, but also made some substantial progress in redefining principles, highlighting pertinent issues and revising approaches based on new knowledge in the area of energy metabolism that have emerged since the 1973 Report.

The most recent joint FAO/WHO/UNU Expert Consultation on Energy in Human Nutrition was convened in October 2001 to review state-of-the-art scientific literature since the 1985 FAO/WHO/UNU Report, and to arrive at recommendations for energy requirements throughout the life cycle (FAO/WHO/UNU, 2004). The 2004 FAO/WHO/UNU Report defined energy requirement as "the amount of food energy needed to balance energy expenditure in order to maintain body size, body

composition and a level of necessary and desirable physical activity, consistent with long term good health". This includes the energy needed for optimal growth and development of children, for the deposition of tissues during pregnancy and for the secretion of milk during lactation consistent with good health of the mother and child.

3.2 Function and Essentiality

A key objective of human nutrition is to ensure that the energy ingested through food consumption is adequate to meet the body's energy demands. The body needs energy to maintain body temperature and metabolic activity, to support growth and for physical work.

A person whose usual food consumption (expressed in terms of dietary energy and quantified in kcal) is below the minimum required level is considered to be undernourished. The focus on dietary energy in assessing food insufficiency or deprivation is justified from 2 perspectives. Firstly, a minimum amount of dietary energy intake is essential for body-weight maintenance and work performance. Secondly, increased dietary energy, if derived from normal staple foods, also increases the intake of protein and other nutrients. However, raising intakes of protein and other nutrients without ensuring a minimum level of energy intake does not necessarily improve a person's nutritional status.

Although food deprivation is still prevalent in many populations around the world, obesity is a rapidly growing problem, particularly in affluent societies. It is therefore also important to minimize excess energy intake over expenditure in order to prevent obesity and its complications.

In determining energy requirements, the first important principle is to use energy expenditure as the basis for estimation. This is due to the recognition that it is energy expenditure, and not energy intake, that drives energy needs. Energy intake does not necessarily reflect energy needs and may vary independently. However, due to the lack of sufficient data on energy expenditure of children under the age of 10 years, the 1985 FAO/WHO/UNU Report relied on energy intake as the basis for estimating the energy requirement for this age group.

The scope for adapting the energy requirement estimates was a prominent issue in the 1985 FAO/WHO/UNU Report. Adaptation was defined as *"a process by which a new and different steady state is reached in response to a change or difference in the intake of food or nutrients"*. 3 methods of adaptation were accepted: (a) metabolic; (b) biological/genetic and (c) social/behavioral. However, the factors acknowledged as most relevant to energy requirement estimates were alterations in body size and a decrease in physical activity.

Other basic principles that were re-confirmed in the 1985 FAO/WHO/UNU Report include the statement that *"Energy requirement estimates refer to groups, not to individuals"* and *"Energy requirement of a group is the mean of the group, and includes no safe margin."* Energy requirement is therefore different from protein and all other nutrient requirements, where a safe margin corresponding to 2 SD above the physiological needs are included. This difference is due to the fact that excess energy cannot be disposed of, accumulating as fat in the body which may lead, in the long-term, to obesity.

Notwithstanding the re-affirmation of certain basic principles, the 1985 FAO/WHO/UNU Report differed from previous reports in one key area. The 1985 FAO/WHO/UNU Report rejected the concept of the "reference individual", thereby allowing for estimates of energy requirements that are more accurate. In short, the statement -*"Requirement for what? Normative versus status quo"*- became a central part of the assessment process, placing a greater responsibility on the user's knowledge and understanding of pertinent energy facts and associated health implications. Energy requirements can thus be estimated for specific and complex situations. Examples include: whether to allow for the extra energy needed by stunted children for catch-up growth; whether the desirable body weight should be the basis for calculating the energy requirement of a community where adult obesity is highly prevalent; or whether it is justifiable to allocate extra energy to allow for physical exercise to be undertaken in currently sedentary communities. However, the 1985 FAO/WHO/UNU Report did not offer explicit advice on when and to what extent the normative or status quo requirement may be applied.

A major innovation of the 1985 FAO/WHO/UNU Report was the adoption of the factorial approach for estimating energy requirements that expresses energy requirement/expenditure, as well as its various components, as multiples of basal metabolic rate (BMR). Besides being the largest component of energy expenditure (as high as 70% in sedentary individuals), expressing energy expenditure/requirements in terms of BMR factors make it unnecessary to correct for body weight, thus simplifying the calculation and allowing easier and more meaningful comparisons among diverse population groups. The report, however, recognized that a residual variability remained of BMR/kg body weight at the diverse weights, with higher values per unit body weight in smaller individuals than in bigger ones. The factorial approach consists of the summation of various activities representing the energy expenditures, such as the costs of the diverse types of physical activity, the extra energy allocated for pregnancy and lactation, and the energy cost of growth.

In line with the new approach of expressing the various components of energy requirement/expenditure as multiples of BMR, it is essential that BMR be measured with utmost care and precision. A variety of predictive equations were previously available, but a validated and unified set of equations was needed to obtain consistent results. The 1985 FAO/WHO/UNU Report provides *"the best estimates at present available for predicting the BMR of healthy people in any population."* These equations are age and sex specific, and use body weight as an independent variable. Height was found not to be a significant contributing factor, except for a small effect in children aged 0 to 3 years and for the older age groups. However, the 2001 FAO/WHO Expert Consultation proposed a new set of equations, now known as the "Oxford equations", based on a large collection of data which included those from healthy Asian populations (Henry, 2001). However, after a lengthy re-analysis of BMR predictive equations, the current 2004 FAO/WHO/UNU Report recommended the continued use of the Schofield BMR predictive equations recommended in the 1985 FAO/WHO/UNU Report.

3.3 Energy Metabolism

The standard unit of energy is the joule, and human energetics are usually expressed in terms of kjoules or KJ (i.e. joules x 1,000). A megajoule (MJ) is 1,000 KJ. One Calorie is equivalent to 4.184

KJ. It is a fundamental principle of thermodynamics that energy cannot 'disappear'. Food energy consumed has to be either excreted in the feces, or absorbed by the body. Once absorbed, a small amount of energy is excreted in the urine as the by-product of protein metabolism and the rest of the absorbed fuel has to be metabolized for energy or stored in the tissue as protein, fat or carbohydrate in the form of glycogen. Metabolized energy supports the making of new chemical compounds within the body, fuels the muscular activity required to breathe, digests food and maintains body posture, and also provides the energy for physical activity.

3.3.1 Macronutrient interactions

Prentice and colleagues have developed a simple conceptual model termed "The Oxidative Hierarchy" which helps in understanding how the body regulates macronutrient balance on any given mixture of fuels consumed (Prentice, 1995). Alcohol is at the top of the hierarchy since the storage capacity is zero. Carbohydrate and protein (for which the body has very limited storage capacities) come next on the hierarchy and both will suppress the oxidation of fat, while fat comes at the base of the hierarchy. The human body has an almost unlimited capacity for fat storage, and both body fat and dietary fat exert very little feedback control on levels of fat oxidation. Knowledge derived from detailed manipulative studies using whole-body calorimeter has advanced our understanding of the extent to which the body can adapt its fuel selection in order to match its rate of utilization of different fuels to the amount provided in the diet (Shetty *et al.,* 1994). Data from Vietnam have indicated that some populations have thrived on fat intakes as low as 6% – 7% of energy intake (FAO/WHO, 1994). At such low intakes of fat, it may be necessary for them to synthesize some fat *de novo* from carbohydrate, a biochemical process which normally occurs at very low flux and which carries the disadvantage of being metabolically wasteful of energy. Such populations are generally extremely lean and may be surviving below their physiological optimum.

3.3.2 Macronutrients and diet-induced thermogenesis

Earlier literature on macronutrients and diet-induced thermogenesis (DIT) induced by different macronutrients tended to emphasize the differences observed when subjects were studied acutely in the period following ingestion of each macronutrient in its pure form. They indicated rather large differences between the so-called 'specific dynamic action' of the macronutrients, with protein having the highest level and fat the lowest. Such studies bear little resemblance to the real life scenarios where diets contain a varied mixture of energy sources. Recent studies, conducted with greater accuracy in whole-body calorimeters, have demonstrated that there is no detectable difference in thermogenesis over a 24-hour period between diets with wide-ranging fat:carbohydrate ratios, as wide-ranging as can be reasonably constructed using mixed meals (i.e. between 7% and 79% energy from fat with reciprocal changes in carbohydrate) (Shetty *et al.*, 1994). Similar studies with whole-body calorimeter have shown that DIT associated with alcohol oxidation is similar to carbohydrate (Westerterp *et al.,* 1999). These studies concluded that within mixed diets, the impact of different macronutrients on DIT can effectively be ignored in relation to energy requirements.

3.4 Effects of Deficiency and Excess

3.4.1 Deficiency

By comparing the distribution of dietary energy supply (DES) with individual energy requirements in different countries, 2 types of food inadequacy measures are obtained, namely the prevalence and the intensity of food inadequacy. The prevalence measure is concerned with the proportion and number of people who have inadequate access to food, i.e. access that falls short of a specified cutoff point; while the estimates of intensity assesses the extent by which access to food falls short of requirement (FAO, 1996).

Energy deficiency can be acute or chronic. Acute energy deficiency (AED) is by nature "episodic", and is characterized by a state of negative energy balance in which the energy expenditure is greater than energy intake. Under these conditions, there is a progressive loss of body weight, along with changes in the pattern of energy expenditure, in an attempt by the body to achieve a new but lower plane of energy equilibrium. If the energy deficiency persists, further weight loss occurs along with deterioration in health, leading ultimately to death.

On the other hand, chronic energy deficiency (CED) is a "steady state" caused by inadequate food energy over a lifetime. Individuals with CED could be in energy balance, although their anthropometric parameters may be less than desirable. This state is achieved by the presence of low body weight and fat stores, but the individual's health is normal and the body's physiological function is not compromised to the extent that the individual is unable to lead an economically productive life. There is good evidence to show that individuals with CED are less productive and that the CED state is associated with higher morbidity and mortality. In addition, the "steady state" referred to above must be appreciated as a theoretical one, subject to periodic fluctuations of physiological and environmental conditions, such as the menstrual cycle and seasons. A high incidence of babies with low body weight has been reported in mothers with low pre-pregnant body mass index (BMI). Milder energy-nutrient deficiency leads to stunting, and is also associated with several functional and behavioral consequences. From a public health viewpoint, it is important to prevent and address CED.

3.4.2 Excessive intake

Excessive energy intake and positive energy balance are usually due to adequate availability of food energy and a sedentary lifestyle. Development in many societies in transition is associated with the adoption of a "western" lifestyle. This process is shifting the nutrition-related disease burden away from under-nutrition and towards death and disability related to energy excess and positive energy balance. Social factors such as income, education, access to information and cultural beliefs, biological factors associated with a genetic predisposition, and metabolic changes associated with diet and physical activity are the main conditioning factors linked to the rising prevalence of positive energy balance and excessive energy stores. Marketing strategies employed by food manufacturers, which stimulate over-consumption of highly palatable energy-dense foods, may also be a contributing factor.

Obesity can lead to both non-fatal and life-threatening diseases. Health problems which are non-fatal (but nevertheless debilitating) associated with chronic energy excess include respiratory difficulties, chronic muscle-skeletal problems, skin problems and infertility.

Life-threatening and chronic health problems can be grouped into 4 main categories (Uauy, 2001):

(a) Type II diabetes (formerly known as Non-Insulin Dependent Diabetes Mellitus), a condition associated with insulin resistance
(b) Cardiovascular problems, including hypertension, stroke and coronary heart disease
(c) Certain types of cancers, such as hormonal-related and large bowel cancers
(d) Gallbladder disease

In many developing societies, populations have experienced better quality of life and increased consumption of calorie-dense foods. This has resulted in an increase in death and disability related to energy excess and positive energy balance, as compared to under-nutrition.

3.5 Macronutrients as Sources of Food Energy

The 4 principle classes of macronutrients that provide food energy to humans are: (a) carbohydrates, (b) fat, (c) protein and (d) alcohol. Each of these macronutrients has numerous sub-types with specific attributes in terms of energy delivery and potential health effects. The gross and metabolizable energy contents of the macronutrients in their natural forms are well established. Carbohydrates, fat and protein have physiological fuel values of 4 kcal/g (17 kJ), 9 kcal/g (37 kJ) and 4 kcal/g (17 kJ) respectively. Alcohol has a caloric value of 7 kcal/g (29kJ/g). Although the 1985 FAO/WHO/UNU Report adopted the Atwater general factor to calculate the energy values of foods, it must be noted that there are several different food energy systems described in current literature, namely, the specific food factors (Merrill & Watt, 1973), the Atwater general factors (FAO/WHO/UNU, 1985) and the net metabolizable energy factors (EC, 1990; Agriculture and Agri-Food Canada, 1996). These various food energy systems are used for food labeling or in food tables, and are a source of confusion to users and consumers. The goal is to establish a single food energy evaluation system for global use; however, to date there are no internationally agreed criteria for the selection of energy factors. A brief summary of the energy-yielding macronutrients is set out below.

3.5.1 Carbohydrates

FAO's existing recommendations on carbohydrate intake in relation to energy requirements suggest that a variety of carbohydrates should contribute at least 55% of total energy in an optimal diet for all ages except children under the age of 2 years. The bulk of carbohydrate-containing foods should be rich in non-starch polysaccharides and have a low glycemic index (FAO/WHO, 1996). A notable omission from these recommendations is any limitation on sucrose or simple sugars intake, as the FAO/WHO Expert Committee has taken the position that there is no evidence of a direct involvement of sucrose, other sugars and starch in the etiology of lifestyle-related diseases. Nonetheless, relationships between the intake of simple sugars and obesity at the population level have been examined in considerable depth (Hill & Prentice, 1995). There is also evidence that very

large intakes of simple sugars, in particular carbonated beverages, observed in some individuals (especially adolescents) may play a role in excess energy consumption (Ludwig *et al.*, 2001).

3.5.2 Fat

The recommendations of the 1994 FAO/WHO Expert Consultation on Fats and Oils in Human Nutrition (FAO/WHO, 1994) are prudent, and the specific considerations of energy balance provide no basis to suggest modifications. The recommendations include the following:

- Dietary fat should supply at least 15% of energy for most adults
- Sedentary individuals should not consume more than 30% of their energy from fat
- Active individuals in energy balance may consume up to 35% of their total energy from fat

It should be noted that there has been a large increase in the global availability of fats and oils, and that fat consumption in many parts of the developing world has shown a large percentage increase. This is especially so in Asia, where intakes were formerly viewed as being well below desirable levels and were associated with a high prevalence of CED.

3.5.3 Protein

Protein requirements are not calculated in the context of protein as a source of dietary energy. There are however, 2 new areas of controversy that have emerged since the 1985 FAO/WHO/UNU Report in respect of the potential impact of protein supply on obesity. Firstly, there is suggestion that high protein intakes in childhood may be causally associated with the development of obesity, although there is now epidemiological evidence to refute the theory. Secondly, there is controversy over whether high protein intakes are useful in down-regulating appetite and hence maintaining energy balance in sedentary societies. Most of the evidence for this comes from short-term studies on satiety and from studies of intentional weight loss using high-protein diets. The latter is probably irrelevant to the general issue of protein as an energy-yielding macronutrient.

3.5.4 Alcohol

It is assumed that there are no circumstances in which alcohol is recommended as an energy-giving substrate. However, in some populations alcohol may contribute up to 5% of total energy or more, and in some individuals it may represent a much greater proportion of food energy. There has been some debate as to whether the energy from alcohol should be considered as having a useful biochemical role or whether it is simply dissipated as heat. Objective evidence derived from calorimetric measurements clearly indicates that alcohol energy must be considered in the overall energy balance equation (Prentice, 1995; Westerterp *et al.*, 1999). Although there is no specific recommendation concerning alcohol intakes, it must be recognized that many people do in practice consume alcohol and that it does contribute to their energy intake.

3.6 Factors Affecting Energy Requirement

Energy requirement is determined based on energy expenditure, and is therefore affected by factors such as BMR and physical activity that affect the major components of energy expenditure.

3.6.1 Age

As the most important component of energy expenditure, BMR depends on the mass of metabolically active tissues in the body, the proportion of each tissue in the body, and the contribution of each tissue to the energy metabolism of the whole body. Changes in body composition due to growth and aging, therefore, markedly affects energy requirements since some organs of the body are much more metabolically active than others. These changes in body composition in children and adults have to be taken into account when calculating the energy requirement of a particular population group. Activity patterns also alter with age. Children become progressively more active once they are able to crawl or walk, while the physical activity pattern of adults are usually dominated by the nature of their work (FAO/WHO/UNU, 1985).

3.6.2 Gender

Men have relatively greater muscle mass than women, which tends to reduce their BMR when expressed in terms of lean body mass since muscle has a low metabolic rate. However, the greater body fat content of women means that the observed BMR per unit total body weight is somewhat lower in women. The energy demand for physical activity will often depend on the different types of employment for men and women. In children, basal energy expenditure on a weight basis differs little between pre-adolescent boys and girls, but since there are differences in body weight and composition from the first few months of life, and different physical demands are made on boys and girls, their energy requirements are considered separately (FAO/WHO/UNU, 1985).

3.6.3 Individual variations

In any assessment of the average requirement, both intra- and inter-individual variability must be recognized. The former results from short-term fluctuations in energy intake and expenditure. It has been argued that intra-individual variations in intakes are more important than inter-individual variations, and that the observed inter-individual variations can largely be explained in terms of the intra-individual variations. However, later evidence supports the conclusion that within-subject variations in BMR are small and insignificant, even when energy intake and physical activity are uncontrolled. It is also generally recognized that in a group of apparently comparable people, there is much inter-individual variation in habitual energy expenditure (Shetty *et al.*, 1986).

3.6.4 Population variations

The differences in BMR between various populations of the world are equivocal. Earlier studies showed that BMR was 8% to 10% lower in populations in tropical climates, while subsequent data suggested no difference in BMR between Indians and Europeans provided the subjects were well-

nourished. Other evidence suggest that the relationship between BMR and standard independent variables such as age, sex and body size may vary among populations including seasonal variations in BMR corresponding with diet and/or climate changes.

3.7 Methods of Measuring Energy Expenditure

Energy needs are determined by energy expenditure. Therefore, in principle, estimates of energy requirements should be based on measurements of energy expenditure.

Energy expenditure can be quantified by direct (measuring the heat output directly) or indirect (measuring oxygen consumption and carbon dioxide production) calorimetric techniques, and converting the values to their energy equivalents. Recently, the indirect technique of doubly-labeled water (DLW) using stable isotopes of hydrogen and oxygen has been widely accepted as the most appropriate and accurate method for assessing energy expenditure (Montoye *et al.*, 1996). The production of carbon dioxide can be calculated from the difference in elimination rates of the 2 isotopes. Based on the respiratory quotient, the oxygen uptake for the time period can be estimated.

Non-calorimetric methods, such as the measurement of heart rate, have also been used to estimate energy expenditure. This method is based on the assumption that heart rate and energy expenditure are linearly related (Warwick, 1989). However, some investigators have shown that the relationship may not be linear over the full range from rest to strenuous activities (Montoye *et al.*, 1996). A refinement of the method has been tested and may prove to be of use in the future (Spurr *et al.*, 1988).

3.7.1 Components of energy expenditure

The components of energy expenditure include BMR, physical activity, metabolic cost of food, and metabolic cost of growth.

(*a*) *Basal Metabolic Rate*

Physiologically, BMR is defined as the lowest rate of energy exchange in the body which is related to the organization of bodily functions and the production of body heat. Technically, it is defined as the rate of energy expenditure of a fasted and fully-rested individual in a thermoneutral environment. It can also simply be defined as the minimal rate of energy expenditure compatible with life.

BMR is usually measured by indirect calorimetry under standard conditions of immobility, in the fasted state (12 to 14 hours after a meal), and in an ambient environmental temperature between 26°C and 30°C, to prevent activation of heat generating processes such as shivering.

BMR can also be predicted with reasonable accuracy (i.e. with a CV of 8%) from predictive equations such as those of Schofield (Schofield *et al.*, 1985) which were used by the FAO/WHO/UNU Expert Consultation to estimate BMR of various age groups (Table 3.1). The Schofield equations were based on extensive and acceptable BMR data from European and North American subjects.

IDECG 1996 compiled and interpreted research data from different parts of the world. Shetty *et al.* (1996) did a critical analysis of BMR data reported by different investigators. The analysis concluded that the appropriateness of the Schofield equations needed to be reviewed because the database used contained a disproportionate number of Italian military subjects which appeared to have a higher BMR per kg than any other Caucasian group. Thus, the data used may have artificially elevated the predictive equations generated by Schofield. Furthermore, some investigators have observed that Asian subjects (Indians and Chinese) have BMR 10% to 12% lower than Europeans, while others have reported the BMR of other populations (Filipino, Indian, Japanese, Brazilian, Chinese, Malay and Javanese) to be 8% to 10% lower in range. (Shetty *et al.*, 1996).

In a draft paper to be used by IDECG working group members in preparation for the FAO/WHO/UNU Workshop on Energy and Protein Requirements in Human Nutrition, Henry (2001) proposed a new set of equations, now known as the Oxford equations, based on a large collection of BMR data using strict selection and exclusion criteria. The criteria included data that were collected only after 1950 and data with complete and adequate details regarding experimental procedures and conditions under which BMR was determined. The data included those from normal healthy populations of Asians such as Chinese, Japanese, Malays, Javanese, Filipinos, etc. These equations tend to produce lower values than the Schofield equations.

In the meantime, Ismail *et al.* (1998) have reported predictive equations for adult Malaysians (Table 3.3), Poh *et al.* (1999) for Malaysian adolescents aged 10 to 14 years old (Table 3.4). and Poh *et al.* (2004) for Malaysian adolescents aged 12 to 18 years old (Table 3.5). These equations also tend to produce lower values than the Schofield equations.

Table 3.1 Equations for predicting BMR from body weight

Age Group (years)	Kcal /day	Correlation coefficient	SD[a]	MJ /day	Correlation coefficient	SD[a]
Males						
0-3	60.9 W – 54	0.97	53	0.255 W – 0.226	0.97	0.222
3-10	22.7 W + 495	0.86	62	0.0949 W + 2.07	0.86	0.259
10-18	17.5 W + 651	0.90	100	0.0732 W + 2.72	0.90	0.418
18-30	15.3 W + 679	0.65	151	0.0640 W + 2.84	0.65	0.632
30-60	11.6 W + 879	0.60	164	0.0485 W + 3.67	0.60	0.686
> 60	13.5 W + 487	0.79	148	0.0565 W + 2.04	0.79	0.619
Females						
0-3	61.0 W - 51	0.97	61	0.255 W – 0.214	0.97	0.2F55
3-10	22.5 W + 499	0.85	63	0.0941 W + 2.09	0.85	0.264
10-18	12.2 W + 746	0.75	117	0.0510 W + 3.12	0.75	0.489
18-30	14.7 W + 496	0.72	121	0.0615 W + 2.08	0.72	0.506
30-60	8.7 W + 829	0.70	108	0.0364 W + 3.47	0.70	0.452
> 60	10.5 W + 596	0.74	108	0.0439 W + 2.49	0.74	0.452

Notes: [a] *Standard deviation of differences between actual BMRs and predicted estimates*
W = body weight in kg
Source: *FAO/WHO/UNU (1985)*

Table 3.2 Descriptive equations and statistics (mean ± SD) of Oxford equations for BMR

Age Group (years)	MJ/d	Kcal/day	SE Mean	N	r
Males					
0-3	0.255W – 0.141	61.0W – 33.7	0.255	277	0.954
3-10	0.0937W + 2.15	23.3W +514	0.328	289	0.827
10-18	0.0769W + 2.43	18.4W +581	0.566	863	0.861
18-30	0.0669W + 2.28	16.0W + 545	0.652	2821	0.760
30-60	0.0592W + 2.48	14.2W + 593	0.693	1010	0.742
60+	0.0563W + 2.15	13.5W + 514	0.685	534	0.776
Females					
0-3	0.246W – 0.0965	58.9W – 23.1	0.042	215	0.960
3-10	0.0942W + 2.12	20.1W + 507	0.360	403	0.820
10-18	0.0465W + 3.18	11.1W + 761	0.525	1063	0.752
18-30	0.0546W + 2.33	13.1W + 558	0.564	1664	0.700
30-60	0.0407W + 2.90	9.74W + 694	0.581	1023	0.690
60+	0.0424W 2.38	10.1W + 569	0.485	334	0.786

Note: W = body weight in kg
Source: Henry (2001), Energy Annex 2 (revised 10 Oct)

Table 3.3 BMR–predictive equations for adult Malaysians

Age Group (years)	N	Formula	r	SE Mean
Men				
18-30	84	0.0550(W) + 2.480	0.644	0.0363
30-60	223	0.0432(W) + 3.112	0.501	0.0189
Women				
18-30	131	0.0535(W) + 1.994	0.511	0.0263
30-60	218	0.0539(W) + 2.147	0.519	0.0200

Notes: BMR is expressed in MJ/day
W = body weight in kg.
Source: Ismail et al. (1998)

Table 3.4 BMR – predictive equations for adolescent Malaysians

Age Group (years)	Regression Equations	No. of Data Points	r^2	s.e.
Boys				
11	BMR = 86.42 W + 2097	83	0.62	390
12	BMR = 93.45 W + 1899	108	0.64	431
13	BMR = 79.75 W + 2377	109	0.66	393
14	BMR = 74.65 W + 2487	56	0.54	429
11 – 15	BMR = 80.38 W + 2319	360	0.70	417
Girls				
10	BMR = 75.29 W + 2118	55	0.62	329
11	BMR = 76.66 W + 2124	118	0.66	365
12	BMR = 52.46 W + 2846	103	0.47	400
13	BMR = 50.86 W + 2736	70	0.43	392
10 – 14	BMR = 54.44 W + 2781	353	0.52	405

Notes: BMR is expressed in kJ/day
W = Body weight in kg
s.e. = standard error
Source: Poh et al. (1999)

Table 3.5 BMR – predictive equations for Malaysian adolescents aged 12 – 18 years

Groups	Regression equations	No. of data points	r	s.e.e.
Boys	BMR = 55.8W + 3187	269	0.54	605
Girls	BMR = 53.4W + 2182	303	0.50	498
Combined	BMR = 54.9W + 1119.6S + 2116	572	0.81	551

Notes: BMR is expressed in kJ/day
W = Body weight in kg
S = sex, where 1 = female, 2 = male
s.e.e. = standard error of estimate
Source: Poh et al. (2004)

(*b*) *Physical Activity*

Energy needs may be calculated based on the amount of time spent and the energy cost of various activities usually determined by indirect calorimetry. To facilitate the calculations, daily activities are divided into 2 broad categories, namely occupational activities and discretionary activities (FAO/WHO/UNU, 1985).

Occupational activities include those activities that are essential for the individual and the community, and can be considered as economic activities that are life-sustaining. The traditional classification of work according to occupation is important, but care must be taken to ensure that there is an adequate description of the occupation.

Discretionary activities are additional activities outside working hours that may be of benefit to the community. The requirement to cover these activities should not be considered as dispensable, since it usually contributes to the physical and intellectual well-being of the individual, household or group. Such activities can be categorized into:

- Optional household tasks
- Socially desirable activities
- Activity for physical fitness and the promotion of health

(*c*) *Metabolic Response to Food*

The increased oxygen uptake after a meal depends on the nutrient composition of the food consumed, and the amount of energy ingested. The measurement of the energy cost of digesting, absorbing and storing ingested nutrients is not easy. It is difficult to separate the energy expended in excess of the BMR after eating a meal, from the energy cost of the physical activity involved in sitting, eating and digesting (FAO/WHO/UNU, 1985).

(*d*) *Growth*

The energy cost of growth includes 2 components: (i) the energy value of the tissue or (ii) product formed and the energy cost of synthesizing it. Although the energy requirement for growth relative to maintenance is small except for the first months of life, satisfactory growth is a sensitive indicator of whether needs are being met. To determine the energy cost of growth, the energetics of growth must be understood, and satisfactory growth velocities must be defined (Butte, 1996). Except in the case of young infants and during lactation, the estimates of energy cost are not very critical, since human growth is a slow process, taking up a small proportion of the energy requirement. (FAO/WHO/UNU, 1985)

3.7.2 Methods of estimating energy requirements

Since BMR is the largest component of energy expenditure, it has been adopted by the FAO/WHO/UNU Expert Consultation as the basis for calculating all components of total energy expenditure (TEE). To obtain the total requirement, the estimate of BMR is multiplied by a factor (Physical Activity Level factor or PAL) that covers the energy cost of increased muscle tone, physical activity, the thermic effect of food, and where relevant, the energy requirements for growth and lactation (FAO/WHO/UNU, 1985). In practice, the PAL is the ratio of TEE to BMR. General estimates of the energy cost of light, moderate and heavy activity for adults have been given by the 2004 FAO/WHO/UNU Report, resulting in estimates of PAL values as shown in Table 3.6.

Table 3.6 Classification of lifestyles in relation to the intensity of habitual physical activity, or PAL

Category	PAL value
Sedentary or light activity lifestyle	1.40 – 1.69
Active or moderately active lifestyle	1.70 – 1.99
Vigorous or vigorously active lifestyle	2.00 – 2.40[1]

Note: [1] PAL values above 2.40 are difficult to maintain over a long period of time.
Source: FAO/WHO/UNU (2004)

3.8 Current RDAs for Energy in Southeast Asia

This discussion is based partly on the review of energy requirements for Southeast Asian countries prepared by Tee (1998) and 3 other national RDAs published after that review. Table 3.7 tabulates the energy requirements for Indonesia, Singapore and Vietnam as reviewed by Tee (1998), and the recommendations for Malaysia (NCCFN, 2005), the Philippines (FNRI, 2002) and Thailand (MPH, 2003).

There are considerable variations in the recommendations because the various countries have used different approaches in arriving at their recommendations, as well as different body weights for the calculations. In Indonesia, energy requirement was estimated by measuring oxygen intake during the performance of activities, and results obtained showed no significant difference from the energy requirement recommendation of the 1985 FAO/WHO/UNU Report (Muhilal, 1998). However, details such as reference and techniques used, in particular those used for children under 5 years old, were not available. In the recently published Malaysian recommended nutrient intakes (RNI) (NCCFN, 2005), local data on BMR were used wherever available and local reference body weights were used for all age groups. For children aged 1 to 9 years, TEE was calculated based on Torun's quadratic polynomial regression equations and the mean body weights of Malaysian children collected from 3 local studies. For adolescents aged 10 to 18 years, the calculations were based on the PAL values of FAO/WHO/UNU (2004) and BMR values as calculated from data obtained from local population groups. The energy requirements recommended for adults and the elderly were based on moderately active lifestyles (PAL 1.75 for adults and PAL 1.60 for elderly) and the average body weight of Malaysians obtained through a national survey. The BMR for adult Malaysians was derived from local studies, while the BMR for the elderly were based on the Schofield equations (FAO/WHO/UNU, 1985).

In the Philippines RNI (FNRI, 2002), the revised RDAs for energy was estimated on the basis of an average weight of 59 kg for young adult men and 51 kg for young adult women. The energy expenditure was derived from the BMR estimated from the Oxford equations (Henry, 2001), multiplied by the PAL value of 1.67 for men and 1.55 for women, corresponding to moderate physical activity level as determined by local studies. For children and adolescents aged 1 to 18 years, the total energy requirement was calculated using Torun's recommendations (Torun *et al.*, 1996), while energy requirements from birth to 12 months were derived from the recommendations of Butte (1996). Singapore has adopted the recommendations of the 1985 FAO/WHO/UNU Report. Vietnam's recommended energy requirement values are also based on the 1985 FAO/WHO/UNU Report with adjustments for the average weight and energy expenditure of Vietnamese according to different categories of physical activities (Lien *et al.*, 1998).

Singapore has the highest recommended energy requirements for infants and children aged 1 to 9 years. Provisions for adolescent boys are higher than those for girls in all the RDAs reviewed (1,700 kcal to 2,840 kcal and 1,600 kcal to 2,300 kcal, respectively). Malaysia, the Philippines and Singapore have the highest energy requirements for adolescent boys. Requirements for adolescent girls are also high for these countries. But the highest recommendations are those of Vietnam.

All countries have recommended higher daily energy requirements for adult men (2,100 kcal to 3,000 kcal) than for women (1,750 kcal to 2,300 kcal) of the same age group of 19 to 59 years. Recommendations for older adults (1,550 kcal to 2,200 kcal) in the age group of 60 years and above are generally lower than for the younger adults (1, 750 kcal to 3,000 kcal). Requirements for adults and elderly men and women in Indonesia, Singapore and Vietnam are the highest in the region.

Provisions have been made for additional energy intake during pregnancy and lactation for all countries. Only Indonesia and Singapore provide for additional amounts during the first trimester. The additional amounts over and above the daily intakes for the second and third trimesters are rather similar among the countries in the region, ranging from 285 kcal to 360 kcal. Malaysia has an exceptionally high recommendation of an additional 470 kcal per day during the third trimester of pregnancy. For lactation, all countries have recommended an additional amount of 500 kcal to 550 kcal per day for the first 6 months, except for the additional 700 kcal recommended by Indonesia. For the second 6 months, except for Malaysia and Vietnam which did not provide for additional amounts, the other countries recommend an additional 500 kcal per day.

Table 3.7 Comparison of current energy RDAs (kcal/day) in selected Southeast Asian countries

Age Group (years)[1]	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 - 1)						
kcal / day	560 – 800	550 – 640	560 – 720	700 – 950	800[a]	620 – 820
Body weight (kg)	5.5 - 8.5	6.0 - 8.0	6.0 - 9.0	7.0 – 9.5	8.0	7.0 - 9.5[b]
kcal / kg	102 – 94	92 – 80	93 – 80	100	100	89 – 86
Children (1 - 9)[c]						
kcal / day	1,250 – 1,900	910 – 1780	1,070 – 1,600	1,150 – 2,100	1,000 - 1,400	1,300 -1,800
body weight (kg)	12 – 24	11 – 26	13 - 24	11 – 27	13 – 23	11-27[b]
kcal / kg	104 – 79	83 – 68	82 - 67	105 – 78	77 – 61	118-67
Boys (10 – 18)[d]						
kcal / day	2,000 – 2,500	2,180 – 2,840	2,140 – 2,840	2,200 – 2,850	1,700 – 2,300	2200-2700
body weight (kg)	30 – 56	36 – 59	34 – 58	34.5 – 64	33 – 57	34.5-64[b]
kcal / kg	67 – 45	61 – 48	63 – 49	64 – 45	52 – 40	64-42
Girls (10 – 18)[d]						
kcal / day	1,900 – 2,000	1,990 – 2,180	1,920 – 2,050	1,950 – 2,150	1,600 – 1,850	2,100 – 2,300
body weight (kg)	35 – 50	37 – 52	35 – 50	34 – 54	34 – 48	36 - 54[b]
kcal / kg	54 – 40	54 – 42	55 – 41	54 – 40	47 – 39	58 - 42
Male (19 - 59)[e]						
kcal / day	3,000	2,440 – 2,460	2,490 – 2,420	2,950 – 2,900	2,100 – 2,150	2,700
body weight (kg)	62	61 – 64	59	63.5	57	50 - 80
kcal / kg	48	40 – 38	42 – 41	46	37 – 38	54 - 34
Male (≥ 60)[f]						
kcal / day	2,200	2,010	2,170 – 1,890	2,450	1,750	2,200
body weight (kg)	62	57	59	63.5	57	50 - 80[b]
kcal / kg	35	35	37 – 32	39	31	44 - 28
Female (19 - 59)[e]						
kcal / day	2,250	2,000 – 2,180	1,860 – 1,810	2,100 – 2,150	1,750	2,300
body weight (kg)	54	52 – 57	51	54	52	40 - 75[b]
kcal / kg	42	38 – 38	36 – 35	39 – 40	34	58 - 31
Female (≥ 60)[f]						
kcal / day	1,850	1,780	1,620 – 1,410	1,900	1,550	1,800
body weight (kg)	54	49	51	54	52	40 - 75[b]
kcal / kg	34	36	32 - 28	35	30	45 - 24
Pregnancy						
1st trimester	+285	+0	+0	+200 – 285	+0	+0
2nd trimester	+285	+360	+300	+200 – 285	+300	+350
3rd trimester	+285	+470	+300	+200 – 285	+300	+350
Lactation						
1st 6 months	+700	+500	+500	+500	+500	+550
2nd 6 months	+500	+0	+500	+500	+500	+0

Notes: [1] *For adults, only RDAs applicable to moderate activity levels are given*
[a] *Figures only for infants 6-11 months*
[b] *body weights not given by Vietnamese RDAs but taken from WHO.*
[c] *1 - 8 years for Thailand*
[d] *10 - 17 years for Singapore; 9-18 years for Thailand*
[e] *19 - 64 years for Indonesia; 19-59 years for Malaysia; 19-64 years for Philippines; 18-59 for Singapore; 19-70 years for Thailand*
[f] *≥ 65 years for Indonesia;≥ 60 years for Malaysia; ≥ 65 years for Philippines; ≥ 60 years for Singapore; ≥ 71 for Thailand*
Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

3.9 Recommended RDAs for Energy for Southeast Asia

3.9.1 Recommended energy requirements of infants (0 – 12 months)

Whitehead *et al.* (1981) compiled energy intakes of infants from literature published between 1940 up to 1980. These data were later used by the 1985 FAO/WHO/UNU Expert Consultation to estimate energy requirements of infants which are set at 5% higher than observed intakes to compensate for underestimation of intake.

Since the 1980's, even though information on the BMR of infants was available, it was inappropriate to estimate energy requirements based on multiples of BMR because reasonable allowances for physical activity were undefined. The 1985 FAO/WHO/UNU recommendations are 9% to 39% higher than those reported by Butte (1996). These discrepancies are significant and could lead to overfeeding of infants. There is now a growing consensus that the recommendations of FAO/WHO/UNU for energy intakes for children less than 2 years of age are over-estimates of young children's energy needs (Butte, 1996; Torun *et al.*, 1996). Past estimates were based on energy intakes by healthy children in affluent countries and included a 5% increment for an assumed underestimation of breast-milk intake.

Concerns have also been raised about the accuracy of the measurement of dietary intake. As a result, estimates of energy requirement based on TEE plus the energy cost of growth (ECG) is still considered the most appropriate measurement. Butte reviewed relevant literature published since 1980 for the IDECG to develop estimates of energy requirements of both breast-fed and formula-fed infants based on energy intake (EI), TEE, and TEE plus the energy required for growth (i.e. tissue deposition). Butte (2001) provided estimates for the total energy requirements of infants derived from TEE measured by the DLW method and energy deposition based on rates of protein and fat gains, which were adopted by the 2000 FAO/WHO/UNU Expert Consultation (FAO/WHO/UNU, 2004). In the absence of primary data from the region on the energy requirements of infants, the following estimates, set out in Table 3.8, have been proposed for the Southeast Asian population.

Table 3.8 Energy requirement of infants during the first year of life[1]

Age (months)	Weight (kg)	Weight gain (g/d)	Total energy expenditure[a]		Energy deposition[b]		Daily energy requirement[c]			
			kcal/d	MJ/d	kcal/d	MJ/d	kcal/d	MJ/d	kcal/kg/d	MJ/kg/d
Boys										
0 – 1	4.58	35.2	306	1.282	211	0.884	518	2.166	113	473
1 – 2	5.50	30.4	388	1.623	183	0.764	570	2.387	104	434
2 – 3	6.28	23.2	457	1.912	139	0.582	596	2.494	95	397
3 – 4	6.94	19.1	515	2.157	53	0.224	569	2.380	82	343
4 – 5	7.48	16.1	563	2.357	45	0.189	608	2.546	81	340
5 – 6	7.93	12.8	603	2.524	36	0.150	639	2.674	81	337
6 – 7	8.30	11.0	636	2.661	17	0.069	653	2.730	79	329
7 – 8	8.62	10.4	664	2.780	16	0.065	680	2.845	79	330
8 – 9	8.89	9.0	688	2.880	14	0.057	702	2.936	79	330
9 – 10	9.13	7.9	710	2.969	21	0.089	731	3.058	80	335
10 – 11	9.37	7.7	731	3.058	21	0.087	752	3.145	80	336
11 – 12	9.62	8.2	753	3.150	22	0.093	775	3.243	81	337

Age (months)	Weight (kg)	Weight gain (g/d)	Total energy expenditure[a]		Energy deposition[b]		Daily energy requirement[c]			
			kcal/d	MJ/d	kcal/d	MJ/d	kcal/d	MJ/d	kcal/kg/d	MJ/kg/d
Girls										
0 – 1	4.35	28.3	286	1.197	178	0.746	464	1.942	107	447
1 – 2	5.14	25.5	356	1.490	161	0.672	517	2.162	107	421
2 – 3	5.82	21.2	416	1.742	134	0.559	550	2.301	94	395
3 – 4	6.41	18.4	469	1.960	68	0.285	537	2.245	84	350
4 – 5	6.92	15.5	514	2.149	57	0.239	571	2.389	83	345
5 – 6	7.35	12.8	552	2.309	47	0.199	599	2.507	82	341
6 – 7	7.71	11.0	584	2.442	20	0.083	604	2.525	78	328
7 – 8	8.03	9.2	612	2.561	17	0.069	629	2.630	78	328
8 – 9	8.31	8.4	637	2.665	15	0.063	652	2.728	78	328
9 – 10	8.55	7.7	658	2.754	18	0.074	676	2.828	79	331
10 – 11	8.78	6.6	679	2.839	15	0.063	694	2.902	79	331
11 – 12	9.00	6.3	698	2.920	14	0.060	712	2.981	79	331

Notes: [1] Calculated from linear regression analysis of total energy expenditure on weight, plus allowance for energy deposition in tissues during growth.
[a] TEE (MJ/day) = -0.416 + 0.371 kg
[b] Weight gain x energy accrued in normal growth
[c] Requirement – total energy expenditure + energy deposition
Source: FAO (2004)

3.9.2 Recommended energy requirements of children and adolescents (1 – 18 years)

There was very little information available in 1981 on the TEE of children. The paucity of information on time allocated to different activities and energy cost of such activities, did not allow reliable estimates of TEE in children below 10 years of age. Consequently, estimates of energy requirements for children aged 1 to 10 years were derived from a review of published dietary intake data involving some 6,500 children, mostly from developed countries (Ferro-Luzzi & Durnin, 1981). The 1985 FAO/WHO/UNU Expert Consultation felt the need to increase the reported energy intake by 5% to accommodate a desirable level of physical activity.

The estimation of energy requirements for children above 10 years of age is based on energy expenditure expressed as multiples of BMR rather than energy intake data. BMR for boys and girls of a given age and weight were calculated using the mathematical equations derived by Schofield *et al.* (1985). The additional energy expended during the day was calculated based on the assumed energy cost of activities performed by the children and adolescents in developing countries. Extra allowance for growth was assumed to be 5.6 kcal (23.4 KJ) per gram of expected weight gain. This corresponds to about 3% of the daily energy requirement at 1 year of age, with a gradual decrease to about 1% at 15 years (Torun *et al.*, 1996).

Table 3.9 provides estimates of energy requirements in children and adolescents for different levels of activity that has been adopted for Southeast Asia. The Southeast Asia recommendations adopted the FAO/WHO/UNU (2004) method of estimating energy requirements for children and adolescents. Energy needs for children and adolescents were calculated from measurements of energy expenditure and the energy needs of growth. For children aged 1 to 9 years, TEE was calculated based on Torun's quadratic polynomial regression equations (Torun, 2001) and the reference body weights proposed for Southeast Asia (Table 2.1). For adolescents aged 10 to 18 years, the calculations were based on

the PAL values of FAO/WHO/UNU (2004) and BMR values as calculated from the Oxford equations (Henry, 2001).

Table 3.9 Estimates of daily TEE of children and adolescents for 3 levels of habitual physical activity

		Habitual Physical Activity[2]					
Age	Weight[1]	Light		Moderate		Heavy	
(years)	(kg)	kcal/day	MJ/day	kcal/day	MJ/day	kcal/day	MJ/day
Boys							
1 – 3	14	[a]	[a]	1,200	5.02	[a]	[a]
4 – 6	20	[a]	[a]	1,475	6.17	[a]	[a]
7 – 9	27	1,565	6.55	1,830	7.66	2,110	8.83
10 – 12	34	1,810	7.57	2,110	8.83	2,410	10.08
13 – 15	47	2,290	9.58	2,650	11.09	3,060	12.80
16 – 18	56	2,495	10.44	2,980	12.47	3,460	14.48
Girls							
1 – 3	14	[a]	[a]	1,165	4.87	[a]	[a]
4 – 6	20	[a]	[a]	1,470	6.15	[a]	[a]
7 – 9	27	1,540	6.44	1,820	7.61	2,100	8.79
10 – 12	36	1,720	7.20	2,010	8.41	2,300	9.62
13 – 15	45	1,890	7.91	2,205	9.23	2,520	10.54
16 – 18	49	1,915	8.01	2,240	9.37	2,565	10.73

Notes: [1] *Reference body weight, please see Table 2.1*
[2] *PAL factors from FAO/WHO/UNU (2004)*
[a] *Assume values similar to moderate physical activity in children 1 to 6 years old*

3.9.3 Recommended energy requirements of adults (18 – >60 years)

The 1985 FAO/WHO/UNU Expert Consultation adopted the principle of relying on estimates of energy expenditure rather than energy intake from dietary surveys to estimate the energy requirements of adults. Since the largest component of TEE is the BMR, which can be measured with accuracy under standardized conditions, the 1985 FAO/WHO/UNU Report estimated all components of TEE as multiples of BMR, also known as the PAL approach.

The equations for calculating BMR from body weight are shown in Tables 3.1 and 3.2. Besides BMR, other components of energy expenditure such as occupational activities, discretionary activities and residual time have been identified and evaluated to derive total energy requirements. Approximate estimates of total daily energy expenditure corresponding to light, moderate and heavy work can be derived as multiples of BMR (PAL values) which are shown in Table 3.5. As discussed above, there is mounting evidence suggesting that the Schofield equation (FAO/WHO/UNU, 1985) may be over-estimating BMR in many populations, leading to an over-estimation of the energy requirements (Shetty *et al.*, 1996). Thus the Oxford equations are adopted in estimating the BMR of adults in Southeast Asia (Table 3.2). This is based on the database used for deriving the equations by Henry (2001), which are the most comprehensive analysis of BMR in Asians that included Southeast Asians, and using a strict criteria on the inclusion and exclusion of data.

Using the Oxford equations to estimate BMR as shown in Table 3.2 and the PAL values corresponding to various levels of physical activity as shown in Table 3.6, the recommended energy intake of an adult man weighing 60 kg and an adult woman weighing 50 kg is shown in Table 3.10 (below). For light physical activity, PAL of 1.45 is used, while PAL of 1.75 is used for moderate physical activity and PAL of 2.05 is used for heavy physical activity.

The final proposed energy requirements for Southeast Asia as shown in Table 3.12 are based on moderate habitual physical activity, whereby PAL is 1.75 for the age groups of 19 to 29 years and 30 to 60 years. However, for those aged 60 years and above, the PAL value used is 1.60. This is due to the fact that older adults and the elderly have lower BMR and decreased physical activity. Nonetheless, it must be noted that the age at which TEE and energy requirements start decreasing depends on individual, social and cultural features that promote or limit habitual physical among older adults.

Table 3.10 Recommended energy intakes of adult men and women at 3 levels of habitual physical activity

Age (years) / Gender	Recommended Energy Intake (kcal/day)		
	Light Habitual Physical Activity (PAL = 1.45)	Moderate Habitual Physical Activity (PAL = 1.75)	Heavy Habitual Physical Activity (PAL = 2.05)
Men (60 kg)			
19 - 29	2,180	2,635	3,085
30 - 60	2,090	2,525	2,955
> 60	1,915	2,310	2,710
Women (50 kg)			
19 - 29	1,755	2,115	2,480
30 - 60	1,710	2,065	2,420
> 60	1,560	1,880	2,205

It must, however, be emphasized that these values are intended to be general guidelines, and it may be useful to make adjustments according to the characteristics of the population concerned (FAO/WHO/UNU, 1985).

3.9.4 Recommended energy requirements during pregnancy and lactation

The 1985 FAO/WHO/UNU recommendations for pregnancy were based on a general acceptance that total energy needs of pregnancy were estimated at 335 MJ (80, 000 kcal) or about 1.2 MJ or 285 kcal/day (Hytten & Chamberlain, 1980). Most reports published after 1985 have recommended lower increments at 0.84 MJ/day or a total of 200 kcal/day for healthy women with reduced activity (Prentice *et al.*, 1996).

Dietary intake during pregnancy must provide the energy that will result in the full-term delivery of a healthy newborn baby of adequate size and body composition. Ideally, women should enter pregnancy with normal weight and good nutrition conditions. Therefore, the energy requirements of pregnancy are those needed for the growth of the fetus, placenta and associated maternal

tissues, and for the increased metabolic demands of pregnancy. This is in addition to the energy needed to maintain adequate maternal weight, body composition and physical activity throughout the gestational period. Special considerations must be made for women who are under- or overweight when they enter pregnancy.

The extra amount of energy required during pregnancy was calculated in association with a mean gestational weight gain of 12 kg by using factorial approaches (FAO/WHO/UNU, 2004). Table 3.11 shows the additional energy cost of pregnancy in women with an average gestational weight gain of 12 kg as suggested by Butte and King (2002), and adopted by the SEA-RDA Committee. The increment in energy requirement is relatively small in the first trimester (350KJ/day or 85 kcal/day), and most women in many communities do not seek nutritional advice before the second or third month of pregnancy. Thus, a practical option is to add the extra requirement of the first trimester to that required in the second trimester.

Table 3.11 Additional energy cost of pregnancy in women with an average gestational weight gain of 12 kg[1]

A. Rates of tissue deposition					
	1st trimester (g/day)	2nd trimester (g/day)	3rd trimester (g/day)	Total deposition (g/280day)	
Weight gain	17	60	54	12,000	
Protein deposition [a]	0	1.3	5.1	597	
Fat deposition [a]	5.2	18.9	16.9	3,741	
B. Energy cost of pregnancy estimated from the increment in BMR and energy deposition					
	1st trimester (KJ/day)	2nd trimester (KJ/day)	3rd trimester (KJ/day)	Total energy cost	
				MJ	kcal
Protein deposition [a]	0	30	121	14.1	3,370
Fat deposition [a]	202	732	654	144.8	34,600
Efficiency of energy utilization [b]	20	76	77	15.9	3,800
Basal metabolic rate	199	397	993	147.8	35,130
Total energy cost of pregnancy (kJ/day)	421	1,235	1,845	322.6	77,100
C. Energy cost of pregnancy estimated from the increment in TEE and energy deposition					
	1st trimester (KJ/day)	2nd trimester (KJ/day)	3rd trimester (KJ/day)	Total energy cost	
				MJ	kcal
Protein deposition [a]	0	30	121	14.1	3,370
Fat deposition [a]	202	732	654	144.8	34,600
Total energy expenditure [c]	85	350	1,300	161.4	38,560
Total energy cost of pregnancy (kJ/day)	**287**	**1,112**	**2,075**	**320.2**	**76,530**

Notes: *[1] Calculated as suggested by Butte and King (2002). Weight gain and tissue deposition in first trimester computed from last menstrual period (i.e. an interval of 79 days). Second and third trimesters computed as 280/3 = 93 days each.*
[a] Protein and fat deposition estimated from longitudinal studies of body composition during pregnancy, and an energy value of 23.6 kJ (5.65 kcal)/g protein deposited, and 38.7 kJ (9.25 kcal)/g fat deposited.
[b] Efficiency of food energy utilization for protein and fat deposition taken as 0.90
[c] Efficiency of energy utilization not included in this calculation, as the energy cost of synthesis is included in the measurement of TEE by DLW.

Source: FAO/WHO/UNU (2004)

The SEA-RDA Committee adopted the recommendations of FAO/WHO/UNU (2004), whereby pregnant women increase their food intake by 1.5 MJ/day (360 kcal/day) in the second trimester, and by 2.0 MJ/day (475 kcal/day) in the third trimester.

3.9.5 Recommended energy requirements during lactation

The 1985 FAO/WHO/UNU recommendations for lactation were based on the median milk consumption of breast-fed Swedish infants for the first 6 months. It was assumed that milk energy was 2.9 kJ/g or 0.7 kcal/g and the efficiency of conversion of dietary to milk energy was 80%. Further more, it was assumed that the average woman would start lactation with 150MJ (36,000 kcal) of additional fat reserves laid down during pregnancy and that these would be used to subsidize the cost of lactation over the first 6 months thus yielding about 0.84MJ/day or 200 kcal/day (Prentice *et al.*, 1996).

The energy requirement of a lactating woman is defined as the level of energy intake from food that will balance the energy expenditure needed to maintain a body size and composition, a level of physical activity, and a breast milk production, which are consistent with good health for the woman and her child, and that will allow performing economically necessary and socially desirable activities (FAO/WHO/UNU, 2004). To operationalise this definition, the energy needed to produce an appropriate volume of milk must be added to the woman's habitual energy requirement, assuming that she resumes her usual level of physical activity soon after giving birth. The energy cost of lactation is determined by the amount of milk that is produced and secreted, its energy content, and the efficiency with which dietary energy is converted to milk energy.

Postpartum loss of body weight is usually highest in the first 3 months, and generally greater among women who practice exclusive breastfeeding, but the extent to which energy immobilized to support lactation depends on the gestational weight gain and the nutritional status of the mother. Thus, the recommendations for lactating women to a large part depend on the women's nutritional status.

For women who feed their infants exclusively with breast milk during the first 6 months of life, the mean energy cost over the 6 month period is: 807g milk/day x 2.8 kJ/d / 0.80 efficiency = 2.8 MJ/day (675 kcal/day). From the age of six month onwards, when infants are partially breast-fed and milk production is on average 550 g/day, the energy cost imposed by lactation is 1.925 MJ/day (460 kcal/day).

Fat stores accumulated during pregnancy may cover part of the additional energy need in the first few months of lactation. Assuming an energy factor of 27.2 MJ/kg, the rate of weight loss in well-nourished women (0.8 kg/month) would correspond to the mobilization of 27.2 x 0.8 kg/month = 21.8MJ/month, or 0.72 MJ/day (170 kcal/day) from body energy stores. This amount of energy can be deducted from the 2.8 MJ (675 kcal) per day needed during the first six months of lactations.

The FAO/WHO/UNU (2004) recommends that well-nourished women with adequate gestational weight gain should increase their food intake by 2.1 MJ/day (505 kcal/day) for the first 6 month of lactation, while undernourished women and those with insufficient gestational weight gain should add to their personal energy demands 2.8 MJ/day (675 kcal/day) during the first semester of lactation. Energy requirements for milk production in the second six months are dependent of rates of milk production, which are highly variable between women and populations. The SEA-RDA Committee decided to adopt the recommendations of the FAO/WHO/UNU (2004) Expert Consultation for lactating women.

3.9.6 Summary of recommended energy requirements

In summary, the SEA-RDAs for energy, by life stages, are shown in Table 3.12

Table 3.12. Recommended RDAs for Energy for Southeast Asia[1]

Age Groups	Reference Weight (kg)	Estimated Energy Requirements	
		Kcal/day	MJ/day
Infant (months)			
0 – 5	6	555	2.32
6 – 11	9	710	2.97
Children (years)			
1 – 3	14	1,180	4.94
4 – 6	20	1,470	6.15
7 – 9	27	1,825	7.64
Boys (years)			
10 - 12	34	2,110	8.83
13 – 15	47	2,650	11.09
16 – 18	56	2,980	12.47
Girls (years)			
10 - 12	36	2,010	8.41
13 – 15	45	2,205	9.23
16 – 18	49	2,240	9.37
Men (years)			
19 – 29	60	2,635	11.02
30 – 59	60	2,525	10.56
≥ 60	60	2,240	8.85
Women (years)			
19 – 29	50	2,115	8.85
30 – 59	50	2,065	8.64
≥ 60	50	1,720	7.20
Pregnancy			
2nd trimester	–	+ 360	+ 1.5
3rd trimester	–	+ 475	+ 2
Lactation (up to 12 months)			
Well-nourished women	–	+ 505	+ 2.1
Undernourished women	–	+ 675	+ 2.8

Note: [1] With the exception of infants, EER is based on moderate physical activity (FAO/WHO/UNU, 2004) (Tables 3.9 and 3.10)

3.10 Guidance On High Energy Intake

Unlike protein and other nutrients where a safe margin of + 2 SD above the physiological needs is included, energy requirement is expressed as the mean of a group and does not include any safe margin. This recognizes the fact that excess energy that is not disposed of is accumulated in the body as fat, and this may lead to the long-term undesirable outcome of obesity (FAO/WHO/UNU, 1985). It is, however, difficult to study the relationships between excess energy intake, the resulting positive energy balance and obesity, particularly during the dynamic phase of weight gain. Overfeeding studies under controlled conditions may provide some insight to the metabolic changes induced by energy excess and the impact of accumulated energy stores.

Bibilography

Agriculture and Agri-Food Canada, 1996. Guide to food labeling and advertising: Nutrient content claims. 25 March. *Health Canada*, Ottawa, Canada.

Butte NF (1996). Energy requirements of infants. *Eur J Clin Nutr* 50:S24-S36.

Butte NF (2001). Energy requirements of infants. Background paper prepared for the joint FAO/WHO/UNU Expert Consultation on Energy in Human Nutrition, June 27-July 25, 2001.

Butte NF & King JC (2002). Energy requirements during pregnancy and lactation. Energy background paper prepared for the joint FAO/WHO/UNU Expert Consultation on Energy in Human Nutrition, June 27-July 25, 2001.

EC (1990). Directives 90/496/EEC: Nutrition labeling for foodstuffs. Official Journal of the European Communities; L276: 40-44.

FAO (1950). *Calorie requirements*. Report of the Committee on Calorie Requirements. FAO Nutritional Studies No.5:1-65.

FAO (1957). *Calorie requirements*. Report of the Second Committee on Calorie Requirements. FAO Nutritional Studies No.15:1-66.

FAO/WHO (1973). *Energy and Protein Requirements*. Report of a joint FAO/WHO Ad Hoc Expert Committee. FAO Nutrition Meetings Report Series No. 52. WHO Technical Report Series No. 522. Food and Agriculture Organization, Rome.

FAO (1996). *The Sixth World Food Survey*. Food and Agriculture Organization, Rome.

FAO/WHO (1994). *Fats and Oils in Human Nutrition*. Report of a Joint Expert Consultation. Food and Agriculture Organization, Rome.

FAO/WHO (1996). *Carbohydrates in Human Nutrition*. Report of a Joint Expert Consultation. Food and Agriculture Organization, Rome.

FAO/WHO/UNU (1985). *Energy and Protein Requirements*. Report of a joint WHO/WHO/UNU Expert Consultation. WHO Technical Report Series No. 724. World Health Organization, Geneva.

FAO/WHO/UNU (2004). *Human Energy Requirements*. Report of a joint FAO/WHO/UNU Expert Consultation. Food and Nutrition Technical Report Series, Food and Agriculture Organization, Rome.

Ferro-Luzzi A & Durnin JVGA (1981). The assessment of human energy intake and expenditure: a critical review of the recent literature. Document ESN: FAO/WHO/UNU/EPR/81/9. Food and Agriculture Organization, Rome.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines*. 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

Henry CJ (2001). Basal metabolic rate studies in humans: Measurement and application. Working draft prepared for the FAO/WHO/UNU Working Groups on Energy and Protein in Human Nutrition, June 27-July 25, 2001. (revised 10 Oct 2001).

Hill JO & Prentice AM (1995). Sugar and body weight regulation. *Am J Clin Nutr* 62:264S-274S.

Hytten F & Chamberlain G (1980). *Clinical Physiology in Obstetrics*. Blackwell Scientific Publication, Oxford.

IDECG (1996). Energy and protein requirements. Proceedings of an IDECG Workshop. NS Scrimshaw, JC Waterlow and B Schurch. *Eur J Clin Nutr* 50 (suppl): S1-S197.

Ismail MN, Ng KK, Chee SS, Roslee R & Zawiah H (1998). Predictive equations for the estimation of basal metabolic rate in Malaysian adults. *Mal J Nutr* 4: 81-90.

Ludwig DS, Peterson KE & Gortmaker SL (2001). Relation between consumption of sugar-sweetened drinks and childhood obesity: a prospective observational analysis. *Lancet* 17: 505-508.

Merrill AL & Watt BK (1973). Energy value of foods: basis and derivation. Agriculture Handbook 74. ARS United States Department of Agriculture, Washington DC.

Montoye HJ, Kemper HCJ, Saris WHM & Washburn RA (1996). Measuring physical activity and energy expenditure. Human Kinetics. Champaign IL

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

Muhilal (1998). Indonesian Recommended Dietary Allowance. *Nutr Rev* 56 (4): S19-S20.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Poh BK, Ismail MN, Zawiah H & Henry CJK (1999). Predictive equations for the estimation of basal metabolic rate in Malaysian adolescents. *Mal J Nutr* 5: 1-14.

Poh BK, Ismail MN, Ong HF, Norimah AK & Safiah MY (2004). BMR predictive equations for Malaysian adolescents aged 12-18 years. Final report for IRPA 06-02-02-0096 research project, Universiti Kebangsaan Malaysia, Bangi.

Prentice AM (1995). Alcohol and Obesity. *Int J Obesity* 19: S44- S50.

Prentice AM, Spaaij CJK, Goldberg GR, Poppitt SD, van Raaij JMA, Totton M, Swann D & Black AE (1996). Energy requirements of pregnant and lactating women. *Eur J Clin Nutr* 50: S82-S111.

Schofield WN, Schofield C & James WPT (1985). Basal metabolic rate – review and prediction, together with an annotated bibliography of source materials. *Hum Nutr: Clin Nutr* 39C(Suppl. 1):5-96.

Shetty PS, Prentice AM, Goldberg GR, Murgatroyd PR, McKenna APM, Stubbs RJ & Volschenk PA (1994). Alterations in fuel selection and voluntary food intake in response to isoenergetic manipulation of glycogen stores in humans. *Am J Clin Nutr* 60:534-543.

Shetty PS, Henry CJK, Black AE & Prentice AM (1996). Energy requirements of adults: an update on basal metabolic rates (BMRs) and physical activity levels (PALs). *Eur J Clin Nutr* 50:S11-S23.

Spurr GB, Prentice AM, Murgatroyd PR, Goldberg GR, Reina JC & Christman NT (1988). Energy expenditure from minute-by-minute heart rate recording:comparison with indirect calorimetry. *Am J Clin Nutr* 48:552-559.

Tee ES (1998). Current status of Recommended Dietary Allowances in Southeast Asia: A Regional Overview. *Nutr Rev* 56 (4): S10-S18.

Torun B (2001). Energy requirements of children and adolescents. Background paperprepared for the joint FAO/WHO/UNU Expert Consultation on Energy in Human Nutrition, June 27-July 25, 2001.

Torun B, Davies PSW, Livingstone MBE, Paolisso M, Sackett R & Spurr GB (1996). Energy requirements and dietary energy recommendations for children and adolescents. 1 to 18 years old. *Eur J Clin Nutr* 50:S37-S81.

Warwick PM (1989). Predicting food energy requirements from estimates of energy expenditure. *Aust J Nutr Diet* 46 (suppl):S3-S28.

Westerterp KR, Prentice AM & Jequier E (1999). Alcohol and body weight. In: Macdonald I, ed. *Health issues related to alcohol consumption*. Oxford: Blackwell Science; 103-23.

Whitehead RG, Paul AA & Cole TJ (1981). A critical analysis of measured food energy intakes during infancy and early childhood in comparison with current international recommendations. *J Hum Nutr* 35:339-348.

4. PROTEIN

4.1 Introduction

The word "protein" was coined from the Greek word proteios, which means "primary" or "of first importance". Protein is any of a large number of organic compounds present in all living organisms that is essential for their function. First discovered in 1838, proteins are now recognized as the predominant component of cells, making up more than 50% of the dry weight of animals.

The building blocks of protein are amino acids and, as such, they are the "currency" of protein nutrition and metabolism. Although there are hundreds of amino acids in nature, only about 20 of these appear in proteins. These amino acids undergo turnover and are constantly degraded and synthesized (non-essential) in the human body according to its needs.

4.2 Characteristics and Functions

4.2.1 Composition of proteins

Proteins in both the diet and body are more complex and variable than the other energy sources, carbohydrates and fats. The defining characteristic of protein is its requisite amino nitrogen group. The average content of nitrogen in dietary protein is about 16% by weight, so nitrogen metabolism is often considered to be synonymous with protein metabolism.

Proteins are highly complex biomolecules composed of amino acids. Amino acids are simple compounds containing carbon, hydrogen, oxygen, nitrogen and occasionally sulfur. They are linked together by peptide bonds between the amino (-NH_3+) and carboxyl (-COO-) group and the products formed by such linkage are called peptides which usually form long strands (polypeptide chains). A critical feature of proteins is the complexity of their physical structure. Many proteins are composed of several polypeptide chains held together by ionic or covalent bonds.

4.2.2 Nutritional classification of amino acids

From a nutritional point of view, the most important aspect of proteins is their amino acid composition. A typical protein may contain 500 or more amino acids but there are only about 20 different amino acids commonly found in plant and animal proteins. L-amino acids (levorotatory) are, however, the metabolically active form found in humans and most animals because of the stereospecificity of enzymes. Thus, only the L-isomer of amino acids is incorporated into amino acids. The almost infinite combinations in which amino acids line up, and the helical and globular shapes in which the strands coil, help to explain the great diversity of tasks that proteins perform in living matter.

Amino acids are categorized into 2 groups:

(a) Those that can be synthesized by the human body if energy and suitable forms of carbon and nitrogen precursors are available are referred to as dispensable (non-essential) amino acids.

(b) Amino acids that cannot be synthesized by the body are referred to as indispensable (essential) amino acids, and must be supplied in the diet. Ordinarily, the 8 indispensable amino acids are isoleucine, leucine, valine, threonine, methionine, phenylalanine, tryptophan, and lysine.

4.2.3 Functions of protein

Proteins play an enormous variety of roles. They make up a large part of the structural framework of cells and tissues. Proteins are produced and secreted to act as cell-cell signals in the form of hormones and cytokines. Hormones like insulin, thyroxin and epinephrine, and most regulatory substances in the body are proteins or derived from proteins. Proteins are also used to synthesize transport molecules like hemoglobin and albumin, and storage molecules like myoglobin. Antibodies, membrane receptors and blood-clotting factors are also proteins (Matthews and van Holde, 1995).

Perhaps the most important of all proteins are the enzymes - the catalysts that promote the tremendous variety of reactions that channel metabolism into essential biochemical pathways. A drastic change in pH in the blood stream can decrease the enzyme's activity to the extent that they no longer function. The presence of proteins in the blood exerts a buffering effect to neutralize too much acidity or alkalinity (Matthews and van Holde, 1995).

4.3 Absorption, Utilization and Excretion

Digestion of proteins begins with pepsin secretion in gastric juices and with proteolytic enzymes secreted from the pancreas and the mucosa of the small intestine. These enzymes are secreted in their "pro" (zymogen) form and become activated by the cleavage of a small peptide portion. The presence of dietary protein in the gut appears to signal the secretion of enzymes (Matthews, 1981).

The proteins and peptides then pass into the small intestine, where the peptide bonds are hydrolyzed by a variety of pancreatic enzymes which include trypsin, chymotrypsin, elastase, carboxypeptidase, and aminopeptidase. Proteins are successively broken down into smaller peptides on the basis of the amino acid residues targeted by these proteolytic enzymes.

After the intracellular hydrolysis of the absorbed peptides, the resulting free amino acids are absorbed by active transport through the mucosal cells into the portal blood system. Some amino acids are immediately metabolized by the gut and the liver during absorption. Absorbed amino acids pass into the liver, where a portion are taken up and used while the remainder passes through the systemic circulation and are utilized by peripheral tissues (Matthews and van Holde, 1996).

Amino acids not immediately used for the synthesis of tissue or essential nitrogenous compounds are catabolized by deamination forming keto-acids which may be converted to other amino acids through transamination or eventually enters the Kreb's cycle for energy production or glycolytic

pathway. The carbon skeleton of amino acids is also available for use in biosynthetic pathways, particularly for glucose (gluconeogenesis) and fat (lipogenesis). Thus, the degradation pathways of many amino acids can be partitioned into 2 groups: amino acids whose carbon skeleton may be used for synthesis of glucose and those whose carbon skeletons degrade for potential use for fatty acid synthesis. This is the basis for the classical nutritional description of amino acids as either "ketogenic" or "glucogenic"(IOM, 2002).

Amino acid nitrogen is excreted in various forms. Most of the nitrogen from protein and amino acid catabolism is lost as urea through urinary excretion. Some amounts are lost as urinary ammonia nitrogen and creatinine. It can also be excreted as uric acid since amino acids can give rise to ammonia, which is used in the synthesis of purine and pyrimidine bases for nucleic acids. The other excretion routes are the feces, skin, sweat and hair (FNRI, 1989).

There is practically no storage of amino acids in the body. They are constantly used to form other compounds, or reformed into other amino acids. The amino acids which arise from intestinal absorption, from tissue protein breakdown, and from other sources form part of the amino acid pool. The amino acid needs of an individual are derived from this pool (FNRI, 1989).

4.4 Effects of Deficiency and Excess

4.4.1 Deficiency

Protein deficiency usually accompanies a deficiency of calories and other nutrients. The effects of protein loss during illness and injury are far-reaching. The most evident result is the wasting of muscle tissue and consequent loss of weight. Other symptoms include anemia and delayed healing of wounds and fractures. A lowering of serum protein levels and hormonal changes may result in edema, and the reduced production of antibodies increases susceptibility to infections (Park and Park, 1985; WHO, 1990; NRC, 1989).

Protein-calorie malnutrition (PCM) refers to a group of diseases caused by deficiencies of both proteins and calories and which are frequently accompanied by infections. A vicious cycle develops in which the malnutrition creates a susceptibility to infection and the infection further aggravates the poor nutritional condition. The 2 major manifestations of PCM are marasmus and kwashiorkor.

Marasmus is caused by a diet that is severely deficient in calories, as well as protein and other nutrients for a prolonged period. Kwahiorkor, on the other hand, primarily results from a deficiency of protein but a deficiency in calories may be a contributing factor.

Although the basic or primary causes of PCM are an inadequate diet, both in quantity and quality, underlying factors include poverty and ignorance; infectious and parasitic diseases, notably diarrhea; respiratory infections; measles and intestinal worms. There are numerous other contributory factors in the web of causation, for instance poor environmental conditions; large family size; poor maternal

health; failure of lactation; premature termination of breast-feeding and adverse cultural practices in child rearing and weaning, such as the use of over-diluted cow's milk and cooking water from cereals and delayed supplementary feeding (Park and Park , 1985).

4.4.2 Excessive intake

There is substantial literature documenting the increase in urinary excretion of calcium with increasing protein intake. In short-term studies, it has been shown that an increase in dietary animal protein intake in healthy subjects increased urinary calcium and oxalate. This has 2 potential detrimental consequences: loss of bone calcium and increased risk of renal calcium stone formation. Although there have been studies demonstrating the increased resorption of bone with increased protein intake, at present the available evidence is not strong enough to suggest limiting protein intake to avoid potential risk of bone loss.

4.4.3 Guidance on high intake

High protein intakes have also been implicated in chronic diseases such as osteoporosis, renal stones, renal insufficiency, cancer, coronary heart disease, and obesity. However, current literature does not permit any recommendation of a tolerable upper intake level (UL) for protein to be made on the basis of chronic disease risk. In addition, data on the potential for high protein diets to produce gastrointestinal effects, changes in nitrogen balance, maximum urea synthesis, or chronic diseases are often conflicting and do not provide dose-response information or clear indications of a lowest-observed-adverse-effect level (LOAEL) or no-observed-adverse-effect level (NOAEL) for these endpoints.

4.5 Food Sources

There are 2 main dietary sources of proteins; (a) animal proteins (e.g., eggs, milk, meat, fish etc); and (b) plant proteins (e.g., pulses, cereals, nuts, beans, soy products etc). In terms of nutrition, animal proteins are rated superior to plant proteins because they are "biologically complete" (i.e. they contain all the essential amino acids needed by the body). Egg proteins are considered to be the best among animal proteins because of their biological value and digestibility. In fact, it is used in nutrition studies as a "reference protein".

Plant proteins, on the other hand, are "biologically incomplete" (i.e. they may be lacking in one or more of the essential amino acids). However, plant proteins are not all lacking in the same amino acids. When a variety of foods such as grains, vegetables, legumes, seeds and nuts are eaten daily, amino acids lacking in certain foods are supplied by other foods.

A food that provides the amino acids that are in short supply in another is said to complement each other. For example, legumes are high in lysine but low in methionine, and grains are low in lysine but essentially high in methionine. These foods complement each other and provide high quality protein.

4.6 Factors Affecting Requirement

4.6.1 Protein quality

Generally, protein quality refers to how well or poorly a given protein is utilized by the body. More specifically, it refers to how well the indispensable amino acid profile of a protein matches the requirements of the body.

The concept of protein quality applies only under conditions in which the amount of protein consumed is equal to or less than the amount needed to meet the requirement for the limiting amino acid. When protein intake exceeds this amount, the efficiency of protein utilization will decline regardless of the balance of the amino acid pattern. This will occur even with the highest-quality proteins because after the requirement for the limiting amino acid has been exceeded, all indispensable amino acids will be present in tissues in excess of the amounts needed to saturate protein-synthesizing system. As amino acids cannot be stored, excess amounts of all amino acids will be degraded and used only as sources of energy (Harper and Yoshimura, 1993).

The common methods of evaluating protein quality are amino acid scoring; biological value; protein efficiency ratio; and protein digestibility/protein digestibility corrected amino acid scoring.

(*a*) *Amino Acid Score*

Amino acid score (sometimes called chemical score) is a method of rating proteins based on its chemical composition, or more specifically, its indispensable amino acid levels. To determine its amino acid score (AA Score), a protein is picked as a reference and other proteins are rated relative to that reference protein. Typically, egg protein has been used as the reference protein, but this assumes that the amino acid profile of eggs is ideal for humans.

The AA Score is determined by dividing the milligram of a particular indispensable acid in one gram of the test protein by the milligram of the same indispensable amino acid in one gram of the reference protein. Thus,

$$\text{AA Score} = \frac{\text{mg of AA in 1 g of test protein}}{\text{mg of AA in 1 g of reference protein}}$$

The amino acid with the lowest AA Score is defined as the limiting amino acid. Typically, the limiting amino acids in dietary proteins are lysine, sulfur-containing amino acids, threonine, and/or tryptophan.

(*b*) *Biological Value*

The biological value of a protein is given as the amount of nitrogen (N) retained in the body divided by the amount of N absorbed from a given protein. Therefore, the digestibility of the protein is taken into account. Thus,

Biological Value = (N retained/ N absorbed) x 100

To measure biological value, subjects are typically first fed a zero-protein diet so that baseline losses of N can be measured (i.e. the amount of N that is lost normally). Then the test protein is fed at varying levels and a N balance study is done.

(*c*) *Protein Efficiency Ratio*

The traditional method for evaluating protein quality has been to calculate the protein efficiency ratio (PER), which has been used since 1919. PER is based on the growth of rats in response to a given amount of protein.

$$\text{PER} = \frac{\text{weight gain of subject (grams)}}{\text{protein intake (grams)}}$$

PER is sometimes used to rate proteins and represents the amount of weight gain (in grams) relative to the amount of protein consumed (in grams). For example, a PER of 2.5 would mean that a weight of 2.5 g was gained by the subject for every gram of protein ingested.

Results from this method will be skewed in applications to humans, depending upon the extent that human requirements for individual amino acids differ from those of the rat. However the method has been very useful in comparing a new protein source against reference proteins, such as egg protein, and does evaluate other factors such as relative digestibility.

(*d*) *Protein Digestibility Corrected Amino Acid Score*

Recently, the protein digestibility corrected amino acid score (PDCAAS) was introduced as a more accurate way to evaluate protein quality for children and adults. The PDCAAS is the AA Score with a correction for food protein digestibility. Thus,

PDCAAS = AA Score of limiting AA X % True Digestibility

Specifically, the amino acid requirements used are those developed by WHO for children of 2 to 5 years old because it is the most demanding group other than infants. PDCAAS goes beyond chemical scoring by factoring in the digestibility of a given protein, giving the amino acid profile more relevance to human needs.

The various methods for determining protein quality are, in essence, methods for determining the proportion of the protein in a food or diet that can be utilized, or more specifically, the proportion of the indispensable amino acid in the protein that can be used for tissue protein synthesis. Although a variety of methods of measuring protein quality has been proposed, none are perfect in rating for human use. PDCAAS has been suggested as the ideal scale

to rate proteins for its ability to meet human requirements, by factoring in the digestibility of a given protein and thereby giving the amino acid profile greater relevance to human needs. It is now adopted as the official method by WHO, the United States Food and Drug Administration and the US Department of Agriculture (IOM, 2002).

4.6.2 Other Factors

In addition, age and body size also greatly affect a person's protein requirements. Protein requirements are highest during the period of rapid growth after birth. For infants, safe intakes of protein from breast milk or infant formulas of comparable quality are estimated to be 2 g/kg of body weight/day shortly after birth and gradually decreases thereafter to the adult RDA value of 0.75 g/kg of body weight/day. Apart from age and body size, and physiological state, factors like infections, worm infestations, injury, emotional disturbances and stress situations can also affect a person's protein requirement.

4.7 Estimating Requirements and Recommended Intakes

4.7.1 Methods for estimating protein and amino acid requirements

The protein requirement of an individual is defined as *"the lowest level of dietary protein intake that will balance the losses of nitrogen from the body in persons maintaining energy balance at modest levels of physical activity"* (FAO/WHO/UNU, 1985). In children and pregnant or lactating women, the protein requirement is taken to include the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health. All requirement estimates refer to needs persisting over moderate periods of time. If more protein is ingested than is needed for metabolic purposes, essentially all the excess is metabolized and the end-products are excreted, since protein is not stored in the body in the way that energy is stored in adipose tissue (WHO, 1990; FAO/WHO/UNU, 1985).

The methods used as bases for estimating protein requirements are the factorial method and the nitrogen (N) balance method. For young infants, estimates of protein requirements are based on breast milk intake (FNRI, 2002).

The factorial method involves the measurement of obligatory losses (the amount of N present in urine, feces, sweat, etc.) when the diet consumed contains no protein, but is otherwise adequate. The requirement is considered to be the amount needed to replace this loss, after adjustments for the efficiency of dietary protein utilization and quality of dietary protein based on its amino acid pattern. For children and pregnant and lactating women, an additional amount of protein required to support tissue growth and milk formation is incorporated into the factorial estimates of requirements (FAO/WHO/UNU, 1985).

The N-balance technique involves the determination of the difference between the intake of nitrogen and the amount excreted in urine, feces, and sweat, together with minor losses by other routes. In most experiments, only the nitrogen content of the diet, urine, and feces has been directly measured.

When the quantity of nitrogen from food proteins is approximately equal to the quantity lost in the feces and urine, the person is said to be in nitrogen balance. N-balance indicates that body tissue is being adequately replaced and repaired and that there is no increase or decrease in total amount of body tissues (Harper and Yoshimura, 1993).

In positive N-balance, more nitrogen is taken in than is lost. Positive N-balance is seen when new tissue is being built, as in infancy and childhood, in adolescence, in pregnancy and lactation, and during recovery from an illness or injury in which protein has been lost.

In negative N-balance, more nitrogen is lost than is consumed in food protein. In other words, body tissue is breaking down faster than it is being replaced. Negative nitrogen balance is seen when protein intake is too low or when the protein is of poor quality. Negative nitrogen balance also develops when insufficient calories are consumed to meet energy needs. In this case, body protein is broken down to supply energy (Harper and Yoshimura, 1993).

4.7.2 Recommendations for protein intake by life stages

3 main sources of references were used in determining the SEA-RDAs for protein. These were the report of the 1985 FAO/WHO/UNU joint Expert Consultation on Energy and Protein Requirements (1985 FAO/WHO/UNU Report), the report of the 1994 IDECG meeting (Dewey *et al.*, 1996) and the 2002 report of the DRI Committee of IOM. The recommended protein intake for each specific population group, as well as the bases for the recommendations, are summarized below.

(*a*) *Infants* (*0 - 6 months*)

For infants aged 0 to 6 months, the 1985 FAO/WHO/UNU Report utilized the data on average breast milk intake of infants to estimate the protein intake of old infants aged 0 to 4 months. A modified factorial approach was used in the 1985 FAO/WHO/UNU Report to estimate protein requirements of infants. This approach requires the estimation of both the maintenance of nitrogen needs and amount of nitrogen required for growth. The explicit assumption of this approach was that the protein needs of an infant will be met if its energy needs are met and the food providing the energy contains protein in quantity and quality equivalent to that of breast-milk (Dewey *et al.*, 1996).

The following assumptions were made by the 1985 FAO/WHO/UNU Report in estimating the protein requirements of breast-fed infants aged 0 to 6 months:

1. The average protein content of breast milk was assumed to be 1.15g/100 ml after the first month post partum, calculated from total nitrogen content x 6.25.
2. The average weights of infants in the NCHS reference (which are based on the Fels Longitudinal study) were used to estimate protein intake per kg body weight.
3. The estimates presented in the 1985 FAO/WHO/UNU Report extended only to infants 4 months of age because there was insufficient information on the intakes of exclusively breast-fed infants beyond that age.

An estimate of the average protein intake per kilogram (kg) of breast-fed infants up to 4 months is shown in Table 4.1.

Table 4.1 Average protein intake of breast-fed infants (0 - 4 months)

Age (months)	Average Protein Intake (g/kg/day)	
	Boys	Girls
0 – 1	2.46	2.39
1 – 2	1.93	1.93
2 – 3	1.74	1.78
3 – 4	1.49	1.53

Source: FAO/WHO/UNU (1985)

In 1994, IDECG (Dewey *et al.*, 1996) critically reviewed the breast-fed infant model of the 1985 FAO/WHO/UNU Expert Consultation in estimating the protein intake of this age group. The following arguments were raised against the recommendations of the 1985 FAO/WHO/UNU Report for infants aged 0 to 4 months:

1. Systematic bias (3% to 6%) associated with the estimation of average breast milk intake resulting in the under-estimation of the amount of milk actually consumed caused by insensible water loss from the infant while test-weighing.
2. The use of NCHS reference (which primarily involves formula-fed infants instead of breast-fed infants) as the basis for the average weight of infants.
3. Failure to account for the "true protein" (i.e. (total N – NPN) x 6.25) concentration of breast milk.
4. Insufficient information on intake of exclusively breast-fed infants who were growing satisfactorily and on intakes of exclusively breast-fed infants after the age of 4 months.
5. Interpolation of the average intake of breast-fed infants as the approximation for the mean requirement.

The DRI Committee has released its report on protein requirements and allowances, which utilized the recommendations of FAO/WHO (2002). The recommended protein intakes for infants aged 0 to 6 months was based on adequate intake (AI) which is estimated from an average breast milk intake of 780 ml/day and the average protein content of breast milk during the first 6 months of lactation (IOM, 2002). The average milk intake was taken to be 11.7 g/L, which produces an AI of 9.1 g/day or 1.52 g/kg/day based on a reference weight of 6 kg.

A comparison of the recommendations of the FAO/WHO/UNU (1985), IDECG (Dewey *et al.*, 1996) and IOM (2000) for infants aged 0 to 6 months is shown in Table 4.2. The estimates of IDECG were based on data taken from 2 studies in the USA using the alternate breast expression method for collecting milk samples over a 24-hour period at each age in which the breast milk intake was carefully measured by test-weighing with electronic balance for periods of 24 or 96 hours.

Most of the differences in the recommended protein intakes are due to the change in assumption regarding the proportion of NPN fraction utilized, and to the higher means for infant weight used by IDECG than in the 1985 FAO/WHO/UNU Report. The decrease in milk protein concentration over time (which was not accounted for in the 1985 FAO/WHO/UNU Report) also contributed to the differences.

Table 4.2 Comparison of recommended intakes of protein for infants (0 - 6 months)

Age (months)	FAO/WHO/UNU 1985			IDECG 1996			IOM 2002
	BF Infant Model	Factorial Approach		BF Infant Model	Factorial Approach		AI
	Protein Intake	Average Intake	Safe level of intake	Protein Intake	Average Intake	Safe level of intake	
	Protein (g/kg body weight/day)						
0 – 1	2.46				1.99	2.69	
1				2.26			
1 – 2	1.93	2.25			1.54	2.04	
2				1.65			
2 – 3	1.74	1.82			1.19	1.53	
3				1.47			
3 – 6			1.86				
3 – 4	1.49	1.47			1.06	1.37	
4				1.30			
4 – 5		1.34			0.98	1.25	
5 – 6		1.30			0.92	1.19	
6				1.23			
0 – 6							1.52

(*b*) *Infants (6 - 12 months)*

A modified factorial approach was used in the 1985 FAO/WHO/UNU Report to estimate protein requirements of infants after the age of 6 months. This approach requires the estimation of both maintenance nitrogen needs and the amount of nitrogen required for growth. The following were the bases of the recommendations of the 1985 FAO/WHO/UNU Report:

1. The only data cited for maintenance protein needs of infants were from a study of children 9 to 17 years of age from which the infants' energy intakes were only 77 kcal/kg/day, far below the recommended level (>100 kcal/kg/day) for this age range.
2. The average maintenance nitrogen requirement was assumed to be 120 mg N/kg/day.
3. Body protein gain during growth was estimated based on formula-fed infants during the first 112 days of life, and on NCHS reference data from 3 to 10 years of age.
4. 50% was added to the theoretical daily body N increment to account for day-to-day variation in the rate of growth, and thus in the need of protein to support that growth.
5. It was assumed that the efficiency of conversion from dietary protein to body protein during growth is 70%, the same efficiency assumed for maintenance needs.
6. The CV in the maintenance requirement was assumed to be 12.5% and 35% for growth, the same as the CV found for adults in numerous short-term N-balance studies.

The IDECG (Dewey *et al.*, 1996) revised the estimates for infants aged 0 to 12 months under the 1985 FAO/WHO/UNU Report, using the following assumptions:

1. There was no additional augmentation for day-to-day (intra-individual) variability in growth; instead, this was considered to be covered by the CV for growth when calculating safe levels.

2. The data on body protein gain was derived from a pooled analysis of studies from the USA, Canada, Denmark, Sweden, Finland and UK (WHO Working Group on Infant Growth, 1994).
3. The efficiency of conversion of dietary protein to body protein was estimated to be 70%.
4. The maintenance requirement was estimated to be 90 mg N/kg/day.

Although the factorial method is not, in fact, used for infants below the age of 6 months, the calculations have been made in order to compare the results with the estimated protein intakes from breast milk. This comparison suggests that the proposed addition of 50% to the nitrogen increment does not increase the estimate, and it may even be insufficient (FAO/WHO/UNU, 1985).

(c) *Children and Adolescents (1 – 18 years)*

In the 1985 FAO/WHO/UNU Report, the factorial approach was used to estimate protein requirements of children and adolescents. For maintenance nitrogen requirements, values were interpolated based on the 2 "anchor" points of 120 mg N/kg/day at 1 year and 100 mg N/kg/day at 20 years of age. The CV was assumed to be 12.5%. The same assumptions as used for infants were included in the calculations:

1. The efficiency of conversion from dietary protein was assumed to be 70%
2. The CV for growth was taken as 35%
3. An additional 50% was added to the growth increment to allow day-to-day variation in growth rate

The IDECG (1996) revised the estimates of protein requirements of the 1985 FAO/WHO/UNU Report for children and adolescents assuming a maintenance requirement of 100 mg N/kg/day (the same as for adults), and without the 50% augmentation for intra-individual variation in growth used in the 1985 FAO/WHO/UHU Report. However, the IDECG (Dewey *et al.*, 1996) used the same assumptions as the 1985 FAO/WHO/UHU Report for the efficiency of conversion of dietary protein to body protein and the CV for growth and maintenance.

The DRI Committee (IOM, 2002) derived the estimated average requirement (EAR) of protein for children and adolescents also by the factorial method, using the following data: (1) estimated maintenance requirements derived from 10 balance studies conducted in China, Chile, Guatemala and the Philippines, and corrected for miscellaneous N losses; (2) rate of protein deposition during growth derived from the studies which used a combination of water dilution technique, whole body potassium analysis and dual-energy x-ray absorptiometry or DEXA; and (3) estimate of efficiency of conversion of dietary protein to body protein derived from the slope of the regression line relating intake and balance. To derive the recommended dietary allowance that will cover 97.5% of the population in each sub-group, the EARs were multiplied by twice the assumed coefficient of variation of 12.5%. A comparison of the recommendations of the FAO/WHO/UNU (1985), IDECG (Dewey *et al.*, 1996) and IOM (2002) for children and adolescents is shown in Table 4.3.

Table 4.3 Comparison of recommended intakes of protein (milk or egg protein) for infants and children*

Age Groups	FAO/WHO/UNU 1985		IDECG 1996		IOM 2002	
	Protein (g/kg body weight/day)					
6 – < 12 months	1.56		1.06		1.50	
1 – 3 years	1.16		0.94		1.10	
4 – 6	1.03		0.87		0.95	
7 – 9	1.00		0.86		0.95	
	Males	Females	Males	Females	Males	Females
10 – 12	0.99	0.98	0.87	0.86	0.95	0.95
13 – 15	0.95	0.90	0.85	0.82	0.88	0.88
16 – 18	0.88	0.82	0.82	0.78	0.85	0.85

Note: * *Values were adjusted for age groups in accordance with Southeast Asian age groups*

(*d*) *Adults (19 - ≥ 50 years)*

The 1985 FAO/WHO/UNU Expert Consultation, which recommended that the intake of protein was similar in elderly and young adults, set the safe level of intake at 0.75 g protein/kg/day, after taking into account the population variability. The protein requirement per kg of body weight is considered to be the same for both sexes at all ages and body weights within the acceptable range (FAO/WHO/UNU, 1985).

EAR used by IOM is 0.66 g/kg/day of protein for male and female adults. To estimate the RDA, the 97.5 percentile of log requirement was calculated from the log median requirement + 2 SD (0.12), deriving an estimate of 46 g/day for a 57 kg reference woman, 19 to 50 years of age, and 56 g/day for a 70kg reference man or 0.80 g/kg/day. The same protein intake level is recommended for older adults, aged 51 to 70 years and 70 years and above.

(e) *Pregnancy*

The 1985 FAO/WHO/UNU Expert Consultation estimated the total protein requirement of a woman gaining 12.5 kg during pregnancy and delivering a 3.3 kg infant to be 925 g, plus 30% (2 SD of birth weight), or 3.3 g per day throughout pregnancy. An efficiency factor of 0.70 is also accepted as applying to pregnant women. The safe levels of additional protein computed in this manner are 1.2 g, 6.1 g, and 10.7 g per day in the first, second and third trimesters respectively.

The DRI Committee (IOM, 2002) estimated the additional requirements during pregnancy from the amount of dietary protein needed for a deposition of 7.2 g protein/day during the third trimester (derived from N and potassium balance studies) and adjusted for efficiency of utilization of dietary protein of 43%. This factor was chosen based on direct studies and because of the closeness to the data on infants and young children (IOM, 2002). The DRI Committee recommends an average total additional protein requirement of 25 g/day for the second and third trimester of pregnancy.

(*f*) *Lactation*

The 1985 FAO/WHO/UNU Expert Consultation suggested a safe level of extra protein intake of about 16 g/day during the first 6 months of lactation, 12 g/day during the second 6 months, and 11 g/day thereafter. These amounts should be added to the normal estimate

of the lactating woman's protein requirement and corrected for the digestibility of the dietary protein. Similarly, as for infants and children, an efficiency factor of 70% for the conversion of dietary protein to milk protein and 12.5% CV of breast milk volume was adopted.

The additional protein requirement for lactation is defined by the DRI Committee (IOM, 2002) as the output of total protein and non-protein nitrogen in milk. To estimate the increase in EAR, the average protein equivalent of breast milk N output during the first 6 months of lactation was divided by the assumed efficiency of dietary protein utilization of 47%. It was assumed that the incremental efficiency of N utilization of 0.47 in adults is the same as that noted for the restoration of N equilibrium in non-lactating women and adolescents (IOM, 2002). The RDA, assuming the same CV as that for total protein in non-lactating women of 12% was an additional protein intake of 25 g/day.

4.8 Current RDAs for Protein in Southeast Asia

Table 4.4 Comparison of current RDAs (g/day) for protein in selected Southeast Asian countries

Age Group (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 – 1)						
g / day	12 – 15	11 – 12	9 – 14	16 – 18	16[a]	21 – 23
body weight (kg)	5.5 – 8.5	6 – 8	6 – 9	7 – 9.5	8	7 – 9.5[b]
g / kg	2.2 – 1.8	1.8 – 1.5	1.5 – 1.6	2.3 – 1.9	2.0	3.0 – 2.4
Children (1 –9)[c]						
g / day	23 – 37	17 – 32	28 – 43	19 – 39	19 – 28	28 – 40
body weight (kg)	12 – 24	11 – 26	13 – 24	11 – 27	13 – 23	11 – 27[b]
g / kg	1.9 – 1.5	1.5 – 1.2	2.1 – 1.8	1.7 – 1.4	1.5 – 1.2	2.6 – 1.5
Boys (10 – 18)[d]						
g / day	45 – 66	45 – 65	54 – 67	49 – 80	42 – 62	50 – 65
body weight (kg)	30 – 56	36 – 59	34 – 58	34.5 – 64	33 – 57	34.5 – 64[b]
g / kg	1.5 – 1.2	1.3 – 1.1	1.6 – 1.2	1.4 – 1.3	1.3 – 1.1	1.5 – 1.0
Girls (10 – 18)[d]						
g / day	54 – 62	46 – 55	49 – 59	51 – 66	42 – 57	50 – 60
body weight (kg)	35 – 50	37 – 52	35 – 50	36 – 54	34 – 48	36 – 54[b]
g / kg	1.5 – 1.2	1.2 – 1.1	1.4 – 1.2	1.4 – 1.2	1.2 – 1.2	1.4 – 1.1
Men (≥ 19)[e]						
g / person	55	62 – 59	67	68	57	60
body weight (kg)	62	61 – 57	59	63.5	57	50 – 80
g / kg	0.9	1.0 – 1.04	1.1	1.1	1.0	1.2 – 0.8
Women (≥ 19)[e]						
g / person	48	55 – 51	58	58	52	55
body weight (kg)	54	52 – 49	51	54	52	40 – 75
g / kg	0.9	1.1 – 1.0	1.1	1.1	1.0	1.4 – 0.7
Pregnancy						
1st trimester	+12	+7.5	+8	+9	+10	+0
2nd trimester	+12	+7.5	+8	+9	+10	+15
3rd trimester	+12	+7.5	+8	+9	+10	+15
Lactation						
1st 6 months	+16	+20	+23	+25	+15	+28
2nd 6 months	+12	+15	+18	+19	+12	+0

Notes: *Protein quality: NPU = 70 for Philippines, Singapore; 80% for Malaysia*
Protein usage = 60 for Vietnam
Protein digestibility of 85% for Thailand
[a] figures only for infants 6 - 11 months
[b] body weights not given by Vietnamese RDAs but taken from WHO.
[c] 1 - 8 years for Thailand
[d] 10 - 17 years for Singapore; 9 - 18 years for Thailand
[e] ≥ 18 for Singapore

Source: *Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)*

All countries listed a protein requirement after having adjusted for protein quality of the diet. Most Southeast Asian countries used a protein quality or net protein utilization (NPU) of 70, while Vietnam used a value of 60 and Thailand used a digestibility value of 85%.

All RDAs for infants are in the range 9 g to 18 g per day, except for Vietnam which provides for a higher intake of 21 g to 23 g per day. For children less than 10 years of age, higher amounts (17 g to 43 g) are recommended by Indonesia, Malaysia, Philippines, Singapore and Vietnam, compared with the lower recommendation of Thailand (17 g to 26 g). Recommended intake for adolescent boys are rather similar for all the countries, ranging from 45 g to 67 g per day, except for Singapore. The latter has a higher RDA for protein of 49 g to 80 g per day. The amounts recommended for adolescent girls are all lower for the adolescent girls (42 g to 62 g per day). Singapore still has a slightly higher RDA for protein for this age group (51 g to 66 g per day).

For all the selected Southeast Asian countries, protein RDA for women are lower than those recommended for men. There are considerable variations in the RDAs for protein, with Indonesia and Thailand having lower values for both men (55 g to 57 g per day) and women (48 g to 52 g per day).

All RDAs tabulated recommend additional amounts of protein during pregnancy, ranging from 7.5 g to 15 g per day. Except for Vietnam, the additional amounts are recommended throughout the duration of the pregnancy. Additional intakes of protein are also recommended for lactating women, with higher amounts suggested for the first 6 months of lactation. Vietnam only recommended additional amounts during the first 6 months of lactation.

4.9 Recommended RDAs for Protein for Southeast Asia

Upon reviewing currently available data, the SEA-RDA Committee decided to adopt the 1985 FAO/WHO/UNU recommendations for all population groups as the SEA-RDAs for protein.

Table 4.5 Recommended RDAs for protein for various age groups

Age Groups	Protein RDA (g/kg body weight/day)		
	For a high quality protein diet	Adjusted for 80% protein quality	Adjusted for 70% protein quality
Infants (months)			
0 – 5	1.86	-	-
6 – 11	1.56	-	-
Children (years)			
1 – 3	1.16	1.45	1.66
4 – 6	1.03	1.29	1.47
7 – 9	1.00	1.25	1.43
Boys (years)			
10 – 12	0.99	1.24	1.41
13 – 15	0.95	1.19	1.36
16 – 18	0.88	1.10	1.26
Girls (years)			
10 – 12	0.98	1.23	1.40
13 – 15	0.90	1.13	1.29
16 – 18	0.82	1.03	1.17
Men (≥ 19 years)	0.80	1.00	1.14
Women (≥ 19 years)	0.80	1.00	1.14
Pregnancy	+ 6	+ 7.5	+ 9
Lactation			
1st 6 months	+ 16	+ 20	+ 23
2nd 6 months	+ 12	+ 15	+ 17

Source: FAO/WHO/UNU (1985)

Based on data in Table 4.5 and using the reference body weights given in Table 2.1, the SEA-RDAs for protein for various age groups are as set out in Table 4.6. RDAs based on 70% protein quality are probably most reflective of the dietary pattern of most population groups in Southeast Asia.

Table 4.6 Recommended RDAs for Protein for Southeast Asia

Age Groups	Protein RDA (g/day)		
	For a high quality protein diet	Adjusted for 80% protein quality	Adjusted for 70% protein quality
Infants (months)			
0 – 5	11	-	-
6 – 11	14	-	-
Children (years)			
1 – 3	16	20	23
4 – 6	21	26	29
7 – 9	27	34	39
Boys (years)			
10 – 12	34	42	48
13 – 15	45	56	64
16 – 18	49	62	71
Girls (years)			
10 – 12	35	44	50
13 – 15	41	51	58
16 – 18	40	50	57
Men (≥ 19 years)	48	60	68
Women (≥ 19 years)	40	50	57
Pregnancy	+ 6	+ 7.5	+ 9
Lactation			
1st 6 months	+ 16	+ 20	+ 23
2nd 6 months	+ 12	+ 15	+ 17

Bibliography

Dewey KG, Beaton G, Fjeld C, Lönnerdal B, Reeds P (1996). Protein requirements of infants and children. *Eur J Clin Nutr* 50(S1):S119-S150.

FAO/WHO/UNU (1985). *Energy and Protein Requirements.* Report of a Joint Expert Consultation. WHO Technical Report Series 724. World Health Organization, Geneva.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

FNRI (1989). *Recommended Dietary Allowances for Filipinos.* Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

Harper AE and Yoshimura NN (1993). Protein quality, amino acid balance, utilization, and evaluation of diets as therapeutic agents. Nutrition 9 (5):

IDECG (1996). Report of a working group on protein and energy requirements. *Eur J Clin Nutr* 50:S93-S95.

IOM (2002). *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein and Amino Acids (Macronutrients).* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington D.C. Chapter 10.

Matthews CK and van Holde KE (1996). *Protein Function and Evaluation in Biochemistry.* 2nd edition. The Benjamin/Cummings Publishing Co, Inc, CA, USA. Chapter 7.

MPH (2003). *Dietary Reference Intake for Thais.* Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia.* A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

NRC (1989). *Recommended Dietary Allowances. 10th Edition.* National Research Council, National Academy Press, Washington DC.

Park JE and Park K (1985). Preventive and Social Medicine. 10th edition. India.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

WHO (1990). *Diet, Nutrition, and the Prevention of Chronic Diseases.* Report of a WHO Study Group. Technical Report Series 797. World Health Organization, Geneva.

5 CALCIUM

5.1 Introduction

Calcium is the most abundant cation in the human body, comprising 1.52% of adult fat-free mass. Essentially all body processes require calcium, thus the level of calcium circulating in the blood is kept remarkably constant by a finely tuned homeostatic mechanism. 60% of serum calcium is ionized and physiologically active. The remaining serum calcium is non-ionized and physiologically inert, 35% of which are bound to protein mainly as albumins and globulins, and 5% are complexed with citrates, carbonates and phosphates.

5.2 Characteristics and Functions

Of the approximately 1.2kg (300 mmol) of calcium present in the human body, about 99% is found in the bones and teeth where its primary role is structural. 1% is present in blood, extracellular fluid, muscle and other tissues, where it plays a role in mediating vascular contraction and vasodilation, muscle contraction, nerve transmission and glandular secretion.

In bone, calcium exists primarily in the form of hydroxyapatite [$Ca_{10}(PO4)_6$ $(OH)_2$], and bone mineral makes up almost 40% of bone weight. Bone is a dynamic tissue that is constantly undergoing osteoclastic bone resorption and osteoblastic bone formation. Bone formation exceeds resorption in growing children, is balanced with resorption in healthy adults, and lags behind resorption after menopause and with aging in men and women. Each year, a portion of the skeleton is remodelled (reabsorbed and replaced by new bone). The rate of cortical (or compact) bone remodelling can be as high as 50% per year in young children and is about 5% per year in adults (Parfitt, 1988). Trabecular (or cancellous) bone remodelling is about five-fold higher than cortical remodelling in adults. The skeleton has an obvious structural role and it also serves as a reservoir for calcium.

5.3 Absorption, Utilisation and Excretion

Calcium absorption occurs predominantly in the jejunum but also, to a lesser extent, in the ileum and colon (Nordin, 1976, 1988). Uptake occurs by active transport and simple passive diffusion. Active transport of calcium into enterocytes and out on the serosal side is dependent on the action of 1,25-dihydroxy vitamin D [1,25$(OH)_2$D], the active form of vitamin D. This metabolite enhances the active phase of absorption by stimulating the biosynthesis of a specific intestinal calcium binding protein which is involved in cellular transport. The active transport mechanism accounts for most of the absorption of calcium at low and moderate intake levels. As calcium intake increases, the active mechanism becomes saturated and any further calcium absorption occurs by passive diffusion. The net result is an increase in the absolute amount of absorbed calcium with increasing intake but a decrease in fractional absorption. These mechanisms may in part account for the different

results from absorption and calcium balance studies on populations with higher or lower customary intakes of calcium. It has, however, been clearly demonstrated that absorbed calcium and calcium intake, throughout a wide intake range, are positively related.

Calcium is lost mainly through renal excretion. A major determinant of urinary calcium excretion is dietary calcium. Losses also occur via feces, sweat, skin, hair, and nails. Calcium also enters the gut via the bile, which is relatively rich in calcium, in the pancreatic secretions, and as part of the desquamated cells from the mucosal lining and may be reabsorbed from the ileum and colon. Because of this endogenous secretion, net absorption is less than gross dietary absorption by roughly 100 mg (2.5 mmol) per day (Nordin, 1976).

Ethnic differences in calcium metabolism have been noted in children and adults. Individuals demonstrate great physiological adaptability in their absorption, excretion and metabolic use of dietary calcium. Little is known about whether the adaptive response is limited by factors such as age, estrogen status and exposure to high or low calcium intakes early in life. Further, there is a lack of information about the range of inter-individual variability in the capacity to adapt to low calcium diets. The major determinant of calcium homeostasis appears to be the plasma level of ionised calcium, sustained in part by the mobilisation and deposition of skeletal calcium. This immediate response to a diminishing calcium supply is thought to precede adaptation to increase calcium absorption and decrease calcium excretion. In adults, adaptation to a change in diet can be very slow; adult men introduced to a diet containing half the amount of calcium compared to their customary diet took several months to regain calcium balance. This is an important qualifying factor in using balance studies to assess dietary calcium needs. These variations in bioefficacy need to be accommodated within any consideration of the nutrient adequacy of the diet (DOH, 1998).

5.4 Effects of Deficiency and Excess

5.4.1 Deficiency

Inadequate intake, poor calcium absorption, and/or excessive calcium losses contribute to inadequate mineralization of bone. Rickets in children occurs when the amount of calcium accretion per unit bone matrix is deficient. Low levels of free iodised calcium in the blood (hypocalcaemia) may result in tetany. Tetany is manifested by intermittent muscle contractions. Muscle pain, muscle spasms and paresthesias (numbness or tingling in the hands and feet) are common signs of tetany.

Long-term deficient calcium intakes are also associated with the development of hypertension and colon cancer. An inverse relationship between calcium and blood pressure had been observed (as intake of calcium decreases, prevalence of hypertension increases), with a steep slope at calcium intakes less than 600 mg/day. Calcium supplementation has been shown to lower blood pressure in some hypertensive people previously ingesting a diet inadequate in calcium. Colon cancer has also been linked with calcium-deficient diets (Barger-Lux & Heaney, 1994). However, evidence in this area remains inconclusive.

Chronic calcium deficiency resulting from inadequate intake or poor intestinal absorption has also been identified as one of several important causes of reduced bone mass and osteoporosis (NIH, 1994; NRC, 1989). A reduction in absorbed calcium causes the circulating ionised calcium concentration to decline, which triggers an increase in parathyroid hormone (PTH) synthesis and release. PTH acts on 3 target organs to restore the circulating calcium concentration to normal. At the kidney, PTH promotes the reabsorption of calcium in the distal tubule. PTH indirectly affects the intestine by stimulating the production of 1,25-dihydroxy vitamin D [1,25$(OH)_2$D]. PTH also induces bone resorption, thereby increasing calcium release in the blood. Through these mechanisms, PTH maintains normal circulating calcium concentration during calcium deprivation, but does so at the expense of skeletal mass.

Osteoporosis is a condition characterised by reduced bone mass, increased bone fragility, and increased risk of fracture (WHO, 1994). Risk of osteoporosis increases with age. Only 26% of all hip fractures in the world occurred in Asia in 1990. However, demographic shifts over the next 50 years will lead to huge increases in the number of elderly in Asia. It has been projected that by the year 2050, about 45% of all hip fractures will occur in Asia (Gullberg *et al.*, 1997).

5.4.2 Excessive intake

Body calcium metabolism is under such close homeostatic control that an excessive accumulation in blood or tissues from over-consumption is rare. Currently, the available data on the adverse effects of excess calcium intake in humans primarily concerns calcium intake from nutrient supplements. Of the possible adverse effects of excessive calcium intake, the 3 most widely studied and biologically important are kidney stone formation (nephrolithiasis), hypercalcaemia and renal insufficiency (milk-alkali syndrome) and interaction of calcium with the absorption of other essential minerals (e.g. iron, zinc, magnesium and phosphorus). Although adverse effects are not restricted only to these 3 conditions, the vast majority of reported effects are related to or result from 1 of these 3 conditions. However, there is general consensus that calcium intake in amounts up to 2,500 mg appear to be safe for most individuals (IOM, 1997).

5.4.3 Guidance on high intake

The DRI Committee has established a tolerable upper intake level (UL) of 2,500 mg/day for all age groups (IOM, 1997).

5.5 Food Sources and Usual Intakes

Foods rich in calcium include milk, cheese, other dairy products, dark green leafy vegetables, bean products (e.g. beancurd) and fish with edible bones (e.g. sardines, anchovies). In recent years, there has also been an increasing number of calcium-fortified food products available in the market, such as some orange juices, breads, biscuits and breakfast cereals.

Table 5.1 shows the usual calcium intakes of adults in some Southeast Asian countries. It can be seen that the average calcium intakes are variable but are typically below 500 mg of calcium per day. Compared to Western countries where dairy products are the major source of calcium, food sources such as beans and bean products, cereals and cereal products, dark green leafy vegetables and fish play major roles in contributing towards calcium intake in Southeast Asian countries.

Table 5.1. Usual calcium intakes (mg/day) of adults in selected Southeast Asian countries

Indonesia	Malaysia	Myanmar	Philippines	Singapore	Vietnam
342 [1]	255-333 [2]	498 [3]	390 [4]	482 [5]	488 [6]

Source: [1] Indonesian Household Survey (1998)
[2] Chee SS, Ismail MN, Ng KK and Zawiah H. (1997). Food intake assessment of adults in rural and urban areas from 4 selected regions in Malaysia. Malaysian Journal of Nutrition, 3:91-102.
[3] Household food security survey, NNC, Myanmar (1998)
[4] National Nutrition Survey, Philippines (1993)
[5] National Nutrition Survey, Singapore (1998)
[6] Report of general nutrition survey data (1987-1989), Ministry of Health, Vietnam (1991)

5.6 Factors Affecting Requirement

5.6.1 Ethnicity

Inadequate calcium intake has been implicated in the development of osteoporosis and the subsequent fracture risks. However, hip fracture rates have often been reported to be highest among Northern Europeans and North Americans despite their high calcium intake, intermediate in Asians with low calcium intake, and lowest among African populations. This apparent discrepancy between habitual calcium intake and reported hip fracture incidence has led to speculation on the influence of ethnicity on calcium requirements. Several possibilities have been put forward to explain the lower incidence of hip fracture among Asians, as compared to Caucasians. These include bio-mechanical factors such as the lower hip axis length in Asians and greater bone strength. For example, the cross-sectional moments of inertia (a measurement of bone rigidity in bending) and safety factor (an index of the strength of the femoral neck during one-legged stance) were found to be higher in Japanese than in American-Caucasians (Nakamura *et al.*, 1993).

In terms of calcium metabolism, fractional calcium absorption was reported to be much higher in Chinese women compared to Caucasians (Kung *et al.*, 1998). This has led to the suggestion that Chinese populations may require lower calcium intakes than Caucasians for equivalent amounts of calcium to be absorbed (Weaver 1998). Whether this represents an ethnic difference or just an adaptation to chronically low dietary calcium intakes since childhood is unknown (Kung *et al.*, 1998).

Other ethnic-related lifestyle factors such as greater physical activity, a diet with less animal protein, greater exposure to sunlight (ultraviolet light) and thus enhanced vitamin D production in the skin, may assist in explaining the lower hip fracture incidence among Asians. Some of these are discussed in greater detail in the following sections.

The exact mechanisms that protect Asians against hip fracture are complicated and merit in-depth investigation. The paucity of published studies on calcium metabolism in Asians makes it difficult

to assess the impact of ethnic-related factors on calcium requirements. Where possible, results from Asian studies have been considered in setting the SEA-RDAs for calcium.

5.6.2 Sunlight exposure

Ultraviolet radiation from sunlight exposure on the skin manufactures vitamin D which plays an important role in enhancing intestinal calcium absorption. Since the lowest incidence rates of hip fractures have been reported in tropical countries and the highest rates are in Scandinavia, it has been postulated that the lack of exposure to sunlight might be associated with fractures. Moreover, a report on the geographic pattern of hip fractures in the United States has shown a positive association with reduced sunlight exposure. However, the lower rates of hip fractures reported in the United Kingdom compared with Scandinavia, and in Canada compared with the USA, do not support this hypothesis.

There is currently inconclusive evidence that the level of sunlight exposure, as an independent factor, explains the discrepancies in results from calcium studies conducted in populations living in different parts of the world. Sunlight exposure probably works with other ethnic-related factors to influence calcium requirements among different populations. Sunlight exposure is only likely to have an impact on calcium requirements among populations in which vitamin D status is limiting. It is plausible that populations with adequate vitamin D status (as in Southeast Asian countries where sunlight is abundant) may be better able to adapt to different levels of calcium intakes compared to populations with less satisfactory vitamin D status (lesser sunlight exposure). However, since the RDA for any one nutrient presupposes that requirement of energy and all other nutrients are met, setting different calcium RDAs based on different levels of sunlight exposure is not warranted.

5.6.3 Bioavailability

When evaluating food sources of calcium, the calcium content is generally of greater importance than bioavailability. Calcium absorption efficiency is fairly similar for most foods including milk, milk products and grains. It should be noted that calcium may be poorly absorbed from foods rich in oxalic acid (spinach, sweet potatoes, rhubarb, and beans) or phytic acid (unleavened bread, raw beans, seeds, nuts, grains, and soy isolates). Soy beans contain large amounts of phytic acid, yet calcium absorption is relatively high from this food (Heaney *et al.*, 1991). Compared to calcium absorption from milk, calcium absorption from dried beans is about half and about one-tenth from spinach.

Bioavailability of calcium from non-food sources or supplements depends on the presence or absence of a meal and the size of the dose. Supplement solubility is not very important, but tablet disintegration (for example, breaking apart) is essential.

As diets used in metabolic studies and in the general population tend to contain calcium from a variety of sources, and because the specific foods used in most of the published studies were not described, bioavailability has not been considered in setting the calcium intake requirements.

5.6.5 Nutrient-nutrient interactions

There is much evidence supporting a positive relationship between urinary sodium and calcium excretion in young and adult free-living individuals consuming a normal diet. Sodium and calcium excretion are linked at the proximal renal tubule. High sodium chloride intake results in increased absorbed sodium, increased urinary sodium, and an increased obligatory loss of urinary calcium. This linkage holds at moderate and high calcium intakes, but some dissociation occurs at low calcium intakes, probably because low calcium intakes induce higher parathyroid hormone levels, and parathyroid hormone promotes the resorption of filtered calcium in the distal renal tubule. Given the low calcium intakes in Southeast Asian countries and the practical constraints in quantifying sodium intake, available evidence does not warrant different calcium intake requirements for individuals according to their sodium consumption.

Increased protein intake has been associated with an increase in urinary calcium excretion, thus shifting calcium balance in a negative direction. While protein increases urinary calcium excretion, its effect on calcium retention is controversial. In addition, it should be recognised that inadequate protein intakes (34 g/day) have been associated with poor general health and poor recovery from osteoporotic hip fractures. Available evidence does not warrant adjusting calcium intake recommendations based on dietary protein intake.

Other nutrients such as fat, phosphate and magnesium have not been found to affect the overall retention or excretion of calcium significantly although they can have short term effects on absorption and excretion rates.

5.6.6 Other food components

Caffeine induces a short-term increase in renal calcium excretion and may modestly decrease calcium absorption, but its effects on dermal calcium loss has not been evaluated. The skeletal effects of caffeine are modest at calcium intakes of 800 mg (20 mmol)/ day and above (IOM, 1997). Available evidence does not warrant different calcium intake recommendations for people with different caffeine intakes.

Increased fibre intake (using wheat bran as the fibre source) has been associated with a decrease in calcium absorption, which can be summarised as a decrease in absorption of about 20% to 30% when bran fibre intake increased from 0.1 g/day to approximately 30g/day. However, there has been very little evidence of the negative effect of increased fibre intakes on bone mass. In fact, negative correlations between trabecular bone density and fibre intake have been reported, while others have found no association. It has been suggested that the effect on calcium absorption may be due to phytate (which is present in substantial amounts in wheat bran), rather than fibre intake per se. Available evidence does not warrant different calcium intake recommendations for people with different fibre intakes.

5.6.7 Physical activity

Although exercise and calcium intake both influence bone mass, it is unclear whether calcium intake influences the degree of benefit derived from exercise. In a review, it was found that high daily calcium intakes (over 1,000 mg (25 mmol)) enhanced the bone mineral density benefits from exercise at the lumbar spine, but enhancement at the radius was less pronounced (Specker, 1996). Additional prospective studies are needed to test and compare individual and combined effects of calcium and exercise. Currently, there is insufficient evidence to justify different calcium intake recommendations for people with different levels of physical activity.

5.6.8 Special populations

Lactose intolerance is common among Asians. Lactose-intolerant individuals often avoid milk products entirely although avoidance may not be necessary. Studies have revealed that many lactose-intolerant people can tolerate smaller doses of lactose. In addition, lactose-free dairy products are also available in the market. Lactose-intolerant individuals absorb calcium normally from milk. Although lactose intolerance may influence intake, there is no evidence to suggest that it influences the calcium requirement (IOM, 1997).

Consumption of vegetarian diets may influence the calcium requirement because of their relatively high contents of compounds that reduce calcium bioavailability such as oxalates and phytates. However, compared to diets containing animal protein, vegetarian diets tend to produce metabolizable anions (e.g. acetate, bicarbonate) that lower urinary calcium excretion. On balance, lacto-ovo-vegetarians and non-vegetarians appear to have fairly similar dietary calcium intakes and on the same intakes, to have similar amounts of urinary calcium excretion (IOM, 1997). Data on strict vegetarians is limited. Available data do not support the need for a different calcium intake recommendation for vegetarians.

5.7 Estimating Requirements and Recommended Intakes

5.7.1 General considerations

There is no biochemical indicator of calcium nutritional status. Calcium levels in blood are regulated within very tight limits in order to maintain normal muscle function, with bone serving as a store-house for calcium. Calcium metabolism is further complicated by the regulation of absorption mediated by vitamin D and by kidney excretion, also affected by vitamin D and parathyroid hormone. Thus, calcium is not a simple nutrient in terms of assessing requirements, because a number of components affect calcium status and therefore calcium requirements. In addition, the very diversity of its functions makes it difficult to define the appropriate end-point for assessing the adequacy of either the dietary supply or of its delivery to the relevant tissues.

5.7.2 Factorial estimates

A factorial approach has commonly been applied when estimating calcium requirements. This approach estimates increasing losses due to growth (if applicable) and then correcting for an expected rate of absorption in the diet. However, there are several limitations to this approach. There are a number of methodological errors in the approach, which makes it difficult to provide assurance that the number is close to actual requirements. The calculation of "obligatory losses" often depends on calcium balance studies. Such studies may be problematic in that it must be measured after equilibrium is established, which has been estimated to occur over weeks to months of adaptation to a new dietary level.

Nonetheless, in the absence of more suitable indicators or methods for estimating requirements, the factorial approach remains one of the most commonly used methods in estimating calcium requirements and it has been used by countries such as UK (DOH, 1991), US (IOM, 1997) and China (Chinese Nutrition Society, 2000).

5.7.3 Calcium intake and fracture risk

Given the important influence of calcium on bone health, it is conceivable that the calcium requirement can be estimated as the optimal calcium intake that would lead to the fewest osteoporotic fractures later in life. Such information could only be obtained through long-term prospective studies to determine the influence of different increments in calcium intakes in young and older subjects with a wide range of usual intakes. Such studies are neither available nor practical to conduct.

Some attempts have been made to compare fracture rates across cultures that have different calcium intakes in order to derive an "optimal calcium intake" associated with the lowest fracture rates. However, this approach has numerous confounding factors such as bone structure, genetic composition, dietary habits (food sources of calcium) and other environmental factors (e.g. length of sunlight exposure).

For the reasons stated above, the use of observational studies relating intakes to fracture risk to estimate calcium requirement has been very limited.

5.7.4 Habitual calcium intake

Data from studies of actual dietary calcium intakes of populations which show no apparent calcium deficiencies can be used to estimate calcium requirements. Such data are, however, more often used as a confirmatory tool rather than a tool from which fundamental estimates are derived. For example, where the actual intakes correspond closely to that derived from the factorial estimates, the requirement of the nutrient can then be determined with greater confidence than with a single measure alone.

It is worth noting that the use of these intakes to estimate apparent requirements are usually only restricted to the particular dietary and environmental circumstances pertaining to the period in which they were measured. As such, application of such intake values to estimate calcium requirements cross-culturally are strongly discouraged.

5.7.5 Calcium retention and bone mass measurements

Since 99% of body calcium is located in the skeleton, bone mass measurements may provide a useful tool to determine calcium retention in order to estimate calcium requirement. To a great extent, the retention of calcium in bone is under strong homeostatic control, which is regulated by genetics, calciotropic hormones and weight-bearing exercise. The target intake of dietary calcium to achieve the desirable and optimal calcium accretion in bone is difficult to estimate because of all the other factors which play a role in bone mineral homeostasis.

Bone mass measurements include bone mineral content (BMC) and bone mineral density (BMD). Any changes in BMC is a useful indicator of calcium retention in children while changes in BMC and BMD are both useful outcome measures of calcium retention in adults. To maximise skeletal size and strength, one must have adequate calcium retention to provide the substrate (along with the other minerals) for bone mineral expansion during growth and maintenance after peak bone mass has been achieved. The use of dual-energy x-ray absorptiometry (DEXA) has enabled precise and reasonably accurate measurements of changes in BMD and BMC to be obtained. Such measurements add valuable refinements to the determination of calcium requirement derived from balance studies. Limited data on BMC and BMD changes were used in the DRI Committee in determining their calcium requirement for the American population (IOM, 1997).

5.7.6 Recommendations for calcium intake by life stages

The main references used in arriving at recommendations for calcium intakes for the Southeast Asian region were the FAO/WHO's recommendations in its 2002 expert consultation report on vitamins and mineral requirements (FAO/WHO, 2002), the UK Department of Health's DRV values (DOH, 1991) and the recommendations of the DRI Committee (IOM, 1997).

(*a*) *Infants* (*0 – 6 months*)

Breast milk has been widely recognised as the most suitable nourishment for infants. Calcium deficiency has not been reported among infants aged 0 to 6 months fed exclusively with breast milk. Hence, the RDA is estimated based on the mean calcium intake of breast-fed infants. Using an average intake of 750 ml of breast milk per day (Chinese Nutrition Society, 2000) and the average calcium content of breast milk as 264 mg/L (average of 10 studies from the US and the UK as summarised in Atkinson *et al.*, 1995), the average calcium intake is estimated to be 198 mg. Taking into account the variability of calcium intakes and requirements, and that factorial estimates of calcium requirement in infants has derived calcium RDA as high as 525 mg in the UK, recommended calcium intake for infants 0 to 6 months of age has been set at 300 mg/day. For formula-fed infants, due to the lower calcium absorption from infant formula (40%) compared to breast milk (66%), the proposed calcium

RDA for formula-fed infants is therefore higher, at 400 mg/day (DOH, 1991). These proposed RDA values for infants aged 0 to 6 months are consistent with the RNI values recommended by FAO/WHO (2002).

(c) *Infants (7 – < 12 months)*

During the ages of 7 to 12 months, the intake of solid foods becomes more significant than during early infancy. The calcium intake contribution from breast milk decreases (partly due to lower calcium concentration of breast milk at 7 to 12 months of lactation and the lower prevalence of breast-feeding during this period) and calcium intake increases substantially from solid foods and formula milk. The average calcium accretion rate was estimated to be 140 mg of calcium per day during the first year of life (IOM, 1997). Taking into account the lower calcium absorption from solid foods and formula milk as compared to breast milk, the proposed RDA for calcium for infants aged between 7 to 12 months is set at 400 mg/day. This value is consistent with the RNI recommended by FAO/WHO (2002).

(d) *Children (1 – 9 years)*

Children with habitually higher calcium intakes through the first 5 years of life (≥ 400mg/day) were found to have significantly higher bone mineral content than their counterparts with lower calcium intake (< 400 mg/day) (Lee *et al.*, 1993), suggesting that for better bone health, daily calcium intake for this age group should exceed 400 mg/day. Children need to absorb 70 mg to 150 mg of calcium per day for growth and bone mineralization (DOH, 1991). The RNI values for children aged 1 to 9 years are proposed by FAO/WHO (2002) to be as follows: 2 to 3 years: 500 mg/day; 4 to 6 years: 600 mg/day; and 7 to 9 years: 700 mg/day.

(e) *Adolescents (10 - 18 years)*

The peak rate of calcium accumulation is 300 mg/day to 400 mg/day, which occurs earlier in girls but continues for longer in boys. Thus, it is difficult to justify any difference between recommended allowances for boys and girls. Assuming a target value of 300 mg/day for the skeleton, urinary loss of 100 mg/day and losses in the dermal and feces of 40 mg/day, a total amount of 440 mg of calcium needs to be absorbed per day. Assuming a high calcium absorption of 35% in a mixed diet (higher than adults), FAO/WHO (2002) has recommended 1,300 mg/day and 1,000 mg/day for populations where animal protein intake is 60 g/day to 80 g/day and 20 g/day to 40 g/day respectively.

(f) *Adults (19 - 49 years)*

Among Asians, calcium balance has been reported to be achieved at intakes of 350 mg/day to 450 mg/day and 540 mg/day in Chinese adults (Chinese Nutrition Society, 2000). Taking the higher value of 540 mg, assuming a CV of 10%, the RDA for calcium is proposed to be 700 mg/day. There were no sufficient data to justify different RDAs for men and women. A study in young Chinese women has shown that an intake above 600 mg would have a beneficial effect on bone mass (Ho *et al.*, 1994). It has been reported that a threshold for calcium exists, so that additional intake has a positive effect on bone mineral density mainly in populations with an intake of approximately 600 mg/day or less (Lau & Cooper, 1996). The proposed RDA of 700 mg/day is consistent with the RDA derived using the factorial approach in the UK (DOH, 1991). A recent review of calcium RDAs in the UK found that

available data were not sufficient to warrant increasing the RDA for adults beyond 700 mg/day (DOH, 1998).

FAO/WHO (2002) has recommended a calcium RDA of 750 mg/day for populations with animal protein intake of 20 g/day to 40 g/day. Studies conducted in Thailand and China have led to a recommendation of 800 mg/day (Chinese Nutrition Society, 2000). Based on these considerations, FAO/WHO (2002) has proposed that the RNI for calcium for adults aged 19 to 50 years be set at 1,000 mg/day for both men and women.

(*g*) *Older Adults* (≥ *50 years*)

Although diminished estrogen after menopause causes accelerated bone loss, estrogen deficiency-related bone loss cannot be prevented by increasing calcium intake (IOM, 1997). Estrogen does to some extent influence calcium absorption, but available evidence is not sufficient to support different RDAs for older women depending on their menopausal status or their use of hormone replacement therapy. Available balance data indicate that the intake requirement of women over the age of 50 is at least 1,000 mg/day and no evidence indicates that it differs substantially from that of similarly aged men (IOM, 1997). The higher RDA in older adults compared to adults aged 19 to 50 years old provides for the fact that calcium absorption is known to decrease with advancing age (Chan *et al.*, 1992; IOM, 1997). The DRI Committee has recommended that the RDA for calcium be 1,200 mg/day for older adults. FAO/WHO (2002) has recommended an even higher intake at 1,300 mg/day for both older men and women.

(*h*) *Pregnancy*

During pregnancy, there are major changes in hormonal patterns and metabolism. There is emerging evidence that during pregnancy, the calcium required for fetal bone mineralization can be obtained by an increased efficiency of maternal calcium absorption, with no detectable mobilization of maternal bones for this purpose. Adaptive maternal responses to fetal calcium needs include an enhanced efficiency of absorption, which is modulated through changes in calciotropic hormones and hence no increment in calcium RDA is warranted during pregnancy (IOM, 1997). However, these findings are usually based on studies done on women with high habitual calcium intake (> 1,000 mg/day), lending uncertainty to whether these results are applicable to women in Southeast Asian countries who typically consume less calcium. In addition, a study on undernourished pregnant mothers found that although calcium supplementation did not increase the metacarpal bone density of mothers during pregnancy, the bone density of the neonates were significantly increased (Raman *et al.*, 1978). FAO/WHO (2002) does not recommend increased calcium intake during pregnancy, except for the third trimester, during which time an additional 200 mg/day of calcium is recommended. The DRI Committee (IOM, 1997) also does not provide for additional calcium intake during pregnancy.

(*i*) *Lactation*

The loss of calcium from the maternal skeleton that occurs during lactation is not prevented by an increase in dietary calcium, and the calcium loss appears to be regained following weaning (IOM, 1997). Recent supplementation studies suggest that lactating women do not have raised dietary calcium requirements (Prentice, 1998). Some studies have found that milk calcium is unaffected by maternal calcium intake (IOM, 1997), but studies among the Chinese

population found that milk calcium was increased by higher maternal calcium intake (Chinese Nutrition Society, 2000). Since most women increase their food intake, and hence their calcium intakes, spontaneously during lactation, a higher RDA during lactation is unlikely to pose any additional difficulty, and may even increase the calcium content of the breast milk. FAO/WHO (2002) does not recommend additional calcium intake during lactation and maintained the calcium RNI at 1,000 mg/day. The DRI Committee (IOM, 1997) also does not provide for additional calcium intake during lactation.

5.8 Current RDAs for Calcium in Southeast Asia

The current RDAs for calcium for 6 Southeast Asian countries are shown in Table 5.2.

It can be seen that there are appreciable differences in the RDAs for calcium for every age group among the selected Southeast Asian countries. It can also be noted that the recommended calcium intake for more recently published RDAs (after 2000) are higher than earlier published ones. For infants, the RDA range from a low of 170 mg/day for Thailand to 600 mg for Singapore. The differences in RDA for children are also evident, ranging from 400 mg/day to 800 mg/day. Adolescent boys and girls have the same RDA within each country. For almost all the countries reviewed, throughout the lifespan (except during pregnancy and lactation), the highest calcium RDAs are those made for this age group. In 3 of the countries (Indonesia, Singapore and Vietnam), the RDA ranged from 500 mg to 700 mg, whereas it is 1, 000 mg in the remaining 3 countries (Malaysia, the Philippines and Thailand). Adult men and women are given the same RDA for calcium in all the countries. Recommended intakes for the more recently published RDAs, ie Malaysia, the Philippines and Thailand) are much higher than the older RDAs. All the countries have included increments in calcium RDAs for pregnancy and lactation and the levels are rather smilar for these two physiological periods. Singapore has the highest RDA for calcium for these groups.

Table 5.2. Comparison of current RDAs (mg/day) for calcium in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 - 1)	300 - 400	300 - 400	200 - 400	500 - 600	270[a]	300 - 500
Children (1 – 9)[b]	500	500 - 700	500 - 700	400 - 500	500 - 800	500
Boys (10 – 18)[c]	600 - 700	1,000	1,000	500 - 600	1,000	700
Girls (10 – 18)[c]	600 - 700	1,000	1,000	500 - 600	1,000	600 - 700
Men (≥ 19)[d]	500	800 - 1,000	750 - 800	400 - 500	800 - 1,000	500
Women (≥ 19)[d]	500	800 - 1,000	750 - 800	400 - 500	800 - 1,000	500
Pregnancy						
1st trimester	900	1,000	800	1,000 - 1,200	800	500
2nd trimester	900	1,000	800	1,000 - 1,200	800	1,000
3rd trimester	900	1,000	800	1,000 - 1,200	800	1,000
Lactation						
1st 6 months	900	1,000	750	1,000 - 1,200	800	1,000
2nd 6 months	900	1,000	750	1,000 - 1,200	800	500

Notes: [a] *Figures only for infants 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9-18 years for Thailand*
[d] *≥ 18 for Singapore*
Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

5.9 Recommended RDAs for Calcium for Southeast Asia

The SEA-RDA Committee made its recommendations for calcium intake after reviewing the available recent international documentation on the subject. The 3 main references used were the FAO/WHO 2002 Expert Consultation Report on Vitamins and Mineral Requirements (FAO/WHO, 2002), the UK Department of Health's DRV values (DOH, 1991) and the recommendations of the DRI Committee (IOM, 1997). The rationale and steps taken in setting requirements and the levels recommended by these organizations were noted. Several studies on calcium intake and dietary pattern of communities in the region, as well as the prevailing socio-economic situations, were considered.

For infants and young children, the SEA-RDA Committee agreed to adapt the recommended intakes of FAO/WHO (2002) for these age groups. Assuming that the animal protein intake of communities in countries in the region is closer to 20 g/day to 40 g/day, the SEA-RDA Committee decided to adopt the lower RNI value of 1,000 mg/day for adolescents aged 10 to 18 years. For adults, the SEA-RDA Committee also decided to recommend a lower calcium intake of 700 mg/day compared to the FAO/WHO (2002) RNI of 1,000 mg/day. Older adults are recommended a higher intake of 1,000 mg/day, which is lower than the RNI of FAO/WHO (2002). Both men and women are given the same recommended intakes.

Given the habitually low calcium intake of Southeast Asian women and the potential benefits of calcium to the fetus, it is proposed that the SEA-RDA for calcium throughout pregnancy be set at 1,000 mg/day. This amount is 300 mg/day above the SEA-RDA for non-pregnancy women, but is the same as that recommended by FAO/WHO (2002) and the DRI Committee for pregnant women. Similarly for lactating women, the SEA-RDA Committee felt that additional calcium intake would improve the calcium content in breast milk. Hence, the SEA-RDA for lactating women is increased to 1,000 mg/day throughout lactation, an increase of 300 mg/day over the RDA for non-lactating women.

The SEA-RDAs for calcium are set out in Table 5.3.

Table 5.3 Recommended RDAs for Calcium for Southeast Asia

Age Groups	Calcium RDA (mg/day)
Infants (months)	
0 – 5	300 [1]
	400 [2]
6 – 11	400
Children (years)	
1 – 3	500
4 – 6	600
7 – 9	700
Boys (10 – 18 years)	1,000
Girls (10 – 18 years)	1,000
Men (years)	
19 – 49	700
≥ 50	1,000
Women (years)	
19 – 49	700
≥ 50	1,000
Pregnancy (throughout)	1,000
Lactation (throughout)	1,000

Notes: [1] *Breast-fed*
[2] *Formula-fed*

Bibliography

Atkinson SA, Alston-Mills BP, Lonnerdal B, Neville MC & Thompson MP (1995). Major minerals and ionic constituents of human and bovine milk. In: *Handbook of Milk Composition.* Jensen RJ (ed). Academic Press, California. Pp. 593-619.

Barger-Lux & Heaney RP (1994). The role of calcium intake in preventing bone fragility, hypertension and certain cancers. *J Nutr* 124: 1406S-1411S.

Chan ELP, Lau E, Shek CC, MacDonald D, Woo J, Leung PC & Swaminathan R (1992). Age-related changes in bone density, serum parathyroid hormone, calcium absorption and other indices of bone metabolism in Chinese women. *Clin Endocrinol* 36: 375-381.

Chinese Nutrition Society (2000). Chinese Dietary Reference Intakes. Chinese Nutrition Society, Beijing (*publication in Chinese*).

DOH (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom.* Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Department of Health, UK. The Stationery Office, London.

DOH (1998). *Nutrition and Bone Health: with particular reference to calcium and vitamin D.* Report on Health and Social Subjects 49. Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population of the Committee on Medical Aspects of Food and Nutrition Policy. Department of Health, UK. The Stationery Office, London.

FAO/WHO (2002). Calcium. In: *Human Vitamin and Mineral Requirements.* Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 151-171.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

Gullberg B, Johnell O & Kanis JA (1997). World-wide Projections for Hip Fracture. *Osteoporosis International* 7: 407-413.

Heaney RP, Weaver CM & Fitzsimmons ML (1991). Soybean phytate content: Effect on calcium absorption. *American Journal of Clinical Nutrition* 53: 745-747.

Ho SC, Leung PC, Swaminathan R, Chan C, Chan SG & Lindsay R (1994). Determinants of bone mass in Chinese women aged 21-40. II. Pattern of dietary calcium intake and association with bone mineral density. *Osteoporosis International* 4: 167-175.

IOM (1997). Calcium. In: *Dietary references for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC; pp 71-145.

Kung AWC, Luk KDK, Chu LW & Chiu PKY (1998). Age-related osteoporosis in Chinese: an evaluation of the response of intestinal calcium absorption and calcitropic hormones to dietary calcium deprivation. *Amer J Clin Nutr* 68: 1291-1297.

Lau EMC & Cooper C (1996). The Epidemiology of Osteoporosis: The Oriental Perspective in a World Context. *Clin Orthopaedics & Related Research* 323: 65-74.

Lee WTK, Leung SSF & Lui SSH (1993). Relationship between long-term calcium intake and bone mineral content of children aged from birth to 5 years. *Bri J Nutr* 70: 235-248.

Martin AD, Bailey DA, Heather AM & Whiting S (1997). Bone mineral and calcium accretion during puberty. *Amer J Clin Nutr* 66: 611-615.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia.* A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Nakamura T, Yoshikawa T & Mizuno Y (1993). Do geometric properties of the femoral neck explain Japanese-American differences in hip fracture incidence? [Abstract]. *J Bone Mineral Res* 8 (Suppl): 867.

NIH (1994). Optimal Calcium Intake. NIH Consensus Statement 12: 4. National Institutes of Health, Bethesda, MC.

Nordin BEC (1976). *Calcium, Phosphate and Magnesium Metabolism.* Churchill Livingstone, London.

Nordin BEC (1988). *Calcium in Human Biology.* Springer Verlag, London.

NRC (1989). *Diet and Health: Implications for Reducing Chronic Disease Risk.* Report of the Committee on Diet and Health, Food and Nutrition Board, Commission on Life Sciences. National Research Council, National Academy Press, Washington. DC.

Parfitt AM (1988). Bone remodelling: Relationship to the amount and structure of bone, and the pathogenesis and prevention of fractures. In: *Osteoporosis: Etiology, Diagnosis and Management.* Riggs BL, Melton LJ III eds. Raven Press, New York, NY.

Prentice A (1998). Calcium Requirements of Breast-feeding Mothers. *Nutr Rev* 56: 124-130.

Raman L, Rajalakshmi K, Krishnamachari KA & Sastry JG (1978). Effect of calcium supplementation to undernourished mothers during pregnancy on the bone density of the neonates. *Amer J Clin Nutr* 31: 466-469.

Specker BL (1996). Evidence for an interaction between calcium intake and physical activity on changes in bone mineral density. *J Bone Mineral Res* 11: 1539-1544.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

Weaver CM (1998). Calcium requirements: the need to understand racial differences. *Amer J Clin Nutr* 68: 1153-1154.

WHO (1994). *Assessment of Fracture Risk and its Application to Screening for Postmenopausal Osteoporosis.* Technical Report Series 843. World Health Organization, Geneva.

6 IRON

6.1 Introduction

Iron is a transition element that exists in several oxidation states, ranging from –2 to +6. In biological systems, the primary forms are ferrous (+2), ferric (+3) and ferryl (+4) states. Iron participates in electron transfer and reversibly binds ligands by the interconversion of these oxidation states. Oxygen, nitrogen and sulfur atoms are important ligands for iron and are involved in oxygen transport and storage, electron transfer and substrate oxidation-reduction (Beard, 2001).

6.2 Characteristics and Functions

There are 4 major classes of iron-containing proteins in mammalian systems (IOM, 2001; Beard, 2001):

(1) Iron-containing heme proteins (hemoglobin, myoglobin and cytochromes). In hemoglobin and myoglobin, iron is the critical ligand for binding di-oxygen. Oxygen is bound to iron-containing porphyrin ring, as part of the prosthetic group of hemoglobin, or part of myoglobin as the facilitator of oxygen diffusion in tissue. In the cytochrome system, heme is the active site where ferric iron is reduced to ferrous iron.
(2) Iron-sulfur enzymes (flavoproteins, heme-flavoproteins) participating primarily in energy metabolism.
(3) Iron storage and transport proteins (transferrin, lactoferrin, ferritin and hemosiderin). The storage iron bound to ferritin is used when iron obtained from diets is inadequate. The transport iron, transferrin, is 25% to 50% saturated with iron.
(4) Other iron-containing or activated enzymes (sulfur non-heme enzymes).

6.3 Absorption, Utilization and Excretion

The concentration of iron in the body varies by age and gender, and its distribution in various tissues and organs is not uniform. As much as 85% to 90% of non-storage iron is in the erythroid mass (Bothwell *et al.*, 1979). 60% of ferritin in the body is contained in the liver, while the rest is found in muscle tissues and the reticuloendothelial system.

Two types of iron compounds are present in foods - heme and non-heme iron. Heme iron is readily absorbed and its absorption is not significantly affected by dietary components. On the other hand, absorption of non-heme iron (which is the more predominant form of iron in foods) is determined by the form of iron, its solubility and interactions with other food constituents. In the gastro-intestinal tract, solubilization of iron, predominantly in ferric form, occurs in the acid milieu of the

stomach where it is reduced to ferrous form, and is then taken up by the enterocytes across the apical membrane and transferred across the basolateral membrane into the plasma. In the duodenum, iron is absorbed via the functional iron-absorbing cells when they reach the tips of the villi. Some of these cells are shed into the lumen together with iron that is not transferred to the plasma. This turnover takes 48 to 72 hours. The primary route of iron uptake into cells is mediated by transferrin receptor on cell surface. Transferrin receptor is regulated by cellular iron status and interaction with iron response elements.

The body recycles iron from destructed senescent red cells (functional lifetime of 120 days). About 85% of iron from degradation of hemoglobin is released as iron-bound transferrin or ferritin.

The storage of iron in the body is approximately 30 mg/kg body weight to 40 mg/kg body weight, of which 60% is stored in the liver, 40% is stored in muscle tissues and cells in the reticuloendothelial system, and 5% is stored in the form of hemosiderin found in Kupffer cell lysosomal remnants.

The primary mechanism to maintain iron homeostasis is the regulation of the amount of absorbed iron to approximate iron losses. In general, iron in the body is highly conserved. Iron balance is maintained by regulating iron absorption in the upper small intestine. Iron losses vary considerably with gender and pathological conditions involving blood loss. Adult men need to absorb about 0.9 mg/day of iron to replace iron loss and maintain iron balance. The primary loss is from the gastrointestinal tract, and amounts to 0.6 mg/day in males. Fecal iron comprises iron from shed enterocytes, extravasated red blood cells and biliary heme breakdown products. Premenopausal females lose more iron due to menstrual loss. Menstrual iron loss is about 1.5 mg/day as estimated from blood loss of 33 mL/month. Due to marked inter-individual variation in menstrual losses, a small proportion of women may require as much as 3 mg to 4 mg iron/day. Use of contraception (such as intrauterine device and oral contraception) can increase or decrease iron losses. Pregnancy adds more demand to iron requirements, and as high as 4 mg/day to 5 mg/day may be needed during the latter half of pregnancy to preserve iron balance. For infants 4 to 6 months old, their iron needs can be met by mobilizing iron stores and from external iron obtained through breast milk. There is no evidence that iron content in milk differs among healthy and anemic lactating mothers.

6.4 Effects of Deficiency and Excess

6.4.1 Causes of iron deficiency

In general, iron deficiency is caused by the inadequacy in iron intake to meet with the requirements (UNICEF/UNU/WHO/MI, 1999). Iron losses associated with physiological process, i.e. menstruation, is probably the most significant loss occurring among reproductive-age women. Pregnancy poses a large burden on iron needs, particularly during the second half of pregnancy. Among growing children, the increased physiological needs for growth (intra-uterine, post-natal and during puberty) is the most important factor affecting their iron status. These high requirements can be met quite readily by diets high in bioavailable iron. However, in most developing countries where plant-based diets are predominant, there are few food sources that contain high amounts of bioavailable iron.

Pathological conditions can also result in increased iron needs. In terms of impact on public health, the most significant conditions are parasitic infections, in particular hookworm and malaria. In addition, Helicobactor pylori (H. Pylori) infection was recently reported to be highly prevalent among populations in developing countries. H. pylori infection has been found to be associated with iron deficiency but the mechanism and the causal relationships have not yet been established. It is postulated that H. pylori infection reduces acid secretion in the gut, resulting in compromising iron availability. Other pathological conditions, such as ulcer and other bleeding conditions of the gastrointestinal tract may also cause iron deficiency and anemia in individuals, but are generally not of public health concern.

6.4.2 Health and functional consequences of iron deficiency/anemia

(*a*) *Pregnancy*

Severe anemia has been reported to be associated with high maternal mortality, accounting for about half of maternal death during child birth (Allen, 2000). Less severe anemia has also been found to have a causal effect on pre-term delivery, low birthweight, and fetal death in several large-scale epidemiological studies. Only anemia associated with iron deficiency appears to result in prematurity. A 4-fold increase in the risk of prematurity when maternal ferritin is less than 12 mg/L compared with those with higher ferritin has been reported. On the other hand, high hemoglobin was found to also adversely affect pregnancy outcomes. There is a U-shaped relationship between hemoglobin and increased undesirable pregnancy outcomes when hemoglobin is either less than 90 g/L or more than 13 g/L. The etiology of these 2 ends of the spectrum is, however, different. While low hemoglobin is related with iron deficiency, high hemoglobin is a result of inadequate expansion of plasma volume associated with hypertension and ecclampsia.

Although it appears that an infant's iron needs are met at the expense of maternal iron needs, fetal iron may be sub-optimal as measured either from cord blood at delivery or later stage in infancy. At 3 and 6 months of age, infants born to mothers who consumed iron supplements have been found to have significantly higher serum ferritin levels, while no difference was found in cord blood. This indicated that maternal iron deficiency anemia may limit infant iron endowment (infant iron storage).

(*b*) *Cognitive Performance and Learning Ability in Children*

Several studies have reported the association of iron deficiency and impaired motor and cognitive development. Iron supplementation in infants, especially, failed to show improvement in cognition despite the improvement in iron status. Longitudinal studies showed that the impairment occurring in early childhood persisted into later childhood. However, definitive conclusion or causal relationship cannot be made due to possible confounding effects of poor socio-economic and other environmental background on child stimulation. Mechanistically, there is a lack of specificity as to which part of the human brain is affected by iron deficiency. In older children, such as in children of school-going age, the reversibility of impaired cognition development was observed when iron treatment was given and anemia was corrected. However, a study in Thailand did not find the reversibility of cognition among school-going children after iron supplementation (Pollitt *et al.*, 1989). It was speculated that

the duration of supplementation might be too short to detect the effect on cognition development, or alternatively, the children may have had iron deficiency at a critical age during their earlier development, which resulted in irreversible impairment.

(c) *Work Performance*

Anemia primarily affects maximal oxygen consumption, whereas cellular iron deficiency markedly impairs endurance as observed in muscle cells. Anemia and tissue iron deficiency are shown to exert independent effects on skeletal muscles. In rats, reduced work performance was related to tissue iron deficiency, not to induced anemia. It was therefore postulated that reduced work performance is related to the lack of enzymes for phosphorylation. Mild anemia can reduce work performance during intense exercise. Haas and Brownlie (2001) reviewed 29 studies and found strong causal effects of severe and moderate iron deficiency anemia on aerobic capacity in both animals and humans. Endurance capacity was also compromised. The reduced work productivity observed in field studies is likely due to anemia and reduced oxygen transport. The evidence of these relationships is very strong, which makes it justifiable to include intervention not only among those showing overt iron deficiency anemia, but also those having iron deficiency without anemia.

(d) *Other Consequences*

The relationship between iron deficiency and morbidity continues to be debated. Iron deficiency has been found to be associated with reversible abnormal immune functions. However, the benefits of iron supplementation on morbidity have not been established.

6.4.3 Guidance on high intake

For the US population, the DRI Committee has set tolerable upper intake levels (ULs) for iron intakes, since a substantial proportion of the US population (21% to 25% of women and 10% of men) take supplements containing iron (IOM, 2001). High iron intake could result in gastrointestinal effects, including nausea, vomiting, diarrhea and constipation, but these side effects can be reduced when the supplements are taken with meals. High iron intake may reduce zinc absorption, especially when the ratio of iron:zinc exceeds 4 : 1. However, this effect was not observed when the iron and zinc supplements are taken with meals.

Attempts have been made to assess the relationships between excessive iron intakes and chronic disease conditions, such as cardiovascular diseases and cancer. Several studies have reported the positive correlations between serum ferritin and these chronic diseases. However, current evidence does not support the causal relationship between dietary iron intakes and risks of coronary heart disease or cancer. It does not, however, exclude the possibility that iron may be a risk factor for coronary heart disease.

Based on these evidence and considerations, the DRI Committee has set the UL for iron for adults on the basis of gastrointestinal effects. The UL is 45 mg/day for adults. The adult figure of 45 mg/day is also used as the UL for adolescents. With regards to children, due to the lack of data, the median intake of children given iron-containing supplements was used, and the UL of 40 mg/day for infants and children was adopted.

6.5 Food Sources

Iron in food is present in either heme or non-heme forms. Heme iron is the form present in animal sources, except in eggs (as phosvidin) and breast milk (as lactoferrin). Heme iron is readily absorbed in the gut, whereas the absorption of non-heme iron depends on the co-presence of iron absorption enhancers and inhibitors. Ascorbic acid, meat protein and organic acids found in fruits and vegetables are reported to enhance the absorption of non-heme iron. Iron absorption inhibitors are commonly found in plant sources. Of special importance are phytate in unpolished rice and various cereal grains, and tannin in some vegetables, tea and coffee. Thus, the amount of iron intake does not necessarily reflect the adequacy of iron from diets.

6.6 Factors Affecting Requirement

6.6.1 Inhibitors of non-heme iron absorption

Most of Southeast Asian diets are plant-based, and rice is the staple of the majority of the population in this region. Rice, as the staple, is the main contributor of dietary energy. Although rice contains some iron, its bioavailability is limited due to high phytate content. The phytate contents may vary according to the variety of rice and the extent of milling (Tuntawiroon *et al.*, 1990). A study of Thai rice from 45 mills throughout the country showed phytate content ranging widely from 11.5 mg/100 g rice to 66 mg/100 g rice, and absorption rates varied considerably. In addition to phytate present in the Asian diet, several indigenous plants which are commonly consumed as vegetables may contain various forms of polyphenols. Tannin is the most potent iron-inhibitor and has been found to be present in several plant leaves. Most of food analysis has been on total polyphenols. However, not all forms of polyphenols have the inhibitory effects on iron absorption. Only tannin, which is present in many plant leaves and possibly in some grains, is of particular concern.

A study of typical Filipino meals representing 3 major islands in the Philippines – Luzon, Visayas, and Mindanao – showed very similar absorption rates. The mean absorption was 6.4 ± 1.2% (Trinidad *et al.*, 1989). Those meals consisted of boiled rice as the staple, eaten with side dishes containing meat and vegetables. Coffee with milk is part of breakfast and fruits (either banana or papaya) are included in the other daily meals. Total iron content ranged from 12 mg/day to 15 mg/day. Non-heme iron formed about 50% of total iron intake in meals from Luzon and Mindanao and about 80% in meals from Visayas. Phytic acid was very high in meals from Luzon, compared with the other two regions (>2,000 mg/day vs >1,000 mg/day), whereas tannic acid was high in both Luzon and Visayan meals, compared with that in Mindanao (approx. 1,000 mg/day to 1,500 mg/day vs 450 mg/day). Vitamin C and meat/fish/poultry (M/F/P) were especially high in meals from Mindanao (F/M 177/207 mg; and F/M 132/154 mg, respectively) compared with that from both Luzon (above 100 mg for both components) and Visayas (F/M 55/94 mg and 80/83 mg).

A series of studies on iron absorption using radioisotopes has been conducted. Typical Thai meals containing rice, vegetables (e.g., string bean, collard and cabbage), chili paste, fish sauce and coconut milk have been tested. The intrinsic iron content was 2 mg to 3 mg, but was as high as

8 mg in 1 report (Hallberg *et al.*, 1974; Hallberg *et al.*, 1986). The absorption of iron from white rice was about 3% and that of the meals that included ascorbic acid was about 8%, providing about 0.16 mg to 0.24 mg of iron. These studies added ascorbic acid in the form of fruits or vegetables, and the amount of iron absorbed increased 2 to 3 times when over 50 mg of ascorbic acid in the form of foods was given. However, it was not possible to ascertain if there was a dose-response relationship between levels of ascorbic acid and absorption rates since the basic meals also contained some ascorbic acid, and was not quantitatively reported (Allen and Ahluwalia, 1997).

In the studies of Thai and Burmese meals which are both rice-based, bioavailability ranged from about 2% to 10%. When 60 mg of fish was added, the bioavailability increased to as high as 21% (FAO/WHO, 2002). Therefore, bioavailability of iron in Asian diets could vary depending on the cereal used as staple and on other dietary components.

A more recent study was done with the inclusion of a local vegetable, lead tree leaf, which contains a high amount of tannin (expressed as tannin equivalent). Iron absorption was suppressed from 12% to below 2% with the inclusion of only about 20 g of this vegetable (contributing 584 mg tannic acid equivalent/meal) to the meal (Tuntawiroon *et al.*, 1991). This amount is typical of the amount consumed in a meal. Unlike the effect of ascorbic acid on phytate, which is more effective at high phytate levels (Allen and Ahluwalia, 1997), the counter-effects of ascorbic acid appeared to level off at high tannic acid level (Tuntawiroon *et al.*, 1991). Verification of doses of ascorbic acid with graded levels of tannin to cover the possible range of intakes from habitual diets will be useful.

In addition to phytate and tannin, calcium which is present in various salt forms in foods and dairy products may interfere with the absorption of both heme and non-heme iron. This inhibitory effect was not observed at level of 40 mg calcium/meal, but became substantial at 300 mg to 600 mg of calcium. Soy products have also been shown to inhibit non-heme iron absorption. However, due to its high iron content, the net effect may remain to be positive. Fermentation of soy is another way to enhance iron absorption.

Cook *et al.* (1991) speculated on the possible exaggeration of the effects of enhancers and inhibitors based on single meals. Both the enhancing and inhibiting effects on non-heme iron absorption were observed from the study using single meals instead of complete meals. Other studies did not verify this contention (Hallberg & Hulten, 2000).

6.6.2 Algorithm for estimating iron bioavailability

Due to the sophistication in conducting isotopic studies to establish non-heme iron bioavailability, attempts have been made to develop algorithms for estimating iron bioavailability from diets.

Monsen and Balintfy (1982) derived iron bioavailability based on contents of iron and enhancers (namely, meat/fish/poultry (MFP) and ascorbic acid (AA)) and levels of iron storage. Diets containing less than 30 g of MFP or less than 25 mg AA is considered low in bioavailability, and absorption ranges from 2% to 5%. Intermediate availability contains 30 g to 90 g MFP or 25 mg to 75 mg AA, and bioavailability lies between 3% to 10%. The high availability meal contains more than 90 g MFP or less than 75 mg AA, or combination of both MFP and AA at levels defined for intermediate bioavailability. The absorption is much higher than 10%.

Despite the limitation of basing bioavailability on the content of MFP and ascorbic acid in the diet, Monsen's work has been adopted as the basis for judging the iron bioavailability of diets. Broad categories of iron bioavailability were suggested, as follows:

Low Bioavailability Diet

- Simple, monotonous diet based on cereals and plant sources, with negligible amount of meat, fish or ascorbic acid.

Intermediate Bioavailability Diet

- Diet containing mainly cereals, but includes some animal sources and ascorbic acid.

High bioavailability

- Diversified diet containing generous amount of meat, poultry, fish and/or foods rich in ascorbic acid.

Murphy *et al.* (1992), using intake data of toddlers in Egypt, Kenya and Mexico suggested a modification of Monsen's algorithm by suggesting 3 levels of grams of meat/fish/poultry/1,000 kcal and mg of ascorbic acid/1,000 kcal. The matrix of 3x3 provided figures for estimating bioavailability. In addition, a "tea factor" was included using 1.00 for no tea and 0.4 if tea consumption is ≥ 600 mL tea/day. The final algorithm is:

Available Iron = (heme iron x 0.25) + (non-heme iron x availability factor x tea factor)

Tseng adjusted Monsen's model based on a study of Russian women and children, by accounting for decreased iron bioavailability due to the amount of tea and phytate consumed in a population, (Tseng *et al.*, 1997). The adjustment for phytate was done by regression analysis based on data from Hallberg's studies. The percentage of iron bioavailability clearly differed when Monsen's model was used compared to the adjustment proposed by Tseng. With Monsen's model, bioavailability of iron in the diets of girls, boys and women was 8% to 11%. With the adjustment for phytate, bioavailability was reduced to only 3% to 4%.

It should be noted that Monsen's model does not account for an inhibitory factor, which may not be of concern in Western diets. However, the model may overestimate bioavailability in diets containing high inhibitors, such as those in developing countries. Murphy *et al.* (1992) attempted to improve the model by adding a tea factor into the model. Nevertheless, it is not applicable in populations where tea or coffee drinking is uncommon. In Southeast Asian diets, several local vegetables are believed to contain a large amount of tannin, but data on these food items remain limited. These data should be made available urgently.

Recently, Hallberg and Hulten (2000) derived an algorithm based on data of absorbed heme and non-heme iron from single meals formulated by different habitual diets of Asia, including Thai diets. This algorithm includes the content of animal tissues, ascorbic acid, phytate, polyphenols, calcium, eggs, soy protein and alcohol in meals. To use these algorithms, however, data on phytate and tannin contents in various foods must be available. At present, most food composition tables or databases in the region do not provide these values. Moreover, analysis of phytate and tannin

contents may be available in some literature, but the analytical methods differ, making the data incomparable. This problem must be resolved before the algorithm can be widely used.

To conclude, due to complexity of diets in Southeast Asia, it is not possible to estimate bioavailability from human studies for all habitual diets. Development of algorithms is a very attractive approach, although much more work is needed in laboratory analyses of various constituents affecting iron bioavailability. Consensus on analytical methods for determinations of phytate and tannin also seems necessary. In the meantime, it is reasonable to assume, based on available scientific data, that bioavailability of iron in Southeast Asia is in the range of 5% to 10%.

6.7 Estimating Requirements and Recommended Intakes

6.7.1 Principal considerations in recommending RDAs for Southeast Asia

The reports of FAO/WHO (1988), FAO/WHO (2002), and the DRI Committee (IOM, 2001), are the major sources from which the requirement figures for Southeast Asia are derived. The requirements as defined by these sources were used as the basis for deriving iron requirements (as median and 95th percentile requirements for absorbed iron) for Southeast Asia. A large body of scientific evidence has been reviewed and considered by experts in the field for setting the SEA-RDAs. Requirements of iron depend on age, sex and physiological conditions, specifically, growth, menstruation, pregnancy and lactation.

It is recognized that although the SEA-RDAs were determined based on the same parameters as those used in the recommendations of FAO/WHO and the DRI Committee (namely, daily iron losses, growth needs, and menstrual losses), the SEA-RDAs may vary from those recommendations due to differences in the factors used for their estimation, such as differences in the reference body weights for specific age-groupings. Most of the available data on requirements are from developed countries such as the US and Sweden. However, the SEA-RDA Committee compared recommendations of both the FAO/WHO and the DRI Committee, and where possible, adopted the requirements on per weight basis (based on reference weight). The FAO/WHO provides reference weight for various age groupings. These figures have been adopted, with some adjustments particularly during puberty and for attained adult body sizes to suit the Southeast Asian populations.

There are differences between the age groupings used by the FAO/WHO and the DRI Committee and that those used by the SEA-RDA Committee. Extrapolation of some figures were necessary and were made to correspond with age groupings under the SEA-RDA framework.

6.7.2 Components of iron requirements

(*a*) *Basal Iron Losses*

Iron is lost from skin and interior surfaces of the body, and iron loss is estimated to be 14 μg/kg/day. A 55 kg non-menstruating woman and 70 kg man loses about 0.8 mg iron/day and 1 mg iron/day, respectively, with 15% individual variation (FAO/WHO, 1988). Iron loss from perspiration was found to be negligible, even in hot, humid climates.

(*b*) *Growth Requirements*

In the first year of life, full-term infants double their iron stores while tripling their body weight (FAO/WHO 2002). The infant is born with excessive circulating iron due to high affinity of fetal hemoglobin for oxygen (i.e. lowered delivery of iron to tissues). Fetal hemoglobin (HbF) is exchanged for production of normal hemoglobin (HbA) at delivery, and oxygen is more readily available from the lungs. Much of this iron is thus released to build the infant's iron stores. Therefore, there is no increase in an infant's iron needs during the first 4 to 6 months of life. Late clamping of umbilical cord at delivery has been shown to add more iron for infant's needs during this period of life.

The increased iron need occurs during 6 to 12 months of age when iron stores are exhausted. It has been estimated that iron requirement per 1,000 kcal of infants aged 6 to 12 months is the highest during the whole lifespan (IOM, 2001). Iron requirement can amount to about 100 mg/kg/day, which is about 4 times that of menstruating women (Hallberg, 2001). After 2 years of age, iron requirement per unit body weight reduces, but total body iron doubles during 1 to 6 years of age.

The last period of rapid growth is during the adolescent growth spurt (Hallberg, 2001). The marked increase in hemoglobin mass and concentration in boys was reported to be about 20% higher than the average requirements in menstruating women. For girls, iron requirement at menarche may be as much as 30% higher than that of their mothers.

(*c*) *Menstrual Blood Losses*

Menstrual blood loss is quite constant from month to month for an individual, but varies considerably among individuals in the same population and across populations in different geographical areas. Menstrual loss among women can vary between less than 10 mL to about 180 mL.

6.7.3 Recommendations for iron intake by life stages

Once the requirement figures are set, bioavailability factor is incorporated to estimate the recommended dietary intakes for each age-sex group. The iron bioavailability of diets in Southeast Asia seems to vary widely, with bioavailability factor possibly within the range of <5% to 10% from rice-based and plant-dominated meals among rural populations, to 10% to 15% from the diets of some sectors of urbanized population where animal sources of iron form a more substantial proportion of the diet. However, the former will more likely be the case for the majority of the population. Reviews of data from the Philippines and Thailand showed that typical meals have iron bioavailability of 5% to 8% (Trinidad *et al.*, 1989; Hallberg *et al.*, 1974; Hallberg *et al.*, 1986; Tuntawiroon *et al.*, 1991). However, for countries with more advanced economic development and dietary patterns that have larger proportions of animal food sources, bioavailability may be higher than 10%. To account for this wide range of bioavailability, the SEA-RDAs provide for 3 different levels of bioavailability, namely 5%, 7.5% and 10%.

(*a*) *Infants (0 – 12 months)*

For infants, iron endowment from mothers has been estimated at about 80 mg/kg (Widdowson

& Spray, 1951). About 50 mg/kg is in the form of hemoglobin, 25 mg/kg is in the form of storage iron and another 5 mg/kg is in the erythroid cells of the marrow, myoglobin and enzymes. During the first 6 to 8 weeks of life, hemoglobin is another reserve of iron. Hemoglobin concentration falls during the first 2 months as newborns adapt to a new environment of oxygen supply. Its concentration declines by 10 g/L/week during this time, resulting in augmentation of iron stores (Dallman, 1992). With this process of redistribution of iron in the body, the total body iron of infants at birth and 4 months of age remain essentially the same. Half of the stored iron at 4 months is mobilized for the production of hemoglobin, myoglobin and enzymes. As a result, iron requirements during the first 4 to 6 months of life of full-term infants can be met by iron provided from breast milk. For infants born prematurely or with low birth weight, it is advisable to give additional iron from supplements starting 2 months after birth.

Iron nutrition of mothers has not been shown to affect the amount of iron endowment, based on supplementation studies (Sturgeon, 1959). Growth of infants can be another factor that determines the need for iron during the first year. Birth weight of Thai infants, for example, have been reported to be at at average of 3,000 g. Thus, iron endowment is approximately 240 mg. Full term infants starting with body iron of 270 mg would need to absorb about 100 mg during the first year (Bothwell *et al.*, 1979) or an average of 0.3 mg/day.

It is assumed that during the first 4 to 6 months of life, breast milk alone can adequately provide additional iron to meet the requirements. Thus the RDA for iron during the first 6 months can be based entirely on the supply from breast milk (IOM, 2001). Iron content in breast milk has been reported to range from 0.2 mg/L to 0.9 mg/L, and is unaffected by maternal iron status. However, this amount from breast milk alone does not provide sufficient iron for the infant's needs. Iron endowed during fetal life is thus very crucial and used to complement the needs during the first six months of life (Butte *et al.*, 2002). Iron deficiency has not been reported to be common among infants before 4 months of age (Dallman, 1992). Supplementation trials among infants from Sweden and Hondurus showed that iron homeostatic control may not be fully functioning until 6 months of life (Domellof *et al.*, 2001). Therefore, for infants aged 0 to 6 months, there is no recommendation for additional iron intake beyond that provided by breast milk. Exclusive breast feeding is thus fully encouraged. This should provide adequate iron to meet the demand.

For infants aged 6 to 12 months, the iron stores that have been endowed since birth is depleted and body iron increases by about 70% from 4 to 12 months of age. Iron needs must be met by diet, in addition to breast milk. Though continued breast-feeding is strongly recommended, and may continue to the end of the second year of life, complementary foods containing high bioavailable iron must be provided. In developed countries, this is easily met by foods containing meat, fish, vegetables, and fruit juices, together with commercial products fortified with iron and vitamin C. In Southeast Asia, the first complementary foods that are traditionally introduced to infants during this period are rice gruel or mashed banana. Food beliefs and taboos may promote or prohibit the introduction of nutritious foods. Experience in Thailand showed that infant feeding guidelines which give information as to when and how much of various foods should be introduced to young children are crucial in increasing

variety of their diets. Thus, the bioavailability of food during the second half of the first year and the second year of life can vary considerably.

FAO/WHO (1988) recommends, for infants 4 to 12 months old, the amount of absorbed iron to meet the requirements of half of the population to be 0.77 mg absorbed iron/day, and 95th percentile (approximately median plus 2 SD) of 0.96 mg absorbed iron/day. The recent recommendation by FAO/WHO (2002) gave slightly lower values of 0.72 mg/day and 0.93 mg/day respectively.

The DRI Committee estimated the recommended level for infants 6 to 12 months old by taking factorial components of iron needs, namely, obligatory losses, increased hemoglobin mass, tissue and storage iron. The EAR was based on growth rate and achieved size of US infants. The body weights used were mid-points between weights at 6 and 12 months for both sexes, which were 9 kg and 8.4 kg for male and female infants respectively. The EAR recommended by the DRI Committee is 0.69 mg/day, which is similar to the median value given by FAO/WHO. The RDA of 1.1 mg/day was set at 97th percentile (approximately median plus 3 SD). The bioavailability of 10% was used, resulting in EAR of 6.9 mg iron intake/day and RDA of 11 mg iron intake/day.

For Southeast Asia, since the median and EAR are similar, and FAO/WHO recommendation may be adopted. Using the median of 0.72 mg/day to cover 95% of the population, the SEA-RDA would be 0.93 mg absorbed iron/day. Since common complementary foods for infants aged 6 to 12 months include some animal sources, especially fish (which is widely available even in rural areas), bioavailability factor of 10% may be applied, resulting in the RDA of 9.3 mg iron intake/day. Regarding the recommended age for introducing complementary foods, WHO/UNICEF most recently issued infant feeding guidelines for exclusive breast feeding during the first 6 months of life. Complementary foods should only begin to be given from 6 months onward. Thus the RDAs for infants 0 to 6 months old and infants 6 to 12 months old are different.

The recommended iron intakes for infants are:

Infants 0 - 5 months, mg/day	0.93 mg/day		
	Iron Bioavailability		
	5%	**7.5%**	**10%**
Infants 6 - 11 months, mg/day	18.6	12.4	9.3

(*b*) *Children (>1 – 6 years)*

Growth rate slows down during the second year of life, with only about 2.5 kg of body mass being added. Iron required to support the additional growth is, on average, 0.4 mg/day. The mean increase of weight from age 2 till onset of puberty averages 2.5 kg/year to 2.75 kg/year (Bothwell *et al.*, 1979). This is equivalent to an iron requirement of 0.3 mg/day. Iron loss during this period also increases with age to about 0.5 mg/day by age 12. The total iron requirement has been estimated to increase from 0.5 mg/day to 0.8 mg/day by about the end of the first decade of life.

The DRI Committee used the factorial modeling of the median components of iron requirements and assumed a small difference between genders. The median requirement was 0.54 mg absorbed iron for children aged 1 to 3 years and 0.74 mg absorbed iron for children aged 4 to 8 years (IOM, 2001). Between the ages of 1 to 6 years old, level of body iron is doubled compared to the level during infancy. On the other hand, FAO/WHO (2002) estimated the median requirement to be 0.46 mg/day and 0.5 mg/day for children aged 1 to years 3 and 4 to 6 years old respectively. The 95th percentile was 0.58 mg and 0.63 mg absorbed iron for the age groups of 1 to 3 years and 4 to 6 years respectively. Therefore, estimates of requirements from the two expert groups are similar. However, the DRI Committee used a very high bioavailability factor, 18%, based on American diets. Thus, the EARs for age groups of 1 to 3 years and 4 to 6 years were 3.0 mg absorbed iron/day and 4.1 mg absorbed iron/day respectively; while RDAs for age groups of 1 to 3 years and 4 to 6 years were 7 mg/day and 10 mg/day for respectively.

However, these recommendations are not appropriate for Southeast Asia. In this region, bioavailability of iron in typical diets ranges from 5% to 10% for diets which consist mainly of vegetables which are high in polyphenols, although the diets may include some animal sources.

The recommended iron intakes for children aged 1 to 6 years are:

	Iron Bioavailability		
	5%	7.5%	10%
Children 1 – 3 years, mg/day	11.6	7.7	5.8
Children 4 – 6 years, mg/day	12.6	8.4	6.3

(c) *Children and Adolescents (6 – 18 years)*

Basal loss of children may be extrapolated from that of adult males. During these growing years, hemoglobin mass, non-storage iron in various tissues, and storage iron are increasing with age. The DRI Committee estimates that hemoglobin mass and non-storage iron increase based on weight change, while storage iron is derived using a fixed factor of 12% of total iron deposition, as is the case for infants (IOM, 2001). By modeling these components, the DRI Committee estimates that EAR for children aged 4 to 8 years to be 4.1 mg. Using a bioavailability factor of 18%, median requirement as absorbed iron is estimated to be 0.74 mg/day.

FAO/WHO (1988) estimated requirements based on basal loss and growth requirements. For children in the age group of 6 to 12 years with mean body weight of 29 kg, median requirement as absorbed iron is 0.94 mg/day, and 95th percentile of requirement is 1.17 mg/day. FAO/WHO recommended the median requirement of 0.71 mg/day, and 95th percentile of 0.89 mg absorbed iron/day, for children in the age range of 7 to 10 years, for both sexes (FAO/WHO, 2002). These recommendations have been adopted for the SEA-RDAs. The slight difference in the recommendations of FAO/WHO and the DRI Committee is likely to be due to the overlapping age groupings and the corresponding differences in body weights used for the estimation of the requirements. For this age group of 6 to 20 years, the requirement is the same for both sexes. Diets of children in this age group are likely to be the same as that for adults, thus a bioavailability factor of between 5% and 10% is applied.

Recommended intakes for children aged 7 to 9 years are:

	Iron Bioavailability		
	5%	7.5%	10%
Children 7 - 9 years, mg/day	17.8	11.9	8.9

For boys aged 9 to 18 years, the DRI Committee used the factorial modeling on components of iron requirements, which included basal iron losses, increased hemoglobin mass, tissue iron (non-storage iron), and menstrual iron losses for girls aged 14 to 18 (IOM, 2001). No provision for storage iron was made, since iron reserve is presumed to be sufficient. The increased hemoglobin mass during this age is significantly associated with growth spurt. The rise of hemoglobin concentration reflects both the expansion of blood volume and the needs associated with sexual maturation (Dallman, 1992). For girls, menarche results in regular iron losses through menstruation. Though the distribution of menstrual losses is very skewed, it is consistent in each individual (Hallberg *et al.*, 1966). In addition, the age at which growth spurt or menarche occurs varies widely among individuals. Blood loss per menstrual cycle was averaged to derive iron loss per day over the menstruation cycle. The mean requirement reaches about 1.5 mg/day at peak growth and settles at a lower level (1.3 mg/day) than prior to growth spurt, to replace menstrual loss.

The DRI Committee estimated median requirements for absorbed iron for each year of age, whereas the variability was determined at mid-point (11 years) for the age group of 9 to 13 years, and 16 years for the age group of 14 to 18 years. The median requirements (EAR) were estimated at 5.89 mg iron intake/day and 7.69 mg iron intake/day for boys of age group 9 to 13 years and 14 to 18 years respectively. The 97.5th percentiles for boys of the same age groups were 7.91 mg/day and 10.83 mg/day respectively. Median requirements for girls of age group 9 to 13 years and 14 to 18 years were 5.66 mg/day and 7.91 mg/day respectively; 97.5th percentiles for girls of the same age groups were 8.34 mg/day and 14.8 mg/day respectively. Using a bioavailability factor of 18%, EARs and RDAs for US boys and girls of the age groups 9 to 13 years and 14 to 18 years are as shown in the table below.

Recommended iron intakes for children and adolescents aged 9 to 18 years are:

Age Groups	Iron intake/day		Absorbed iron required/day	
	EAR	RDA	EAR	RDA
Boys				
9 - 13	5.9	8	1.06	1.44
14 - 18	7.7	11	1.4	1.98
Girls				
9 - 13	5.7	8	1.02	1.44
14 - 18	7.9	15	1.42	2.7

Source: IOM (2001)

During adolescence, growth spurt accounts for the mean increase of about 4.6 kg body mass/year and 4 kg body mass/year for boys and girls respectively (Bothwell *et al.*, 1979). The requirements for growth amount to 0.7 mg/day for boys and 0.45 mg/day for girls. Adding obligatory loss in boys, the total iron requirement during the growth spurt is about 1.6 mg/day. After the growth spurt, additional iron is also required to increase iron stores,

from 200 mg to 1,000 mg, resulting in a requirement of 1.2 mg/day for late adolescent males. For girls, menstruation starts as the accelerated growth spurt declines. The median daily requirement has been estimated at about 1.4 mg/day, but inter-individual variability of iron loss due to menstruation is high, and a small proportion will need much higher iron than the median requirement to meet the demand.

Using the same basis for establishing requirements for adolescents, FAO/WHO (1988) and FAO/WHO (2002) arrived at slightly different figures of requirements from the DRI Committee, as shown in Table 6.1 below.

Table 6.1 FAO/WHO iron intake requirements for adolescents

Age (years)/Sex	Body Weight (kg)	Growth (mg/day)	Basal Loss (mg/day)	Menstruation (mg/day)*	Absorbed Iron RDA (mg/day)	
					Median	95th Percentile
FAO/WHO (1988)						
Boys						
12-16	53	0.66	0.8	-	1.46	1.82
Girls						
12-16	51	0.36	0.79	0.47	1.62	2.02
FAO/WHO (2002)						
Boys						
11-14	45	0.55	0.62	-	1.17	1.46
15-17	64.4	0.6	0.9	-	1.50	1.88
Girls						
11-14 (non-menstruating)	46.1	0.55	0.65	-	1.20	1.40
11-14 (menstruating)	46.1	0.55	0.65	0.48/1.9	1.68	3.27
15-17	56.4	0.35	0.79	0.48/1.9	1.62	3.1

Note: Values are median/95th percentile of menstrual loss.*

Again, the discrepancy between the recommendations of the DRI Committee and FAO/WHO may be due to different age groupings used. Starting at the age of 11 years, there are separate RDAs for boys and girls. Since the differences in the 2 recommendations are likely to be mathematical, the more recent FAO/WHO (2002) values at 95th percentile are adopted for the SEA-RDAs.

The recommended intakes (mg/day) for adolescents in the age group of 10 to 18 years are:

	Iron Bioavailability		
	5%	7.5%	10%
Adolescent boys (years)			
10 – 14	29.2	19.5	14.6
15 – 18	37.6	25.1	18.8
Adolescent girls (years)			
10 – 14 (non-menstruating)	28.0	18.7	14.0
10 – 14 (menstruating)	65.4	43.6	32.7
15 – 18	62.0	41.3	31.0

When the bioavailability factor is applied, it is obvious that at low bioavailability (5%), iron needs are significantly higher than the amount of iron that can be obtained from habitual plant-based diets typical of Southeast Asia. For practical reasons, it may be advisable that RDA relating to a bioavailability factor of close to 10% be adopted. However, this recommendation is conditioned upon the need to ensure that dietary advice on quality of diets be provided, i.e., to include reasonable amounts of meat and/or ascorbic acid food sources along with the RDA.

(*d*) *Adults (≥ 19 years)*

While physiological loss in adult males is estimated at 1 mg/day, about 1.2 mg/day of iron may be required between ages 18 and 30 years old to replete iron stores. Figures for women are extrapolated from isotopic studies in men with the addition of menstrual loss (FAO/WHO, 1988). The median menstrual loss in women is about 30 mL/month, equivalent to 0.45 mg/day of iron. It has been estimated that 25% of women lose more than 0.8 mg/day, 10% lose over 1.3 mg/day, and 5% lose more than 1.6 mg/day (FAO/WHO, 1988). Due to the high variation in menstrual loss, iron requirement may need to be set as high as 2 mg/day for adult women.

The DRI Committee modeled the iron requirement separately for men and women, using factorial modeling of components (IOM, 2001). For men, only basal loss was estimated as the total need for absorbed iron. The median estimate was 1.08 mg and at 97.5th percentile the estimate was 1.53 mg absorbed iron/day. Using bioavailability factor of 18%, the EAR and RDA for adult males are 6 mg/day and 8 mg/day respectively for men aged 19 and above. For adult menstruating women, in addition to basal loss, menstrual loss was pro-rated as daily iron loss. The skewed distribution of menstrual loss was accounted for in estimating median and 97.5th percentile requirements. The bioavailability factor of 18% was used to derive at the distribution of requirements. The EAR and RDA were set at 8 mg/day and 18 mg/day respectively for menstruating adult women. Similar to the method used for men, only basal iron loss was used to estimate requirements of post-menopausal women (assuming the age range of 50 and above). The median requirement and 97.5th percentile of requirement are 0.896 mg absorbed iron/day and 1.42 mg absorbed iron/day respectively. Applying the bioavailability of 18%, the EAR and RDA were set at 5 mg/day and 8 mg/day respectively.

FAO/WHO (2002) recommended the same values of median requirements and 95th percentile requirements for all ages above 18. No specific age was defined for post-menopause, but lower values of requirements were recommended. The median requirements for men, women, and post-menopausal women are 1.05 mg absorbed iron/day, 1.46 mg absorbed iron/day and 0.87 mg absorbed iron/day. The requirements for the 95th percentile of these respective groups are 1.37 mg absorbed iron/day, 2.94 mg absorbed iron/day, and 1.13 mg absorbed iron/day. Applying bioavailability factor of 5% to 10%, the recommended iron intake for adults are given in the table below. While typical diets in Southeast Asia can meet the RDAs for men and menopausal women, the RDA for menstruating women is high and cannot be met by diet alone.

The recommended intakes (mg/day) for adults aged 19 years and above are:

	Iron Bioavailability		
	5%	7.5%	10%
Adult men	27.4	18.3	13.7
Adult women			
Menstruating	58.8	39.2	29.4
Menopause	22.6	15.1	11.3

(e) *Pregnancy*

The DRI Committee took into account basal losses, iron deposited in fetus and related tissues, and iron used for expansion of hemoglobin mass as factors in determining the iron requirement for pregnant women. Basal loss was estimated from that of non-pregnant women. During the first trimester, iron requirement may remain at the level for replacing basal loss since there is no menstrual loss. FAO/WHO (1988) has estimated the iron deposited in fetus and placenta to be 315 mg partitioned by trimester. The hemoglobin mass increases drastically from the second trimester onwards, but the expansion also depends on the extent of iron supplements provided. The total iron cost of pregnancy was estimated to be approximately 1,000 mg, of which about 250 mg to 350 mg is returned after child delivery. The net cost of pregnancy is estimated to be 700 mg/pregnancy to 800 mg/pregnancy. Provision for adolescent pregnancy was made by including iron deposition in tissue into the model, as is the case for adolescents. A bioavailability factor of 25% is used since the absorption increases as pregnancy progresses. The EAR is slightly higher for pregnant women aged 14 to 18 years (23 mg/day) compared with older women (22 mg/day). The RDA is 27 mg/day for pregnant women. These figures can be converted to median and 97th percentile of requirement for pregnant women as absorbed iron, EAR of 5.7 mg and 6.75 mg respectively, and RDA of 27 mg absorbed iron/day.

FAO/WHO (2002) did not specify the RDA for iron for pregnant women, considering that the needs are beyond what can be provided from diets alone. The FAO/WHO recommended that iron supplements in tablet form be given to all pregnant women because of the difficulties in correctly evaluating iron status in pregnancy. In non-anemic pregnant woman, daily supplements of 100 mg of iron (e.g. as ferrous sulphate) given during the second half of pregnancy are adequate. In anemic women, higher doses are usually required. WHO recommends hemoglobin level of 110 g/L as the cut-off for anemia during pregnancy.

Recently, Beaton argued that the current method used for estimating iron needs during pregnancy is seriously overestimated (Beaton, 2000). The critical question raised is whether reaching the target level of hemoglobin has significant health benefits.

(*f*) *Lactation*

At child birth, blood loss during delivery and in the puerperium is more than can be compensated by iron from declining red cell mass. For lactating women, iron is also secreted in breast milk. Adding these to the basal loss, the estimated iron requirement during lactation is about 1.1 mg/day (FAO/WHO, 1988).

The DRI Committee, assuming lactation amenorrhea for 6 months after child delivery with exclusive breast feeding, considered the components of iron requirement of lactating women to include iron secreted in breast milk plus basal losses. The median requirement was estimated to be 1.17 mg/day. For adolescent lactating women, provision for tissue iron deposition was also made, resulting in the median requirement of 1.26 mg/day. Using 18% iron bioavailability factor, EAR and RDA are slightly higher for lactating women aged 14 to 18 years. EAR for lactating women aged 14 to 18 years is 7 mg iron/day, and 6.5 mg/day for those above 18 years old. Similarly, RDA for lactating women aged 14 to 18 years is 10 mg/day, and 9 mg/day for older women. The intake recommended by FAO/WHO (2002) is 1.15 mg/day and at the 95th percentile, 1.50 mg/day. These values are very close to the estimates of the DRI Committee. Thus, the FAO/WHO (2002) figures have been adopted as the SEA-RDAs.

Recommended iron intakes for lactating women are:

	Iron Bioavailability		
	5%	7.5%	10%
Lactating women, mg/day	30	20	15

6.8 Current RDAs for Iron in Southeast Asia

The current RDAs for iron in the Southeast Asian region are set out in Table 6.2.

Table 6.2 Comparison of current RDAs (mg/day) for iron in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 - 1)	3 – 5	9[a]	0.38 – 10	7	9.3[a]	10 – 11
Children (1 – 9)[b]	8 – 10	6 – 9	8 – 11	7	5.8 - 8.1	6 – 12
Boys (10 – 18)[c]	13 – 23	15 – 19	13 – 20	6 – 12	11.8 - 16.6	11 – 12
Girls (10 – 18)[c]	14 – 25	14 – 33	19 – 27	7 – 19	19.1 - 28.2	12 – 24
Men (≥ 19)[d]	13	14	12	6	10.4	11
Women (≥ 19)[d]	14 – 26	11 – 29	10 – 27	6 – 19	9.4 - 24.7	9 – 24
Pregnancy						
1st trimester	56	29	27	19	[f]	24
2nd trimester	56	[e]	34	19	[f]	30
3rd trimester	56	[e]	38	19	[f]	30
Lactation						
1st 6 months	28	15	27	19	15	24
2nd 6 months	28	15 – 32	30	19	15	24

Notes: *RDAs based on iron bioavailability of 10% for Malaysia, 8.5% for the Philippines; bioavailability for other countries not mentioned*
[a] *Figures only for infants 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[d] *≥ 18 for Singapore*
[e] *Iron supplements in tablet form recommended for all pregnant women; in the non-anaemic women, daily supplements of 100 mg iron given during second half of pregnancy are adequate*
[f] *Pregnant women should receive iron supplements of 60mg/day*
Source: *Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)*

RDAs for iron in the Southeast Asian countries reviewed differ considerably, probably reflecting the different bioavailability factors that were applied in setting the RDAs in these countries. The RDAs for Singapore are generally the lowest whilst those of Indonesia are the highest. The largest differences are observed in the recommended intakes for pregnant and lactating women, ranging from 19 mg to 56 mg per day. The recommended intakes for men are less variable than those for women, from adolescents and throughout the reproductive years. As expected, the RDA for postmenopausal women decreased considerably from those for younger women.

6.9 Recommended RDAs for Iron for Southeast Asia

The SEA-RDAs for iron were set after a review of state-of-the-art knowledge and information. The 2 key sources for the review were the recommendations of the DRI Committee (IOM, 2001) and the 2002 recommendations of the FAO/WHO. Southeast Asian populations are similar in body size, ethnic background and environmental factors. Nevertheless, country-specific RDAs may vary considerably depending on the dietary components of typical diets. The SEA-RDAs for iron are as summarized in Table 6.3.

Table 6.3 Recommended RDAs for Iron for Southeast Asia

Age Groups	Iron RDA (mg/day)		
Infants (months)			
0 - 5		0.93	
		Iron Bioavailability	
	5%	7.5%	10%
6 - 11	18.6	12.4	9.3
Children (years)			
1 - 3	11.6	7.7	5.8
4 - 6	12.6	8.4	6.3
7 – 9	17.8	11.9	8.9
Boys (years)			
10 – 14	29.2	19.5	14.6
15 – 18	37.6	25.1	18.8
Girls (years)			
10 - 14 (Not menstruating)	28.0	18.7	14.0
10 - 14 (Menstruating)	65.4	43.6	32.7
15 - 18	62.0	41.3	31.0
Men (≥ 19 years)	27.4	18.3	13.7
Women (Menstruating, 19 – 49 years)	58.8	39.2	29.4
Women (Menopause, ≥ 50 years)	22.6	15.1	11.3
Pregnancy	*	*	*
Lactation	30.0	20.0	15.0

Note: ** It is recommended that iron supplements in tablet form be given to all pregnant women because of the difficulties in correctly evaluating iron status in pregnancy. In the non-anemic pregnant woman, daily supplements of 100 mg of iron (e.g., as ferrous sulphate) given during the second half of pregnancy are adequate. In anemic women, higher doses are usually required*

In recommending RDAs, there are special concerns for 4 population groups, namely, infants, menstruating adolescents and adults, and pregnant women.

Using WHO-estimated breast milk intakes during the first 6 months, iron intake was only about 0.3 mg/day. Traditionally, solid food was introduced to infants since the first few months of life. Although recent feeding guidelines by UN expert groups now recommend the feeding of complementary foods after 6 months, it is inevitable that in many countries, complementary foods are still introduced at a much earlier age. These foods are likely replacing breast milk, resulting in possibly lower than expected iron intake from breast milk. Two studies of infants around 4 to 6 months of age in rural Thailand revealed intake of about 1 mg iron/day from complementary foods at 3 to 5 months of age (Viriyapanich et al., and Winichagoon *et al.*, unpublished data). This warrants concern and the need for more efforts to revisit and strengthen strategies to promote exclusive breast feeding. Since the consequence of iron-deficiency anemia on child development and cognition can be irreversible, especially if it occurs during infancy, studies to understand how to meet iron requirements of infants during the second 6 months through diet is critical and urgently needed.

In addition, it is assumed that by the second half of the second year of life, most children adopt adult diets, which are generally more diverse, hence, decreasing the risk of developing iron deficiency. However, where the bioavailablility factor of iron in the diets is not high, and parasitic infestation is common, the risk of iron deficiency among toddlers and pre-school children may be high. There is currently insufficient information on infants and young children.

The SEA-RDAs figures relate to 3 different levels of iron bioavailability, which are expected to cover the range of iron bioavailability in typical Southeast Asian diets. Each country may consider using this information as a guide and reference for its country-specific RDAs. It must be recognized that in populations where iron deficiency is prevalent, iron absorption also increases. As stated by Hallberg (Hallberg, 2002), the body has several ingenious systems that control iron in the body to ensure optimal use of available iron, adjust absorption within a limit to meet body needs and prevent excessive iron absorption. Iron content and forms in the diets sets the limits of this control mechanism. These factors must be considered in choosing bioavailability figures and deriving RDAs that can reasonably be met by improving diets. For practicality, adjustment of RDA figures by comparing with the actual intake data, if available, may be needed.

Finally, it must be recognized that RDAs are recommendations for population groups, and are meant to meet the needs of 97.5% of the population and are levels at which the probability of having inadequate intakes are very low. Interactions among micronutrients are now well-recognized. Improving iron status may be achieved, not only by recommending higher intake of iron, but also other related micronutrients. The diversity of Southeast Asian diets is an advantageous quality and should be capitalized in the application of RDAs for specific nutrients.

Bibliography

Allen LH, Ahluwalia N. 1997. *Improving Iron Status through Diet. The Application of Knowledge Concerning Dietary Iron Bioavailability in Human Populations.* OMNI Technical Papers, No. 8. John Snow International, Arlington, VA.

Allen LH (2000). Anemia and iron deficiency: effects on pregnancy outcome. *Am J Clin Nutr* 71:1280S-1284S.

Beard JL (2001). Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr* 131: 568S-580S.

Beaton GH (2000). Iron needs during pregnancy: do we need to rethink our targets? *Am J Clin Nutr* 72(suppl): 265S-271S.

Bothwell TH, Charlton RW, Cook JD & Finch CA (1979). *Iron Metabolism in Man*. Blackwell Scientific Publications. Chapter 1.

Butte N, Lopez-Alarcon MG & Garza C (2002). Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life. World Health Organization, Geneva.

Cook JD, Dassenko S & Skikne BS (1991). Assessment of the role of nonheme-iron availability in iron balance. *Am J Clin Nutr* 54: 717-722.

Dallman PR (1992). Changing iron needs from birth through adolescence. In: *Nutritional Anemia.* Fomon SJ and Zlotkin S (eds). Nestle Nutrition Workshop Series, v. 30. Raven Press Ltd., New York, 1992. pp29-38.

Domellof M, Cohen RJ, Dewey KG, Hernell O, Rivera LL & Lonnerdal B (2001). Iron supplementation of breast-fed Honduran and Swedish infants from 4 to 9 months of age. *J Pediatr* 138:679-687.

FAO/WHO (1988). *Requirements of vitamin A, iron, folate and vitamin B12.* Report of a joint FAO/WHO expert consultation. Rome, Italy.

FAO/WHO (2002). Iron. In: *Human vitamin and mineral requirements.* Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 195-221.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

Haas JD & Brownli T (2001). A critical review of the research to determine a causal relationship. *J Nutr* 131(2s-11): 676S-690S.

Hallberg L, Hogdahl A, Nilsson L & Rybo G (1966). Menstrual blood loss – a population study. Variation at different ages and attempts to define normality. *Acta Obst Gynec Scandinav* 45: 320-351.

Hallberg L, Garby L, Suwanik S & Bjorn-Rasmussen E (1974). Iron absorption from southeast Asian diets. *Am J Clin Nutr* 27: 826-836.

Hallberg L, Brune M & Rossander L (1986). Effect of ascorbic acid on iron absorption from different types of meals. Studies with ascorbic acid rich foods and synthetic ascorbic acid given in different amounts with different meals. *Hum Nutr: Appl Nutr* 40A: 97-113.

Hallberg L and Hulten L (2000). Prediction of dietary iron absorption: An algorithm for calculating absorption and bioavailability of dietary iron. *Am J Clin Nutr* 71: 1147-1160.

Hallberg L (2001). Perspectives on nutritional iron deficiency. *Annu Rev Nutr* 21: 1-21.

Hallberg L (2002). Advantages and disadvantages of iron-rich diet. *Eur J Clin Nutr* 56 (supp): S12-S18.

IOM (2001). Iron. In: *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC; pp 290-393.

Monsen ER and Balintfy JL (1982). Calculating dietary iron bioavailability: Refinement and computerization. *J Am Diet Assoc* 80: 307-311.

Murphy SP, Beaton GH & Calloway D (1992). Estimated mineral intakes of toddlers: predicted prevalence of inadequacy in village populations in Egypt, Kenya and Mexico. *Am J Clin Nutr* 56: 565-577.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Pollitt E, Hathirat P, Kotchabhakdi NJ, Missell L & Valyasevi A (1989). Iron deficiency and educational achievement in Thailand. *Am J Clin Nutr* 50(3)(supp): 687-697.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

Tseng M, Chakraborty H, Robinson DT, Mendez M & Kohlmeier L (1997). Adjustment for iron intake for dietary enhancers and inhibitors in population studies: Bioavailable iron in rural and urban residing Russian women and children. *J Nutr* 127: 1456-1468.

Sturgeon P (1959). Studies of iron requirement in infants and children. III. Influence of supplemental iron during normal pregnancy on mother and infants. B. The infant. *Brit J Haemat* 5: 45-55.

Trinidad TP, Madriaga JR, Valdez DH, Cruz EM, Mallillin AC, Sison CC and Kuizon MD (1989). Iron absorption from adequate Filipinos meals. *The Nucleus* xxvii: 39-46.

Tuntawiroon M, Sritongkul N, Rossander-Hulten, Pleehachinda R, Suwanik R, Brune A & Hallberg L (1990). Rice and iron absorption in man. *Euro J Clin Nutr* 44: 489-497.

Tuntawiroon M, Sritongkul N, Brune M, Rossander-Hulten L, Pleehachinda R, Suwanik R & Hallberg L (1991). Dose-dependent inhibitory effect of phenolic compounds in foods on nonheme iron absorption in men. *Am J Clin Nutr* 53: 554-557.

UNICEF/UNU/WHO/MI (1999). Preventing iron deficiency in women and children. Technical consensus on key issues. UNICEF/UNU/WHO/MI techinical workshop, UNICEF New York, NY, October 7-9, 1998.

Widdowson EM & Spray CM (1951). Chemical development in utero. *Arch Dis Child* 26:205-214.

7 IODINE

7.1 Introduction

Iodine, an essential trace element, is present in the human body only in minute amounts of around 15 mg to 20 mg (WHO, 1994). About 70% to 80% percent of the iodine in the human body is in the thyroid gland. The rest is found in highest concetrations in the salivary glands, gastric glands and dense connective tissues, while a small amount is distributed evenly throughout the body.

7.2 Characteristics and Functions

The primary function of iodine in the body is as a constituent of thyroid hormones, thyroxin (T_4) and triiodotyrosine (T_3), both of which are secreted by the thyroid gland and affect growth, development and the metabolic rate of the body. Thyroid hormones are involved in the regulation of various enzymes and metabolic processes, and control how iodine moves throughout the body (IOM, 2001).

7.3 Absorption, Utilization and Excretion

The thyroid gland needs approximately 60 μg of iodine per day to maintain an adequate supply of thyroxine. As reviewed by the DRI Committee, iodine is ingested in various chemical forms (IOM, 2001). Some iodine-containing compounds (thyroid hormones) are absorbed intact. The administration of iodinated preparations such as Lipiodol, increases iodine stores, and has been successfully used for therapy of Iodine Deficiency Disorders (IDD). Iodate, which is widely used as an additive to salt, is rapidly reduced to iodide and completely absorbed. Iodide, which is chemically-bound iodine, is rapidly absorbed in the stomach. Inorganic iodine is efficiently absorbed from the normal diet.

About half of the iodine in the diet that is absorbed from the intestine as inorganic iodide is normally taken up by the follicular cells of the thyroid gland. It is transported across the follicular cell to the apical microvillae where it is oxidized to iodine. It is then incorporated into protein (thyroglobulin) to form mono- and di-iodothyrosine (MIT and DIT), precursors of T_4 with 4 iodine atoms per molecule, and T_3 with 3 iodine atoms. These hormones are then released into the blood in response to stimuli controlled by the Thyroid Stimulating Hormone (TSH). TSH, which is secreted by the anterior pituitary, is the body's principal active regulator of thyroid hormone production, accelerating iodoprotein synthesis. The release of TSH is stimulated by the Thyrotropin Releasing Hormone (TRH), which is produced by the hypothalamus. Both TRH and TSH secretions are triggered by a decline in blood thyroid hormone level, so that the system maintains the blood concentration of thyroid hormone at functional levels over a wide range of iodine status. Almost all of T_4 and T_3 in the blood are bound to carrier proteins, and only about 0.5% remains in isolated (free) form in

human serum called FT_4 and FT_3, both of which possess metabolic activity (Hetzel, 1989; Todd, 1998).

Iodide ions are concentrated by the thyroid and to a lesser extent by the salivary and gastric glands, and distributed throughout body tissues. About 20% of the iodine in the blood is in the form of iodide, and the levels do not vary with different thyroid states.

The excretion of iodine is mainly through urine. The level of excretion correlates with the level of intake so that urinary iodine level can be used to assess the level of iodine intake (WHO, 1994).

7.4 Effects of Deficiency and Excess

7.4.1 Deficiency

When iodine requirements are not met, a series of functional and developmental abnormalities occur. Hypothyroidism (low thyroid hormone level) caused by iodine deficiency, results in physical and mental retardation in infants and children. In adults, it causes reduction in mental function, sluggishness and lethargy. Hypothyroidism may also cause enlargement of the thyroid gland, a condition called goiter (IOM, 2001).

Iodine deficiency lowers IQ by an average of 13.5 points (Gerasimov, 1998) and is the most common preventable cause of mental deficiency in the world. The WHO has estimated that the elimination of iodine deficiency would prevent brain damage that has caused irreversible mental handicap in at least 43 million people throughout the world. In the neonate, iodine deficiency causes increased perinatal mortality, infant mortality and low birth weight. The incidence of neonatal hypothyroidism is high in iodine deficient areas and roughly correlates with the severity of other features of IDD.

In areas where iodine intake is abnormally low, adequate secretion of thyroid hormones may still be achieved by increasing the secretion of TSH. However, if iodine deficiency is severe, obvious alterations in thyroid function are common, such as inverse relationship between serum thyroxine (T_4) and TSH, although this correlation is not found with serum triiodothyronine (T_3) (Ermans, 1980).

Goiter has long been the most recognized and prominent effect of iodine deficiency. However, the understanding of iodine deficiency has now reached far beyond goiter. Iodine deficiency affects growth and development, particularly brain development. Use of the term Iodine Deficiency Disorders, or IDD, reflects a new understanding of the full spectrum of the effects of iodine deficiency on different population groups (Hetzel, 1989; Dunn and van der Haar, 1990).

Among WHO's 191 member states, IDD is known to be a significant public health problem in 130 countries, while it has been eliminated in the remaining 61 countries (WHO/UNICEF/ICCIDD, 1999). It is estimated that around 740 million people (13%) are affected by goiter. Meanwhile, 2,225 million people (38%) are at risk from IDD, i.e. they live in areas with goiter prevalence greater than 5%. Of the people at risk from IDD, 13.3% live in Africa, 8.8% in the Americas, 15.6% in the Eastern

Mediterranean, 12.4% in Europe, 23.1% in the Western Pacific, and 26.8% in Southeast Asia. In Southeast Asia, it is estimated that 172 million people (12%) are suffering from goiter, while 599 million (41%) are at risk (Dunn and van der Haar, 1990).

Goitrogenic substances (anti-thyroid substances) play a role in the etiology of iodine deficiency. Some foods, such as brassica/cabbage, sprouts, cassava, sweet potato, bamboo shoots, maize and lima beans, contain goitrogenic substances (DOH, 1995a). The anti-thyroid action is related to the presence of thioglucosides, which release thiocyanate and isothiocyanate after digestion, and the presence of cyanoglycosides, which produce cyanide after ingestion. If large quantities of these foods are eaten and the level of dietary iodine is marginal, goiter could develop (Gaitan, 1988). Excessive amounts of calcium, fluorine, magnesium, and manganese ions in water may also be goitrogenic (DOH, 1995a).

7.4.2 Excessive intake

Excessive amounts of iodine can also lead to goiter. It should be noted that, in addition to food, many cough medicines and milk contaminated with iodine-containing sanitizing agents also contribute to iodine intake. However, it is unlikely that any harmful effect would occur with habitual intakes of up to 300 micrograms per day (Griggs and Wahlqvist, 1988).

7.5 Food Sources

The iodine content in foods depends on the iodine content in the soil and water where the foods are grown or cultivated. Foods rich in iodine include seafood, seaweed, vegetables, meat, eggs, milk and dairy products. Their iodine content ranges from 13 μg/100g (milk) to 66 μg/100g (seafood) (WHO, 1994). Some dried seaweed may even contain up to 500 μg/100g. Iodine occurs in foods largely as inorganic iodine (iodates). In the UK, where cattle feed is supplemented with iodine, particularly during winter, milk has become a source of iodine with iodine content of up to 28 μg/100 g (DOH, 1995a).

Iodized salt is a major source of iodine in countries where universal salt iodization is practiced as the main strategy to control iodine deficiency. Currently, it is estimated that approximately 65% of all edible salt is iodized in Southeast Asian countries. In fact, the level of salt iodization by some countries is above that which is recommended by WHO/UNICEF/ICCIDD (20 to 40 ppm iodine).

A total diet study conducted by the United States Food and Drug Administration in 1984 revealed that intake of iodine among infants, children and teenagers was 140 μg/day, 160 μg/day and 210-360 μg/day, respectively. Based on total diet survey conducted in 1985 to 1986, iodine intake (including iodine from iodized salt) among adult men and women was 250 μg/day and 170 μg/day, respectively (NRC, 1989). In a survey conducted in the UK, the intake of iodine was found to be 243 μg/day and 176 μg/day for men and women, respectively (Wenlock *et al.*, 1982). In most European countries, iodine intake is in excess of requirements (DOH, 1995a).

Currently, there is no available data from Southeast Asian countries on the intake of iodine obtained from total diet surveys. One explanation is the lack of data on iodine content of Asian foods. A study of iodine intake based on the main food sources of iodine among pregnant women in a coastal area of Maluku, Indonesia, where goitrogenic foods are commonly consumed, showed that the average intake of iodine was 395.2 μg/day (Picauly *et al.*, 2000).

7.6 Factors Affecting Requirement

Iodine is ingested in a variety of chemical forms in food. Most ingested iodine is reduced in the gut and absorbed almost completely. Some iodine-containing compounds are absorbed intact. The thyroid selectively concentrates iodide in the amounts required for adequate thyroid hormone synthesis. The thyroid gland needs approximately 60 μg of iodine per day to maintain an adequate supply of thyroxine (IOM, 2001). Several other tissues also concentrate iodine, including salivary glands, breast, choroids plexus and gastric mucosa.

Under normal circumstances, the absorption of dietary iodine is greater than 90%. When thyroxine is given orally, the bioavailability is approximately 75%. Certain micronutrients are necessary for the utilization of iodine for thyroid hormone synthesis. Selenium deficiency for example, inhibits the conversion of T_4 to T_3 in the liver. Iron deficiency impairs thyroid metabolism leading to an inability to control body temperature. Protein malnutrition affects iodine absorption and metabolism by decreasing thyroidal iodine clearance (Pennington, 1988). Soy flour has been shown to inhibit iodine absorption, and when iodine was added to this formula, goitre did not appear (IOM, 2001).

Some foods contain goitrogens, substances that interfere with thyroid hormone production or utilization. Examples include cassava, crucifera vegetables (cabbage, broccoli, cauliflower), bamboo shoots, maize, lima beans and millet. Most of these substances are not of major clinical importance unless there is co-existing iodine deficiency (IOM, 2001).

7.7 Estimating Requirements and Recommended Intakes

7.7.1 Indicators for estimating requirements for iodine

Over 90% of dietary iodine eventually appears in the urine. Data on urinary iodine excretion are variously expressed as a concentration (μg/L), in relationship to creatinine excretion (μg iodine/g creatinine), or as 24-hour urine collections (μg/day). Most studies have used the concentration in casual samples because of the obvious ease of collection. In populations with adequate general nutrition, urinary iodine concentration co-relates well with the urine iodine/creatinine ratio. Urinary iodine excretion is recommended by the WHO, the International Council for the Control of Iodine Deficiency Disorders, and UNICEF for assessing iodine nutrition worldwide. Simple methods are available for measuring urinary iodine. The urinary iodine concentration reflects very recent iodine nutrition (days) in contrast to indicators such as thyroid size and serum thyroid stimulating hormone (TSH) and thyroglobulin concentrations.

The size of the thyroid gland increases in response to iodine deficiency, mediated at least in part by increased serum TSH concentration. This earliest clinical response to impaired iodine nutrition reflects an adaptation to the threat of hypothyroidism. Excess iodine can also produce goiter because large amounts inhibit intrathyroidal hormone production, again leading to increased TSH stimulation and thyroid growth. Traditionally, goiter was assessed by neck palpation with each lobe of the normal thyroid being regarded as no larger than the terminal phalanx of the subject's thumb. Thyroid size is recommended by WHO/UNICEF/ICCIDD for assessing iodine nutrition worldwide (WHO/UNICEF/ICCIDD, 1994). Ultrasonography defines thyroid size much more precisely and reliably. The technology - safe, practical, and easily performed in the field - is replacing palpation in most studies. Most data come from surveys on school-age children.

As serum TSH concentration responds to circulating levels of thyroid hormone, which in turn reflect adequate production of thyroid hormone, it is an excellent indicator of altered thyroid function in individuals. Sensitive assays have been widely available for about 2 decades, and serum TSH concentration is now the preferred test for assessing thyroid function in individuals. It is also used on blood spots by filter paper methodology in most countries for the routine screening of neonates to detect congenital hypothyroidism (WHO/UNICEF/ICCIDD, 1994).

7.7.2 Recommendations for iodine intake by life stages

Recommendations for the iodine RDA for Southeast Asia as based on a review of recommendations of 3 major sources, namely FAO/WHO (FAO/WHO, 2002), the DRI Committee (IOM, 2001) and the UK Department of Health (DOH, 1995b). These organizations had undertaken extensive reviews of iodine requirement studies and provided updated recommendations on iodine requirements and intakes.

(*a*) *Infants (0 – 12 months)*

The review by FAO/WHO indicated that the iodine content of breast milk varies markedly as a function of the iodine intake of the population (FAO/WHO, 2002). For example, it ranges from 20 μg/L to 330 μg/L in Europe and from 30 μg/L to 490 μg/L in the US. It is as low as 12 μg/L under conditions of severe iodine deficiency. An average breast milk intake of 750 ml/day would give an intake of iodine of about 60 μg/day in Europe and 120 μg/day in the US. The upper US value (490 μg/L) would provide 368 μg/day or 68 μg/kg/day for a 5-kg infant. Positive iodine balance in the young infant, which is required for the increasing iodine stores of the thyroid, is achieved only when the iodine intake is at least 15 μg/kg/day in full-term infants. This corresponds approximately to an iodine intake of 90 μg/day. However, for pre-term infants, the requirement is increased to 30 μg/kg/day.

For infants 0 to 6 months old, the DRI Committee's recommendation was based on median iodine intake of infants from breast milk of women consuming iodized salt, as well as iodine balance studies in infants (IOM, 2001). The adequate intake (AI) of iodine for infants aged 0 to 6 months was set at 110 μg/d. The AI of iodine for infants 7 to 12 months old was extrapolated from the AI of infants 0 to 6 months old, to 130 μg/day. These recommendations are higher than the 2002 recommendations of FAO/WHO.

To estimate the Reference Nutrient Intake (RNI) of iodine for infants, the UK Department of Health used the level of iodine intake of infants from breast milk without signs of iodine deficiency (30 μg/day to 40 μg/day) plus a safety margin, resulting in the RNI of iodine of 50 μg/day for infants less than 3 months old (DOH, 1995b). The RNI of iodine for older infants was extrapolated from RNI of iodine for adults.

(*b*) *Children and Adolescents (1 – 19 years)*

The daily iodine requirement on a body weight basis decreases progressively with age. A study by Tovare and colleagues (Tovare *et al.*, 1969) co-relating 24-hour thyroid radioiodine uptake and urinary iodine excretion in 9 to 13 year old school children in rural Mexico suggested that an iodine intake in excess of 60 μg/day is associated with a 24-hour thyroidal radioiodine uptake below 30%. Lower excretion values are associated with higher uptake values. This would approximate 3 μg/kg/day in an average-sized 10 year old child (approximate body weight of 20 kg), so that an intake of 60 μg/day to 100 μg/day for children aged 1 to 10 years seems appropriate. These requirements were based on the body weight of Mexican children who participated in this study. The average body weight of a 10-year old child, as per the FAO references, is 25 kg. Thus, the iodine requirement for a 1 to 10-year old child would be 90 μg/day to 120 μg/day.

The iodine RDA for children aged 1 to 13 years recommended by the DRI Committee is 90 μg/day to 120 μg/day (IOM, 2001). The iodine EAR for these children was based on iodine balance studies for each specific age groups. The EAR of iodine for teenagers (aged 14 to 18 years) was based on extrapolation from adult data because of the absence of available data in this age group. The RDA for children and teenagers (1 to 18 yrs) of 150 μg/day was calculated by multiplying each EAR with 1.4 (assuming 40% as the value of 2 SD).

The RNI of iodine for children and teenagers recommended by the UK Department of Health were extrapolated from RNI of iodine of adults. This was done using EAR for energy, which was based on basal metabolic rate (DOH, 1995b).

(*c*) *Adults*

Iodine intake at 150 μg/day for adults is justified by the fact that it corresponds to the daily urinary excretion of iodine and to the iodine content of food in non-endemic areas (areas where iodine intake is adequate) (FAO/WHO, 2002). A urinary iodine concentration of 100 μg/L corresponds to an intake of about 150 μg/day in adults. Median urinary iodine concentrations below 100 μg/L in a population are associated with increases in median thyroid size and in serum TSH and thyroglobulin values. Correction of the iodine deficiency will bring all these measures back into the normal range.

The DRI Committee calculated the EAR of iodine for adults based on thyroid iodine accumulation and turnover (IOM, 2001). According to the DRI Committee, there is no evidence to show that the EAR of iodine is different among age groups of adults, or among men and women. Similar to the calculation of iodine RDAs for children and adolescents, the RDA for adults was calculated by multiplying each EAR with 1.4 (assuming 40% as the value of 2 SD).

The UK Department of Health proposed iodine reference values (intake) based on the following observations:

1. There are no data on iodine requirements for UK, therefore an EAR cannot be calculated;
2. A urinary iodine excretion of less than 50 μg/g creatinine is usually associated with high incidence of goiter in a population;
3. Increasing iodine intake from 100 μg/day to 500 μg/day did not effect different results in the incidence of goiter; and
4. At the iodine intake level of 100 μg/day, the level of iodine in the thyroid gland has been shown to be normal, and iodine intake of up to 300 μg/day did not result in any further increase in the iodine level in the thyroid gland (DOH, 1995b)

Based on the above considerations, the UK Department of Health recommended a Lower Reference Nutrient Intake (LRNI) for adults of 70 μg/day as a minimum requirement of iodine, as well as a RNI of 140 μg/day as a safety margin to allow the effects of different dietary patterns. A similar concept was also applied in the formulation of iodine RDA for Americans by the DRI Committee (IOM, 2001).

(*d*) *Pregnancy and Lactation*

Iodine requirement during pregnancy is increased to provide for the needs of the fetus and to compensate for the increased loss of iodine in the urine resulting from an increased renal clearance of iodine during pregnancy (Aboul-Khair *et al.*, 1964). Serum TSH and thyroglobulin are still higher in the neonates than in the mothers. These abnormalities are prevented only when the mother receives a daily iodide supplementation of 161 μg/day during pregnancy (derived from 131 μg potassium iodide and 100 μg T_4 given daily). These data indicate that the iodine intake required to prevent the onset of sub-clinical hypothyroidism of mother and fetus during pregnancy, and thus to prevent the possible risk of brain damage of the fetus, is approximately 200 μg/day (FAO/WHO, 2002). The same amount has been recommended for lactating women.

The EAR of iodine for pregnant women was estimated based on the results of studies on thyroid iodine content of the newborn, iodine balance in infants, and iodine supplementation during pregnancy (IOM, 2001). A value of 160 μg/day and 220 μg/day was recommended as the EAR and RDA, respectively, for pregnant women. For lactating women, the EAR of iodine was calculated based on EAR of teenage girls plus EAR for producing breast milk, resulting in an EAR of 290 μg/day. A factor of 1.4 was also used to convert EAR into RDA for pregnant women and lactating women.

The UK Department of Health did not specify additional iodine requirements for pregnant and lactating women, despite the need to increase iodine requirement during pregnancy to provide for the needs of the fetus and to compensate for the increased loss of iodine in the urine resulting from an increased renal clearance of iodine during pregnancy (Aboul-Khair *et al.*, 1964).

7.8 Current RDAs for Iodine in Southeast Asia

Table 7.1 Comparison of current RDAs (μg/day) for iodine in selected Southeast Asian countries

Age Groups (years)	Indonesia (2000)	Malaysia (2005)	Philippines (2002)	Thailand (2003)
Infants (0 - 1)	50 – 70	90 – 120	90	90[a]
Children (1 – 9)[b]	70 – 120	72 – 108	90 – 120	90 – 120
Boys (10 – 18)[c]	150	106 – 144	120 – 150	120 – 150
Girls (10 – 18)[c]	150	98 – 148	120 – 150	120 – 150
Men (≥ 19)	150	114 – 124	150	150
Women (≥ 19)	150	98 – 110	150	150
Pregnancy				
1st trimester	175	200	200	200
2nd trimester	175	200	200	200
3rd trimester	175	200	200	200
Lactation				
1st 6 months	200	200	200	200
2nd 6 months	200	200	200	200

Notes: [a] *Figures only for infants aged 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *9 - 18 years for Thailand*
Source: Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)
The review of Tee (1998) indicates that the Singapore (1998) and Vietnam (1996) RDAs do not include iodine.

The RDAs for iodine in 4 Southeast Asian countries are tabulated in Table 7.1. The recommended intakes for Indonesia, Philippines and Thailand are rather similar. The Indonesian RDAs for infants and pregnant women, are however, clearly lower than those for the other 2 countries. In all the countries mentioned, there are no differences in the recommended intake for adolescent boys and girls and for men and women. The slightly lower values for females in the Malaysian RDA is probably due to their slightly lighter weight. The Malaysian recommended intake for iodine for all age groups are generally lower than those for the other 3 countries. All countries recommend additional intakes of iodine during pregnancy and lactation, up to 33% in most cases.

7.9 Recommended RDAs for Iodine for Southeast Asia

Since there is a lack of scientific studies on iodine requirements of Southeast Asian populations, the SEA-RDAs were determined after reviewing the recommendations of the DRI Committee (IOM, 2001) and the FAO/WHO Expert Consultation (FAO/WHO, 2002). The reference body weight of the respective Southeast Asian countries and the level of salt iodization in these countries were also taken into consideration. These factors are important due to the co-relation between thyroid hormones and metabolic rate, and the fact that the level of salt iodization in many Southeast Asian countries is above the level of 20 ppm to 40 ppm iodine currently recommended by WHO/UNICEF/ICCIDD.

The iodine RDAs recommended by the the DRI Committee (IOM, 2001) and FAO/WHO (2002) for the various age groups, calculated on a per kilogram body weight basis, are similar. Therefore, for harmonization of iodine RDAs in Southeast Asia, each country can calculate their respective RDAs by applying their respective reference body weights. After reviewing the recommendations of the DRI Committee and FAO/WHO, the recommendations of FAO/WHO (2002) were adopted by the SEA-RDA Committee. The SEA-RDAs are set out in Table 7.2.

Table 7.2 Recommended RDAs for Iodine for Southeast Asia

Age Groups	Iodine RDA (μg/day)
Infants (months)	
0 – 5	90
6 – 11	90
Children (years)	
1 – 3	90
4 – 6	90
7 - 9	120
Boys (years)	
10 – 12	120
13 - 15	150
16 – 18	150
Girls (years)	
10 – 12	120
13 - 15	150
16 – 18	150
Men (years)	
19 – 65	150
> 65	150
Women (years)	
19 – 65	150
> 65	150
Pregnancy	200
Lactation	200

Bibliography

Aboul-Khair SA, Turnbull AC & Hytten FE (1964). The physiological changes in thyroid function during pregnancy. *Clin Sci* 27: 195-207.

DOH (1995s). Dietary reference values for food energy and nutrients for the United Kingdom. Report on Health and Social Subjects number 41. Department of Health, London.

DOH (1995b). Dietary reference values for food energy and nutrients for the United Kingdom. Department of Health, London.

Dunn JT & F.van der Haar (1990). A practical guides to the correction of iodine deficiency. ICCIDD/UNICEF/WHO. The Netherlands.

Ermans AM (1980). Etio-pathogenesis of endemic goiter. In: *Endemic Goiter and Endemic Cretinism*. Stanbury JB. and BS. Hetzel (eds.). John Wiley. p. 287-301.

FAO/WHO (2002). Iodine. In: *Human vitamin and mineral requirements*. Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 181-194.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines*. 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

Gaitan E (1988). Goitrogens. *J Clin Endocrinol Metab* 2:683-702.

Gerasimov G (1998). In: *Elimination of iodine deficiency disorders (IDD) in Central and Eastern Europe, the Commonwealth of States, and the Baltic States*. Delange F, A.Robertson, E.McLoughney and G. Gerasimov (eds). WHO/UNICEF/ICCIDD, WHO/EURO/NUT/98.1.

Griggs D & Wahlqvist M (1988). Food Facts: the complete no-fads-plain-facts guide to healthy eating. Penguin Books.

Hetzel BS (1989). The story of iodine deficiency: an international challenge in nutrition. Delhi. Oxford University Press.

IOM (2001). Iodine. In: *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC; pp 258-289.

NRC (1989). *Recommended Dietary Allowances*. National Research Council, National Academy Press, Washington, DC.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Pennington JT (1988). Iodine. In: *Trace minerals in Foods*. Smith KT (ed). 1st ed. Marcel Dekker, Inc. New York. Pg 249-289.

Picauly I, Hardinsyah & Rimbawan (2000). Kebiasaan pengolahan pangan, konsumsi pangan dan status yodium ibu hamil di daerah endemic GAKY, Saparua, Maluku Tengah. Media Gizi dan Keluarga.

Tee ES (1998). Current status of recommended dietary alowances in Asia: A regional overview. *Nutr Rev* 56(4): S10-S18.

Todd CH (1998). Hyperthyroidism and other thyroid disorders: a practical handbook for recognition and management. WHO/ICCIDD. WHO/NHD/99.1.

Tovare E, Maisterrena JA & Chavez A (1969). Iodine nutrition levels of school children in rural Mexico. In: *Endemic Goitre*. Stanbury JB (ed.). Pan American Health Organization, Washington, D.C., Sc. Publication no. 193. p. 411-15.

Wenlock RW, Buss DH, Moxon RE & Bunton NG (1982). Trace elements 4: Iodine in British Foods. *British Journal of Nutrition* 7:381-390.

WHO (1994). *Trace Element in Human Nutrition*. World Health Organization, Geneva.

WHO/UNICEF/ICCIDD (1994). Indicator for assessing iodine deficiency disorders and their control through salt iodization. Report of a Joint Consultation. Document WHO/NUT/94.6. Geneva.

WHO/UNICEF/ICCIDD (1999). Progress towards the elimination of iodine deficiency disorders (IDD). WHO/NHD/99.4.

8 ZINC

8.1 Introduction

Micronutrient deficiency remains widespread in Asia. While the deficiencies of micronutrients such as iron, vitamin A and iodine are well-established, there is a lack of a universally accepted single measure to assess zinc status in humans. Measurement of serum zinc levels is often used but it is a poor indicator of marginal zinc deficiency, as the body has the ability to adapt to a wide range of zinc intakes.

The risk of zinc deficiency among children in developing countries may be widespread due to food composition and dietary intake data. The major sources of zinc are animal products, which are generally consumed in small amounts by children from low-income households. Moreover, the Asian diet is based, to a large extent, on whole-grain cereals and other plant foods containing phytate, which chelates zinc and inhibits its absorption. Thus, infants and young children who are fed complementary foods that are wholly plant-based face the risk of zinc deficiency. Consequences of zinc deficiency in children include growth failure, immuno-suppression and increased risk of infections.

8.2 Characteristics and Functions

The wide distribution of zinc in all body tissues and fluids reflects its essential role in metabolic activity as a component of more than 300 key enzymes. Most of the zinc in the body is found in bone, liver, kidney, muscle and skin. The body's total zinc content ranges from about 2.3 mmol (1.5 g) in women to 3.8 mmol (2.5 g) in men (King and Keen, 1994).

Zinc can exist in several different valence states, but the divalent ion (Zn+) is the most prevalent. It is a vital micronutrient required throughout the life stages. Zinc has biochemical functions that determine physiological effects (e.g. tissue or cell growth, cell replication, bone formation, skin integrity, cell-mediated immunity and host defense) which can be divided into 3 categories:

1. Catalytic function;
2. Structural function; and
3. Regulatory function.

8.2.1 Catalytic function of zinc

Zinc has a catalytic function as it plays a central role in metabolism, including the assimilation of metabolic fuel and maintenance of immune function. It is a critical component of various metalloenzymes in humans. Nearly 100 specific enzymes, including alcohol dehydrogenase, alkaline phosphatase and carbonic anhydrase, depend on zinc for catalytic activity.

The immune system comprises a series of multifaceted inter-related activities involving essential amino acids, essential fatty acids, vitamins and minerals including zinc. In zinc deficiency, there is a suppressive effect on thymic function, T-lymphocyte development, lymphocyte proliferation, and T-cell dependent B-cell functions, leading to a decrease in resistance to respiratory and gastrointestinal infections.

8.2.2 Structural function of zinc

Zinc plays a structural role in proteins and biomembranes. One key example that has engendered much recent interest is the zinc finger motif. Zinc fingers are small protein domains in which zinc plays a structural role contributing to the stability of the domain. Zinc fingers are structurally diverse and are present among proteins that perform a variety of functions in several cellular processes, including replication and repair, transcription and translation, metabolism and apoptosis (Krishna *et al.*, 2003). The linking of these zinc fingers to corresponding sites on DNA initiates the transcription process and gene expression. Hence, zinc fingers appear as potential targets for therapeutic interventions (Wu *et al.*, 1995).

8.2.3 Regulatory function of zinc

Zinc also functions as a regulator of gene expression. For example, in metallothionein expression, failure of the regulatory mechanism involving zinc can be lethal to fetal development. Zinc acts as a regulator in influencing apoptosis and protein kinase C activity (Thompson, 1995).

In general, zinc, as a component of numerous enzymes, participates in the reaction at catalytic sites, the maintenance of the structural integrity of proteins and the regulation of gene expressions. While much is known of the biochemical functions of zinc, important aspects remain unclear, including the regulation of zinc metabolism and the maintenance of zinc homeostasis at a molecular level.

8.3 Absorption, Utilization and Excretion

Understanding the complexities of zinc metabolism and homeostasis is important for the understanding of zinc dietary requirements. Presently, there is a lack of adequate epidemiological data, including laboratory, functional and clinical biomarkers of zinc nutritional status. However, with the advent of modern metabolic and tracer techniques in recent years, progress has been made in eliciting pertinent information on zinc metabolism.

The subcellular mechanisms of zinc absorption remain to be explicated, but both saturable and unsaturable processes are thought to be involved. It is believed that zinc is predominantly transported via a saturable, specific transport mechanism. The transport proteins involved under conditions of a range of dietary zinc intake are being identified and characterized.

Zinc absorption is influenced by several factors, including the individual's physiological state and dietary components. In general, fractional absorption of zinc increases during physiological states that have high requirement for zinc. These include infancy, pregnancy and lactation. Exclusively breast-fed infants have very high fractional absorption, due to several factors including the complex organic matrix of breast milk, the modest concentrations of zinc and other minerals, and the high physiologic requirement for zinc during a period of rapid growth.

Animal and human studies indicated that zinc homeostasis is maintained through the synergistic action of gastrointestinal absorption from dietary sources and endogenous fecal zinc excretion. Potential endogenous sources include pancreatic and biliary secretions, gastroduodenal secretions, and sloughing of mucosal cells. The excretion of endogenous zinc is crucial in maintaining zinc balance just above and below optimal intakes. As dietary zinc intake increases, endogenous fecal zinc excretion increases immediately but only by a relatively small amount. The absorption of zinc however, responds more slowly but has the capacity to cope with larger changes in dietary zinc (King *et al.*, 2000).

At low dietary zinc intakes, zinc absorption becomes more efficient, and the endogenous excretion of zinc is reduced to conserve more zinc for tissues use. In addition, there is increased reabsorption of the endogenous zinc due to up-regulation of the carrier-mediated process. At low zinc intake levels, absorption occurs primarily by a carrier-mediated process, which is proposed to be a ligand, probably a metallothionein that enhances jejunal zinc absorption (King *et al.*, 2000). Adjustments in urinary zinc excretion are relatively minor compared to the gastrointestinal shifts in the endogenous fecal zinc excretion and zinc absorption.

In populations with chronically low dietary zinc intake, it is probably more critical to conserve endogenous zinc for maintenance of zinc homeostasis than adaptation in fractional absorption. In studies on populations experiencing long-term poor zinc intakes, fractional and total zinc absorption remained relatively constant, but the endogenous fecal zinc losses continued to decline, thus allowing the subjects to achieve a positive crude zinc balance on a very low zinc diet.

8.4 Effects of Deficiency and Excess

8.4.1 Deficiency

Adequate zinc intake is crucial for health. As zinc has a central role in cell division, protein synthesis and growth, zinc is particularly important in infancy, childhood, pregnancy and lactation. While severe zinc deficiency is rare, mild-to-moderate forms of zinc deficiency may be relatively common worldwide.

The observed signs and symptoms of dietary zinc deficiency in humans include loss of appetite, growth retardation, immune dysfunction or abnormalities, delayed sexual maturation, skin changes, altered cognition, diarrhea, reproductive teratogenesis and behavioral abnormalities. In addition,

individuals with malabsorption syndromes (sprue, Crohn's disease and short bowel syndrome) may also be at risk of zinc deficiency due to malabsorption of zinc and increased urinary zinc losses.

Impaired growth velocity is a primary clinical feature of mild zinc deficiency and can be corrected with zinc supplementation. A meta-analysis of 25 zinc intervention studies showed that in stunted children, zinc supplements have small but significant height improvements (average of +0.46 SD in height gain) (Brown *et al.*, 1998). For children with initial low plasma zinc, zinc supplementations produced a smaller weight gain (+0.26 SD). No significant effect of zinc supplementation was observed on weight-for-length.

Zinc deficiency affects cells of the immune system. It causes a reduction in the number of B and T lymphocytes through increased apoptosis, and also reduces their functional capacity. The functions of the macrophages that normally destroy bacteria are also compromised. The production and potency of several cytokines, which are the central messenger of the immune system, are also disturbed. While the mechanisms underlying the relationship between zinc and immunity remain unclear, it is hypothesized that zinc may be involved in one of these 4 ways (Dardenne, 2002):

(1) Through its functions as enzymes - zinc is an essential factor for more than 300 metalloenzymes;
(2) In the activity of some immunity mediators, e.g. thymulin hormone that promotes T lymphocyte maturation;
(3) Contribution to membrane stabilization that otherwise could lead to reduced phagocytosis; or
(4) As a major intracellular regulator of lymphocyte apoptosis.

Thus, zinc supplementations have been found to improve the incidence, duration and severity of diarrheal disease and reduce the incidence and rates of acute lower respiratory tract infections, parasitic infections and malaria among children in developing countries.

Severe maternal zinc deficiency has been associated with spontaneous abortion and congenital malformation, while milder forms of zinc deficiency have been associated with low birth weight, intrauterine growth retardation, preterm delivery and complications during labour and delivery (Jameson, 1993). The results of various studies on maternal zinc supplementation during pregnancy have been inconclusive, perhaps due to the studies being conducted in developed and relatively well-nourished populations. The preliminary findings of 8 randomized and controlled intervention trials in less developed countries reported that maternal zinc supplementation has a beneficial effect on neonatal immune status, early neonatal morbidity and infant infections (Osendarp *et al.*, 2003).

8.4.2 Excessive intake

Excessive zinc intakes can produce acute and chronic effects of toxicity. These deleterious toxic effects occur mostly via over-supplementation with zinc. Acute effects of zinc toxicity (2 g or more of zinc sulfate) can produce metallic taste, gastric distress, dizziness, vomiting, nausea, abdominal cramps and bloody diarrhea. Chronic effects (18.5 mg/day or 25 mg/day) can result in reduction

of immune functions and HDL cholesterol and impairment of copper status. Consequently, chronic ingestion of zinc supplements exceeding 15 mg/day is not recommended without adequate medical supervision (Pluhator *et al.*, 1996).

High doses of zinc intake can impair copper absorption, as indicated by a decrease in erythrocyte copper-zinc superoxide dismutase activity, leading to immunosuppression, especially for the mother and fetus during pregnancy.

8.4.3 Guidance on high intakes

While there is no evidence of adverse effects from intake of naturally occurring zinc in food, the upper tolerable intake level (UL) for zinc applies to total zinc intake from food including fortified food, water and supplements. Table 8.1 shows the UL values suggested by the FAO/WHO, IOM and IZiNCG (International Zinc Nutrition Consultative Group). Reports of zinc toxicity have focused on the role of zinc in causes of copper deficiency, changes in the immune system, and alterations in blood lipids. Reported adverse effects of excesssive zinc intake include gastrointestinal pain, diarrhea, functional impairment in immunological response, and significant decreased concentrations of HDL-cholesterol.

While there is no evidence of adverse interactions between zinc and other nutrients when zinc is found in food, the UL values for zinc for various age groups have been established to safeguard possible toxic effects arising from excess zinc intake. These UL values are based on the consistency of results from studies on interaction of zinc and copper. Findings on the effects of zinc on lipoproteins are deemed not consistent and therefore not used to derive the UL for zinc.

Table 8.1 Comparison of ULs or no observed adverse effects levels (NOAEL) for zinc

FAO/IAEA/WHO		IOM		IZiNCG	
Age/Sex	UL (mg/day)	Age/Sex	UL (mg/day)	Age/Sex	NOAEL (mg/day)
Infants (months)		Infants (months)		Infants (months)	
0 – 6	-	0 - 6	4	0 - 5	-
7 – 12	13	7 - 12	5	6 - 11	6
Children (years)		Children (years)		Children (years)	
1 – 3	23	1 - 3	7	1 - 3	8
3 – 6	23	4 - 8	12	4 - 8	14
6 – 10	28				
Boys (years)		Boys (years)		Boys (years)	
10 – 12	34	9 - 13	23	9 - 13	26
12 – 15	40	14 - 18	34	14 - 18	44
15 – 18	48				
Girls (years)		Girls (years)		Girls (years)	
10 – 12	32	9 - 13	23	9 - 13	26
12 – 15	36	14 - 18	34	14 - 18	39
15 – 18	38				
Adults (years)		Adults (years)		Adults (years)	
Men (18 - ≥ 60)	45	Men (> 19)	40	Men (> 19)	40[a]
Women (18 - ≥ 60)	35	Women (> 19)	40	Women (> 19)	40[a]

Note: [a] *Represents upper limits*
Source: *IZiNCG (2004)*

8.5 Food Sources

Zinc is widely distributed in foods and is typically associated with the protein fraction and/or nucleic fraction of food. The zinc content in foods varies widely. Seafood (especially oysters and mollusks), lean red meat and eggs are rich sources of zinc. Other good sources include poultry, pork, and dairy products. Whole grains (especially bran and germ), legumes and vegetables (leafy and root) contain zinc, but the presence of high phytate content in these plant foods reduces the zinc bioavailability. Fruits and refined cereals are poor sources of zinc. Some food sources of zinc are set out in Table 8.2.

Table 8.2 Selected foods and their zinc content

	Zinc (mg/100g) Lowest		Zinc (mg/100g) Highest
Cereal and grain products			
Polished rice	0	Soda cream biscuit	3.1
Starchy roots, tubers and their products			
Potato, raw and with skin	0	Tapioca, raw & peeled	2.4
Vegetables and vegetable products			
Cabbage, boiled	0.1	Chilli, dried	8.2
Eggplant, raw	0.1		
Tomato, raw	0.1		
Chinese radish, boiled	0.1		
Fruits and fruit products			
Apple, raw and peeled	0	Dragon fruit, raw	3.0
Pink grapefruit	0		
Honey mango, raw	0		
Meat, poultry, fish and their products			
Beef sausage	1.7	Beef brisket	6.2
Chicken breast, raw, lean & skin	0.4	Chicken liver	7.3
Duck, canned	0.6	Goose liver, raw	3.6
Mutton, cooked	2.1	Lamb shank, simmered lean & fat	9.0
Pig's trotter, cooked	0.8	Pork liver, boiled	6.7
Indian Halibut, raw	trace	Anchovy, whole & dried	
Whole stingray, raw			
Cuttlefish, raw	0	Oyster	65.6
Egg and egg products			
Whole hen egg, hard boiled	0	Hen egg yoke, fried	4.4
Dairy and dairy products			
Cheese cottage, creamed	0.1	Parmesan cheese	5.8
Legumes, nuts, seeds and their products			
Jackfruit seed	0.4	Peanuts, roasted	6.6
Long beans, boiled	0.4		

Source: HPB Singapore (2003)

8.6 Factors Affecting Requirement

Various dietary factors affect the absorption of zinc and consequently zinc requirements (Lonnerdal, 2000; Krebs, 2000).

8.6.1 Amount and form of zinc intake

The amount of zinc in a meal will, in itself, affect absorption. Zinc absorption will decrease with increasing amounts of zinc intake. Intestinal perfusion studies in humans showed linear increases in zinc absorption from 0.1 mmol/L to 1.5 mmol/L zinc, with rates leveling at higher concentrations. Data compiled from zinc absorption studies showed that the amount of zinc absorbed from single meals usually levels off at approximately 18 μmol to 20 μmol.

Intraluminal zinc is present in different forms after intake. The free zinc forms complexes with ligands such as amino acids and organic acids. The type of complex formed influences the absorbability of the zinc. For example, zinc sulfate and zinc acetate have higher absorption than zinc oxide and zinc carbonate. These findings have implications on zinc supplementation programs.

Long-term zinc intake or zinc status can also affect absorption of dietary zinc. Individuals with poor zinc status absorb more efficiently than those with good zinc status. It appears that homeostatic mechanisms up-regulate zinc absorption and retention at low levels of zinc intake.

8.6.2 Protein quantity and quality

The amount and type of protein in a meal will affect zinc bioavailability. In general, fractional zinc absorption increases linearly with increasing protein content. The presence of animal protein substantially enhances the efficiency of absorption. Soluble low molecular-weight organic substances such as sulfur amino acids bind zinc and facilitate its absorption.

Zinc bioavailability from breast milk is greater than that of cow's milk. Casein has been shown to have a negative effect on zinc absorption, due to the binding of zinc by phosphorylated serine and threonine residues on partially undigested casein subunits. Studies on the effect of various protein sources are often confounded by the presence of other constituents that may inhibit zinc absorption. For example, zinc absorption from infant formula based on dephytinized soy protein was as good as that from milk-based infant formula.

8.6.3 Phytate and fiber

Phytates are present in plant foods, particularly cereals such as maize, bran and legumes, and phytic acid serves as a storage of phosphorous for plants. Inositol hexaphosphates and pentaphosphates (phytic acids) bind cations such as zinc and form poorly soluble complexes that result in reduced absorption of zinc. It has been suggested that the phytate-to-zinc molar ratio can be used to estimate zinc bioavailability from the diet. The effect of phytate on zinc absorption can be modified by the presence of dietary protein in the meal.

It is often suggested that fiber has a negative effect on zinc absorption. However, this is usually due to the fact that most fiber-containing foods also contain phytate. Reducing the phytate content of bread, for example, by leavening using enzymatic action of yeast and other fermentation means, markedly increase zinc absorption.

8.6.4 Minerals

Bivalent cations tend to compete with zinc for binding ligands in the intestine lumen and for receptor sites in the enterocytes.

Based on tracer and supplementations studies, it appears unlikely that calcium per se has a negative effect on zinc absorption. Dietary calcium content may, however, affect zinc absorption from phytate–containing meals. Calcium has the propensity to form complexes with phytate and zinc that are insoluble and consequently have an inhibitory effect on zinc absorption. The form of calcium also has an effect, e.g. calcium phosphate decreases zinc absorption while calcium citrate-malate has no negative effect on zinc absorption.

Very high ratio of iron to zinc and in water solutions were found to reduce zinc absorption. However, the interaction of iron on zinc was found to be less pronounced when zinc intake is closer to a "physiological" level. Studies have also shown that iron in fortified foods had no significant negative effect on zinc absorption. Long-term use of iron supplements has not been found to impair zinc absorption or zinc status.

Interaction between copper and zinc has been described in experimental animals albeit at high ratios. Modest increased intake of copper was not found to interfere with zinc absorption in humans. However, it remains to be studied whether high copper intake affects zinc absorption when zinc intake is low. Such a situation may exist in countries where the drinking water is contaminated with copper and the dietary intake of zinc is low.

8.6.5 Other food components

Polyphenols including tannins that are found in tea (green and black), coffee (regular and decaffeinated), wines, various herbs and fruits are known to inhibit zinc absorption. Their hydroxyl radicals give them a strong affinity for minerals such as zinc, iron, and copper. Oxalates in spinach and rhubarbs are also known to chelate minerals including zinc, thus reducing the absorption of the minerals.

The content of promoters and inhibitors of zinc absorption in the diet therefore determines the fraction of dietary zinc that is potentially absorbable. The efficiency of absorption of potentially available zinc is inversely related to the zinc content in the diet.

8.7 Estimating Requirements and Recommended Intakes

The main references used in deriving recommended zinc intakes for Southeast Asian countries are the FAO/WHO Expert Consultation report of 2002 (FAO/WHO, 2002), the DRI Committee report of 2001 (IOM, 2001) and the IZiNCG report of 2004 (IZiNCG, 2004).

Due to the lack of specific and sensitive indices for zinc status that reflect a response to dietary intake, determination of zinc requirements cannot be derived directly from present epidemiologic data. Thus, FAO/WHO (2002), IOM (2002) and IZiNCG (2004) utilized an indirect factorial approach for most age and physiologic groups to estimate the average physiologic zinc requirement, which is defined as the amount of zinc that must be absorbed to match the amount of endogenous zinc losses. Total endogenous zinc losses comprise (a) intestinal excretion, which is a major factor in the maintenance of zinc homeostasis, and (b) non-intestinal excretion i.e. through the kidney (urine) and integument (sweat) with smaller quantities in semen and menstrual losses. Non-intestinal losses of endogenous zinc are generally constant across a wide range of zinc intake (4 mg/day to 15 mg/day). In contrast, intestinal excretion of endogenous zinc varies widely in relation to the amount of absorbed zinc. In growing children and pregnant women, the rate of zinc retention in the newly accrued tissues were also included in the calculation of total physiologic zinc requirements. In lactating women, the zinc concentration in breast milk at different stages is also added to the requirements.

8.7.1 Conceptual differences between FAO/WHO, IOM and IZiNCG in considering zinc requirements

(*a*) *Basis of Studies*

In determining the total non-intestinal and intestinal endogenous losses, FAO/WHO, the DRI Committee of IOM and IZiNCG differed to some extent in their estimations of zinc losses from urine, semen, menses and body surface area. For example, IZiNCG concurred with the conceptual approach of the DRI Committee, which estimated the mean zinc urinary excretion of 0.63 mg/day and 0.44 mg/day for adult men and women respectively, based on the larger number of published studies reviewed and studies in which the zinc intakes were unlikely to influence urinary zinc excretion. In contrast, FAO/WHO derived its estimation from only 2 studies of men and 1 study of women with restricted zinc intakes and subsequently factored in 40% to adjust for the degree of reduction in urinary zinc excretion that occurred in these low zinc intakes. Unlike FAO/WHO, both the DRI Committee and IZiNCG included zinc loss in semen of 0.1 mg/day. In estimating the surface losses of zinc, IZiNCG and the DRI Committee applied similar values of 0.0065 mg/kg body weight for adult men and women. FAO/WHO, however, reported a lower value for surface losses of zinc in men and women. Both FAO/WHO and IZiNCG did not estimate menstrual zinc losses while the DRI Committee estimated an average menstrual zinc loss to be 0.1 mg/day. For the estimation of intestinal zinc losses, the 3 organizations applied different conceptual approaches based on the number and scope of the studies reviewed and consequently recommended different estimates.

(*b*) *Sources of Zinc Loss*

Based on these different approaches, FAO/WHO, the DRI Committee and IZiNCG estimated the total endogenous zinc losses in adult men and women by source of loss. The sources of zinc loss which these organizations took into consideration are set out in Table 8.3. Thus, these total endogenous zinc losses represent the estimated physiological requirements for zinc absorption. The estimated amount of zinc required to meet the total endogenous losses in men ranges from 1.40 mg/day by WHO to 3.84 mg/day by IOM, while the respective values for women (non-pregnant, non-lactating) range from 1.00 mg/day to 3.30 mg/day. The estimation by IZiNCG lies in between these values for both men and women.

Table 8.3 Comparison of zinc intake requirements based on endogenous zinc losses

Sources of Endogenous Zinc Losses (mg/day)	FAO/IAEA/WHO (1996)	IOM (2001)	IZiNCG (2004)
Men			
Reference body weight (kg)	65	75	65
Urinary excretion	0.30	0.63	0.63
Integument	0.30	0.54	0.42
Semen	–	0.10	0.10
Total non-intestinal endogenous losses	0.60	1.27	1.15
Intestinal excretion of endogenous zinc	0.80	2.57	1.54
Total endogenous losses	1.40	3.84	2.69
Women			
Reference body weight (kg)	55	65	55
Urinary excretion	0.30	0.44	0.44
Integument	0.20	0.46	0.36
Menstrual blood	-	0.10	0
Total non-intestinal endogenous losses	0.50	1.00	0.80
Intestinal excretion of endogenous zinc	0.50	2.30	1.06
Total endogenous losses	1.00	3.30	1.86
Additional requirements for pregnancy			
1st trimester	0.1	0.16	0.70[a]
2nd trimester	0.3	0.39	
3rd trimester	0.7	0.63	
Additional requirements for lactation			
0 - 3 months	1.4	1.35[b]	1.0[b]
3 - 6 months	0.8		
> 6 months	0.5		

Notes: [a] *A single estimate for additional zinc requirements is applied throughout pregnancy*
[b] *A single estimate for additional zinc requirements is applied throughout lactation*
Source: IZiNCG (2004)

(c) *Reference Body Weights*

FAO/WHO, the DRI Committee of IOM and IZiNCG also differ in their consideration with regards to the reference average body weight. Whilst IOM used body weights that are more appropriate for North American population, FAO/WHO used the NCHS/CDC 1997 growth reference data. For example, the body weight for men and women applied by IOM are 75 kg and 65 kg respectively, while the values used by FAO/WHO are 65 kg and 55 kg. IZiNCG believes the values of FAO/WHO are more suitable for the general population worldwide.

(d) *Bioavailability*

In considering zinc absorption, bioavailability is a key factor and FAO/WHO, the DRI Committee and IZiNCG also differ in their approaches to this issue. Table 8.4 sets out the estimates of varying dietary zinc absorption based on different types of diets as categorized by these 3 organizations.

Table 8.4 Comparison of estimated dietary zinc absorption

	FAO/IAEA/WHO (1996)		IOM (2001)		IZiNCG (2004)	
Diet Type	Highly Refined[a]	Mixed/ Refined Vegetarian[b]	Unrefined[c]	Mixed, Semi-purified, EDTA-washed soy protein	Mixed, Refined vegetarian	Unrefined, cereal-based
Study Type	Single Meal and Total Diet		Total Diet		Total Diet	
Subjects	NA[d]	NA	NA	Men (19-50 yrs)	Men and Women (>20 yrs)	
Phytate:Zinc Molar ratio	< 5	5 – 15	> 15	NA	4 – 18	> 18
Zinc Absorption [e]	50%	30%	15%	41%	26% M 34% F	18% M 25% F

Notes: [a] *Refined diets low in cereal fiber and animal foods provide the principle source of protein. Includes semi-purified formula diets.*
[b] *Mixed diets and lacto-ovo-vegetarian diets that are not based on unrefined cereal grains or high extraction rate (> 90%) flours.*
[c] *Cereal-based diets with > 50% of energy intake from unrefined cereal grains or legumes and negligible animal protein intake.*
[d] *NA = not available*
[e] *Critical level of zinc absorption or level of zinc intakes are just sufficient to meet physiologic requirements for absorbed zinc*
Source: IZiNCG (2004)

(*i*) *FAO/WHO (2002)*

In determining zinc absorption according to its bioavailability, FAO/WHO used data from a combination of single meal studies and total-diet studies. Based on these considerations and other data from zinc absorption studies, 3 categories of diets were identified as having high, moderate and low zinc bioavailability. The potential bioavailability of zinc is described according to the type of diet:

- High zinc bioavailability
  The diet consists of refined foods low in cereal fiber, phytic acid content and phytate-zinc molar ratio less than 5; has non-plant sources of zinc such as meats, fish, certain seafoods and poultry. This type of diet is assumed to have 50% bioavailability.

- Moderate zinc bioavailability
  Mixed diet with animal or fish protein; lacto-ovo, ovovegetarian or vegan diets not based primarily on unrefined cereals or high-extraction-rate flours; the range of phytate-zinc molar ratio is 5-15 or not exceeding 10 if more than 50% of the energy intake is from unfermented, unrefined cereals and flours; the diet is fortified with inorganic calcium salts (> 1 g Ca^{2+} / day). This type of diet is assumed to have 30% bioavailability.

- Low zinc bioavailability
  The diet consists of highly unrefined, unfermented and ungerminated cereals with fortification of inorganic calcium salts (> 1 g Ca^{2+} / day) and negligible intake of animal food sources; the diet includes high-phytate soy-protein, cereal (wheat, rice, maize, oatmeal, millet), legume and lentil products. The assumed bioavailability of this type of diet is 15%.

Average normative zinc requirements in terms of μg/kg body weight/day for each age group and gender were computed for populations consuming high, moderate and low levels of zinc bioavailability diets (FAO/WHO, 2002). For example, an adult female aged 18 to 60 years would require 36 μg/kg body weight/day, 59 μg/kg body weight/day or 119 μg/kg body weight/day of zinc, depending upon whether her diet has high, moderate or low zinc bioavailability, respectively.

(ii) DRI Committee (IOM, 2001)

The DRI Committee used only total-diet studies to estimate dietary zinc absorption. These studies however, were limited to North American or Western European adult male subjects and the diet types were described as both mixed types and semi-purified formula diets. In estimating zinc absorption, the DRI Committee used the regression relationship between zinc intake and absorbed zinc. For example, given that the total absorbed zinc of an adult man is 3.84 mg/day, he would thus need to ingest 9.4 mg/day. Similarly for women, 6.8 mg/day of zinc needs to be consumed to provide 3.3 mg/day of absorbed zinc that would, in turn, match the total endogenous losses. These values provided fractional absorptions of 0.41 and 0.48 for men and women, respectively.

(iii) IZiNCG (2004)

While the IZiNCG concurred with IOM that total–diet studies provide the most valid estimates of dietary zinc absorption, it however did not agree with the use of absorption studies that included semi-purified diets or other diets that did not represent typical diets consumed by populations. The IZiNCG selected studies that had applied radio- or stable-isotopes to estimate true zinc absorption, and studies of typical mixed, refined vegetarian, or unrefined, cereal-based diets. For each type of diet, IZiNCG provided different zinc absorption estimates for men and women. However, for children 1 to 18 years of age, the mean of zinc absorption estimates for men and women from each diet type was applied (i.e. 31% absorption from mixed/refined vegetarian diets and 23% from unrefined, cereal-based diets). The estimates for zinc absorption are applied to compute the physiologic requirements for absorbed zinc, and from which the EAR and RDA values are further derived.

(e) *Estimating Recommended Intakes*

The RDA (recommended nutrient intake level which theoretically will meet almost all (97.5%) individuals' physiologic requirement) is commonly set at 2 SD above EAR (at which 50% of individuals should meet their physiologic requirements). To estimate variability in zinc requirement (i.e. the CV of the requirement distribution), the 3 organizations adopted different values for the CV. FAO/WHO (2002) assumed the CV for zinc requirements as 25% and thus the RDA was set at 150% of EAR. IOM (2001) assumed the CV as 10% and thus the RDA was set as EAR plus two times of CV (2 x 10%) or 120% of EAR. IZiNCG (2004) estimated 12.5% variability in zinc requirement and calculated the RDA as the EAR plus 2 times the CV (2 x 12.5%) or 125% of EAR. However, the difference in the RDA with CV of 10% or 12.5% is negligible.

8.7.2 Recommendations for zinc intake by life stages

(*a*) *Infants* (0 – 6 *months*)

Breast milk is assumed to have the highest zinc bioavailability compared to other types of milk or milk mixtures. For example, breast milk supplemented with whey-adjusted milk formula, and low-phytate food supplements with other liquid milk have moderate zinc bioavailability, while phytate-rich vegetable protein based formula (e.g. soy) with or without whole grain cereals have low zinc bioavailability.

The DRI Committee did not have estimations for the physiologic requirement for zinc for infants. Instead, it proposes an AI value, based on the content of zinc in breast milk at different ages and the average amount of milk consumed. The DRI Committee assumed that 50% of the zinc in breast milk is available for absorption and that the average breast milk consumption is 0.76L/day. The DRI Committee set the AI level at 2.0 mg/day (2.5 mg/L x 0.78 L/day) in order to provide adequate zinc in the early weeks of life. The AI figures match those computed by factorial estimates of requirements for infants 0 to 6 months old, i.e. 2.1 mg/day, at 1 month and 1.54 mg/day at 5 months. The factorial estimates are based on measurements of zinc intake of breast-fed infants, fractional absorption, and endogenous losses.

Like the DRI Committee, IZiNCG assumed 50% absorption of zinc from breast milk but differed in the estimated volume of breast milk consumed. IZiNCG estimated the amount of breast milk consumed to be 714 ml/day at 1 to 2 months, 784 ml/day at 3 to 5 months. The amount of breast milk consumed decreases to 549 ml/day by the ages of 12 to 23 months. IZiNCG is of the opinion that breast milk can provide sufficient amount of zinc for the first 6 months of life.

In contrast, FAO/WHO attempted to estimate the physiologic zinc requirements of young infants by extrapolating from data for adults in relation to metabolic rate and thereafter adding the amount of zinc in the newly deposited tissue. The endogenous zinc losses were assumed to be about 20 μg/kg/day to 40 μg/kg/day for breast-fed infants and infants fed with formula or weaning foods, respectively. The estimated zinc required for growth in the first 3 months of life were set at 120 μg/kg/day for male infants and 140 μg/kg/day for female infants. The FAO/WHO assumed that the absorption of zinc from breast milk was 80% but this figure was derived from a single study.

(*b*) *Infants* (6 - 12 *months*)

For infants aged 7 and 12 months, breast milk provides only 0.5 mg/day and 0.39 mg/day of zinc respectively. The NHANES III data showed that the median zinc intake from complementary foods is 1.48 mg/day for older infants consuming breast milk. Thus, the average zinc intake from breast milk and complementary foods is estimated to be about 2 mg/day (0.5 + 1.48).

The DRI Committee (IOM, 2002) and IZiNCG (2004) used a factorial method to estimate the physiologic zinc requirement for infants in this age group. Total endogenous zinc losses were estimated to be 0.064 mg/kg/day (i.e. fecal excretion 0.050 mg/kg/day, non-intestinal losses 0.014 mg/kg/day). Zinc requirement for growth was set at 0.020 mg/g of tissue gained. These figures for endogenous losses and zinc required for growth were then multiplied, respectively, by the reference body weight and the expected rate of weight gain for this age range.

To estimate the EAR for breastfeeding infants of 6 to 11 months of age, IOM (2002) and IZiNCG (2004) utilized the same fractional zinc absorption from breast milk as 50%. However, the 2 organizations differ in their estimates of average breast milk consumption and zinc concentrations for each age group, therefore their estimated average zinc intake from breast milk are also different. The amount of absorbed zinc required from complementary foods is determined as a difference between the required absorbed zinc (0.836 mg/day) and the amount of zinc ingested from the milk (fractional absorption of 0.5 x average zinc intake from human milk). The EAR for breast-fed infants is then calculated as the amount of zinc from breast milk plus the amount of zinc from complementary foods, assuming fractional absorption of zinc is 30% from complementary foods.

FAO/WHO (2002) estimated the endogenous zinc losses in this age group (0.57 μg/basal kcal) from the estimates in adults. The estimated zinc increase for infants aged 6 to 12 months was set at 0.033 mg/kg/day. Assuming that infants in this age group are already supplemented with foods of moderate bioavailability, the physiologic requirement for zinc is set at 0.311 mg/kg body weight/day as recommended by FAO/WHO (2002).

(c) *Children and Adolescents (1 – 19 years)*

Physiologic zinc requirements for children and adolescents are estimated from the factorial method which included intestinal and urinary losses and requirements for growth. For children and adolescents, the DRI Committee (IOM, 2002) estimated the total endogenous zinc losses to be 0.048 mg/kg/day. Zinc requirements for growth of 0.020 mg/g of tissue gained is then added to these endogenous losses to derive at physiologic zinc requirements. An additional 0.1 mg/day was included in the estimated physiologic requirements for male adolescents aged 14 to 18 years to account for zinc losses in the semen (IOM, 2002).

IZiNCG (2004) estimated the non-intestinal zinc losses (0.014 mg/kg/day) and zinc content for tissue accrual (0.020 mg/g) to be similar to the values indicated by the DRI Committee. However, a lower estimate of intestinal losses of endogenous zinc (0.02 mg/kg/day) was utilized to estimate the total endogenous losses for children of 0.034 mg/kg/day.

FAO/WHO (2002) estimated the endogenous losses for older infants and children (0.57 ug/basal kcal) from the estimates in adults. For children aged 1 to 10 years, growth requirements were based on the assumption that new tissue contains 30 μg/g (0.030 mg/g) zinc. During adolescence, a zinc content of 23 μg/g (0.023 mg/g) increase in body weight was assumed. For the various age groups, FAO/WHO (2002) set the physiologic requirements for zinc as follows:

Age Groups (years)	Zinc Requirement (mg/kg body weight/day)
Children (years)	
1 – 3	0.230
4 – 6	0.190
7 – 9	0.149
Boys (years)	
10 – 12	0.133
13 – 15	0.126
16 – 18	0.102
Girls (years)	
10 – 12	0.113
13 – 15	0.107
16 – 18	0.093

It is noted that the DRI Committee's RDA values for zinc for children and adolescents are closest to the FAO/WHO values in the moderate zinc bioavailability category. In the case of Southeast Asian countries, with diets that tend to include lower amounts of animal products and higher amounts of plant-based foods containing phytate, as compared to Western diets. It is thus prudent for these countries to adopt the amounts in the moderate or low bioavailability categories.

(*d*) *Adults (≥ 19 years)*

The physiologic requirement for zinc by adult men is defined as the amount of zinc that must be absorbed to counterbalance the sum of endogenous zinc lost through all routes of excretion. The DRI Committee (IOM, 2002) and IZiNCG (2004) estimated mean urinary zinc excretion to be 0.63 mg/day based on recommended zinc loss through the body surfaces to be 6.5 μg/kg. Both organizations took into consideration zinc loss of 0.1 mg/day in the semen. As for intestinal zinc losses, the DRI Committee and IZiNCG differed in their estimations, with the DRI Committee recommending a higher estimate of 2.57 mg/day compared to IZiNCG's recommendation of 1.54 mg/day.

FAO/WHO (2002) estimated a lower level of 0.3 mg/day for urinary zinc losses. Intestinal and integument zinc losses were estimated by FAO/WHO to be 0.8 mg/day and 0.3 mg/day, respectively. However, FAO/WHO did not estimate zinc loss in the semen.

All 3 organizations applied the same principles of computing endogenous zinc losses in men to estimate the total endogenous zinc losses in women. While the DRI Committee accounted for menstrual zinc loss in the total endogenous zinc losses, IZiNCG and FAO/WHO did not include the estimate of zinc loss in menstrual fluid. Similarly for men, the differences in the calculation of total endogenous losses for women by the 3 organizations resulted in different estimates of physiologic zinc requirement and the consequent recommended intake of zinc.

FAO/WHO (2002) is of the opinion that there is no consistent evidence to indicate that aging adversely affects absorption of zinc. Therefore, zinc requirements for the elderly are assumed to be similar to those for other adults. Nevertheless, zinc requirement for the elderly may be different due to lower efficiency of zinc absorption and possible differences in zinc metabolism among the elderly.

(e) *Pregnancy*

Additional zinc is required during pregnancy by maternal and embryonic tissues. The physiologic zinc requirement during the third trimester is double that of non-pregnant women (FAO/IAEA/WHO, 1996). Estimated increases in dietary zinc requirements (mg/day) during pregnancy are set out as follows:

Trimester	FAO/WHO (2002)	IOM (2001)	IZiNCG (2004)
1st	0.1	0.16	0.70
2nd	0.3	0.39	0.70
3rd	0.7	0.63	0.70

These additional zinc requirements for each trimester of pregnancy should be added to the zinc physiologic requirements for absorbed zinc of adolescent or adult women.

(*f*) *Lactation*

Zinc requirements for lactating women vary according to the zinc content of breast milk and the amount of milk produced during the first and second 6 months of lactation. Zinc content in breast milk is high in early lactation, 4 mg/L in the first 2 weeks, and 2 mg/L to 3 mg/L in the second month. It declines to 0.9 mg/L after 3 months. Based on mean daily output of zinc in milk (1.4 mg/L) during the first 3 months of lactation, it is estimated that the physiologic zinc requirements for lactating women would be 3 times that of non-lactating, non-pregnant women.

Using the age specific information on zinc content in breast milk at 4, 8 and 12 weeks of lactation, and the estimated volume of 0.78 L/day of breast milk measured during the first year post-partum, the DRI Committee (IOM, 2002) estimated a total of 1.35 mg/day as the average increased requirements for absorbed zinc during lactation. This estimate has accounted for the subtraction of 1.0 mg/day of zinc loss during the first 4 weeks of lactation. This amount of zinc, approximately 30 mg or averaging 1.0 mg/day for the first month of postpartum, has been accumulated during pregnancy and is available for re-utilization.

IZiNCG (2004) estimated an additional amount of 1.0 mg/day for absorbed zinc during lactation. This value is derived from estimates of zinc transfer in breast milk using the milk output data of women from the developing countries. For example, the amount of zinc transferred from mother to child in breast milk for infants aged 0 to 2 months (1.64 mg/day) is calculated by multiplying the estimated milk volume of 0.714 L/day with zinc concentration in breast milk of 2.3 mg/L.

FAO/WHO (2002) estimated zinc concentrations in breast milk as 2 to 3 mg/L at 1 month, 0.9 mg/L at 3 months and 0.7 mg/L at 4 months. Based on information on maternal milk volume and zinc concentration in breast milk, the expert committee estimated the average additional zinc amounts during the first year of post partum as 1.4 mg/day (0 to 3 months), 0.8 mg/day (3 to 6 months) and 0.5 mg/day (more than 6 months). For early lactation, both FAO/WHO and IZiNCG concurred with the DRI Committee that part of the requirement

for early lactation is covered by postnatal involution of the uterus, skeletal resorption and decreased maternal blood volume (King and Turnlund, 1989; FAO/IAEA/WHO, 1996). The SEA-RDA for lactating women is based on the additional requirement for absorbed zinc for lactation by FAO/WHO (2002) and on diets of moderate zinc bioavailability.

Table 8.5 summarizes the intakes for zinc recommended by FAO/WHO, IOM and IZiNCG.

Table 8.5 Comparison of recommended intake values for zinc

IOM (2001)		FAO/WHO (2002)				IZiNCG (2004)		
Age Groups	AI (mg/day)	Age Groups	RDA (mg/day)			Age Groups	AI (mg/day)	
			High Bioavailability	Moderate Bioavailability	Low Bioavailability			
Infants (months)		Infants (months)				Infants (months)		
0 – 6	2.0	0 – 5	1.1[a]	2.8[b]	6.6[b]	0 – 2	1.64	
					3 – 5		1.06	
RDA (mg/day)								
7 – 12	3.0	6 – 11	0.8[a] 2.5[c]	4.1	8.4		RDA (mg/day)	
							Mixed or refined vegetarian diets	Unrefined, cereal-based diets
						6 – 11	4.0	5.0
Children (years)		Children (years)				Children (years)		
1 – 3	3.0	1 – 3	2.4	4.1	8.3	1-3	3.0	3.0
4 – 8	5.0	4 – 6	2.9	4.8	9.6	4-8	4.0	5.0
		7 – 9	3.3	5.6	11.2	9-13	6.0	9.0
Male		Male				Male		
9 – 13	8.0	10 – 18	5.1	8.6	17.1	10-18	10.0	14.0
14 – 18	11.0							
Female		Female				Female		
9 – 13	8.0	10 – 18	4.3	7.2	14.4	14-18	9.0	11.0
14 – 18	9.0							
Male		Male				Male		
19 – 70	11.0	19 – 65	4.2	7.0	14.0	≥19 yrs	13.0	19.0
> 70	11.0	> 65	4.2	7.0	14.0			
Female		Female				Female		
19 – 70	8.0	19 – 65	3.0	4.9	9.8	≥19	8.0	9.0
> 70	8.0	> 65	3.0	4.9	9.8			
Pregnancy (Age, yrs)		Pregnancy				Pregnancy (Age, yrs)		
14 – 18	12.0	1st trimester	3.4	5.5	11.0	14-18 yrs	11.0	15.0
19 – 30	11.0	2nd trimester	4.2	7.0	14.0	≥19 yrs	10.0	13.0
31 – 50	11.0	3st trimester	6.0	10.0	20.0			
Lactation (Age, yrs)		Lactation (months)				Lactation (Age, yrs)		
14 – 18	13.0	0 – 3	5.8	9.5	19.0	14-18	10.0	11.0
19 – 30	12.0	4 – 6	5.3	8.8	17.5	≥19	9.0	10.0
31 – 50	12.0	7 – 12	4.3	7.2	14.4			

Notes: [a] *Breast-fed*
[b] *Formula-fed*
[c] *Not applicable to infants consuming breast milk only*

8.8 Current RDAs for Zinc in Southeast Asia

Current recommended daily zinc intakes for Indonesia, Malaysia, the Philippines, Singapore and Thailand are set out in Table 8.6.

Table 8.6 Comparison of current RDAs for zinc (mg/day) in selected Southeast Asian countries

Age Groups (years)	Indonesia (2002)	Malaysia (2005)	Philippines (2002)	Singapore (2003)	Thailand (2003)
Infants (0 – 1)	3 – 5	1.1 – 3.7	1.4 – 4.2	[a]	3[b]
Children (1 – 9)[c]	10	4.1 – 5.8	4.5 – 5.4	10	2 – 4
Boys (10 – 18)[d]	15	9.0	6.8 – 8.9	10 – 15	5 – 9
Girls (10 – 18)[d]	15	7.5	6.0 – 7.9	10 – 12	5 – 7
Men (≥ 19)[e]	15	6.2 - 6.7	6.4	15	13
Women (≥ 19)[e]	15	4.3 - 4.9	4.5	12	7
Pregnancy					
1st trimester	20	5.5	5.1	15	9
2nd trimester	20	7.0	6.6	15	9
3rd trimester	20	10.0	9.6	15	9
Lactation					
1st 6 months	25	8.8	11.5	19	8
2nd 6 months	25	7.2	11.5	19	8

Notes: [a] *No values given*
[b] *Figures only for infants 6-11 months*
[c] *1 - 8 years for Thailand*
[d] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[e] *≥ 18 for Singapore*
Source: Indonesia: MOH Indonesia (2002); Philippines: FNRI (2002); Malaysia: NCCFN (2005); HPB Singapore (2003); Thailand: MPH (2003)

There are considerable differences in the RDAs for zinc in the 5 Southeast Asian countries reviewed. The Indonesian recommended intakes for zinc for all age groups, including during pregnancy and lactation, tend to be higher than those of the other 4 countries. Singapore's recommended intakes are slightly lower than those of Indonesia, followed by Thailand. The Malaysian and Philippines RDA for zinc are rather similar. Except for Indonesia, girls and women are generally recommended lower intakes compared to the boys and men.

All of these countries have recommended additional zinc intake during pregnancy, with Indonesia, Singapore and Thailand recommending an increase in zinc intake of about 30% throughout pregnancy. Malaysia and Philippines have recommended increases in zinc intake with advancing pregnancy, with an addition of 13% above the requirement before pregnancy in the first trimester to 100% in the third trimester. For lactation, Indonesia, Philippines and Singapore recommended a further increase of 20% of zinc intake above the RDA during pregnancy. Malaysia and Thailand also recommend additional amounts during lactation; a marginal amount of 14% above non-lactating women in the case of Thailand whereas Malaysia recommends a large increase of 60%.

8.9 Recommended RDAs for Zinc for Southeast Asia

While the conceptual approach in estimating human physiological needs of zinc differs somewhat among FAO/WHO, the DRI Committee and IZiNCG, their recommendations for life stages from birth through adolescence are quite similar. However, in adulthood and for pregnancy and lactation, the recommended values of the DRI Committee and IZiNCG are generally considerably higher than those of FAO/WHO. In the younger age groups, the recommendations of the DRI Committee and IZiNCG tend to lie between the low to moderate zinc bioavailability values of FAO/WHO.

Based on these considerations and the recommended values of some countries in Southeast Asia, the SEA-RDA Committee proposed that Southeast Asian countries in general follow the recommendations of FAO/WHO (2002), using the values in the low to moderate zinc bioavalability values that are indicative of the socio-economic and cultural situation in the region. Using the reference body weights adopted by the SEA-RDA Committee (Table 2.1), the recommended RDAs for zinc for Southeast Asia are summarized in Table 8.7.

Table 8.7 Recommended RDAS for Zinc for Southeast Asia (assuming moderate bioavailability[a])

Age Groups	Zinc RDA (mg/day)
Infants (months)	
0 – 5	1.1[b], 2.9[c]
6 – 11	4.2
Children (years)	
1 – 3	4.8
4 – 6	5.7
7 – 9	6.0
Boys (years)	
10 – 12	6.8
13 – 15	8.9
16 – 18	8.6
10 – 18	8.1[d]
Girls (years)	
10 – 12	6.1
13 – 15	7.2
16 – 18	6.8
10 – 18	6.7[d]
Men (years)	
19 – 65	6.5
> 65	6.5
Women (years)	
19 – 65	4.4
> 65	4.4
Pregnancy	
1st trimester	5.5
2nd trimester	7.0
3rd trimester	10.0
Lactation	
0 – 3 months	9.5
4 – 6 months	8.8
7 – 12 months	7.2

Notes: [a] *Moderate bioavailability level of 30%: mixed diets containing animal or fish protein; phytate-zinc molar ratio of 5 : 15.*
[b] *Exclusively breast-fed infants ; assumed 80% availability; assumed CV of 12.5%; based on average of 3 values of normative requirements of 175, 200 and 79 µg/kg/day*
[c] *Formula-fed infants: moderate bioavailability for whey-adjusted milk formula and for infants partly human-milk-fed or given low-phytate feeds supplemented with other liquid milks; CV of 12.5%; based on average of 3 values of normative requirements of 547 µg/kg/day, 514 µg/kg/day and 204 µg/kg/day.*
[d] *Mean of the RDA for the 3 age groups, 10 to 12 years, 13 to 15 years and 16 to 18 years.*

Bibliography

FAO/IAEA/WHO (1996). *Trace Elements in Human Nutrition and Health*. World Health Organization, Geneva.

Brown KH, Peerson JM and Allen LH (1998). Effect of zinc supplementation on children's growth: a meta-analysis of intervention trials. *Biblio Nutr Diet* 54: 76-83.

Dardenne M (2002). Zinc and immune function. *Eu J Clin Nutr* 56, Suppl 3: S20-S23.

FAO/WHO (2002). Zinc. In: *Human Vitamin and Mineral Requirements*. Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 257-270.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines*. 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

IOM (2001). Zinc. In: *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC; pp 442-501.

IZiNCG (2004). Assessment of the Risk of Zinc Deficiency in Populations and Options for its Control. International Zinc Nutrition Consultative Group Technical Document #1. *Food Nutr Bull* 25(1): 94S-203S.

Jameson S (1993). Zinc status in pregnancy: the effect of zinc therapy on perinatal mortality, prematurity and placental ablation. *Ann NY Acad Sci* 678: 178-192.

King JC, Shames DM and Woodhouse LR (2000). Zinc homeostasis in humans. *J Nutr* 130:1360S-1366S.

King JC and Keen CL (1994). Zinc. In: Modern Nutrition in Health and Disease. Shils, ME, Olson JA and Shike M (eds). 8th Edition, Vol. 1. Lea and Febiger, Philadelphia.

Krebs NF (2000). Overview of zinc absorption and excretion in the human gastrointestinal tract. *J Nutr* 130: 1374S-1377S.

Krishna SS, Majumdar I and Grishin NV (2003). Structural classification of zinc fingers. *Nucleic Acids Res* 31:532-550.

Lonnerdal B (2000). Dietary factors influencing zinc absorption. *J Nutr* 130: 1378S-1383S.

MOH Indonesia (2002). Angka kecukupan gizi rata-rata yang dianjurkan (per orang per hari). Keputusan Menteri Kesehatan RI, Nomor 913/MEN/KES/VIII/2002, Ministry of Health, Indonesia.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Osendarp SJM, West CE and Black RE (2003). The need for maternal zinc supplementation in developing countries: an unresolved issue. *J Nutr* 133(suppl.): 817S-827S.

Pluhator MM, Thomson ABR and Fedorak RN (1996). Clinical aspects of trace elements: zinc in human nutrition and toxicity. *Canadian J Gastroenterol* 10:97-103.

HPB Singapore (2003). Food Composition Guide. Health Promotion Board, Singapore. pp 162.

Thompson CB (1995). Apoptosis in the pathogenesis and treatment of disease. *Science* 267: 1456-1462.

Wu H, Yang WP and Barbas CF (1995). Building zinc fingers by selection: toward a therapeutic application. *Proc Natl Acad Sci USA* 92: 344-348.

9 SELENIUM

9.1 Introduction

Interest in human nutritional requirements for selenium initially stemmed from the recognition of its essential role in animal nutrition. Thus, in the past, estimates for selenium requirements for humans were derived from animal data. For example, in the 1980 edition of the United States (US) RDA, recommendations for selenium were extrapolated from the selenium requirements of mammalian animal species (NRC, 1980). From 1980 onwards, human selenium nutrition research accelerated markedly with 2 studies, one from China and the other from New Zealand. These studies made a profound impact on the course of human selenium research during the subsequent decades.

The role of selenium in human nutrition, particularly its function as part of the enzyme glutathione peroxidase, which protects vital components of the cell against oxidative damage, is well recognized. FAO/WHO, the US, Canada, Australia, New Zealand, Japan and the European Commission now include dietary recommendations for selenium.

9.2 Characteristics and Functions

Selenium is an essential, tightly bound, cofactor for glutathione peroxidase (GPX). GPX requires reduced glutathione (GSH) as a cosubstrate. GSH, a tripeptide of glycine, cysteine and glutamate, is found in most cells of the body and furnishes reducing equivalents in reactions. GPX enzymes designated as GPX1, 2, 3, and 4 are all selenium-dependent. GPX catalyzes the reduction of both organic peroxides and hydrogen peroxides which, if not removed, can damage cellular membranes.

Selenium is also necessary for iodine metabolism. 5'-Iodothyronine deiodinase (type I) has been shown to be a selenoprotein with a single selenium atom at its active site. It is found in the endoplasmic reticulum of the liver, kidney and muscle.

Some of the less defined roles of selenium include its involvement in the maintenance or induction of the cytochrome P450 system, in pancreatic function, in DNA repair and enzyme activation, immune system function, and detoxification of heavy metals.

9.2.1 Forms of Selenium

Selenium is a non-metal that exists in many ionic forms. The chemistry of selenium is similar to that of sulfur; consequently, selenium can substitute for sulfur in amino acids such as methionine, cysteine, and cystine (Groff and Grooper, 1997). These forms are readily absorbed from the diet. Selenium has a high toxic potential since there appears to be no physiological control of how much can be absorbed by humans.

9.2.2 Selenium forms in foods

Selenium occurs naturally in foods almost exclusively in the form of organic compounds, primarily selenomethionine, selenocystine, selenocysteine, and Se-methyl selenomethionine. These organic forms represent the selenium analogs of sulfur-containing amino acids which are incorporated into the plant proteins. The inorganic forms of selenium, which include selenite (H_2SeO_3) and selenate (H_2SeO_4), may also be found in some vegetables.

In animal foods, there are specific selenium proteins where selenium is incorporated via selenide as selenocysteine, while selenomethionine, and possibly also selenocysteine to some extent, are non-specifically incorporated as analogues to methionine and cysteine in foods of both animal and plant origin. Selenomethionine, as well as the inorganic forms selenite and selenate, are the most common forms of selenium in food supplements and fodder additives. It is uncertain if the inorganic forms occur in foods. A number of uncharacterized forms exist (e.g., in fish), but their contribution to total dietary selenium is unknown (EC, 2000).

Selenium forms used in supplements are inorganic selenite and selenate and organic selenium in the form of selenomethionine, selenocysteine and selenium-enriched yeast. The forms of selenium found in yeast vary according to production processes and it has been suggested that selenomethionione comprises 20% to 50% of the selenium and that some is bound as selenotrisulphides (EC, 2000).

9.2.3 Selenium forms in blood and tissues

Most selenium in animal tissues is present in 2 forms: (a) selenomethionine, which is incorporated in place of methionine in a variety of proteins; and, (b) selenocysteine in selenoproteins such as glutathione peroxidase, iodothyronine deiodinase, and selenoprotein P. Other forms may be present since tissue selenium has not yet been fully characterized (Levander and Burk, 1996).

Selenomethionine in tissues is derived from the diet because it cannot be synthesized in the body. This form of selenium is not regulated by selenium status and can be regarded as an unregulated storage compartment. When dietary selenium supply is uninterrupted, the turnover of the selenomethionine pool provides selenium to the organism. This has been demonstrated in individuals who moved from areas of high selenium intake to areas of low intake. Blood selenium levels declined slowly for a year before reaching levels typical of populations in the low-selenium area. Glutathione peroxidase activity in red blood cells was similar in subjects residing in the 2 areas, suggesting that the fall in selenium concentration was at the expense of selenomethionine, with selenocysteine being maintained (Levander and Burk, 1996).

Selenocysteine is the form of selenium known to account for its biological activity. Most evidence suggest that the selenocysteine compartment is tightly regulated. Such regulation is necessary because this reactive compound would likely interfere with biochemical function if it were free in the cell. Selenocysteine is incorporated into proteins by a specific mechanism, and there is no evidence that it substitutes for cysteine in animal systems. Selenium incorporation into transfer

ribonucleic acid (tRNA) has been demonstrated, indicating that there may be other biologically active forms of selenium other than selenocysteine (Levander and Burk, 1996).

9.3 Absorption, Utilization and Excretion

The organic and inorganic forms of selenium are all efficiently absorbed to different extents from the gastrointestinal tract. The duodenum appears to be the primary absorptive site. Less absorption occurs in the jejunum and ileum and none is absorbed in the stomach.

Factors enhancing selenium absorption include vitamins C, A and E, as well as the presence of reduced glutathione in the intestinal lumen. Heavy metals, such as mercury and phytates, are thought to inhibit selenium absorption through chelation and precipitation. A more detailed write up on the bioavailability of different forms of selenium is given in a subsequent section.

Within tissues such as the liver, selenomethionine (derived from diet, mainly from plants) may be stored as selenomethionine in an amino acid pool used for protein synthesis, just as the amino acid methionine is used or catabolized to Se-adenosylmethionine (SeAM) to yield selenocysteine and selenocystine, similar to methionine metabolism, to generate cysteine. Selenocysteine (derived from selenomethionine metabolism or from the diet) may be degraded by selenocysteine B-lyase to yield free elemental selenium. Free elemental selenium may be attached to transfer RNA charged with serine and incorporated into the selenium-dependent enzymes for functional uses. The selenium not used as a cofactor for enzymes may be converted to selenide or to selenite, may be stored for future use, or may be excreted (Groff and Grooper, 1997).

The selenate from the diet may be converted in the body to selenite, which is further metabolized to selenodiglutathione and subsequently to selenide. Selenide may be degraded to methylselenide for excretion or may be converted into selenophosphate. Selenophosphate can be metabolized, and its selenium attached to tRNA for synthesis of 5'-deiodinase or glutathione peroxidase (Groff and Grooper, 1997).

Selenium is excreted from the body almost equally in the urine and feces. About 50% to 60% of selenium, or about 50 μg, is excreted in the urine, while the remaining 40% to 50% is excreted in the feces. Selenium losses via the lungs and skin contribute to daily selenium excretion. Renal clearance comparison studies on people with low body stores of selenium and on those with much higher levels indicate that kidneys play an important role in selenium homeostasis in humans. However, only a few (e.g., trimethyl selenonium ion) of the several metabolites of selenium have been identified in the urine.

9.3.1 Interactions with other nutrients

Lead and selenium appear to interact such that sub-clinical amounts of lead intake are found to significantly lower the tissue concentration of selenium. The speculated reason for this mechanism is that both elements bind to sulfhydryl groups. Iron deficiency in rats has been shown to decrease

the synthesis of hepatic glutathione peroxidase and to decrease hepatic selenium concentrations. The mechanism of action is believed to occur in part due to decreased transcription of the gene for glutathione peroxidase. Alternately, selenium absorption or use may be responsible.

Copper deficiency has been shown to decrease the activity of the selenium-dependent enzymes glutathione peroxidase and 5'-deiodinase. Arsenic is thought to competitively inhibit the uptake of selenium. The significance of this interaction in human nutrition has not yet been determined.

An interaction also occurs between selenium and the amino acid methionine. Dietary selenium is present in the form of selenomethionine and is readily available in most organisms. The potency of selenium in this form may be reduced if there is methionine deficiency. In a methionine-deficient state, selenomethionine substitutes for it in the synthesis of body proteins. Selenium becomes available only as these proteins become degraded in the course of normal turnover.

9.4 Effects of Deficiency and Excess

9.4.1 Deficiency

The metabolic effects of selenium deficiency include susceptibility to certain types of oxidative injury, alteration in thyroid hormone metabolism, increased susceptibility to injury by mercury, alterations in activities of biotransformation enzymes, and an increase in plasma glutathione concentration (Levander and Burk, 1996).

Selenium deficiency has been linked to regional human diseases such as Keshan disease and Kashin-Beck's disease in China. Keshan disease is an endemic cardiomyopathy that primarily affects children and women of childbearing age in some areas of China. It is characterized by cardiomyopathy involving cardiogenic shock and/or congestive heart failure, along with multifocal necrosis of heart tissue, which becomes replaced with fibrous tissue (Ge and Yang, 1993). It can be categorized clinically into 4 different types depending on its severity, namely: acute, sub-acute, chronic and insidious (IOM, 2000). Once the disease is established, selenium therapy is of little or no value. Although Keshan disease has been associated with selenium deficiency, the deficiency cannot account for all the other aspects of the disease. Conditions indicate that selenium deficiency alone seldom causes illness, but it may lead to biochemical changes that predispose to illness associated with other stresses. It has also been associated with low selenium levels in staple cereals and in samples of human blood, hair and tissue (IOM, 2000).

Keshin-Beck disease is another disease that has been linked with low-selenium status in China. It is characterized by osteoarthropathy involving degeneration and necrosis of the joints and epiphyseal-plate cartilages of the legs and arms (Ge and Yang, 1993). It is an endemic osteoarthritis that occurs during the preadolescent or adolescent years. Enlargement and deformity of the joints characterize advanced cases and the principal pathological change is multiple degeneration and necrosis of hyaline cartilage tissue.

Clinical thyroid disorders have not been reported in selenium-deficient individuals with adequate iodine intake, but based on observations in Africa, it has been postulated that infants born to mothers deficient in both selenium and iodine are at increased risk of cretinism (IOM, 2000). Predominant symptoms of deficiency included poor growth, muscle pain and weakness, loss of pigmentation of hair and skin, and whitening of nail beds. Poor growth may also be related to the role of selenium in thyroid hormone metabolism. In some patients undergoing total parenteral nutrition (TPN) devoid of selenium, several cases of biochemical selenium deficiency have been reported. Such patients have been found to have low erythrocyte glutathione peroxidase activities and low levels of selenium in plasma and red cells; and in some cases, cardiomyopathy. Patients who experienced muscular discomfort or weakness responded to selenium therapy.

In several epidemiological studies, the incidence of cardiovascular disease and cancer has been postulated to be related to selenium status. However, such studies remain inconclusive (EC, 2000; Levander and Burk, 1996).

Earlier reports from China indicated that women of childbearing age were susceptible to developing Keshan disease, whereas men were resistant. However, cases of the disease reported in the past 20 years appear to be limited to children, with equal prevalence in boys and girls. Thus, a gender effect in susceptibility to this disease may be present at extremely low selenium intakes, but no such effect has been demonstrated at current intakes.

Fertilizers used in Finland were supplemented with selenium in 1984 (Levander and Burk, 1996) to prevent any possible deleterious effects of poor selenium status on human health. As a result, selenium intake from the Finnish diet increased from 30 μg/day to 40 μg/day to almost 100 μg/day and plasma selenium concentrations in the population increased accordingly.

9.4.2 Excessive intake

Selenium toxicity or selenosis has been observed both in miners and individuals with excess selenium intake from supplements. Intakes of 750 μg or up to 27.3 mg or more per day have produced physical manifestations, as well as biochemical abnormalities (NRC, 1989). Signs of selenosis included loss of hair and nails, skin lesions, tooth decay, and abnormalities of the nervous system. The most common symptoms of human selenium poisoning are nausea, vomiting, hair loss, nail changes, irritability, increasing fatigue, peripheral neuropathy and sour milk or garlic-odor breath.

Acutely poisoned persons who experienced hair loss in 3 to 4 days may have consumed as much as 38 mg selenium per day. Human selenium poisoning from consumption of toxic foods containing high levels of selenium was also reported in China, where chronically intoxicated individuals ingested an average of 4.99 mg selenium per day in a vegetable diet. In the US, 13 reportedly poisoned persons consumed a "health food" supplement that contained about 182 times (27-2387 mg) more selenium than the amount stated in the label (Levander and Burk, 1996).

The biochemical basis of selenium toxicity is not fully understood, but several possible reaction mechanisms that have been suggested include: interference with sulfur metabolism, catalytic oxidation of sulfhydryl groups, and inhibition of protein synthesis (Levander and Burk, 1996).

The reference dose (an estimate of exposure that is likely to be without appreciable deleterious consequences over a lifetime) has been calculated to be 5 μg/kg/day. This was based on a no-observed-adverse effect-level (NOAEL) of 853 μg/day (calculated from a regression equation relating whole blood selenium concentrations to dietary selenium intakes in persons exhibiting no clinical signs of selenosis), or 15 μg/kg body weight and included an uncertainty factor of 3 (to account for insensitive individuals).

9.4.3 Guidance on high intake

A reference intake level for safety is now being introduced because of the increased use of fortification or enrichment of foods and/or increased consumption of nutritional supplements. It would thus be very useful to have information on tolerable upper intake levels (ULs). The European Commission (EC, 2000) and the DRI Committee (IOM, 2000) have set ULs, both using essentially the same database.

The Scientific Committee on Food, European Commission derived its UL using the NOAEL of 850 μg/day for clinical selenosis reported in the 1989 study on 349 subjects of Yang et al. (EC, 2000). An uncertainty factor of 3 was used to allow for remaining uncertainties in the studies used in deriving an upper level. An UL of 300 μg selenium/day derived for adults covers selenium intake from all sources, i.e., food and supplements.

According to the European Commission, no data are available to suggest that other life-stage groups have increased susceptibility to adverse effects of high selenium intakes. There are no reports of adverse effects on infants born from mothers with high intakes of selenium or adverse effects on lactating women with dietary selenium intakes below the UL for adults. Therefore, the UL of 300 μg per day should also apply for pregnant and lactating women, as well as for non-pregnant and non-lactating women. While there may be no data to support a derivation of an UL for children, there are no reports indicating that children are more susceptible to adverse effects from selenium. The European Commission thus found it appropriate to extrapolate the UL from adults to children on a body weight basis.

The DRI Committee on the other hand, derived the UL for selenium of 400 μg/day based on a NOAEL of 800 μg Se/day and an uncertainty factor of 2 (IOM, 2000). There are no reports of teratogenicity or selenosis in infants born to mothers with high (but not toxic) intakes of selenium. The UL was thus set at 400 μg/day for adults including non-pregnant and non-lactating women. The UL of 47 μg/day (approximately 7 μg/kg/day) for infants was based on the NOAEL of 47 μg Se/day divided by an uncertainty factor of 1. The NOAEL in turn was based on the study by Shearer and Hadjimarkos (1975) which showed that breast milk selenium concentration of 60 μg/day was not associated with known adverse effects. Thus, the infant UL was adjusted for older infants, children and adolescents on the basis of relative body weights. The ULs set by the European Commission and the DRI Committee are summarized in Table 9.1.

Table 9.1. Comparison of ULs for Selenium

Age/Physiologic Group	Selenium UL (μg /day)	
	European Commission	DRI Committee
Infants (months)		
0 – 6	-	45
7 – 12	-	60
Children (years)		
1 - 3	60	90
4 – 6	90	-
4 – 8	-	150
7 – 10	130	-
9 – 13	-	280
11 – 14	200	-
Adolescents (years)		
14 – 18	-	400
15 – 17	250	-
Adults	300	400
Pregnany	300	400
Lactation	300	400

In the absence of sensitive biochemical markers of impending selenium intoxication, the 1998 FAO/WHO Expert Consultation provisionally set the UL for selenium at 400 μg/day for adults (FAO/WHO, 2002). A maximum tolerable dietary concentration of 2 μg/kg dry diet was suggested for all classes of domesticated livestock and has proved satisfactory in use. These findings, according to the FAO/WHO, suggests that the proposed UL of 400 μg/day for human subjects provides a fully adequate margin of safety. The UL for children and for pregnant and lactating women has not yet been determined.

9.5 Food Sources

Seafoods, kidney, liver (0.4 μg/g to 1.5 μg/g), and to a lesser extent, other meats (0.1 μg/g to 0.04 μg/g) are consistently good sources of selenium, whereas the amounts in grains and other seeds are more variable (<0.1 μg/g to >0.8 μg/g), depending on the selenium content of the soils in which they are grown. Dairy products contain about <0.1 μg/g to 0.3 μg/g, while fruits and vegetables generally contain little selenium (<0.1 μg/g) (WHO, 1996; Levander and Burk, 1996).

An analysis of foods conducted in Thailand by flourometric method showed high levels of selenium in fish and seafoods (45 ± 20.8 μg/100 g) and eggs (40.2 ± 14.0 μg/100 g); moderate levels in meat and poultry (18.2 ± 5.8 μg/100 g) and pulses (13.1 ± 13.4 μg/100 g) and low levels in cow's milk (6.4 ± 2.4 μg/100 g) and cereals (5.0 ± 1.1 μg/100 g), vegetables (1.2 ± 2.0 μg/100 g) and fruits (0.6 ± 0.5 μg/100 g) (Sirichakwal *et al.*, 2005).

Analysis of drinking water done in the US and several other countries showed that it does not supply nutritionally significant amounts of selenium. In specific areas however, water wells were shown to supply much greater amounts of selenium, and according to the investigators, this could be a result of irrigation practices, mining, or the presence of selenium-containing rocks (IOM, 2000).

Dietary intakes of selenium differ markedly among different populations (IOM, 2000). Factors that affect the intake include the natural differences in the selenium content of foods, food consumption patterns and the geographic origin of the food supply (i.e. whether it comes from regions with selenium-rich or selenium-poor soils) (WHO, 1996). Predominantly vegetarian populations living in areas with soils of low selenium content or availability are particularly at risk of developing a low selenium status. This is because vegetarians, aside from missing out on the richer food sources such as organ meats, fish and muscle meats, also consume plant foods of low selenium content if produced in such areas. Food consumption patterns also affect the proportion of dietary selenium that is bioavailable. For example, if most of the selenium is derived from cereals, its bioavailability will be higher than when it is derived mostly from certain fish, such as tuna.

The mean per capita/day intakes of adults in most countries (except China) ranged from a low of 28 μg/day to a high of 220 μg/day (NRC, 1989; EC, 2000; IOM, 2000). In one region of China where human selenosis was endemic, intakes as high as 6690 μg/day have been reported (Levander and Burk, 1996). High dietary selenium intakes occur in areas with naturally seleniferous soils.

Analyses of national food composites in the US indicated that the overall mean dietary selenium intake of adults between 1974 and 1982 was 108 μg/day. The daily means for each year ranged from 83 μg to 129 μg (NRC, 1989). Another study done in the US reported selenium intakes of 81 μg/day while a Canadian survey reported higher selenium intakes of 113 μg to 220 μg/day (IOM, 2000).

In the Southeast Asian region, a study in Singapore has reported mean intakes of 96 ± 2 μg selenium per day among adults, with cereals and cereal products (rice, noodles), fish (mackerel, trevally) and other meats (e.g., chicken) as the main contributors to this intake (Tan, 1998). A study in Thailand has reported selenium intake of about 54 μg/day.

9.6 Factors Affecting Requirement

9.6.1 Bioavailability

The absorption of selenite selenium was found to be greater than 80%, while that of selenium as selenomethionine or as selenate was greater than 90%. These findings suggest that the absorption of selenium does not appear to be under homeostatic control. The rate-limiting step in determining the overall bioavailability of dietary selenium is thus not likely to be its absorption but rather its conversion within tissues to its metabolically active forms (e.g. its incorporation into glutathione peroxidase or 5'-deiodinase) (WHO, 1996).

A number of depletion-repletion experiments based on restoration of gluthathione peroxidase activity in depleted rats showed the bioavailability of selenium to be 80% in wheat; and 90% in Brazil nuts and beef kidney to be 90%. Bioavailability of selenium in tuna was only 20% to 60% of the bioavailability of selenium from selenite, while that of selenium from other seafoods (shrimp, crab and Baltic herring) was high (WHO, 1996).

Data on bioavailabilty of selenium in humans are scarce (NRC, 1989; WHO, 1996). However, bioavailability trials conducted on subjects with poor selenium status have indicated that organically bound forms of selenium are retained better than inorganic selenium, but all forms tested caused similar increases in glutathione peroxidase activity. A supplementation study among Finnish men of relatively low selenium status showed the selenate selenium was as effective as the selenium in seleniferous wheat in increasing glutathione peroxidase activity. The wheat, however, increased plasma selenium levels more than selenate selenium. When the supplements were withdrawn, platelet glutathione peroxidase activity declined less in the group given wheat. Results of this study suggest that it is important to estimate not only short-term availability, but also long-term retention and the convertibility of tissue selenium stores into the biologically active form (WHO, 1996).

Among the few studies comparing selenium bioavailability from different foods is the 1991 experiment of van der Torre cited by the European Commission (2000) which showed that supplementation with selenium-rich foods such as bread and meat gave similar increases in circulating selenium levels. The bioavailability of selenium from fish, especially fish containing mercury was low and, according to the investigators, this may be due to formation of unabsorbable mercury selenium complexes.

9.6.2 Gender

Earlier reports from China, at a time when selenium deficiency was more severe than in recent years, indicated that women of childbearing age were susceptible to developing Keshan disease, whereas men were resistant. However, cases of the disease reported in the past 20 years appear to be limited to children, with equal prevalence among boys and girls. Thus, a gender effect in susceptibility to this disease may be present at extremely low selenium intakes, but no such effect has been demonstrated at current intakes. Given women's apparently increased susceptibility to Keshan disease, selenium requirements for the various age groups are based on male reference weights (IOM, 2000).

9.7 Estimating Requirements and Recommended Intakes

Several studies on status of selenium nutriture in Asia, particularly in China, have been reported. These are used for references in this chapter. The few studies carried out in countries in the Southeast Asian region are also referred to. The main references used when considering recommended intakes for selenium for Southeast Asian countries are the FAO/WHO (2002) expert consultation report and the report of the DRI Committee (IOM, 2000).

9.7.1 Indicators for estimating requirement for selenium

Indicators that could be considered as the basis for deriving estimated requirements for selenium in adults include concentration of selenium in blood, hair, and nails; concentration of glutathione peroxidases and selenoproteins in blood; and urinary excretion of the element (IOM, 2000).

Some co-relations between dietary intake of the element and hair and nail concentrations of

selenium have been demonstrated. However, the use of hair and nail selenium as markers of selenium status has been limited because factors such as the form of selenium fed, the methionine content of the diet, and the color of the hair affect the deposition of selenium in these tissues. Furthermore, the forms of selenium in hair and nails have not been characterized, Hence, these markers are of little value in determining selenium requirements across population groups.

Several forms of selenium are present in blood and in metabolizing tissues. Physiologically active forms include the selenoproteins and some as yet uncharacterized forms that are present in low abundance. These forms of selenium are under physiological regulation. Within a specific range of dietary selenium intakes, selenoprotein concentrations are a function of selenium intake. Above this range of intakes, selenoprotein concentrations become regulated only by genetic and environmental factors. This lack of selenium effect implies that the selenium requirement for selenoprotein synthesis has been met (Yang *et al.*, 1987). At this plateau point, human plasma selenoproteins contain 0.8 μmol/L to 1.1 μmol/L (7 to 9 μg/dL) of selenium (Hill *et al.*, 1996). Thus, when tissue concentrations of selenium are below the level at which selenoproteins have plateaued, it can be stated with confidence that selenium supplies are limiting. Under these conditions, tissue (plasma) concentrations of the element are useful as indices of nutritional selenium status.

Several selenoproteins are present in blood. Plasma contains the extracellular glutathione peroxidase (GSHPx-3) and selenoprotein P. Erythrocytes and platelets contain the most abundant form of selenium-containing glutathione peroxidase, intracellular glutathione peroxidase (GSHPx-1). Other selenoproteins have not been identified in blood. All 3 of these blood selenoproteins (GSH-Px-3, selenoprotein P, and GSHPx-1) have been used to assess selenium status, but plasma GSHPx-3 has been preferred in recent years because its determination is more accurate than the determination of the erythrocyte enzyme GSHPx-1. Since hemoglobin interferes with the measurement of GSHPx-1 in the erythrocyte, use of this marker is problematic and consequently few data are available that can be used to set a selenium requirement. Furthermore, studies indicate that plasma GSHPx-3 activity reflects the activity of tissue selenoenzymes better than does GSHPx-1 activity in erythrocytes.

The limited available information on selenoprotein P indicates that it is the major form of selenium in plasma and suggests that it will be as good an indicator of selenium status as plasma GSHPx-3. However, since an assay for it is not widely available at present, the data for selenoprotein P are insufficient to support its use to estimate a dietary requirement.

Attempts have been made to use urinary selenium excretion as an index of selenium status. While excretion of the element is proportional to selenium status, excretion is also sensitive to short-term changes in selenium intake. Thus, urinary excretion in selenium deficiency may reflect immediate selenium intake more than nutritional selenium status. This limits the utility of urinary selenium measurements.

Fluorometric analysis of serum selenium in 297 apparently healthy Filipino subjects, yielded values ranging from 37.5 μg Se/L to 340 μg Se/L, with a mean of 158.7 μg Se/L (Alberto *et al.*, 1985). The range of values was lower among females compared to males, but the means were not significantly different. Values tended to be higher in the older age group. The levels for adolescents (143.8 μg Se/L) and adult males (141.4 μg Se/L) corresponded closely with the mean concentration reported for Canadian men (142.9 μg Se/L) using the same fluorometric procedure (Alberto *et al.*, 1985). Selenium status could have been better characterized had glutathione peroxidase activity

determination been done and/or information on dietary intake were available. The Philippine Food Composition Table however, to date, does not include selenium content of Philippine foods.

Information from NHANES III showed that the median serum selenium concentration was 12.4 μg/L for 17,630 American subjects aged 9 to more than 70 years. Serum or plasma selenium concentrations greater than 7 μg/L to 9 μg/L are associated with maximization of plasma selenoproteins (IOM, 2000).

9.7.2 *Methods for estimating requirements*

There are 3 common methods to estimate selenium requirement:

1. Metabolic balance studies
2. Comparison of dietary intakes in populations with and without selenium deficiency
3. Depletion-repletion techniques

The balance method, which was used in a number of studies in China, New Zealand and the US, revealed that balance could be achieved over a wide range of selenium intakes, from 9 μg/day to 90 μg/day (Levander and Burk, 1996). Analysis of the data indicated that the intake needed for balance is a function of body weight, suggesting that the size of the body pool could influence balance requirement. Results further suggested that the body compensates for a low selenium intake by decreased excretion of the element, pointing to the inappropriateness of this method for determining selenium requirements.

Another approach used to estimate selenium requirement is comparing dietary intakes in geographical areas in which selenium deficiency occurs with those in areas without such deficiency. Chinese scientists have established that Keshan disease, which is widely accepted as a selenium-responsive juvenile cardiomyopathy, is not found in those areas where selenium intakes are at least 19.4 μg/day and 14.1 μg/day for male and female adults respectively (Levander and Whanger, 1996). These intakes were presumed to represent the minimum requirement for good health.

In depletion-repletion techniques, subjects are depleted by feeding low selenium diets. The subjects are then repleted with graded amounts of selenium until glutathione peroxidase activity is restored to its full activity. The minimum dose of selenium required to restore biochemical function is then taken as the physiological requirement. The measurement of glutathione peroxidase activity is chosen as a marker of selenium status because the activity of the enzyme is proportional to dietary selenium intake at varying nutritional levels of exposure (Levander and Whanger, 1996).

In a study among a group of healthy North Americans fed a low selenium diet for 45 days, plasma selenium concentration decreased, but red cell selenium concentration and glutathione peroxidase activity were unchanged. These results suggest that the depletion-repletion technique is difficult to use in populations with adequate selenium status as it takes a considerable amount of time to fully deplete selenium-replete subjects. Using this same technique among subjects already depleted of selenium in a Keshan disease area of China, 5 groups of 8 or 9 healthy male adults, ranging from 18 to 42 years of age, were given graded doses of selenium (0, 10 μg/day, 30 μg/day, 60 μg/day and 90 μg/day) orally in the form of selenomethionine (Yang *et al.*, 1987) Plasma glutathione peroxidase activity responded similarly to the 3 highest levels of selenium supplementation

after 5 to 8 months. These results suggest that 30 μg Se/day in the form of selenomethionine, in addition to their usual dietary intake of about 11 μg/day, was sufficient to maximize glutathione peroxidase activity. Based on the criteria of maximizing a known biochemical function, the Chinese scientists thus concluded that a dietary intake of 41μg Se/day was sufficient to satisfy the physiological requirement of adults for selenium.

A study was conducted in New Zealand among 52 adult men and women aged 19 to 59 years with a mean selenium intake of 28 ± 15 μg/day, and initial plasma glutathione peroxidase activities that were approximately 75% of the values after selenium supplementation. The subjects were divided into 5 groups and were given 0, 10 μg/day, 20 μg/day, 30 μg/day, or 40 μg of selenium as selenomethionine per day for 20 weeks (Duffield *et al.*, 1999). While the supplemented groups had higher plasma glutathione peroxidase activities than the placebo group, the increase at the lowest level (10 μg/day) was statistically different from the increase at the highest level (40 μg/day). A conservative value of 38 μg/day (28 μg/day from food) plus 10 μg/day, (the lowest level of supplementation) was taken as the requirement of adults.

Another study compared plasma selenium indices (plasma selenium concentration, glutathione peroxidase activity, and selenoprotein concentration) among boys (aged 8 to 12 years) and adult males residing in a Keshan disease area and a disease-free area (IOM, 2000). Boys and adult males in the disease-free area were given supplements of 100 μg/day and 200 μg/day, respectively, for 14 days. Selenoprotein concentrations in boys and adult males in the endemic area were 13% and 23%, respectively, of the levels in the selenium supplemented area; glutathione peroxidase activities were 26% and 37%; and plasma selenium concentration were 21% and 24%. These results indicate that the biochemical functions of selenium are compromised in populations where Keshan disease occurs, and that it is possible that the disease can occur in populations in which glutathione peroxidase activities in adults are as high as 37% of supplemented values. This would also mean that there is very little margin of error left if less than 100% of the enzyme activity is accepted as sufficient.

9.7.3 Recommendations for selenium intake by life stages

(*a*) *Infants* (*0 – 12 months*)

There are no functional criteria that reflect response to dietary intakes of selenium in infants (IOM, 2000). The DRI Committee thus based its recommendations on an AI that reflects the observed mean selenium intake of infants fed principally with breast milk (IOM, 2000). Furthermore, there are no reports of signs of selenium deficiency manifested among American or Canadian infants who are exclusively breast-fed. The AI for infants aged 0 to 6 months was thus based on an average volume of milk intake for this age group of 0.78 L/day and an average concentration of selenium in human milk of 18 μg/L (0.23 μmol/L).

For infants aged 7 to 12 months, 2 methods for estimating the AI were suggested by the DRI Committee, one method by extrapolation (metabolic scaling), the other by estimating selenium intake from breast milk and complementary foods, and both resulted in similar values (IOM, 2000).

The FAO/WHO felt that the RNIs for infants are compatible with estimates of the international reference range of the selenium content of breast milk (18.5 μg/L), with data from an extensive international survey of breast milk selenium and with WHO data on milk consumption of exclusively breast-fed infants in developed and developing countries (FAO/WHO, 2002). Data from the WHO-IAEA survey from 6 countries suggest that the breast milk from all countries met the RNI for infants aged 0 to 6 months. In 2 of 6 countries, Hungary and Sweden, the breast milk selenium was marginal with respect to the RNI for infants aged 7 to 12 months.

The FAO/WHO has recommended that intakes for infants aged 0 to 5 months and 6 to 11 months be calculated based on the estimated selenium requirements of 0.85 μg/kg/day and 0.91 μg/kg/day, respectively (FAO/WHO, 2002).

(*b*) *Children (1 – 9 years)*

In the absence of specific data on children, the EARs for selenium for children were extrapolated from adult values. Selenium requirements for the various age groups were based on the higher reference weights for males, since females were reportedly slightly more susceptible to developing Keshan disease (IOM, 2000). Keshan disease was found to occur in young selenium-deficient Chinese children, suggesting that the children have the greatest need for selenium. Thus, the EARs for children were calculated to be sufficient to prevent Keshan disease. A China study showed that Keshan disease did not occur in populations with a per capita adult selenium intake of 17 μg/day (Yang *et al.*, 1987) and thus the EARs for children were set at levels equal to or more than 17 μg Se/day.

The FAO/WHO recommended intakes for children were calculated based on the factors derived from studies done in Keshan, China, on the basis of body weight and a factor to allow for growth. Thus, for children aged 1 to 3 years, 4 to 6 years and 7 to 9 years, the estimated selenium requirements are 1.13 μg/kg/day, 0.92 μg/kg/day and 0.68 μg/kg/day respectively (FAO/WHO, 2002).

(*c*) *Adolescents (10 – 18 years)*

The requirement for selenium for adolescents are calculated on the basis of body weight and a factor to allow for growth. If the protein requirement for the adolescent is adequate, then automatically the selenium needs will be met. For male and female adolescents aged 10 to 18 years, the estimated selenium requirements are based on 0.50 μg/kg/day and 0.42 μg/kg/day respectively (FAO/WHO, 2002).

(*d*) *Adults (≥ 19 years)*

In 1996, the WHO had set the average normative selenium requirement (intake needed to maintain a level of tissue storage or other reserve that is judged to be desirable) of adults at 26 μg/day. Although the 1987 study of Yang et al. estimated that an intake of 40 μg/day was required to maintain maximal glutathione reductase activity in most adult males in the population, the WHO recommendation of 26 μg/day was deemed sufficient to achieve two-thirds of the maximum attainable activity glutathione peroxidase which was set as a goal by the WHO. The WHO accepted this level of activity based on observations that abnormalities in the ability of blood cells to metabolize hydrogen peroxide become apparent only when the

gluthathione peroxidase activity in these cells declines to one-quarter of the maximum or less. The WHO, however, emphasized that their estimates are applicable only to diets containing selenium of relatively high bioavailability, since selenomethionine, the selenium form used in the study of Yang *et al.* (1987) is very efficiently absorbed and utilized by humans.

The above criteria satisfying the definition of average normative requirement for selenium was used as the basis for calculating the RNI values for selenium in the 2002 FAO/WHO Expert Consultation Report. The estimates of average requirements from the 1996 Report were interpolated by allowing for differences in weight and basal metabolic rate of age groups up to 65 years and adding a 25% increase (2 x assumed SD) to allow for individual variability of recommended nutrient intake. The FAO/WHO estimated selenium requirement for adult men aged 19 to 65 years to be 0.42 μg/kg/day, while those the requirement for adult men above 65 years old is 0.41 μg/kg/day. For adult women, aged 19 to 65 and above, the requirement is set at 0.37 μg/kg/day (FAO/WHO, 2002).

The DRI Committee, on the other hand, has set its EAR at 45 μg/day for both males and females, based on the average of intervention studies conducted in China and New Zealand, both of which used maximization of gluthathione peroxidase activity as a criterion of adequacy (IOM, 2000). As mentioned earlier, the China study (Yang *et al.*, 1987) showed that full activity of gluthathione peroxidase was reached at an intake 41 μg/day, which when adjusted for weight of North Americans, gives a value of 52 μg/day. The New Zealand study on the other hand, suggests an EAR of 38 μg/day (Duffield *et al.*, 1999). The selenium requirement for women were based on the higher reference weight of men because of the reported greater susceptibly of women in developing the Keshan disease. Also, the data used to set the EAR came largely from men. After taking into account variation in requirements (a CV of 10% was assumed as information on the SD of selenium requirements is not available), the RDA was set at 55 μg/day for both male and female adults. Adults aged 51 years and over appeared to have the same selenium requirement as younger adults. No pathological conditions related to selenium insufficiency have been reported in older individuals, and markers of selenium status in blood did not differ by age or gender. The EAR and RDA for this age group are thus the same as those for younger adults.

(*f*) *Pregnancy and Lactation*

The additional selenium requirement during pregnancy was predicted by factorial estimation of the likely quantity of selenium incorporated into the tissues of the fetus. If the selenium content of the protein (the assumed total products of conception amount to 4.6 kg to 6 kg of lean tissue with a protein content of approximately 18.5% to 20%) resemble that of skeletal muscle, the growth of these tissues could require between 1.0 μg/day to 4.5 μg/day of selenium, depending on whether the analysis reflects consumption of diets from a low-selenium (but non-pathogenic) environment or from a region with relatively high selenium intakes. FAO/WHO estimates that an increase of 2 μg/day for the 2nd trimester and 4 μg/day for the 3rd trimester, assuming 80% absorption and utilization of dietary selenium and allowing 12.5% for variability of estimates would be appropriate. During lactation, the increase in maternal dietary selenium required to produce 6 μg/day of selenium for 0 to 6 month old infants would be 9 μg/day, assuming 80% efficiency of utilization; and an increase of 16 μg/day to produce 10 μg/day for

infants aged 7 to 12 months. The total RNI would be 35 μg/day and 42 μg/day for the first and second 6 months of lactation respectively (FAO/WHO, 2002).

The DRI Committee estimated additional requirement for pregnant women based on a fetal deposition using the estimated selenium content of 250 μg/kg body weight for a 4-kg fetus. This need could be met by an additional 4 μg/day over 270 days of the pregnancy (1000 μg / 270 days). The requirements for lactating women are based on the loss of selenium in breast milk during lactation. No adjustment is made for absorption, since the bioavailability of selenium in breast milk is greater than 90% (IOM, 2000).

9.8 Recommended RDAs for Selenium for Southeast Asia

The estimates derived or arrived at by the FAO/WHO are applicable to Southeast Asian populations with a proportionately lower weight range than most populations in Western and developed countries. As accepted by WHO, FAO and IAEA, it is neither essential nor desirable to maintain selenium status at a level which fully saturates blood GSHPx activity when, on current evidence, this is not an advantage for health. For these reasons, the FAO/WHO's RNI for selenium for all population groups are adopted as the SEA-RDAs (FAO/WHO, 2002).

The RDAs for specific population groups are summarized in Table 9.2.

Table 9.2 Recommended RDAs for Selenium for Southeast Asia

Age Groups	Assumed weight (kg)[a]	Average normative requirement[b]		RDA[c]
		per kg/day	total/day	mg/day
Infants (months)				
0 – 5	6	0.85	5.1	6
6 – 11	9	0.91	8.2	10
Children (years)				
1 – 3	12	1.13	13.6	17
4 – 6	19	0.92	17.5	22
7 – 9	25	0.68	17.0	21
Boys (10 – 18 years)	51	0.50	22.5	32
Girls (10 – 18 years)	49	0.42	20.6	26
Men (years)				
19 – 65	65	0.42	27.3	34
> 65	64	0.41	26.2	33
Women (years)				
19 – 65	55	0.37	20.4	26
> 65	54	0.37	20.2	25
Pregnancy				
1st trimester	-	-	-	26
2nd trimester	-	-	-	28
3rd trimester	-	-	-	30
Lactation				
1st 6 months	-	-	-	35
2nd 6 months	-	-	-	42

Notes: [a] *Weight (kg) interpolated from FAO/WHO/IAEA (1996)*
[b] *Derived (by interpolation) from FAO/WHO/IAEA (1996) values*
[c] *Recommended nutrient intake derived from average normative requirement + 2SD*
Source: FAO/WHO (2002)

Bibliography

Alberto SP, Desnacido JA, Ruta ES & Natera MG (1985). Establishment of Normal Levels of Serum Selenium in Different Filipino Population Groups. *Phil J Nut* 38(3 & 4): 169-174.

Duffield AJ, Thomson CD, Hill KE & Williams S (1999). An estimation of selenium requirements for New Zealanders. *Am J Clin Nutr* 70: 896-903.

EC (2000). Opinion of the Scientific Committee on Food on the Tolerable Upper Intake of Selenium. Health & Consumer Protection Directorate-General. Scientific Committee on Food. SCF/CS/NUT/UPPLEV/25 Final. 28 November 2000. European Commission.

FAO/WHO (2002). Selenium. In: *Human Vitamin and Mineral Requirements*. Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 235-255.

Ge K & Yang G (1993). The epidemiology of selenium deficiency in the etiologic study of endemic diseases in Chainna. *Am J Clin Nutr* 57: 259S-263S.

Groff JL & Grooper DS (1997). Microminerals (Selenium). In: *Advanced Human Nutrition and Human Metabolism*. 3rd ed. Woodsworth Thomas Learning. pp.440-446.

IOM (2000). Selenium. In: *Dietary Reference Intakes for Ascorbic acid, Vitamin E, Selenium, and Carotenoids*. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC; chapter 7, pp 284-324.

Levander O (1991). Scientific rationale for the 1989 recommended dietary allowance for Selenium. *J Am Diet Assoc* 91:1572-1576.

Levander OA & Burk RF (1996). Selenium In: *Present Knowledge in Nutrition*. EE Ziegler and Filer LJ (eds) 7th Ed ILSI Press. Washington DC.

Levander OA & Whanger PD (1996). Deliberations and Evaluations of the Approaches, Endpoints and paradigms for selenium and iodine Dietary Recommendations. *J Nutr* 126: 2427S-2434S.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

NRC (1980). *Recommended dietary allowances*. Ninth Revised Edition. National Research Council. National Academy of Sciences, Washington. DC,

NRC (1989). *Recommended dietary allowances., 10th ed*. Washington. DC, National Research Council. National Academy of Sciences.

Shearer RR & Hadjimarkos DM (1975). Geographic distribution of selenium in human milk. *Arch Environ Hlth* 30: 230-233.

Sirichakwal PP, Puwastien P, Polngam J & Kongkachuichai R (2005). Selenium content of Thai Foods. *J Fd Comp Anal* 18: 47-59.

Tan Wei Ling et al. (1998). Usual Selenium Intakes of Adult Singaporeans (1988 Survey), Unpublished Report

WHO (1996). *Trace Elements in Human Nutrition and Health*. World Health Organization, Geneva, pp 105-122.

Yang GQ, Zhu LZ, Liu SJ, Gu LZ, Qian PC, Huang JH & Lu MD (1987). Human Selenium Requirements in China. In: *Selenium in Biology and Medicine*. Combs GR, Spallholz JE, Levander OA, Oldfield JE (Eds). Van Nostrand Reinhold Co., New York. pp 589-607.

10 VITAMIN A

10.1 Introduction

"Vitamin A" is the generic term for compounds that possess the biological activity of retinol. The term "retinoids" refers to both naturally occurring forms of vitamin A and the many synthetic analogs of retinol, with or without biological activity. It has the distinction of being the first fat-soluble vitamin to have been recognized.

Vitamin A comes in a variety of forms: retinal (aldehyde form), retinol (alcohol form), retinoic acid (acid form), and others in animal products. These forms are interconvertible to some extent.

Potential vitamin A activity is present in certain carotenoids, which are plant pigments and are also called provitamin A. Carotenoids, being the original source of all vitamin A, must first be converted to the retinoid form in order to possess vitamin A functions. Of the over 600 carotenoids found in nature, 50 have potential vitamin A activity, of which the most potent is beta-carotene.

10.2 Characteristics and Functions

Vitamin A performs many functions in the body. It is an essential micronutrient throughout the life cycle and is required for vision, regulation of cell proliferation and differentiation and reproduction, especially for embryonic development, growth and tissue maintenance (FAO/WHO, 2002; IOM, 2001).

10.2.1 Vision

The 11-cis form of retinol is a constituent of rhodopsin, the visual pigment (also called visual purple) in the retina, which is critically important for vision in dim light. When struck by light, the 11-cis retinaldehyde is isomerized into the all-trans form. The speed at which rhodopsin is regenerated, hence the speed of dark adaptation, depends on the continuous availability of vitamin A.

10.2.2 Epithelial cell integrity and protection against infection

In vitamin A deficiency, goblet cell numbers are reduced in epithelial tissues, resulting in diminished mucous secretions with their anti-microbial components. Furthermore, the cell lining's protective tissue surfaces fail to regenerate and differentiate, leading to the accumulation of keratin. Both factors – diminished mucous secretion and keratinization of epithelial cells – diminish resistance to invasion by potentially pathogenic bacteria.

Vitamin A deficiency also compromises the immune system by interfering with the production of protective tissue secretions and cells. Retinoic acid is important in maintaining an adequate level of circulating natural killer cells that have anti-viral and anti-tumor activity.

Several human studies have linked impairment in immunity to low level of vitamin A in plasma or serum. Immune function tests however, could not be used as indicators for setting intake requirements due to the lack of human studies using controlled diets that have evaluated immune function tests as a means to assess the adequacy of different levels of dietary vitamin A. Most changes in immune functions that have been associated with a nutrient deficiency are not specific to the nutrient under study. For example, low T cell-mediated immunity may be caused by a lack of vitamin A, protein, energy, zinc or other specific nutrient deficiency or imbalance.

10.2.3 Skeletal growth

Vitamin A is required for growth and differentiation of cells throughout the body. Specific cellular proteins combine with retinoids within the cells during metabolism and with nuclear receptors that mediate retinoid action of genome and modulate the transcription of several hundreds of genes. Retinal is the form required in the reproductive systems and in embryonic development.

10.2.4 Embryogenesis

Retinoic acid is present in temporally specific patterns in the embryonic regions known to be involved in the development of structures posterior to the hindbrain (e.g., vertebrae and spinal cord). It is also involved in the development of the limbs, heart, eyes and ears.

10.3 Absorption, Utilization and Excretion

10.3.1 Absorption

Vitamin A sources in the human diet include preformed vitamin A from animal foods as well as provitamin A carotenoids from vegetables and animal foods.

The absorption of both preformed vitamin A and provitamin A compounds occur mainly in the duodenum. The overall absorptive efficiency of preformed vitamin A is high (70% to 90%) and remains high (60% to 70%) as intake increases. On the other hand, the absorption of beta-carotene is only 40% to 60% and highly variable in normal subjects. Lower absorption values of 20% to 50% have also been reported which may be further reduced to as low as 10% with increasing intake of dietary carotenoids.

Preformed vitamin A in food occurs mainly as retinyl esters which, after ingestion, are hydrolyzed to retinol in the intestinal lumen. Free retinol is then taken up by mucosal cells, bound to a specific cellular retinol binding protein (CRBP II) and esterified to long chain fatty acids which are then incorporated into chylomicrons for lymphatic transport to the liver and other tissues.

Being fat-soluble, the absorption of both vitamin A and provitamin A carotenoids appears to be dependent on the amount and type of fat in the diet.

10.3.2 Utilization and Excretion

The chylomicrons, containing the retinyl esters together with some intact beta-carotene, leave the mucosal cells through the lymphatic system and enter the systemic blood via the thoracic duct. All vitamin A compounds, including retinoic acid which passes into portal blood directly, meet in the liver where transformations similar to those in the intestinal mucosal cells take place.

The liver is the main storage organ for vitamin A compounds. Whereas most of the body's stores of retinyl esters are found in the liver, significant amounts of retinoids are stored in fatty tissues throughout the body. Both hepatic and extrahepatic reserves of carotenoids can be mobilized to yield retinol in times of need.

Retinyl esters are hydrolyzed as needed in the liver to retinal, which then combines with a plasma-specific retinol-binding protein (RBP). The RBP-retinol complex (holo-RBP) thus formed is secreted into the blood where it associates with another protein, transthyretin. The transthyretin-RBP-retinol complex circulates in the blood and delivers retinol to the target cells.

Oxidatively chain-shortened products of vitamin A metabolism are excreted in the urine, while vitamin A products with intact chains are excreted in the bile.

10.4 Effects of Deficiency and Excess

10.4.1 Deficiency

The consequences of vitamin A deficiency (VAD), based on various studies conducted in Indonesia, are as follows (Muhilal, 2001):

- Flattening, enlargement and reduction in number of the epithelial cells, as well as a marked reduction or absence of the goblets' cells
- Xerophthalmia in the forms of Bitot's spot (XIB), xerosis of the cornea and keratomalacia (X2/X3) and corneal scars (XS)
- Depressed immune response in children
- Higher morbidity among children
- Higher mortality among children
- Slower growth among children

Early VAD might even affect the mental development of children when they reach school-going age.

10.4.2 Excessive intake

Vitamin A is fat-soluble and can be stored. Routine consumption of large amounts of vitamin A over a period of time may result in toxic symptoms, including liver damage, bone abnormalities and joint pain, headaches and vomitting, and skin desquamation. In a case in Indonesia, 2 children each consumed 5 to 10 capsules of vitamin A, each capsule containing 60,000 μg (200,000 IU), by mistake.

The 2 children suffered from headaches, vomiting and skin rashes. Their serum vitamin A levels only returned to normal value (around 30 μg/dL) 2 months after their excessive intake of vitamin A.

A careful review of the latest available information by a WHO Expert Group recommended that daily intakes in excess of 3,000 μg (10,000 IU) or weekly intakes in excess of 7,500 μg (25,000 IU) should not occur at any period during gestation. It is unlikely that vitamin A intake through daily diet will cause adverse effects. However, due to the availability of synthetic vitamin A as supplements, which can be purchased commercially or obtained through vitamin A fortification programs, there are possibilities of excessive consumption. Vitamin A overdoses usually happen by accident or by misinformation (e.g. taking more vitamin A will improve health). Excessive intake of vitamin A by pregnant women may have a teratogenic effect. Retinol that is transferred via the placenta into the fetal circulation will cause the teratogenic effect. Reported birth defects arising from excessive vitamin A consumption include abnormalities in the face and head (e.g. cleft palate, low ear), cardiac, genitourinary, central nervous, muscular and skeletal systems. The reported toxic dose for pregnant women is around 150 mg (500,000 IU) as a single dose or over 7.5 mg (25,000 IU) for daily dose. Women who are or who might become pregnant should carefully limit their total daily vitamin A intake to a maximum of 3,000 μg (10,000 IU) to minimize risk of fetal toxicity.

High doses of vitamin A (60,000 μg or 200,000 IU) can be safely given to breast-feeding mothers for up to 2 months post-partum and for 6 weeks for non-breast-feeding women. Daily prophylactic or therapeutic doses should not exceed 900 μg, which is well above the mean requirement of about 200 μg daily for infants. Most children 1 to 6 years of age tolerate single oral doses of 6000 μg (200,000 IU) vitamin A in oil at intervals of 4 to 6 months without adverse symptoms. Older children seldom experience toxic symptoms unless they habitually ingest vitamin A in excess of 7,500 μg (25,000 IU) for prolonged periods of time.

10.4.3 Guidance on High Intake

The upper tolerable intake level (UL) is the highest level of daily vitamin A intake that is likely to pose no risk of adverse health effects in almost all individuals. Members of the general population should be advised not to routinely exceed the UL. For the purpose of deriving at a UL, 3 primary adverse effects of chronic vitamin A intake are: (a) reduced bone mineral density, (b) teratogeneciy and (c) liver abnormalities. The ULs recommended by the DRI Committee (IOM, 2001) are slightly different from those of FAO/WHO and are set out in Table 10.1.

Table 10.1 ULs of vitamin A for various age groups

Age Groups	Preformed Vitamin A UL (μg/day)
Infants	600
Children / Adolescents (years)	
1 – 3	600
4 – 8	900
9 – 13	1,700
14 – 18	2,800
Women (≥ 19 years)	3,000
Men (≥19 years)	3,000
Pregnancy	2,800
Lactation	2,800

Source: IOM (2001)

10.5 Food Sources

Animal foods are the source of preformed vitamin A or retinol, mostly in the form of retinyl ester. Since the liver is the site of vitamin A storage, it's retinol content is the highest. The fat of meat, poultry, fish and egg may also contain substantial amounts of vitamin A.

Sources of provitamin A carotenoid are red palm oil and other vegetable oils, green leafy vegetables and yellow fruits, roots and tubers. Some animal products like milk, cream, butter and eggs also contain carotenoids.

10.6 Factors Affecting Requirement

10.6.1 Bioavailability

Bioavailability is the proportion of an ingested nutrient that is available for utilization in normal physiologic functions and for storage. The bioavailability of preformed vitamin A is very high. More than 90% of retinol added to food as a fortificant is absorbed.

The bioavailability of carotenoids in green leafy vegetables is low because the carotenoids are incorporated in the matrix of the food, and therefore are enclosed within the cell walls. Furthermore, vegetables contain limited amounts of substances that affect absorption, such as the lack of fat. The nutritional status of the host and the presence of parasites such as round worm and giardia may also reduce bioavailability.

The most important factors that contribute to the poor bioavailability of carotenoids from dark-green leafy vegetables seem to be the matrix, the absorption modifiers, and host-related factors, especially parasitic infestations. The matrix of dark-green leafy vegetables is characterized by many membranes and a lack of fat inside the chloroplast, the organel of the plant cell which contains the carotenoids. Dark-green leafy vegetables also contain a relatively large amount of absorption inhibitors such as fibre and other carotenoids which could compete for absorption. When parasitic infestations or other factors that interfere with the digestive system are added to the effect of the difficult-to-digest matrix and absorption inhibitors of vegetables, the bioavailability of carotenoids from dark-green leafy vegetables may become very poor.

10.6.2 Bioconversion of carotene to retinol

Bioconversion is the proportion of absorbed provitamin A carotenoids which is converted to retinol in the body. The FAO/WHO has proposed that 3.3 μg beta-carotene has the same vitamin A activity as 1 μg retinol. Bioefficacy of beta-carotene dissolved in oil, studied in Indonesian children, using isotopic label beta-carotene and retinol, revealed that 2.4 μg beta carotene is required to form 1 μg retinol. (van Lieshout *et al.*, 2001). When determining the bioefficacy of carotenoids in food, the bioavailability and bioconversion the carotenoids should also be considered.

Since there are several provitamin A compounds with differing biological activities, the FAO/WHO introduced the concept of retinol equivalents (RE) and established the following relationships among food sources of vitamin A (FAO/WHO, 2002).

1 μg retinol	=	1 RE
1 μg beta-carotene	=	0.167 μg RE
1 μg other provitamin A carotenoids	=	0.084 μg RE

In other words,

1 retinol equivalent (RE)	=	1 μg of all-trans retinol
	=	6 μg all trans-b-carotene
	=	12 μg of other provitamin A carotenoids

The DRI Committee cited several studies which indicate lower biological activities of the carotenoids and suggested lower conversion factors. The FAO/WHO (2002) examined the impact of using the newer factors that have been suggested. It was noted that when the food supplies of several countries were analyzed for RE using the new conversion factors, it would seem that some countries are in short supply of vitamin A. According to the FAO/WHO, this is inconsistent with the preponderance of epidemiologic evidence that the prevalence of vitamin A deficiency in these countries is low. The Expert Consultation thus concluded that the 1:6 bioconversion factors originally derived from balance studies be retained until there is firm confirmation from ongoing studies that are using more precise methodologies (FAO/WHO, 2002).

It has been strongly recommended that weight or molar units should replace the use of IU to reduce confusion and overcome limitations in the non-equivalence of the IU values for retinol and beta-carotenes. The conversion factors to be used are as follows:

1 IU retinol	=	0.3 μg retinol
1 IU beta–carotene	=	0.6 μg beta–carotene
1 IU retinol	=	3 IU beta-carotene

10.7 Estimating Requirements and Recommended Intakes

10.7.1 Indicators for estimating requirement for vitamin A

Ocular signs of VAD are measured by clinical examination and history, and can be quite specific to preschool-age children. However, in rare occasions, examination of large populations may be required to obtain incidence and prevalence data. Sub-clinical VAD is more prevalent, requiring smaller sample sizes to obtain valid prevalence estimates.

Direct measurement of concentrations of vitamin A in the liver where it is stored or in the total body pool relative to known specific vitamin A–related functions (e.g., night blindness) would be

the indicator of choice for determining intake requirements. This cannot be done with the methodology now available for population use. There are several practical biochemical methods for estimating sub-clinical vitamin A status but all have limitations. Each method is useful in identifying deficient populations, but none of these indicators are definitive or directly related quantitatively to disease occurrence. Useful indicators include a plasma retinol concentration above 0.70 μmol/L, that is associated with a relative dose response below 20%, or a modified relative dose response below 0.06. For lactating women, breast-milk retinol levels above 1.05 μmol/L (or above 8 μg/g milk fat) are considered to reflect minimal maternal stores because levels above 1.75 μmol/L are common to populations known to be healthy and without evidence of insufficient dietary vitamin A

These indicators are less specific to VAD than clinical eye signs and less sensitive for measuring sub-clinical vitamin A status. WHO recommends that where feasible, at least 2 sub-clinical biochemical indicators, or 1 biochemical and a composite of non-biochemical risk factors, should be measured and that both types of indicators should point to deficiency in order to identify populations at high risk of VAD (FAO/WHO, 2002).

Although all biochemical indicators currently available have limitations, the biochemical indicator of choice for population assessment is the distribution of serum levels of vitamin A (serum retinol). Only at very low blood levels (<0.35 μmol/L) is there an association with corneal disease prevalence. Blood levels between 0.35 μmol/L and 0.70 μmol/L are likely to characterise sub-clinical deficiency, but sub-clinical deficiency may still be present at levels between 0.70 μmol/L and 1.05 μmol/L and occasionally above 1.05 μmol/L. The prevalence of values below 0.70 μmol/L is a generally accepted population cut-off for preschool-age children to indicate risk of inadequate vitamin A status and above 1.05 μmol/L to indicate an adequate status. Clinical and sub-clinical infections are also known to lower serum levels of vitamin A on average as much as 25 percent independently of vitamin A intake. Therefore, at levels between about 0.5 μmol/L and 1.05 μmol/L, the relative dose response or modified relative dose response test on a subsample of the population can be useful for identifying the prevalence of critically depleted body stores when interpreting the left portion of serum retinol distribution curves.

10.7.2 Recommendations for vitamin A intake by life stages

The 1998 FAO/WHO Expert Consultation felt that the requirement and safe level of intake for vitamin A recommended by the 1988 FAO/WHO Expert Consultation was still acceptable and hence adopted those recommendations (FAO/WHO, 2002). The term "safe level of intake" used in the 1988 report was also retained because it was felt the levels recommended do not strictly correspond to the definition of a recommended nutrient intake.

The mean requirement for an individual is defined by the FAO/WHO as the minimum daily intake of vitamin A to prevent xerophthalmia in the absence of clinical or sub-clinical infection. This intake should account for proportionate bioavailability of preformed vitamin A (about 90%) and pro-vitamin A carotenoids from a diet that contains sufficient fat (e.g., at least 5 g to 10 g). The required level of intake is set to prevent clinical signs of deficiency, allow for normal growth, and reduce

the risk of vitamin A–related severe morbidity and mortality on a population basis. It does not allow for frequent or prolonged periods of infections or other stresses.

The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to permit adequate growth and other vitamin A–dependent functions, and to maintain an acceptable total body reserve of the vitamin. This reserve helps offset periods of low intake or increased need resulting from infections and other stresses.

(*a*) *Infants* (*0 – 12 months*)

No functional criteria of vitamin A status have been demonstrated that reflect response to dietary intake in infants. Thus, recommended vitamin A intakes for infants are calculated from the vitamin A provided in breast milk. This is based on the assumption that during at least the first 6 months of life, exclusive breast-feeding can provide sufficient vitamin A to maintain health, permit normal growth, and maintain sufficient stores in the liver.

Reported retinol concentrations in breast milk varies widely from country to country. Because breast-fed infants in endemic vitamin A-deficient populations are at risk of death from 6 months onward, the safe levels of intake for infants should be based on observations of breast-fed infants in communities where good nutrition is the norm. Hence the FAO/WHO's safe level for infants up to 6 months of age is based on observations of breast-fed infants in such communities. Average consumption of breast milk by such infants is about 750 ml/day during the first 6 months. Assuming an average concentration of vitamin A in breast milk of about 1.75 μmol/L, the mean daily intake would be about 375 μg RE, which is therefore the recommended safe level. From 7 to 12 months, breast milk intake averages at 650 ml, which would provide 325 μg vitamin A daily. As breast-fed infants in endemic vitamin A–deficient populations are at increased risk of death from 6 months onward, the recommended safe intake is increased to 400 μg.

Both the FAO/WHO and the DRI Committee have adopted the same approach, although the concentration of vitamin A and volume of breast milk varied slightly. As a result, the DRI Committee's recommended intakes are slightly higher.

(*b*) *Children and Adolescents* (*1 - 18 years*)

For older children, the FAO/WHO recommended intakes were estimated from those derived from infancy, i.e. 39 μg RE/kg body weight/day. Providing for allowances for storage requirements and variability, the safe intake for children 1 to 9 years was estimated to range from 400 μg/day to 500 μg/day. The safe intake for adolescents was stepped up from that for children to 600 μg/day.

No data are available to estimate an average requirement for children and adolescents. The DRI Committee had therefore employed a computational method that included an allowance for adequate liver vitamin A stores to set the EAR. The EAR for children and adolescents is extrapolated from adults by using metabolic body weight. The resulting RDA computed for children were slightly lower, compared with the recommendation of FAO/WHO. But for older adolescents, the DRI Committee's recommendations were significantly higher.

(c) *Adults (19 - 64 years)*

The mean dietary requirement estimate of set by the FAO/WHO (2002) was derived from information on cures achieved in a few vitamin A-deficient adult men and on the vitamin A status of groups receiving intakes that are low but adequate to prevent the appearance of deficiency-related syndromes, and that said definition is taken with the understanding that the curative dose is higher than the preventive dose. On the other hand, the DRI Committee's EAR corresponds to the amount of dietary vitamin A that will maintain a given body pool size in well-nourished subjects.

Both the FAO/WHO and the DRI Committee estimated the average requirement using the Olson formula (A x B x C x D x E x F) although there are slight differences in some of the parameters used. The data used and the results obtained for a male adult is shown below:

Table 10.2 Parameters of Olsen formula to calculate vitamin A EAR for male adults

Factors	FAO/WHO 2002	IOM 2001
• Percent of body vitamin A stores lost per day when ingesting a vitamin A-free diet	0.5% (0.005)	0.5% (0.005)
• Minimum acceptable liver vitamin A reserve	20 μg/g	20 μg/g
• Liver weight to body weight ratio	1:33 (0.03)	1:33 (0.03)
• Reference weight for a specific age group and gender	65 k	76 k
• Total body to liver vitamin A reserves ratio	10:9 (1.1)	10:9 (1.1)
• Efficiency of storage of ingested vitamin A	40%(2.5)	50% (2.0)
Requirement	**434 μg**	**627 μg**
Safe level of intake/RDA	**600 μg**	**900 μg**

The portion of body vitamin A stores lost per day has been estimated to be 0.5% based on the rate of excretion of radioactivity from radiolabeled vitamin A and by the calculation of the half life of vitamin A (IOM, 2001; FAO/WHO, 1988). The minimal acceptable liver reserve is estimated to be 20 μg/g and is based on the concentration at which no clinical signs of a deficiency are observed, adequate plasma retinal concentrations are maintained, induced biliary excretion of vitamin A is observed, and protection against vitamin A deficiency for approximately 4 months while the person consumes a vitamin A-deficient diet. The liver weight:body weight ratio is 1:33 (0.03), and is an average of ratios for infants and adults. The ratio of total body vitamin A stores: liver vitamin A reserves is 10:9 (1.1) and is based on individuals with adequate vitamin A status. FAO/WHO estimated storage efficiency at 50%, while the IOM report estimated storage efficiency at 40%, as suggested by more recent studies. Both FAO/WHO and the DRI Committee used the same factors, except for reference body weight and efficiency of storage. Both also used 20% CV to arrive at the final recommendations.

Due to differences in some of the parameters used, the DRI Committee's recommended intakes are much higher.

(*d*) *Pregnancy and Lactation*

The DRI Committee noted that direct studies of the requirement for vitamin A during pregnancy are lacking. The model used by the DRI Committee to establish EAR for pregnant women was thus based on the accumulation of vitamin A in the liver of the fetus during gestation and an assumption that approximately half of the body's vitamin A is contained in the liver when liver stores are low, as in the case of newborns. Liver vitamin A concentrations for full term stillborn infants have ranged from less than 10 to greater than 100 μg/g liver, with values tending to be skewed towards the lower range. A vitamin A concentration of 1800 μg/liver for fetus of 37 to 40 weeks of gestation age was used to calculate a concentration of 3600 μg. Assuming the efficiency of maternal vitamin A absorption at an average rate of 70%, and vitamin A to be accumulated mostly in the last 90 days of pregnancy, the mother's requirement would be increased by approximately 50 μg/day during the last trimester. Due to the fact that vitamin A in the mother's diet may be stored and mobilized later as needed and some vitamin A may be retained in the placenta, the EAR is estimated to be approximately 50 μg/day in addition to the EAR for non-pregnant adolescent girls and women for the entire pregnancy period. The resulting RDA estimated in this manner was an additional 50 to 70 μg/day for pregnant women.

The FAO/WHO's recommendations were also based on the same considerations that during pregnancy, additional vitamin A is needed for the growth and maintenance of the fetus for providing a limited reserve in the fetal liver and for maternal tissue growth. It was noted however that there are no reliable figures available for the specific vitamin A requirements for these processes.

The FAO/WHO also estimated that newborn infants need around 100 μg of retinol daily to meet their needs for growth. During the third trimester, the fetus grows rapidly and, although obviously smaller in size than the infant born at full term, the fetus presumably has similar needs. Incremental maternal needs associated with pregnancy are assumed to be provided from maternal reserves in populations of adequately nourished healthy mothers. In populations consuming at the basal requirement, an increment of 100 μg/day during the full gestation period should enhance maternal storage during early pregnancy and allow adequate amounts of vitamin A for the rapidly growing fetus in late pregnancy. However, this increment may be minimal for women who normally ingest only the basal requirement level of vitamin A inasmuch as the needs and growth rate of the fetus will not be affected by the mother's initial vitamin A reserves. Recognising that a large portion of the world's population of pregnant women lives under conditions of deprivation, the FAO/WHO increased the recommended safe level by 200 μg to ensure adequacy of intake during pregnancy. Because therapeutic levels of vitamin A are generally higher than preventive levels, the safe intake level recommended during pregnancy is 800 μg RE/day. As an additional note, the FAO/WHO advised that women who are or who might become pregnant should carefully limit their total daily vitamin A intake to a maximum of 3,000 μg RE (10 000 IU) to minimise risk of fetal toxicity.

If the amounts of Vitamin A recommended for infants are supplied by breast milk, mothers should absorb at least as much Vitamin A from their diets to replace maternal losses. Thus, an amount of 350 μg was recommended to be added to the safe intake for women, bringing the total requirement during lactation to 850 μg RE.

(e) *Older Adults (65 years and above)*

The FAO/WHO felt that there is no indication that the vitamin A requirement of healthy elderly individuals differs from those of other adults. It is however also noted that the elderly are more commonly affected by several disease conditions that impede vitamin A absorption, storage, and transport and hence affected their requirements for the vitamin.

10.8 Current RDAs for Vitamin A in Southeast Asia

The current vitamin A RDAs for 6 southeast Asian countries are set out in Table 10.3.

Table 10.3 Comparison of current RDAs (μg RE/day) for vitamin A in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (1975)	Philippines (2002)	Singapore (1988)	Thailand (1989)	Vietnam (1996)
Infants (0 – 1)	350	375 – 400	375 – 400	300	400[a]	325 – 350
Children (1 – 9)[b]	350 – 400	400 – 500	400	250 – 400	400 – 500	400
Boys (10 – 18)[c]	450 – 600	600	400 – 600	575 – 750	600 – 700	500 – 600
Girls (10 – 18)[c]	500	600	400 – 450	575 – 750	600	500 – 600
Men (≥ 19)[d]	600	600	550	750	700	600
Women (≥ 19)[d]	500	500 – 600	500	750	600	500
Pregnancy						
1st trimester	+200	+300	+300	+0	+200	+0
2nd trimester	+200	+300	+300	+0	+200	+100
3rd trimester	+200	+300	+300	+0	+200	+100
Lactation						
1st 6 months	+350	+350	+400	+450	+375	+300
2nd 6 months	+300	+350	+400	+450	+375	+0

Notes: [a] *Figures only for infants aged 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[d] *≥ 18 for Singapore*

There are no major differences in the RDAs for vitamin A of the countries reviewed, except during pregnancy and lactation. Overall, the RDAs for Malaysia and the Philippines, both revised in recent years, are rather similar for all age groups. Generally, adolescent boys and men are recommended slightly higher vitamin A intake compared to the adolescent girls and women.

For infants, the Singapore RDA is slightly lower compared with the other countries listed. For children 1 to 9 years of age, the RDAs of Malaysia and Thailand are slightly higher compared to that of the other countries. RDAs for adolescent boys and girls, and adult men and women are marginally higher in Singapore and Thailand.

Recommendations for additional retinol during pregnancy vary considerably among the countries reviewed. Singapore does not recommend additional intakes. Indonesia, Thailand and Vietnam recommend an additional 100 μg/day to 200 μg/day, whereas an additional 300 μg/day is recommended under the Malaysian and Philippines RDA. All the RDAs reviewed provide for additional vitamin A intake during lactation, with amounts ranging from 300 μg/day to 450 μg/day throughout the 12-month lacation period. Vietnam's RDAs do not recommend additional vitamin A intake during the second 6 months of the lactation period.

10.9 Recommended RDAs for Vitamin A for Southeast Asia

The SEA-RDA Committee reviewed the recommendations and documents of the FAO/WHO and the DRI Committee, and decided to adopt the recommendations of FAO/WHO (2002) for vitamin A as the SEA-RDAs. The SEA-RDAs for vitamin A are summarized in Table 10.4.

Table 10.4 Recommended RDAs for vitamin A for Southeast Asia

Age Groups	Vitamin A RDA (μg/day)
Infants (months)	
0 – 5	375
6 – 11	400
Children (years)	
1 – 3	400
4 – 6	450
7 – 9	500
Boys (10 – 18 years)	600
Girls (10 – 18 years)	600
Men (years)	
19 – 65	600
> 65	600
Women (years)	
19 – 65	500
> 65	600
Pregnancy	800
Lactation	850

The SEA-RDAs are slightly different from some of the current RDAs. For infants, the revised RDA is slightly higher compared with the current RDAs in Malaysia and Singapore, but are close to those in Indonesia, the Philippines, Thailand and Vietnam. The recommended intakes for children in the region are marginally higher than the current RDA. This is also the case for RDAs for adolescent boys and girls. For adult men and women, the SEA-RDAs are lower than the current RDAs in Malaysia and Singapore.

The SEA-RDA for additional amounts recommended during pregnancy is also markedly higher than current RDAs for pregnancy in all the countries in the region, except for the Philippines where the RDA is the same. For lactation, the additional amount recommended is slightly lower or the same as the existing RDAs.

Bibliography

FAO/WHO (1988). *Requirements of vitamin A, iron, folate and vitamin B12*. Report of a Joint FAO/WHO Expert Consultation. FAO Food & Nutrition Ser. No. 23. Food and Agriculture Organization, Rome.

FAO/WHO (2002). Vitamin A. In: *Human Vitamin and Mineral Requirements.* Report of a Joint FAO/WHO Expert Consultation. Food and Agriculture Organization, Rome; pp 87-107.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

IOM (2001). Vitamin A. In: *Dietary References Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; pp 82-161.

Muhilal (2001). The consequences of vitamin A deficiency in children. *Gizi Indonesia*; 25: 30-36.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Solomons NW (2001). Vitamin A and carotenoids. In: *Present Knowledge in Nutrition*. Bowman BA & Russel RM (eds). ILSI Press, Washington DC; pp. 127-145.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

van Lieshout M, West CE, Muhilal, Permaesih D, Wang Y, Xu X, van Breemen RB, Creemers AFL, Verhoeven MA & Lugtenburg J (2001). Bioefficacy of β-carotene dissolved in oil studied in children in Indonesia. *Am J Clin Nutr* 2001; 73: 949-958.

11 VITAMIN D

11.1 Introduction

The first scientific description of rickets, which is the hallmark of a vitamin D deficiency, was provided in the 17th century by both Dr Daniel Whistler in 1645 and Professor Francis Glisson in 1650. The major breakthrough in understanding the causative factors of rickets was the development of nutrition as an experimental science and the recognition of the existence of vitamins. However, vitamin D was classified as a vitamin through a historical error. It is now accepted that the biologically-active form of vitamin D is, in fact, a steroid hormone. Norman (2001) recalled that it was in 1919-1920 that Sir Edward Mellanby, working with dogs raised exclusively indoors (in the absence of sunlight or ultraviolet light), devised a diet that allowed him to establish that rickets was caused by a deficiency of a trace component present in the diet. He felt that this component is probably identical with a fat-soluble vitamin and went on to establish that cod liver oil was an excellent antirachitic agent. Ultimately, this antirachitic factor became classified as a vitamin.

11.2 Characteristics and Functions

Vitamin D (calciferol), which comprises a group of fat-soluble seco-sterols that are found in very few foods naturally, is photosynthesized in the skin of vertebrates by the action of solar ultraviolet B radiation. Vitamin D comes in many forms, but the 2 major physiologically relevant ones are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D_2 originates from yeast and the plant sterol, ergosterol; vitamin D_3 originates from 7-dehydrocholesterol, a precursor of cholesterol, when synthesized in the skin. From a nutritional perspective, the 2 forms are metabolised similarly in humans, are equal in potency, and can be considered equivalent.

Vitamin D without a subscript represents either D_2 or D_3 or both and is biologically inert. It is now established that vitamin D is metabolized first in the liver to 25-hydroxyvitamin-D (25-OH-D or calcidiol) and subsequently in the kidneys to 1,25-$(OH)_2$D to produce a biologically-active hormone. Because vitamin D can be made in the skin, it should not strictly be called a vitamin, and some nutritional texts refer to the substance as a prohormone and to the 2 forms as cholecalciferol (D_3) or ergocalciferol (D_2).

Vitamin D is essential for life in higher animals. It is one of the most important biological regulators of calcium metabolism. As pointed out in the review of the DRI Committee (IOM, 1997), the major biologic function of vitamin D in humans is to maintain serum calcium and phosphorus concentrations within the normal range by enhancing the efficiency of the small intestine to absorb these minerals from the diet. 1,25$(OH)_2$D enhances the efficiency of intestinal calcium absorption along the entire small intestine, but primarily in the duodenum and jejunum. 1,25$(OH)_2D_3$ also enhances dietary phosphorus absorption along the entire small intestine but its major influence is in the jejunum and ileum. When dietary calcium intake is inadequate to satisfy the body's calcium requirement,

$1,25(OH)_2D$, along with parathyroid hormone (PTH), mobilizes monocytic stem cells in the bone marrow to become mature osteoclasts. The osteoclasts, in turn, are stimulated by a variety of cytokines and other factors to increase the mobilization of calcium stores from the bone. Thus, vitamin D maintains blood calcium and phosphorus at supersaturating concentrations that are deposited in the bone as calcium hydroxyapatite.

A multitude of other tissues and cells in the body can recognize $1,25(OH)_2D$. Although the exact physiologic function of $1,25(OH)_2D$ in the brain, heart, pancreas, mononuclear cells, activated lymphocytes, and skin remains unknown, its major biologic function has been identified as a potent antiproliferative and prodifferentiation hormone. There is little evidence that vitamin D deficiency leads to major disorders in these organ and cellular systems.

11.3 Absorption, Utilization and Excretion

Because dietary vitamin D is fat-soluble once it is ingested, it is incorporated into the chylomicron fraction and absorbed through the lymphatic system. It is estimated that approximately 80% of ingested vitamin D enters the body via this mechanism. Vitamin D is principally absorbed in the small intestine.

Vitamin D is principally excreted in the bile. Although some of it is reabsorbed in the small intestine, the enterohepatic circulation of vitamin D is not considered to be an important mechanism for its conservation. However, since vitamin D is metabolized to more water-soluble compounds, a variety of vitamin D metabolites, most notably calcitroic acid, are excreted by the kidney into the urine.

Once vitamin D enters the circulation from the skin or from the lymph via the thoracic duct, it accumulates in the liver within a few hours. In the liver, vitamin D undergoes hydroxylation at the 25-carbon position in the mitochondria, and soon thereafter, it appears in the circulation as 25-hydroxyvitamin D (25(OH)D). In order to have biologic activity at physiologic concentrations, 25(OH)D must be hydroxylated in the kidney in the 1-carbon position to form $1,25(OH)_2D$. It is thought that $1,25(OH)_2D$ is the biologically-active form of vitamin D and that it is responsible for most, if not all, of the vitamin's biologic functions.

The production of $1,25(OH)_2D$ in the kidney is tightly regulated principally through the action of PTH in response to serum calcium and phosphorus levels. The half-life of $1,25(OH)_2D$ in the circulation of humans is approximately 4 to 6 hours. Because of the tight regulation of the production of $1,25(OH)_2D$ and its relatively short half-life, it has not proven to be a valuable product to tackle vitamin D deficiency, adequacy, or excess.

Although the kidney supplies the body with $1,25(OH)_2D$ to regulate calcium and bone metabolism, it is recognized that activated macrophages, some lymphoma cells, and cultured skin bone cells also make $1,25(OH)_2D$. Although the physiologic importance of locally produced $1,25(OH)_2D$ is not well understood, the excessive unregulated production of $1,25(OH)_2D$ by activated macrophages and

lymphoma cells is responsible for the hypercalciuria associated with chronic granulomatous disorders and the hypercalcemia seen with lymphoma.

11.4 Effects of Deficiency and Excess

11.4.1 Deficiency

Vitamin D deficiency is characterized by inadequate mineralization or by demineralization of the skeleton causing rickets, which is characterized by widening at the end of the long bones, rachitic rosary, deformations in the skeleton including frontal bossing legs and knocked knees, respectively. In adults, vitamin D deficiency leads to a mineralization defect in the skeleton during osteomalacia. In addition, the secondary hyperparathyroidism associated with vitamin D deficiency enhances mobilization of calcium from skeleton, resulting in portico (IOM, 1997).

Any alteration in the cutaneous production of vitamin D_3, the absorption of vitamin D in the intestine or the metabolism of vitamin D to its active form, 1,25-$(OH)_2$D can lead to vitamin D deficiency. In addition, an alteration in the recognition of 1,25-$(OH)_2$D by its receptor can also cause vitamin D deficiency and metabolic bone disease accompanying biochemical abnormalities.

It is well-recognized that vitamin D deficiency causes abnormalities in calcium and bone metabolism. There is a possibility that vitamin D deficiency is also associated with an increased risk of colon, breast and prostate cancer, as suggested by epidemiological surveys of people living at higher latitudes. At present, it is premature to categorically suggest that vitamin D deficiency increases cancer risk. Prospective studies need to be carried out to asses the hypothesis.

11.4.2 Excessive intake

Serum 25(OH)D is a useful indicator of vitamin D status, both under normal conditions and in the context of hypervitaminosis D. The latter is characterized by a considerable increase in plasma 25(OH)D concentration to a level of approximately 160 ng/ml to 500 ng/ml. Because changes in circulating levels of 1,25$(OH)_2$D are generally small and unreliable, the elevated levels of 25(OH)D are considered the indicator of toxicity. Serum levels of 25(OH)D have diagnostic value, particularly in distinguishing the hypercalcemia due to hypervitaminosis D from that due to other causes, such as hyperparathyroidism, thyrotoxicosis, humoral hypercalcemia of malignancy and lymphoma (IOM, 1997).

Excessive amounts of vitamin D are not normally available from usual dietary sources, and thus reports of vitamin D intoxication are rare. However, there is always the possibility that vitamin D intoxication may occur in individuals who take excessive amounts of supplemental vitamins. Symptoms of vitamin D intoxication include hypercalcemia, hypercalciuria, anorexia, nausea, vomiting, thirst, polyuria, muscular weakness, joint pains, diffuse demineralization of bones and general disorientation. If vitamin D intoxication is allowed to continue unchecked, death will eventually occur (Norman 2001).

11.4.3 Guidance on high intake

The upper tolerable intake levels (ULs) for vitamin D recommended by the DRI Committee (IOM, 1997) are set out in Table 11.1.

Table 11.1 ULs of vitamin D for various age groups

Age Groups	Vitamin D UL (μg/day)
Infants	25
Children (1 – 18 years)	50
Adults (> 18 years)	50
Pregnany	50
Lactation	50

Source: IOM (1997)

11.5 Food Sources

In nature, very few foods contain vitamin D. Those that do include some fish liver oils, the flesh of fatty fish, the liver and fat of aquatic mammals such as seals and polar bears, and eggs from hens that have been fed vitamin D. Almost all human intake of vitamin D from foods comes from fortified milk products and other fortified foods such as breakfast cereals. The vitamin D content of unfortified foods is generally low, with the exception of fish (many of which contain 5 μg/100 g to 15 μg/100 g (200 IU/100 g and 600 IU/100 g respectively). Atlantic herring contains up to as much as 40 μg /100 g (1,600 IU/100 g) (IOM, 1997).

11.6 Factors Affecting Requirement

The DRI Committee has summarized the 2 main factors that may affect intake requirements, namely aging and malabsorption disorders (IOM, 1997).

Aging is known to significantly decrease the capacity of human skin to produce vitamin D_3. In adults over the age of 65 years, there is a fourfold decrease in the capacity to produce vitamin D_3 as compared to younger adults aged 20 to 30 years. Although 1 study suggested that there may be a defect in intestinal calcium absorption of trace quantities of vitamin D_3, in the elderly, 2 other studies demonstrated that aging does not significantly affect absorption of pharmacological doses of vitamin D. It is not known whether the absorption of physiologic amounts of vitamin D is altered in the elderly.

Patients suffering from various intestinal malabsorption syndromes such as severe liver failure, Crohn's disease, Whipple's disease, and spure often suffer from vitamin D deficiency due to their inability to absorb dietary vitamin D. Thus, patients who are unable to secrete adequate amounts of bile or who have a disease of the small intestines are more prone to develop vitamin D deficiency owing to their inability to absorb this fat-soluble vitamin.

11.7 Estimating Requirements and Recommended Intakes

11.7.1 Indicators for estimating requirement for vitamin D

(*a*) *Serum 25(OH)D*

The DRI Committee was of the opinion that serum 25(OH)D concentration is the best indicator for determining an individual's adequacy of vitamin D intake since it represents a summation of the total cutaneous production of vitamin D and the oral ingestion of either vitamin D_2 or vitamin D_3. Thus, serum 25(OH)D is used as the primary indicator of vitamin D adequacy (IOM, 1997).

The normal range of serum 25(OH)D concentration is the mean serum 25(OH)D ± 2 SD of a group of healthy individuals. The lower limit of the normal range can be as low as 8 g/ml (20 mol/liter) and as high as 15 g/ml (37.5 mol/liter) depending on the geographic location where the blood samples were obtained. A 25(OH)D concentration below 11 ng/ml (27.5 mol/liter) is considered to be consistent with vitamin D deficiency in infants, neonates, and young children, and is therefore used as the key indicator for determining the vitamin D reference value.

Little information is available about the level of 25(OH)D that is essential for maintaining normal calcium metabolism and peak bone mass in older children and in young and middle-aged adults. For the elderly, there is mounting scientific evidence to support their increased requirement for dietary vitamin D in order to maintain normal calcium metabolism and maximize bone health. Therefore, the serum 25(OH)D concentration was utilized to evaluate vitamin D deficiency in this age group, but it was not the only indicator used to determine the vitamin D reference value for elderly.

Serum PTH concentrations are inversely related to 25(OH)D serum levels. Therefore, the serum PTH concentration, in conjunction with 25(OH)D, has proven to be a valuable indicator of vitamin D status.

(*b*) *Serum Vitamin D*

The serum concentration of vitamin D is not indicative of vitamin D status. Its half-life is relatively short, and the blood concentration can range from 0 to greater than 100 ng/ml (0 nmol/liter to 250 nmol/liter) depending on an individual's recent ingestion of vitamin D and exposure to sunlight.

(*c*) *Serum 1,25(OH)$_2$D*

Similarly, serum 1,25(OH)$_2$D level is not a good indicator of vitamin D status. This hormone's serum concentration is tightly regulated by a variety of factors, including circulating levels of serum calcium, phosphorus, PTH, and other hormones.

(*d*) *Evaluation of Skeletal Health*

The ultimate effect of vitamin D on human health is maintenance of a healthy skeleton. Thus, in reviewing the literature for determining vitamin D status, one of the indicators that

has proven to be valuable is an evaluation of skeletal health. In neonates and children, bone development and the absence of rickets, either in combination with serum 25(OH)D and PTH concentration, or on their own, are good indicators of vitamin D status. For adults, bone mineral content (BMC), bone mineral density (BMD) and fracture risk, in combination with serum 25(OH)D and PTH concentrations, have proven to be the most valuable indicators of vitamin D status.

11.7.2 Recommendations for vitamin D intake by life stages

The recommendation for how much vitamin D is required to maintain adequate calcium metabolism and good bone health for all ages may be considered the easiest, albeit at times the most difficult, to determine. Humans of all ages, races, and both sexes can obtain all of their body's requirement for vitamin D through exposure to an adequate amount of sunlight. However, the sunlight-mediated synthesis of vitamin D in the skin is profoundly affected by a wide variety of factors, including degree of skin pigmentation, latitude, time of day, season of the year, weather conditions, and the amount of body surface covered with clothing or sunscreen. Therefore, it is very difficult to determine an accurate value for an EAR as most of the studies are subject to one or more of these variables which is difficult to quantify, in particular the amount of exposure to sunlight.

The following estimates of requirements and intakes have been summarized from the DRI Committee (IOM, 1997). The FAO/WHO did not provide details of the methodology or rationale for the recommended intakes, which are the same as those of the DRI Committee for all age groups, including pregnancy and lactation.

(*a*) *Infants (0 to 12 months)*

The vitamin D available to the infant during the first 6 months of life depends initially on the vitamin D status of the mother during pregnancy and subsequently on the infant's exposure to sunlight and its diet. With habitual small doses of sunshine, breast- or formula-fed infants do not require supplemental vitamin D. For infants who live in far northern latitudes or who are restricted in exposure to sunlight, a minimal intake of 2.5 μg/day of vitamin D will likely prevent rickets. However, at this intake and in the absence of sunlight, many infants will have serum 25(OH)D concentrations within the range often observed in cases of rickets. For this reason, and assuming that infants are not obtaining any vitamin D from sunlight, an adequate intake (AI) of at least 5 μg/day is recommended.

Similarly, for infants 7 to 12 months old, it has been observed that in the absence of any sunlight exposure, an intake of 5 μg/day will result in most of the infants with serum 25(OH)D concentration above the 11 ng/ml level. This amount was thus accepted as the adequate intake for infants 7 to 12 months old.

(*b*) *Children and Adolescents (1 to 18 years)*

There are no data on how much vitamin D is required to prevent vitamin D deficiency in children aged 1 through 8 years. Extrapolating from available data in slightly older children and data from different continents for children who are not exposed to adequate sunlight, most children who had a mean dietary intake of 1.9 μg/day to 2.5 μg/day showed no evidence

of vitamin D deficiency and had normal serum 25(OH)D values. To cover the needs of almost all children aged 1 through 8 years, the DRI Committee doubled the above value to arrive at an AI of 5 μg, regardless of exposure to sunlight.

In the absence of additional data, the DRI Committee felt that 27.5 nmol/liter (11 ng/ml) is the appropriate cut-off for children aged 9 to 18 years. Most children in this age group who ingested 2.5 μg/day of vitamin D from fortified margarine had no evidence of vitamin D deficiency and had a normal serum 25(OH)D level. To cover the needs of all children in this age group, regardless of exposure to sunlight, the above value is doubled for an AI of 5 μg/day.

(*c*) *Adults (19 to 65 years and above)*

Based on the available literature, both sunlight and diet play an essential role in providing vitamin D to this age group. Individuals who are not exposed to sufficient sunlight, for example those who are home bound, can become vitamin D deficient. In a study during the winter months, it was observed that most women with an average intake of 3.3 μg/day to 3.4 μg/day of vitamin D had serum 25(OH)D concentrations greater than 30 nmol/liter (12 ng/ml). Because there is limited data specifically relating to men, the DRI Committee had assumed that the AI for men is similar to that for women. To cover the needs of adults aged 19 through 50 years, the above value is rounded down to 2.5 μg and then doubled for an AI of 5.0 μg/day, regardless of exposure to sunlight.

Studies reviewed by the DRI Committee showed that there is evidence, at least in women, that dietary intakes of vitamin D higher than 2.5 μg/day are necessary at ages 51 through 70 years to prevent higher rates of bone loss during periods of low sun exposure. It is further assumed that the dietary vitamin D requirement for men at this age range is the same as that for women. At a vitamin D intake greater than 5.5 μg/day, there was no seasonal variation in serum PTH concentration. Given that there are few data from individuals with limited but uncertain sun exposure and stores to precisely determine a value between 2.5 μg and 17.5 μg, the DRI Committee recommended 5 μg. To cover the needs of all adults aged 51 through 70 years, the above value was doubled for an AI of 10 μg.

The DRI Committee felt that evidence is strong that the elderly are at higher risk of vitamin D deficiency. Vitamin D deficiency can lead to secondary hyperparathyroidism and osteomalacia, and can also exacerbate osteoporosis, resulting in increased risk of skeletal fractures. Based on examination of available data obtained from various studies, including supplementation carried out among the elderly, the DRI Committee concluded that a value of 7.5 μg/day may be prudent for individuals over 70 years of age with limited sun exposure and stores. In order to cover the needs of adults over age 70, regardless of exposure to sunlight and stores, the above value is doubled for an AI of 15 μg/day.

(*d*) *Pregnancy and Lactation*

Although there is ample evidence of placental transfer of serum 25(OH)D from the mother to the fetus, the quantities are relatively small and do not appear to affect the overall vitamin D status of pregnant women. Women, whether pregnant or not, who receive regular exposure to sunlight do not need vitamin D supplementation. It was also noted that at vitamin D

intakes of less than 3.8 μg/day, pregnant women residing at high latitudes during the winter months had a mean 25(OH)D concentration of 9.1 ng/ml during delivery. Thus, the DRI Committee felt that there is no need to increase vitamin D AI during pregnancy above that required for non-pregnant women, maintaining the adequate intake at 5 μg/day.

There is no scientific literature that recommends a minimum vitamin D intake in order to maintain serum 25(OH)D concentration in the normal range during lactation. There is also no evidence that lactation increases a mother's AI for vitamin D. Therefore, the DRI Committee felt that it is reasonable to extrapolate from observations in non-lactating women that when sunlight exposure is inadequate, a vitamin D intake of 5 μg/day is sufficient.

11.8 Current RDAs for Vitamin D in Southeast Asia

The current RDAs for vitamin D in selected Southeast Asian countries are tabulated in Table 11.2. According to the review of Tee (1998), Vietnam did not list RDAs for vitamin D under its recommendations of 1996.

Table 11.2 Comparison of current RDAs (μg/day) of vitamin D in selected Southeast Asian countries

Age Groups (years)	Indonesia (2002)	Malaysia (2005)	Philippines (2002)	Singapore (2003)	Thailand (2003)
Infants (0 - 1)	5.0	5.0	5.0	10.0	5.0[a]
Children (1 - 9)	5.0	5.0	5.0	2.5 – 10.0	5.0
Boys (10 - 18)	5.0	5.0	5.0	2.5	5.0
Girls (10 - 18)	5.0	5.0	5.0	2.5	5.0
Men (≥ 19)	5.0 – 15.0	5.0 – 15.0	5.0 – 15.0	2.5	5.0 – 10.0
Women (≥ 19)	5.0 – 15.0	5.0 – 15.0	5.0 – 15.0	2.5	5.0 – 10.0
Pregnancy					
1st trimester	+ 0	+0	+0	+7.5	+0
2nd trimester	+ 0	+0	+0	+7.5	+0
3rd trimester	+ 0	+0	+0	+7.5	+0
Lactation					
1st 6 months	+ 0	+0	+0	+7.5	+0
2nd 6 months	+ 0	+0	+0	+7.5	+0

Notes: [a] *Figures only for infants aged 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[d] *≥ 18 for Singapore*
Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

With the exception of Singapore, the RDAs for vitamin D are rather similar for the other 4 countries reviewed. The Singapore recommendations tend to be higher for infants and young children whilst lower for the other age groups. None of the RDAs proposed different intakes for the two sexes. The amounts recommended for all age groups, from infants to adults, are the same, ie 5 μg/day. For older adults and elderly, Indonesia, Malaysia and the Philippines have recommended increasingly higher amounts of vitamin D, from 10-15 μg/day. The Thai RDA recommends the same amount of 10 μg/day vitamin D for the older adults and elderly.

Except for Singapore, all the 4 countries did not recommend additional amounts of vitamin D for pregnant and lactating women. Singapore provides for an addition of 7.5 μg/day of the vitamin.

11.9 Recommended RDAs for Vitamin D for Southeast Asia

Upon reviewing the RDAs of FAO/WHO (2002) and the DRI Committee (IOM, 1997), the SEA-RDA Committee decided to adopt the recommendations of these 2 organizations, which have the same recommended intakes (Table 11.3).

Table 11.3 Recommended RDAs for vitamin D for Southeast Asian countries

Age Groups	RDAs for Vitamin D (μg/day)
Infants (months)	
0 – 6	5
7 – 12	5
Children (years)	
1 – 3	5
4 – 6	5
7 – 9	5
Boys (10 – 18 years)	5
Girls (10 – 18 years)	5
Men (years)	
19 – 50	5
51 – 65	10
> 65	15
Women	
19 – 50	5
51 – 65	10
> 65	15
Pregnancy	5
Lactation	5

The SEA-RDAs are significantly different from the current RDAs in selected Southeast Asian countries. This is true for all the selected countries set out in Table 11.2, except for the Philippines, whose recommended intakes are very similar to the SEA-RDAs.

The SEA-RDAs for infants and young children are much lower (half) than the current RDAs. For adolescents and adults, the situation is reversed, with higher intakes recommended by the SEA-RDAs. Another significant difference for the adult group is that while the same RDA was provided for all ages of adults, the SEA-RDAs recommend increasing amounts of vitamin D intake with advancing age (5 μg/day, 10 μg/day and 15 μg/day for young adults, older adults and elderly, respectively). The SEA-RDAs for pregnant and lactating women is also different from current RDAs in that no additional amount of vitamin D is recommended for these groups.

Bibliography

DOH Thailand (1989). Recommended daily dietary allowances and dietary guidelines for Thais. Department of Health, Ministry of Public Health, Bangkok, Thailand.

FAO/WHO (2002). Vitamin D. In: *Human Vitamin and Mineral Requirements*. Report of a joint FAO/WHO Expert consultation. FAO, Rome, pp 109-118.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

IOM (1997). Vitamin D. In: *Dietary References for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington DC, pp 250-287.

MOH Indonesia (1994). Recommended daily dietary allowances for Indonesians. Ministry of Health Indonesia, Jakarta.

MOH Singapore (1988). Recommended daily dietary allowances for normal healthy persons in Singapore. Food and Nutrition Department, Ministry of Health Singapore.

MOH Vietnam (1996). Vietnamese recommended dietary allowances. Ministry of Health Vietnam, Hanoi, Vietnam.

MPH (2003). *Dietary Reference Intake for Thais.* Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Norman AW (2001). Vitamin D. In: *Present Knowledge in Nutrition*. 8th Edition. Bowman BA and Russel RM (eds). International Life Sciences Institute Press, Washington, DC; chapter 13, pp. 146-163.

Teoh ST (1975). Recommended daily dietary intakes for Peninsular Malaysia. *Med J Mal* 30:38-42.

12 VITAMIN C

12.1 Introduction

Vitamin C is a micronutrient required for normal metabolic functioning of the body. Humans (and other primates) have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin C via the glucoronic acid pathway. Humans thus rely on dietary sources to meet the needs for vitamin C.

Vitamin C is an acidic molecule with strong reducing activity, derived from hexose sugars and is an essential component of most living tissues. It exists in 2 major forms, both present in biological tissues and food: ascorbic acid and dehydroascorbic acid. These 2 forms are interconvertible through an intermediate free radical. The term "vitamin C" is usually used to include all forms with its biological activity, although strictly speaking the individual chemical names should be used for the individual vitamers. The terms "vitamin C" and "ascorbic acid" however, have frequently been used synonymously.

12.2 Characteristics and Functions

Some functions of vitamin C remain poorly defined, but what is clear is that the central features of its biological activity are its reducing potential and its conversion to free radicals. The oxidation of vitamin C (L-ascorbic acid) sequentially releases two donor electrons that become available for biochemical reactions observed *in vivo* and/or *in vitro*.

12.2.1 Enzyme cofactor

Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, carnitine, and neurotransmitters. Proline hydroxylase and lysine hydroxylase, the 2 enzymes involved in the biosynthesis of procollagen, require vitamin C for maximal activity. Posttranslational hydroxylation of proline and lysine residues by these enzymes is essential for the formation and secretion of stable collagen helixes. A deficiency of vitamin C results in weakening of collagenous structures, causing tooth loss, joint pains, bone and connective tissue disorders and poor wound healing, all of which are characteristics of scurvy.

Vitamin C is also required for maximal activity of the 2 enzymes (dioxygenases) involved in the biosynthesis of carnitine, which is essential for the transport of activated long-chain fatty acids into the mitochondria. Vitamin C deficiency results in fatigue and lethargy, the early symptoms of scurvy. Vitamin C is also used as a cofactor for catecholamine biosynthesis, particularly the conversion of dopamine to norepinephrine catalyzed by dopamine beta–monooxygenase. Depression, hypochondria, and mood changes, all of which frequently occur in scurvy cases, could be related to deficient dopamine hydroxylation.

The activities of mono- and dioxygenases involved in peptide amidation and tyrosine metabolism are known to be dependent on vitamin C, but their connection to scurvy has not been clearly established.

12.2.2 Antioxidant (chemical reducing agent)

Ascorbic acid is an important scavenger for free radicals, such as the reactive oxygen species (ROS) which may cause tissue damage resulting from lipid peroxidation, DNA strand breakage or base alterations and may lead to degenerative diseases such as heart disease or cancer. Degradation (usually by lactone ring opening) produces non-biologically active species, such as diketogulonic acid, which is then converted into a number of further degradation products, including oxalic acid. This represents the turnover pathways of vitamin C and thus may help determine the dietary requirements of those species which need a dietary source of the vitamin.

One example of vitamin C's antioxidant function is its prevention of the oxidation of low-density lipoprotein (LDL). LDL is atherogenic after structural modification. It is still uncertain which factors are responsible for the oxidation of LDL *in vivo* but *in vitro* techniques show that LDL can be oxidized into a potentially atherogenic form through metal-ion-dependent oxidation of its lipid component with subsequent modification of apolipoprotein by reactive aldehyde products of lipid peroxidation. The regeneration of oxidized a-tocopherol (vitamin E) on LDL by vitamin C has also been proposed, although this may have less importance for LDL protection. Vitamin C could also decrease oxidative damage in vascular walls.

As an extracellular reducing agent, vitamin C may reduce harmful oxidants in gastric juice. Gastric juice may provide protection against gastric cancers, because vitamin C in the stomach or duodenum could quench reactive oxygen metabolites or prevent formation of mutagenic N-nitroso compounds. Vitamin concentrations however, were found in 1 study to be normal in the gastric juice of patients at risk for familial gastric cancer. In another study, high dietary vitamin C intakes were found to correlate with reduced gastric cancer risk. It is difficult to determine from epidemiological observations, whether vitamin C itself or other components of foods confer protection.

12.2.3 Chemical reductant

Vitamin C has an important relationship with the oxidation of transition metal ions, especially iron and copper. Ascorbic acid modulates iron absorption, transport and storage by keeping it in its reduced form or by preventing its chelation by phytates or other food ligands. This renders the iron much more soluble in the small intestine alkaline environment and this increases iron absorption from non-heme iron.

12.3 Absorption, Utilization and Excretion

Ascorbic acid is absorbed in the intestines through a sodium-dependent active transport process that is saturable and dose-dependent. Active transport predominates at low gastrointestinal

ascorbate concentrations, while simple diffusion occurs at high concentrations. After absorption, ascorbic acid is then transported as a free acid in the plasma into the cells, including leukocytes and red blood cells. Absorbed ascorbic acid is present as an anion in blood plasma, unbound to plasma proteins. Over a range of usual adult dietary vitamin C intakes (30-60 mg), 80% to 90% is absorbed. At doses of 1 gram or more, absorption efficiency drops to about 20%.

Relatively small amounts of vitamin C are lost through catabolism since the immediate oxidized forms of vitamin C are readily reduced back to ascorbic acid. The primary products of oxidation beyond dehydroascorbic acid (DHA) include oxalic and threonic acids, L-xylose, and ascorbate 2-sulfate. Unabsorbed ascorbate is degraded in the intestines, which may account for the diarrhea and intestinal discomfort sometimes reported by persons ingesting large doses.

The second primary mechanism for regulation of body ascorbate content is renal action to conserve or excrete unmetabolized ascorbate. Little unmetabolized ascorbate is excreted with dietary intakes of up to 80 mg/day while renal excretion of ascorbate increases proportionately with higher intakes.

Dose-dependent absorption and renal regulation of ascorbate allow for the conservation of vitamin C during low intakes and limitation of plasma levels at high intakes. Tissue-specific cellular transport systems allow for wide variation of tissue ascorbate concentrations. High levels are maintained in the pituitary and adrenal glands, leukocytes, eye tissues and humors, and the brain, while low levels are found in plasma and saliva. Due to homeostatic regulation, the biological half-life of ascorbate varies widely from 8 to 40 days and is inversely related to the ascorbate body pool. Similarly, catabolic turnover varies widely, about 10 to 45 mg/day, over a wide range of dietary intakes due to body pool size. A total body pool of less than 300 mg is associated with symptoms of scurvy, while maximum body pools are limited to about 2 g. At very low ascorbate intakes, essentially no ascorbate is excreted unchanged and a minimal loss occurs.

12.4 Effects of Deficiency and Excess

12.4.1 Deficiency

The first symptom of vitamin C deficiency is fatigue, the “lassitude” of scurvy. Fatigue is subtle and precedes other symptoms such as bleeding gums. Scurvy, a potentially fatal disease, can be prevented with as little as 10 mg vitamin C per day, an amount easily obtained through consumption of fresh fruits and vegetables. However, daily ingestion of only this amount for several weeks results in a body pool that is not substantially above 300 mg. On the other hand, when 77.5 mg of ascorbate is ingested daily, a pool of approximately 1500 mg can be maintained. Such level can prevent clinical scurvy from developing for 1 to 1.5 months in men given a diet low in ascorbic acid. Steady-state plasma concentrations achieved by 60 mg/d will prevent deficiency for 10 to 14 days if vitamin C ingestion suddenly ceased. Steady state plasma concentrations of 55 μmol/L to 60 μmol/L, achieved by 100 mg/day, will probably prevent deficiency for 1 month if vitamin C ingestion suddenly ceases.

12.4.2 High intake

(*a*) *Beneficial Effects*

Vitamin C enhances iron absorption from non-heme iron. Several human absorption studies conducted by several investigators indicate that each main meal should preferably contain at least 25 mg to 50 mg of ascorbic acid. Higher ascorbic acid intakes should be considered if meals contain higher amounts of factors inhibiting iron absorption, such as phytates and tannins.

Dietary intakes of 200 mg or more of vitamin C from fruits and vegetables have been associated with lower cancer risk especially for cancers of the oral cavity, esophagus, stomach, colon, and lung. 5 servings of fruits and vegetables appear to be protective. However, consumption of vitamin C as a supplement in experimental trials did not decrease the evidence of colorectal adenoma and stomach cancer. These results would suggest that fruit and vegetable intake may be associated with lower cancer risk not because of interactions between ascorbate and bioactive compounds in these foods, but due to non-dietary characteristics of people who eat fruits and vegetables.

The totality of evidence from human studies reviewed by Carr and Frei (1999) suggest that a dietary of intake of 90 mg to 100 mg of vitamin C per day is associated with reduced risk of cardiovascular disease and cancer. Other than to treat deficiency, beneficial effects of vitamin C on clinical outcomes have not been conclusively demonstrated.

It is commonly believed that high doses of vitamin C protect against or cure colds. A review of studies conducted on the common cold over the past 20 years concludes that, in general, large doses of vitamin C do not have a significant effect on the incidence of the common cold. However, a few studies have indicated that certain susceptible groups (e.g., individuals with low dietary intake and marathoners) may be less susceptible to the common cold when taking supplemental vitamin C. Additionally, large doses of vitamin C have been found to decrease the duration and severity of colds, an effect that may be related to the antihistamine effects found to occur with large doses (2 grams) of vitamin C.

(*b*) *Excessive Intake*

Vitamin C has little toxicity, although there are adverse effects that are often dose-dependent. IOM has recently evaluated the safety of vitamin C and decided on an upper tolerable intake level (UL) of 2 g/day for adults, the amount of intake where no risk of adverse effects is observed. Available data indicate that oral intakes of very high amounts of vitamin C (2 g/day to 4 g/day) are well-tolerated biologically.

Nausea, abdominal cramps, and osmotic diarrhea are the most common adverse effects of high vitamin C intake. These effects are attributed to the osmotic effect of unabsorbed vitamin C passing through the intestine and eliminated in the stool. Some studies conducted to evaluate gastrointestinal effects reported osmotic diarrhea, transient colic, and flatulent distention in normal healthy volunteers at doses of 3 g/day to 4 g/day. Abdominal bloating and osmotic diarrhea were observed to occur after doses of more than 2 g are taken at once.

Diseases like hemochromatosis, thalassemia major, sideroblastic anemia or other diseases requiring multiple red blood cell transfusions may result in iron overload. While it is theoretically possible for vitamin C to enhance iron overload or harm individuals with these disorders, patients with hemochromatosis should not be discouraged from eating fruits and vegetables. Although uncertain, it is unlikely that vitamin C induces iron over-absorption in healthy people.

A product of vitamin C catabolism is oxalate. Results of several studies show that oxalate excretion is probably increased at vitamin C doses of 1 g or more daily in some people, although the consequences are not clear. Reports of kidney stone formation associated with excess ascorbic acid intake are limited to individuals with renal disease, and not associated with apparently healthy individuals. The DRI Committee has evaluated the scientific evidence and concluded that there is no evidence for kidney stone formation at high intakes of ascorbic acid. Patients with hyperoxaluria should take high doses of vitamin C only under medical supervision.

Data are conflicting concerning the effect of ascorbate on urate excretion. This may be due to lack of steady-state for vitamin C, differences in plasma concentrations, or duration of vitamin C administration. The DRI Committee has evaluated the scientific evidence and concluded that there is no evidence for hyperuricosuria at high intakes of ascorbic acid.

Increasing vitamin C levels have been reported to destroy vitamin B_{12}; however, the findings are controversial.

(c) *Guidance on High Intake*

Based on considerations of causality, relevance and the quality and completeness of the databases, the DRI Committee selected osmotic diarrhea and related gastrointestinal disturbances as critical endpoints on which to base the UL of vitamin C. UL is the highest level of daily nutrient intake that is not likely to pose risk of adverse health effects in almost all individuals.

As per extensive review of the DRI Committee, in vivo data do not clearly show a causal relationship between excess vitamin C intake by apparently healthy individuals and other adverse effects (i.e., kidney stone formation, excess iron absorption, reduced vitamin B_{12} and copper levels, increased oxygen demand, systemic conditioning, pro-oxidant effects, dental enamel erosion, or allergic response) in adults and children. The UL values, as estimated by the DRI Committee are summarized in Table 12.1.

According to FAO/WHO (2002), 1 g of vitamin C appears to be the advisable UL of dietary intake.

Table 12.1 UL of vitamin C for various age groups

Age Groups (years)	Vitamin C (mg/day)
Infants	Not possible to establish; sources of intake should be formula and food only
Children	
1 – 3	400
4 – 8	650
9 – 13	1,200
Adolescents (14 – 18)	1,800
Pregnant women (years)	
14 – 18	1,800
> 19	2,000
Lactating women (years)	
14 – 18	1,800
> 19	2,000
Women (≥ 19)	2000
Men (≥ 19)	2000

Source: IOM (2000)

12.5 Food Sources

While relatively few foods contain high concentrations of ascorbic acid, there are many that contain moderate amounts. Fresh fruits and leafy vegetables, which are plentiful in Southeast Asian countries, contain the highest concentration. Of the fruits, the best sources of vitamin C include ripe papaya, guava, mango, citrus fruits and cantaloupe. Among vegetables, the best sources are ripe tomato, bell peppers, bean sprouts and leafy vegetables. Meat, fish, poultry, eggs and dairy products contain smaller amounts of vitamin C and are not significant sources, unless large quantities are included in the diet.

Fruits, leafy vegetables and tubers contribute significantly to Vietnam's reported usual intake of 71.5 mg. In Singapore, the mean ascorbic acid intake of adults is 88 mg/day and the food sources includes vegetables such as *chye sim* (mustard green/flowering cabbage), cabbage, *kailan* (Chinese kale) and fruits such as papaya, orange and guava. In the Philippines, intake of vitamin C appeared to be lower, at around 50 mg in the late 1980s.

These reported intakes were calculated from food nutrient contents based on food composition tables. Actual intakes may be considerably lower than the reported amounts, considering the variable losses in cooking and processing. In a study conducted in the Philippines, losses due to cooking ranged from 33% to 82 %; an additional 3% to 25% of vitamin C was lost when foods were subjected to over-cooking or reheating. On the other hand, actual intakes could also be higher than computed amounts if these do not include vitamin C from supplements, fortified foods, and ascorbic acid added to some processed foods because of its antioxidant or other properties.

Vitamin C is very labile, and the loss of vitamin C upon the boiling of milk provides an example of a cause of infantile scurvy. The vitamin C content of food is strongly influenced by season, transport to market, shelf life, time of storage, cooking practices and chlorination of water.

Dietary availability is thus not a constraint in setting recommended intakes of vitamin C. Since dietary sources of vitamin C are readily available, intake is governed by food selection. Most food-based dietary guidelines are similar in that all recommended consumption of five servings of fruits and vegetables daily. If this recommendation is followed, daily vitamin C intake can be as high as 210 mg to 280 mg, depending on food content factors and food choices.

12.6 Factors Affecting Requirement

Bioavailability, nutrient-nutrient interactions, smoking and gender are important factors that affect vitamin C requirement.

At levels of usual dietary intakes, some 70% to 90% of vitamin C consumed is absorbed, giving a total daily intake of 30 mg to 180 mg. Absorption decreases to about 50% or less with single doses above 1 g. The type of food consumed has not been shown to have a significant effect on absorption of vitamin C, either those intrinsic in food or supplemental vitamin C. The bioavailability of the vitamin naturally found in foods or in the form of a supplement has not been shown to be significantly different from that of pure synthetic ascorbic acid.

Vitamin C participates in redox reactions with many other dietary and physiological compounds, including glutathione, tocopherol, flavonoids, and the trace metals iron and copper. Interactions of ascorbate with the endogenous antioxidant glutathione have been shown in both rodents and humans. In human feeding trials, it has been shown that glutathione status is very much affected by dietary ascorbate intake. Thus, ascorbate may contribute to antioxidant protection by maintaining reduced glutathione.

Evidence from *in vitro* and animal studies have shown that vitamin C can regenerate or spare alpha-tocopherol but studies in guinea pigs and humans have not confirmed that this interaction occurs to a significant extent *in vivo*. Calculation of redox potentials indicates that ascorbate can recycle the flavonoid radical. It has also been shown that ascorbic acid acts synergistically with the flavonoid quercetin, to protect cutaneous tissue cells in culture against oxidative damage induced by glutathione deficiency.

A variety of interactions of ascorbate with the redox-active trace metals iron and copper have been reported. Ascorbic acid is involved in the regulation of iron metabolism at a number of points. Ascorbate-related reduction of iron to the ferrous state is involved in iron transfer and storage pathways. Vitamin C is the most potent enhancer of non-heme iron absorption, possibly due to lowering of gastrointestinal iron to the more absorbable ferrous state or amelioration of the effect of dietary iron absorption inhibitors. A study by Hallberg (1987) showed that iron absorption from non-heme food sources can be increased significantly with a daily vitamin C intake of at least 25 mg during each meal (estimated for 3 meals/day). Higher vitamin C intakes should be considered if meals contain higher contents of nutrient inhibitors such as phytates and tannins.

Some evidence indicates that excess ascorbic acid intake may affect copper metabolism in a variety of ways, including inhibition of intestinal absorption and ceruloplasmin oxidase activity and labilization of ceruloplasmin-bound copper for cellular transport. However, the significance of these effects

in humans is questionable, because high ascorbate intakes among men on a metabolic unit did not inhibit copper absorption.

Nearly all studies show that smokers have decreased plasma and leukocyte ascorbate levels compared to nonsmokers. Part of this difference may be attributable to a lower intake of fruits and vegetables among smokers than among nonsmokers. However, studies that have adjusted for differences in vitamin C intake and those which have assessed populations with similar fruit and vegetable intakes still find that smokers have lower plasma vitamin C concentrations than nonsmokers. This indicates that smoking per se predisposes to lower vitamin C status.

In both observational and intervention studies, human plasma or serum ascorbate levels are usually found to be higher in females than in males of the same population at a given vitamin C intake. Although studies that directly compare the vitamin C requirements for men and women were not found, a difference in average vitamin C requirements of men and women is assumed based on mean differences in body size, total body water, and lean body mass.

12.7 Estimating Requirements and Recommended Intakes

12.7.1 Indicators for estimating requirements for vitamin C

Functional measures for vitamin C status are not currently available, and vitamin C status is generally based on vitamin C concentrations in plasma or leucocytes. Although these indexes are closely related over a wide range of vitamin C intakes, leukocyte concentrations are considered to be the more sensitive indicator of vitamin C status (Johnston, 2001). However, the measurement of leukocyte vitamin C is technically complex and data interpretation is complicated by the variable vitamin C content in different leukocyte fractions and the lack of standardized reporting procedures. Hence, the measurement of plasma vitamin C concentration is currently the most practiced and widely applied test for vitamin C status. Plasma concentrations of less than 11 μmol/L (0.2 mg/dL) indicate vitamin C deficiency, and concentrations between 11 μmol/L and 28 μmol/L (0.2 and 0.5 mg/dL respectively) represent marginal vitamin C status, defined as moderate risk for developing vitamin C deficiency. Intakes at the recommended level of 75 mg/day to 90 mg/day are associated with plasma vitamin C concentrations near 34 μmol/L to 45 μmol/L (0.6 mg/dL to 0.8 mg/dL respectively). Tissue saturation is said to be achieved at intakes slightly more than 100 mg/day, corresponding to plasma concentrations near 60 μmol/L (1.0 mg/dL).

12.7.2 Recommendations for vitamin C intake by life stages

The main references used in arriving at recommendations for riboflavin intakes for the Southeast Asia region were the 2002 report of the FAO/WHO Expert Consultation on vitamins and mineral requirements (FAO/WHO, 2002) and the recommendations of the DRI Committee (IOM, 2000). The rationale and steps taken in setting requirements and the levels recommended by these organizations were considered. Existing RDAs of countries in the Southeast Asian region were additional references; there were no studies of vitamin C requirements in the region.

(*a*) *Infants (0 to 12 months)*

In infants, no functional criteria of vitamin C status that reflect response to dietary intake have been demonstrated. Thus, the DRI Committee proposed that recommended intakes of vitamin C for infants be based on an AI that reflects the observed mean vitamin C intake of infants fed principally with breast milk.

Breast milk is recognized as the optimal milk source for infants throughout at least the first year of life. It is recommended as the sole nutritional milk source for infants during the first 4 to 6 months of life. The DRI Committee estimated the AI for infants based on the average volume of milk intake of 780 ml and an average concentration of vitamin C of 50 mg/L in breast milk. For infants 0 to 6 months old, 40 mg/day was the estimated AI.

During the second 6 months of life, solid foods become a more important part of an infant's diet and add a significant, but poorly-defined, amount of vitamin C to the diet. Although limited data are available for typical vitamin C intakes from foods by infants fed breast milk, mean vitamin C intakes from solid foods are 22 mg/day for formula-fed infants. For purposes of developing an AI for this age group, it is assumed that infants who are fed breast milk have intakes of solid food similar to formula-fed infants of the same age group. Based on a mean breast milk intake during the second 6 months of life at 0.6 L/day and a vitamin C concentration of about 45 mg/L at 9 months (the midpoint of this age group) of lactation, intake of vitamin C from breast milk would be approximately 27 mg/day. Adding up the intake from milk (27 mg/day) and food (22 mg/day), the total AI for vitamin C is rounded to 50 mg/day.

The FAO/WHO estimated the mean vitamin C concentration in breast milk at 40 mg/L (FAO/WHO, 2002). However, it was felt that the amount of vitamin C in breast milk appears to reflect maternal dietary intake rather than the infant's needs. Moreover, it was noted that 8 mg/day of vitamin C is sufficient to prevent scorbutic signs in infants. The FAO/WHO therefore set the recommended intake for infants aged 0 to 6 months at 25 mg/day. The recommended intake for older infants were gradually increased to 30 mg per day.

(*b*) *Children and Adolescents (1 to 18 years)*

In the absence of direct data on which to determine EARs for vitamin C for children and adolescents, the DRI Committee extrapolated the requirement from those of adults based on relative body weight. The resulting RDAs for children aged 1 to 3 years and 4 to 8 years are lower than the adequate intakes established for infants. The recommended intakes for vitamin C for children and adolescents in the 2002 FAO/WHO Report were gradually increased from the recommended intake for infants.

(*c*) *Adults (19 - 64 years)*

Historically, the human requirements for vitamin C have been estimated from the amount needed to prevent the classic disease of scurvy, the amount metabolized by the body and/or the amount that will maintain adequate body stores of the vitamin. Scurvy is now rare in most countries of the world. Other human experimental data that can be utilized to set a vitamin C requirement, based on a biomarker other than scurvy, are also limited. The DRI Committee's recommended intakes of vitamin C are based on an amount of the vitamin that

is thought to provide antioxidant protection as derived from the correlation of such protection with neutrophil ascorbate concentrations (IOM, 2000).

The DRI Committee did, however, acknowledge that there are no human data to quantify directly the dose-response relationship between vitamin C intake and *in vivo* antioxidant protection. The DRI Committee cited only one study (Levine *et al.*, 1996) with 7 apparently healthy males that reported plasma, neutrophil, and urinary ascorbate concentrations during vitamin C depletion and repletion to steady state. Thus, there are wide uncertainties in the data utilized to estimate the vitamin C requirements. However, in the absence of other data, the DRI Committee felt that maximal neutrophil concentration with minimal urinary loss appears to be the best biomarker at the present time.

Based on vitamin C intakes sufficient to maintain near-maximal neutrophil concentrations with minimal urinary loss, the DRI Committee set an EAR of 75 mg/day of vitamin C for men (IOM, 2000). Based on this, the RDA for men was computed to be 90 mg/day. Since no similar data were available for women, it is assumed that women will have a lower requirement due to their smaller lean body mass, total body water, and body size. The RDA for women was thus set at 75 mg/day.

The DRI Committee also noted that at a vitamin C intake of 90 mg/day, the plasma ascorbate concentration reaches 50 μmol/L, which has been shown to inhibit LDL oxidation *in vitro* systems. Although it is not known whether vitamin C prevents LDL oxidation *in vivo*, such a function might be relevant in the prevention of heart disease. Furthermore, since neutrophils are at 80% saturation at an EAR of 75 mg/day, this should potentially protect intracellular proteins from oxidative injury when these cells are activated during infectious and inflammatory processes.

In the 2002 FAO/WHO Report, dietary intake was calculated from physiologic requirements. At saturation, the whole body content of ascorbate in adult males is approximately 20 mg/kg, or 1500 mg. Clinical signs of scurvy appear when the whole body content falls below 300 mg to 400 mg, and the last signs disappear when the body content reaches about 1,000 mg. In these experiments, ascorbate in the whole body was catabolised at an approximate rate of 2.9%/day.

There is a sigmoidal relationship between intake and plasma concentrations of vitamin C. At low doses, dietary vitamin C is almost completely absorbed, but over the range of usual dietary intakes (30 mg/day to 180 mg/day), absorption may decrease to 75% due to competing factors in the food source.

A body content of 900 mg falls half way between tissue saturation and the point at which clinical signs of scurvy appear. Assuming an absorption efficiency of 85%, and a catabolic rate of 2.9, the average intake of vitamin C can be calculated as:

$$900 \times 2.9/100 \times 100/85 = 30.7 \text{ mg/day}$$

(which can be rounded off to 30 mg/day)

The recommended nutrient intake (RNI) would therefore be:

900 x (2.9 + 1.2)/100 x 100/85 = 43.4 mg/day
(which can be rounded off to 45 mg/day)

No turnover studies have been done in women, but from the smaller body size and whole body content of women, requirements might be expected to be lower. However, in depletion studies, plasma concentrations fell more rapidly in women than in men. FAO/WHO therefore made the same recommendation for non-pregnant, non-lactating women as for men (FAO/WHO, 2002).

An intake of 45 mg/day will ensure that measurable amounts of ascorbate will be present in the plasma of most people and will be available to supply tissue requirements for metabolism or repair at sites of depletion or damage. A whole body content of around 900 mg of vitamin C would provide at least 1 month's safety interval, even for a zero intake, before the body content falls to 300 mg.

(*d*) *Older Adults* (*65 years and above*)

Upon reviewing available data, the DRI Committee concluded that no consistent differences in the absorption or metabolism of ascorbic acid due to aging have been demonstrated at median vitamin C intakes. This suggests that the reports of low blood vitamin C concentrations in elderly populations may be due to poor dietary intakes, chronic disease or debilitation or other factors, rather than an effect of aging per se. Therefore, the DRI Committee did not provide for additional vitamin C allowance for older adults beyond that recommended for younger adults, being 90 mg/day for men and 75 mg/day for women.

The FAO/WHO also felt that the requirements of elderly people do not differ substantially from those of younger people in the absence of pathology, which may influence absorption or renal functioning. The FAO/WHO recommended intakes for the elderly are therefore the same as those for adults (45 mg/day for both sexes) (FAO/WHO, 2002).

(e) *Pregnancy and Lactation*

Maternal plasma vitamin C concentration has been known to decrease with the progression of pregnancy due to hemodilution as well as active transfer to the fetus. Therefore, in order to transfer adequate vitamin C to the fetus, additional vitamin C is needed during pregnancy. In the absence of data on near maximal neutrophil saturation during pregnancy, the method of determining the EAR for pregnancy is based on adding the EAR for near-maximal neutrophil concentration of the non-pregnant woman to the amount of vitamin C necessary to transfer adequate vitamin C to the fetus. In the absence of precise data regarding transfer of maternal vitamin C to the fetus, and with the knowledge that intakes of 7 mg/day of vitamin C will prevent young infants from developing scurvy, the DRI Committee estimated that the average requirement for pregnant women (19 – 30 years) be increased by 10 mg/day over the vitamin C requirement for non-pregnant woman. To obtain the EAR for lactation, a further 40 mg/day, being the average vitamin C level in breast milk, during the first 6 months of lactation, is added to the EAR for the non-lactating women. Based on this EAR, the RDA for vitamin C for lactating women is obtained by adding 45 mg/day to the recommended intake of non-lactating women.

The FAO/WHO also recognized that during pregnancy, there is a moderate extra drain on vitamin C, particularly during the last trimester and that 8 mg/day of vitamin C has been reported to be sufficient to prevent scorbutic signs in infants aged 4 to 17 months. The FAO/WHO therefore provided an extra 10 mg/day throughout pregnancy, bringing the recommended intake up to 55 mg/day, to enable reserves to accumulate to meet the extra needs of the growing fetus during the last trimester (FAO/WHO, 2002).

FAO/WHO estimates that during lactation, 20 mg/day of vitamin C is secreted in milk. For an assumed absorption efficiency of 85%, an extra 25 mg will be needed by the lactating mother. The FAO/WHO Expert Consultation therefore concluded that the recommended intake should be set at 70 mg/day to fulfil the needs of both the mother and infant during lactation (FAO/WHO, 2002).

12.8 Current RDAs for vitamin C in Southeast Asia

A tabulation of the current RDAs for vitamin C in 6 Southeast Asian Countries is set out in Table 12.2.

Table 12.2 Comparison of current RDAs (mg/day) for vitamin C in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 - 1)	30 - 35	25 - 30	30	20	35[a]	30
Children (1 - 9)	40 - 45	30 - 35	30 - 35	20	40	35 - 55
Boys (10 - 18)	50 - 60	65	45 - 75	20	45 - 90	65 - 80
Girls (10 - 18)	50 - 60	65	45 - 70	20	45 - 75	70 - 80
Men (≥ 19)	60	70	75	30	90	75
Women (≥ 19)	60	70	70	30	75	70
Pregnancy						
1st trimester	+10	+10	+10	+20	+10	+0
2nd trimester	+10	+10	+10	+20	+10	+10
3rd trimester	+10	+10	+10	+20	+10	+10
Lactation						
1st 6 months	+25	+25	+35	+20	+35	+30
2nd 6 months	+10	+25	+30	+20	+35	+0

Notes: [a] *figures only for infants aged 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[d] *≥ 18 for Singapore*
Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

Recommendations for vitamin C in the countries in the region reviewed are rather similar, with the exception of recommendations for Singapore. RDAs for vitamin C in Singapore are clearly lower that those of other countries for all age groups, up to as much as 50%. On the other hand, the recently updated Thai RDA appear to be the highest. Philippines, Thailand and Vietnam have recommended slightly lower amounts of the vitamin for girls and women, compared to RDAs for boys and men.

All countries recommended additional vitamin C intake throughout pregnancy, except for the Vietnamese RDA which does not recommend additional amounts of the vitamin during the first trimester. With the exception of Singapore which recommends an additional 20 mg/day, all other countries recommend an addition of 10 mg/day. Except for Vietnam which only recommends additional vitamin C during the first 6 months of lactation, all other countries have recommended

additional intakes during the whole lactation period. The additional amounts recommended are generally higher than those recommended during pregnancy and range from 20 mg/day to 35 mg/day for the first 6 months and 0 mg/day to 35 mg/day during the second 6 months.

12.9 Recommended RDAs for vitamin C for Southeast Asia

After thorough review of all of the above information and the recommendations of both IOM and FAO/WHO, the SEA-RDA Committee adopted the recommendations of the FAO/WHO (2002) for vitamin C. However, the SEA-RDA Committee also considered the high prevalence of iron-deficiency anemia among many communities in the region, and the observation that the additional intake of at least 25 mg of vitamin C enhances absorption of soluble non-heme iron (Hallberg, 1987). Furthermore, recent studies have indicated a possible antioxidant role for vitamin C. The SEA-RDA Committee therefore decided to add 25 mg vitamin C per day to all age groups from children 10 years and above. The SEA-RDA Committee's recommended RDAs for the Southeast Asian population are summarized in Table 12.3.

Table 12.3 Recommended RDAs for vitamin C for Southeast Asia

Age Groups	Vitamin C RDA (mg/day)
Infants (months)	
0 – 5	25
6 – 11	30
Children (years)	
1 – 3	30
4 – 6	30
7 – 9	35
Boys (10 – 18 years)	65
Girls (10 – 18 years)	65
Men (years)	
19 – 65	70
> 65	70
Women (years)	
19 – 65	70
> 65	70
Pregnancy	80
Lactation	95

The SEA-RDAs are much higher than the levels recommended by Malaysia and Singapore, published at least 16 years ago. This is especially so for adolescents and adults, where the SEA-RDAs are at least twice the earlier values. In comparison with Indonesia and Thailand, the SEA-RDAs are slightly lower for infants and children, but are slightly higher for adolescents and adults. The revised Philippines RDAs for vitamin C are similar to the proposed RDAs for most age groups. The former has a higher recommended intake during lactation, reaching up to 105 mg/day during the first 6 months.

While usual intakes in the region are generally lower, the SEA-RDA levels are thought to be desirable in a region where iron-deficiency anemia is widespread. The low vitamin C intakes observed in some Southeast Asian countries are due more to poor food choices rather than unavailability of food sources. Every effort should thus be made to encourage increased consumption of fresh fruits and vegetables, as well as home food production if cost is a constraint.

Bibliography

Carr AC & Frei B (1999). Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am J Clin Nutr* 69:1086-1101.

FAO/WHO (2002). Vitamin C. In: *Human Vitamin and Mineral Requirements*. Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 73-86.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines*. 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

Hallberg L. (1987) Wheat fiber, phytates and iron absorption. *Scand J Gastroenterol Suppl* 129:73-79.

IOM (2000). Vitamin C. In: *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; chapter 5, pp 95-185.

Johnston CS (2001). Vitamin C. In: *Present Knowledge in Nutrition*, 8th Edition. Bowman BA and Russel RM, eds. International Life Sciences Institute Press, Washington, DC; chapter 16, pp. 175-213.

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

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

13.THIAMIN (VITAMIN B_1)

13.1 Introduction

Thiamin, also known as vitamin B_1 or aneurin, was one of the earliest vitamins recognized. In the late 1890's, Dutch medical officers Eijkman and Grijns, working in Java, showed that a paralytic condition resembling beri-beri could be produced in chickens by feeding them a diet consisting solely of polished rice. They recognized the similarities between polyneuritis in birds and the disease beri-beri in humans. Human experiments were carried out in a mental asylum and in railroad labour camps in the Malay States, whereby half of the subjects received polished or white rice, and the other half received brown rice, from which the bran had not been removed. Beri-beri always appeared to affect the subjects who were given only polished rice.

In 1926, Jansen and Donath, working in the laboratory formerly occupied by Eijkman, isolated the anti-beri-beri factor, vitamin B_1, as crystals from a water extract of rice bran. In 1936, Williams identified and published the chemical formula and named it Thiamin, referring to the thiazole and amino groups in the molecule. A year later, improved methods of synthesis led to the first commercial production of the vitamin, thiamin.

13.2 Characteristics and Functions

The thiamin molecule consists of one pyrimidine and one thiazole ring, linked by a methylene bridge. The molecule is a water-soluble white crystalline solid made up of carbon, hydrogen, nitrogen, oxygen and sulfur. In the crystallized state or in an acid solution, the stability of thiamin is good, even when heated. In a neutral or alkaline solution, thiamin is unstable and sensitive to heat, oxygen and ultraviolet light.

Thiamin serves as an important co-enzyme in metabolism. It combines with pyruvic acid to form a co-enzyme thiamin pyrophosphate (TPP) necessary for the metabolism of carbohydrates and branched-chain amino acids. Hence, when there is insufficient thiamin, the overall decrease in carbohydrate metabolism and its inter-connection with amino acid metabolism (via a-keto acids) have severe consequences, such as a decrease in the formation of acetylcholine for neural function. Because transketolase activity decreases early in thiamin deficiency, its determination in erythrocytes is used as a reliable tool to assess thiamin nutritional status.

13.3 Absorption, Utilization and Excretion

Following ingestion, absorption of thiamin occurs primarily in the jejunum, involving mainly 2 mechanisms. At low concentrations, the vitamin is absorbed by an active, carrier-mediated system involving phosphorylation. At higher concentrations, the main absorption mechanism is passive diffusion.

In the blood, thiamin is transported both in erythrocytes and plasma. Only a small percentage of a high dose of thiamin is absorbed, and elevated serum levels result in active urinary excretion of the vitamin. After an oral dose of thiamin, peak excretion occurs in about 2 hours, and excretion is nearly completed after 4 hours.

Total thiamin content of the adult human has been estimated to be approximately 30 mg and the biological half-life is approximately 9 to 18 days. Body storage of thiamin is minimal, the liver being the main extra-muscular storage site. The limited stores may be depleted within 2 weeks or less on a thiamin-free diet, with clinical signs appearing shortly thereafter. The body is readily depleted of thiamin by fever and other metabolic stress.

13.4 Effects of Deficiency and Excess

13.4.1 Deficiency

Thiamin deficiency results in the disease called beri-beri, which has been classically considered to exist in dry (paralytic) and wet (oedematous) forms. Beri-beri occurs in breast milk-fed infants whose nursing mothers are deficient in the vitamin. It also occurs in adults with high carbohydrate intakes mainly from milled rice and intakes of anti-thiamin factors.

The clinical signs of deficiency include anorexia; weight loss; mental changes such as apathy, decrease in short-term memory, confusion and irritability; muscle weakness; and cardiovascular effects such as an enlarged heart. In wet beri-beri, edema occurs; in dry beri-beri, muscle wasting is obvious. In infants, cardiac failure may occur rather suddenly. In relatively industrialized nations, the neurologic reflections of Wernicke-Korsakoff syndrome are frequently associated with chronic alcoholism with limited food consumption.

Thiamin deficiency can develop within 2 to 3 months of a deficient intake and can cause disability and death. In young and healthy non-alcoholic individuals, subjective symptoms appear after 2 to 3 weeks of a deficient diet.

Frank thiamin deficiency is rare today, although some population segments could be on marginal or sub-marginal intakes of the vitamin. Symptoms are less prominent in sub-clinical deficiencies and may include tiredness, headache and reduced productivity.

13.4.2 Excessive Intake

Toxicity of thiamin does not appear to be a problem because renal clearance of ingested amounts is rapid. There are no reports of adverse effects from consumption of excess thiamin by ingestion of food and supplements. This, however, does not mean that there is no potential for adverse effects resulting from high intakes, especially in view of the increasing practice of supplements of up to 50 mg/day of thiamin.

There is some evidence of toxicity from large doses given parenterally. Varying reports have been made on these doses. Large parenteral doses (100 mg to 500 mg) have been reported to be generally well-tolerated. Only after several injections, by different routes, of doses exceeding by more than 100-200 fold the recommended daily intake have toxic effects been observed.

13.4.3 Guidance on High Intake

Due to inadequate data for a quantitative risk assessment, no tolerable upper intake level (UL) has been proposed for thiamin.

13.5 Food Sources

Thiamin is present in all plant and animal tissue, but most contain only low concentrations of the vitamin. In plants, thiamin occurs predominantly as free thiamin and in animals almost entirely (95% to 98%) in phosphorylated forms, the predominant form being thiamin pyrophosphate. The richest source of thiamin is yeast. Cereal grains, however, comprise the most important dietary source of thiamin in most human diets. Pork is the richest source of naturally occurring thiamin.

Several anti-thiamin factors have been recognized. Thiamin-degrading enzyme or thiaminase is present in the raw tissue of many fishes, chiefly fresh water fishes but also in Atlantic herring. These are heat labile and can be effective antagonists of the vitamin when consumed without heat treatment. Other thiamin antagonists include polyphenols (eg. caffeic acid, chlorogenic acid, tannic acid) present in tea, coffee, betel nuts and red cabbage and flavonoids (eg quecetin and rutin) widely distributed in edible fruits and vegetables.

13.6 Factors Affecting Requirement

Experimental studies with adults and infants have indicated that thiamin requirement is related to the dietary ratio of carbohydrates to fat. However, a major increase in the proportion of fat in the diet was found to have a comparatively small effect on thiamin requirements. Thus diets exceedingly high in carbohydrates appear to increase the requirement for thiamin. Protein is intermediate between fats and carbohydrates with regards to its metabolic influence on thiamin requirements.

Thiamin requirement will vary with body weight because energy requirements are related to body weight. A small (10%) difference in the average thiamin requirements for men and women is assumed based on mean differences in body size and energy utilization.

Heavy exercise under certain conditions may increase the requirement for thiamin, but the observations on the effects of physical activity on thiamin requirement have been inconsistent, the effects small, and the experimental conditions highly variable. It is assumed that under normal conditions, physical activity does not appear to influence thiamin requirements to a substantial

degree. However, those who are engaged in physically demanding occupations or who spend much time training for active sports may require additional thiamin.

Minimal requirements for dietary thiamin may increase with age, particularly for active individuals. Studies have shown that older women (over 50 years of age) have lower thiamin excretion at all levels of intake and have slower response to partial thiamin repletion compared to younger women. This would suggest that a higher thiamin-calorie ratio may be needed by older than by younger individuals.

Thiamin is said to be sufficient provided the recommended thiamin to calorie ratio is maintained. However, it has been reported that in abnormal physiological conditions such as intravenous feeding, heavy alcoholism, liver disease, excessive carbohydrate intake, and use of powerful diuretics, clinical thiamin deficiency can develop despite daily administration of 1.2 mg or more of the vitamin. The need for additional thiamin increases during severe diarrhea, fever, stress and surgery.

13.7 Estimating Requirements and Recommended Intakes

13.7.1 Indicators for estimating requirement for thiamin

Biochemical changes in thiamin status occur well before the appearance of clinical signs of deficiency. Thiamin status can thus be assessed by determining these biochemical changes, for example, by determining the erythrocyte transketolase activity (ETKA); by measuring the concentration of thiamin and its phosphorylated esters in blood or serum components or by measuring urinary thiamin excretion.

13.7.2 Erythrocyte transketolase activity

Erythrocyte transketolase activity (ETKA), the activity of the thiamin-requiring enzyme transketolase, appears to provide information as to tissue reserves of thiamin and reflects a direct functional evaluation at the cellular level. It is generally regarded as the best functional test of thiamin status. ETKA is measured in hemolyzed erythrocytes by determining the rate of disappearance of pentose or the appearance of hexose. Usually, ETKA is determined without (basal) and with (stimulated) addition of TPP in vitro and is expressed as basal activity (ETKA) or as the difference between stimulated and basal activity as a percentage of the basal activity (ETKA-AC activation coefficient or TPP effect). Thiamin deficiency is associated with decreased ETKA and increased TPP effect: the higher the value of TPP effect, the greater the degree of thiamin deficiency.

The biochemical diagnostic criteria of thiamine deficiency consist of low ETKA (normal values range from 42.1 to 86.2 mu/ litre/min) and high TPP effect (normal range 0% to 14%; marginally deficient 15% to 24%; severe deficiency ≥ 25%). ETKA has been reported to be poorly correlated with dietary thiamin intake. Therefore, evaluation of thiamin status should consider other indicators along with ETKA.

13.7.3 Urinary thiamin

Urinary thiamin levels can provide information as to the adequacy of the dietary intakes. At recommended intakes, urinary excretion of thiamin ranges from 40 μg to 90 μg per day. When intake is deficient, urinary excretion falls below 25 μg per day. A correlation between the urinary excretion of thiamin per gram of creatinine and thiamin intake has been observed. Analysis of 24 hour urine collection provides more reliable information than random sample collections. In cases of clinical thiamin deficiency, 24 hour urinary excretion of 0 μg to 15 μg of thiamin have been reported.

Additional information as to the physiological state with respect to thiamin can be obtained from the test-dose procedure. The most commonly used procedure is to administer parenterally 5 mg of thiamin and measure the urinary excretion of thiamin over the following 4-hour period Although the test may not specifically identify clinical thiamin deficiency or indicate the severity of the deficiency, it can be used as an indicator of low intake and tissue deficits of the vitamin. The 2 main limitations for the use of this indicator are:

(a) They do not provide the desired information regarding the state of deficiency or the degree of depletion of tissue thiamin reserves.

(b) The test-dose procedure has not been used extensively in the field because of the inconvenience of obtaining 4-hour urine collections following the administration of the thiamin test load.

13.7.4 Erythrocyte thiamin

Blood contains only about 0.8% of the total body thiamin, and the concentration is too low to allow precise extrapolation of the total thiamin status. There is, however, a relatively accurate microbiological method which can be used with whole blood, red and white blood cells, or any other body fluids and tissues.

As thiamin status declines, the concentration of TPP in the red cell decreases at approximately the same rate as occurs in other tissues.

13.7.5 Pyruvate and lactate

Thiamin is required for pyruvate metabolism; increased blood pyruvate and lactate levels can be caused by thiamin deficiency. In thiamin deficiency, the fasting level of blood pyruvate has frequently been found to be normal and only rises above the normal following a glucose load. The estimation of blood pyruvate can be of help in the diagnosis of suspected thiamin deficiency.

This indicator is not appropriate for the detection of marginal thiamin deficiencies in view of limits in the sensitivity of this index. An elevated pyruvate level is also not always attributable to thiamin deficiency.

13.7.6 Recommendations for Thiamin Intake by Life Stages

The main references used in arriving at recommendations for thiamin intakes for the Southeast Asian region were the FAO/WHO report on vitamins and mineral requirements (2002) and the recommendations of the DRI Committee of IOM (1998). The rationale and steps taken in setting requirements and the

levels recommended by these organizations were considered. Existing RDAs of countries in the region were additional references; there were no studies of thiamin requirements in the region.

(*a*) *Infants* (*0 - 5 months*)

Both the FAO/WHO (2002) and the DRI Committee (IOM, 1998) had used the observed mean thiamin intake of infants fed principally with breast milk as the goal for intake by infants. The mean concentration of thiamin in mature breast milk is 0.21±0.035 mg/L (SD). Using the mean value for intake of breast milk of 0.78 L/day and average content of 0.21 mg of thiamin/L, the total thiamin intake is 0.16 mg/day for infants aged 0 to 6 months, which is rounded to 0.2 mg/day. It has also been estimated that the minimum daily requirement needed to protect against deficiency is approximately 0.17 mg/day.

(*b*) *Infants* (*6 - 11 months*)

The FAO/WHO had recommended an intake of 0.3 mg/day for this group of infants; no details were however provided. IOM had also estimated the AI of thiamin as 0.3 mg/day, extrapolating from the EAR for adults and adjusting for the expected variance.

The DRI Committee had also calculated the AI for thiamin for infants aged 6 to 11 months using the estimated thiamin content in 600 ml of breast milk (0.13 mg) plus the amount of thiamin provided by solid foods (0.5 mg). The result was approximately 0.6 mg of thiamin per day which was felt to be unreasonably higher than the extrapolated value (IOM, 1998).

(*c*) *Children* (*1 - 9 years*)

As there was no data on which to base an estimated requirement for this age group, the DRI Committee estimated the EAR and RDA by extrapolation from adult values. The resultant estimates are 0.5 mg/day for children aged 1 to 3 years and 0.6 mg/day for those aged 4 to 8 years. FAO/WHO (2002) did not provide details of the recommendations for these age groups, but they were generally similar to those of the IOM (1998).

(*d*) *Adolescents* (*10 - 18 years*)

Some studies to estimate requirements of thiamin have been reported. These include studies relating dietary intake of thiamin to several indicators of thiamin status and a controlled-diet, dose-response experiment. The DRI Committee however felt that the data were insufficiently definitive and recommended that the EARs and RDAs for thiamin for these age groups be extrapolated from the adult values.

The FAO/WHO (2002) recommendations for these ages were rather similar to the DRI Committee's recommendations. The recommended intake was 1.2 mg/day for males, and 1.1 mg/day for females.

(e) *Adults* (*> 19 years*)

The DRI Committee reviewed data obtained from several studies to estimate requirements for this population group. Great reliance was given to the carefully controlled, thiamin depletion-repletion experiment of Sauberlich *et al.* (1979) in a metabolic unit. The investigators concluded that thiamin at 0.30 mg/1,000 kcal (approximately 1.0 mg/day) met the minimum requirement for young men. Findings from several other studies supported this estimation.

Based on above observations, it was concluded that the EAR for thiamin was 1.0 mg/day for men and 0.9 μg/day for women, which represents about 10% decrease for women based on body size and energy needs. The RDA for adults was then set by taking the EAR plus twice the CV to cover the needs of 97% to 98% of the individuals in the group. The CV was assumed to be 10% because information was not available on the standard deviation of the requirement for thiamin. RDA for adults was thus calculated to be 1.2 mg/day for men and 1.1 mg/day for women. The same recommendations were also made by the FAO/WHO and are adopted for the Southeast Asia region.

Although there are more data to suggest that requirements might be somewhat higher in the elderly than in younger adults, there is also a concomitant decreased energy utilization that may offset this. Therefore, the proposed RDA is the same for adult males and females of all ages.

(*f*) *Pregnancy*

For pregnancy, the requirement is estimated to increase by about 30%, based on increased growth in maternal and fetal compartments (approximaly 20%) and a small increase in energy utilization (about 10%). Both IOM (1998) and FAO/WHO (2002) have proposed an addition of 0.3 mg/day of thiamin during pregnancy, ie 1.4 mg/day.

(*g*) *Lactation*

Lactating women are estimated to transfer 0.2 mg thiamin in their milk each day, and an additional 0.2 mg is estimated for the increased energy cost of lactation. The FAO/WHO (2002) had proposed an addition of 0.4 mg per day or a total thiamin intake of 1.5 mg per day.

13.8 Current RDAs for Thiamin in Southeast Asia

Table 13.1 sets out the current RDAs for thiamin in 6 countries in the Southeast Asian region.

Table 13.1 Comparison of current RDAs (mg/day) for thiamin in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 – 1)	0.3 – 0.4	0.2 – 0.3	0.2 – 0.4	0.2 – 0.38	0.3[a]	0.3 – 0.4
Children (1 – 9)	0.5 – 1.0	0.5 – 0.9	0.5 – 0.9	0.46 – 0.72	0.5 – 0.6	0.8 – 1.3
Boys (10 – 18)	1.0	1.2	1.2	0.88 – 1.14	0.9 – 1.2	1.0 – 1.2
Girls (10 – 18)	1.0	1.1	1.1	0.78 – 0.86	0.9 – 1.0	0.9 – 1.0
Men (≥ 19)	1.0 – 1.2	1.2	1.2	0.98 – 1.18	1.2	1.2
Women (≥ 19)	1.0	1.1	1.1	0.80 – 0.84	1.1	0.9
Pregnancy						
1st trimester	+0.2	+0.3	+0.3	+0.09	+0.3	+0
2nd trimester	+0.2	+0.3	+0.3	+0.09	+0.3	+0.2
3rd trimester	+0.2	+0.3	+0.3	+0.09	+0.3	+0.2
Lactation						
1st 6 months	+0.3	+0.4	+0.4	+0.2	+0.3	+0.2
2nd 6 months	+0.3	+0.4	+0.4	+0.2	+0.3	+0

Notes: [a] *Figures only for infants 6-11 months*
[b] *1-8 years for Thailand*
[c] *10-17 years for Singapore; 9-18 years for Thailand*
[d] *≥ 18 for Singapore*

Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

Recommendations for thiamin intake in selected countries in the region are rather similar, although the RDAs for Singapore are marginally lower. For all countries, levels recommended for boys (0.9 mg to 1.2 mg) are marginally higher than for girls (0.8 mg to 1.1 mg). Similarly, in all the countries, recommendations for men are slightly higher than the RDAs for women.

All countries recommend additional thiamin intake throughout pregnancy, except for Vietnam which does not provide for additional intakes during the first trimester. An additional amount ranging from 0.2 mg/day to 0.3 mg/day is recommended for all the 5 countries reviewed except for Singapore which has recommended 0.09 mg as the additional intake.

All countries recommended additional thiamin intake during lactation although Vietnam only recommends this for the first 6 months of lactation. The additional amount recommended is generally slightly higher than those suggested for pregnancy.

13.9 Recommended RDAs for Thiamin for Southeast Asia

After careful consideration of all the above information and the recommendations in the two major reports (IOM, 1998 and FAO/WHO, 2002), The SEA-RDA Committee proposed that the FAO/WHO (2002) recommendations for the requirements for thiamin (Table 13.2) be adopted as the SEA-RDAs.

These recommendations are also the same as the RDAs proposed by the DRI Committee (IOM, 1998). Compared with the current RDAs in selected countries, the SEA-RDAs for all the age groups are not markedly different.

Table 13.2 Recommended RDAs for Thiamin for Southeast Asia

Age Groups	Thiamin RDA (mg/day)
Infants (months)	
0 – 6	0.2
6 – 12	0.3
Children (years)	
1 – 3	0.5
4 – 6	0.6
7 – 9	0.9
Boys (years)	
10 – 18	1.2
Girls (years)	
10 – 18	1.1
Men (years)	
19 – 65	1.2
> 65	1.2
Women (years)	
19 – 65	1.1
> 65	1.1
Pregnancy	1.4
Lactation	1.5

Bibliography

FAO/WHO (2002). Thiamin, riboflavin, niacin, vitamin B6, pantothenic acid and biotin. In: *Human Vitamin and Mineral Requirements*. Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; Chapter 3, pp 27-51.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

IOM (1998). Thiamin. In: *Dietary References Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; chapter 4, pp 58-86.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Rindi G (1996). Thiamin. In: *Present Knowledge in Nutrition.* 7th edition. Ziegler EE and Filer LJ Jr (eds). ILSI Press, Washington, DC, pp 160-166.

Sauberlich HE, Herman YF, Stevens CO & Herman RH (1979). Thiamin requirement of the adult human. *Am J Clin Nutr* 32:219-222.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

14 RIBOFLAVIN (VITAMIN B_2)

14.1 Introduction

Subsequent to the discovery of thiamine, riboflavin (vitamin B_2) was also discovered as a more heat-stable factor vitamin. It was isolated from milk and shown to be part of the B complex in 1933 by Gyorgy and co-workers. The primary form of riboflavin is as an integral component of the co-enzymes flavin mononucleotide (FMN) and flavin-adenine dinucleotide (FAD). It is in these bound co-enzyme forms that riboflavin functions as a catalyst for redox reactions in numerous metabolic pathways and in energy production.

14.2 Characteristics and Function

Riboflavin is an orange-yellow crystalline compound, consisting of carbon, hydrogen, oxygen, and nitrogen. It is less soluble in water than thiamine. It is stable to heat, oxidation and acid, although it disintegrates in the presence of alkali or light, especially UV light.

Riboflavin is necessary for growth and reproduction. Riboflavin functions as part of a group of enzymes that are involved in the breakdown and utilization of carbohydrates, fats and proteins. Riboflavin is necessary for cell respiration because it works with enzymes in the utilization of cell oxygen. It is also necessary for the maintenance of good vision, skin, nails, and hair.

14.3 Absorption, Utilization and Excretion

Most dietary riboflavin is consumed as a complex of food protein with FMN and FAD. In the stomach, gastric acidification releases most of the co-enzyme forms of riboflavin (FAD and FMN) from the protein. Primary absorption of riboflavin occurs in the proximal small intestine via a rapid, saturable transport system. A small amount of riboflavin is absorbed in the large intestine.

The rate of absorption is proportional to intake, and it increases when riboflavin is ingested along with other foods and in the presence of bile salts. At low intake levels, most absorption of riboflavin is via an active or facilitative transport system. In plasma, some riboflavin is complexed with albumin; however, a large portion of riboflavin associates with other proteins, mainly immunoglobulins, for transport.

At physiological concentrations, the uptake of riboflavin into the cells of organs such as the liver is facilitated and may require specific carriers. At higher levels of intake, riboflavin can be absorbed by diffusion.

Riboflavin is converted into co-enzymes within the cellular cytoplasm of most tissues, but mainly in the small intestine, liver, heart and kidney. The metabolism of riboflavin begins with the adenosine

triphosphate (ATP)-phosphorylation of the vitamin to FMN. Flavokinase, the catalyst for this conversion, is hormonal control. FMN can then be complexed with specific apoenzyme to form a variety of flavoproteins; however, most is converted to FAD by FAD synthetase. As a result, FAD is the predominantly flavoco-enzyme in body tissues. Production of FAD is controlled by product inhibition such that an excess of FAD inhibits its further production.

When riboflavin is absorbed in excess, very little is stored in the tissues. The excess is excreted, primarily in the urine. In healthy adults consuming well-balanced diets, riboflavin accounts for 60% to 70% of the excreted urinary flavins. Urinary excretion of riboflavin varies with intake, metabolic events, and age. In newborns, urinary excretion is slow; however, the cumulative amount that is excreted is similar to the amount excreted in older infants.

14.4 Effects of Deficiency and Excess

14.4.1 Deficiency

Deficiency may result from one or several of these factors:

- Long-established faulty dietary habits
- Food idiosyncrasies
- Strict vegetarian diet
- Alcoholism
- Arbitrarily selected diets for relief of symptoms of digestive trouble
- Prolonged following of a restricted diet in the treatment of a disease such as peptic ulcer or diabetes

The clinical features of riboflavin deficiency do not have the specificity that may characterize deficits of some other vitamins. Isolated deficiency is rarely encountered. Early symptoms may include weakness, fatigue, mouth pain and tenderness and burning and itching of the eyes. More advanced deficiency may give rise to cheilosis, angular stomatitis, dermatitis, corneal vascularization, anaemia and brain dysfunction (Rivlin, 1996).

14.4.2Excessive Intake

There appears to be general agreement that dietary riboflavin intake at many times the RDA, either from food or supplements, is without demonstrable toxicity (Rivlin, 1996; FAO/WHO, 2002). IOM (1998) has cautioned that this does not mean that there is no potential for adverse effects resulting from high intakes. Because data on the adverse effects of the vitamin intake are limited, caution may be warranted.

The apparent lack of harm resulting from high oral doses of riboflavin may be due to its limited solubility, the body's limited capacity to absorb it from the gastrointestinal tract and its rapid excretion in the urine. There is a limited absorption of 50 mg to 500 mg of riboflavin with no adverse effects. However, prolonged ingestion of large doses of any one of the B complex vitamins, including riboflavin, may result in high urinary loss of other B vitamins.

14.4.3 Guidance on High Intake

The DRI Committee felt that the data on adverse effects from high riboflavin intake are not sufficient for a quantitative risk assessment, thus a tolerable upper intake level (UL) cannot be derived (IOM, 1998).

14.5 Food Sources

The food sources of riboflavin are similar to those of other B vitamins. Therefore, it is not surprising that if a given individual's diet has inadequate amounts of riboflavin, it will very likely be deficient in other B vitamins as well. Most plant and animal tissues contain at least small amounts of riboflavin. Outstanding sources of riboflavin are organ meats, milk, leafy vegetables, cheese and eggs. Other good sources are enriched bread, lean meat, legumes, whole grains, and dried yeast. Natural grain products tend to be relatively low in riboflavin, but fortification and enrichment of grains and cereals has led to a great increase in riboflavin intake from these sources.

14.6 Factors Affecting Requirement

14.6.1 Bioavailability estimates

There is a considerable diversity of flavins in natural foods, but over 90% of riboflavin is estimated to be in the readily digestible form of flavoco-enzymes (mainly FAD and, to a lesser degree, FMN), with smaller amounts of the free vitamin and only traces of glycosides and esters that are also hydrolysed during absorption from the gut. The overall bioavailability of riboflavin in foods, mostly as digestible flavoco-enzymes, has been reported to be excellent at nearly 95%, but absorption of the free vitamin is limited to about 27 mg per single meal or dose in an adult.

14.6.2 Nutrient-nutrient interactions

The proportions of fat and carbohydrates in the diet appear to influence the riboflavin requirements of the elderly: a lower fat to carbohydrate ratio decreases the requirement.

Riboflavin interrelates with other B vitamins, notably niacin, which requires FAD for its formation from tryptophan, and vitamin B6, which requires FMN for conversion to the co-enzyme pyridoxal 5'-phosphate. These interrelationships are not known to affect the requirement for riboflavin.

14.6.3 Energy intake

No studies were found that examined the effect of energy intake on the riboflavin requirement. Despite the lack of experimental data, the known biochemical function of riboflavin in the utilization of energy suggests at least a small (10%) adjustment to reflect differences in the average energy utilization and size of men and women, a small increase in the requirement to cover increased energy use during pregnancy, and a small increase to cover the inefficiencies of milk production.

14.6.4 Physical activity

Riboflavin status measurements seem to be affected by physical activity. Some studies have demonstrated a moderate rise in the erythrocyte glutathione reductase activity coefficient (EGRAC) as well as a decrease in urinary riboflavin excretion with an increase in physical activity. It is possible that the riboflavin requirement is increased for those who are ordinarily very active physically (e.g., athletes or those who carry heavy packs much of the day), but data are not available on which to quantify the adjustment that should be made.

14.7 Estimating Requirements and Recommended Intakes

14.7.1 Indicators for estimating requirement for riboflavin

(*a*) *Erythrocyte Glutathione Reductase*

The erythrocyte glutathione reductase (EGR) value is an enzymatic and hence functional indicator that is conventionally run with and without the addition of FAD, the co-enzyme required for the activity of EGR. Results are expressed as an activity coefficient (EGRAC), which is the ratio of activities in the presence and absence of FAD. For reference intervals, an EGRAC ratio of 1.0 indicates no stimulation by FAD and the presence of holoenzyme only, indicating that more than adequate amounts of FAD (and riboflavin) were present in the original erythrocytes.

Since FAD is a labile compound, the EGRAC must be obtained using fresh red cells that are washed, lysed, and measured promptly for enzymatic activity. Because the glutathione reductase in the red cells of individuals with G6PD deficiency has an increased avidity for FAD, this test is not valid in individuals with that condition.

(*b*) *Red Cell Flavin*

Red cell flavin has been used as an indicator of the cellular concentration of riboflavin in its co-enzyme forms, since these co-enzymes comprise over 90% of flavin. Due to the instability of the predominant FAD, which is rapidly hydrolysed enzymatically upon rupture of cells, red cell flavins are deliberately hydrolysed and measured either microbiologically or fluorometreically as riboflavin.

Since the margin of difference between adequacy and inadequacy is rather small, sensitivity and interpretation of results may be problematic. This is a useful indicator that reflects the functional, cellularly trapped forms of riboflavin.

(*c*) *Urinary Flavin*

Levels of urinary flavin can be measured using fluorimetric HPLC (high pressure liquid chromatographic) methods as well as by microbiological procedures. Flavin metabolites can comprise as much as one third of total urinary flavin.

Under conditions of sufficiency (i.e., a riboflavin intake of approximately 1.5 mg/day), the amount of riboflavin excreted per day exceeds 120 μg total, or 80 μg/g of creatine. The amount of riboflavin excreted per gram of creatinine is greater for children than adults.

Load tests may be used to gauge the degree to which the body is saturated with riboflavin; the results generally agree with those obtained by using other indicators. Subcutaneous administration of 1 mg of riboflavin followed by assessment of urinary flavin output for a 4 hour period was found to correspond well with riboflavin excretion over 24 hours. A break point for increased urinary excretion of riboflavin occurred with or without the load when adult males received more than 1.1 mg/day of dietary riboflavin. Above this level, there is a sharp linear increase in the slope for riboflavin intakes up to 2.5 mg/day. Their results were adapted to suggest a reference value of 1.4 mg or more for the normal 4 hour urinary excretion of riboflavin after a 5 mg load.

14.7.2 Recommendations for riboflavin intake by life stages

The main references used in arriving at recommendations for riboflavin intakes for the SEA-RDAs were the recommendations of the FAO/WHO in its 2002 expert consultation report on vitamins and mineral requirements and the 1998 recommendations of the DRI Committee of IOM (1998). The rationale and steps taken in setting requirements and the levels recommended by these organizations were considered. Existing RDAs of countries in the region were additional references; there were no studies of riboflavin requirements in the region.

(*a*) *Infants* (*0 - 5 months*)
Since there are insufficient data that reliably reflect response to dietary riboflavin intake in infants, AI has been used as the goal for intake by infants (IOM, 1998; FAO/WHO, 2002). The AI reflects the observed mean riboflavin intake of infants fed principally with breast milk. Based on a mean value for intake of breast milk of 750 ml/day and estimated average riboflavin concentration in breasat milk of 0.35 mg/l, the total riboflavin content is 0.26 mg/day or 0.3 mg/day after rounding.

(*b*) *Infants* (*6 - 11 months*)
By extrapolation up from AI for infants ages 0 through 6 months, the AI for riboflavin for older infants would be 0.35 mg/day. Rounding this to 0.4 mg/day, this amount has been accepted as the recommended intake.

Alternatively, the AI for riboflavin for infants aged 6 through 11 months could be calculated using the estimated riboflavin content of 600 ml of human milk (0.21 mg) plus the amount of riboflavin provided by solid foods (0.6 mg). It was felt that the result, 0.8 mg of riboflavin per day, is unreasonably high because it is twice the extrapolated value.

(*c*) *Children and Adolescents* (*1 - 18 years*)
Since there are no sufficient data to set EAR and RDAs for children aged 1 through 18 years, the DRI Committee has extrapolated these from adult values using a metabolic body weight ratio multiplied by a growth factor. The EAR for children aged 1 through 3 years was determined to be 0.4 mg/day. RDA for riboflavin was determined as equal to EAR plus twice the CV to cover the needs of 97% to 98% of the individuals in the group, resulting in a RDA of 0.5 mg/day for children aged 1 to 3 years. RDA for children aged 4 to 8 years was similarly calculated to be 0.6 mg/day.

By using the same methodology, the RDA for adolescent boys, aged 9 to 13 years and 14 to 18 years were calculated to be 0.9 mg/day and 1.3 mg/day respectively. For adolescent girls, the corresponding recommended intakes were 0.9 mg/day and 1.0 mg/day respectively.

No detailed computations were provided, but the FAO/WHO recommended very similar intakes for these age groups:

Table 14.1 Recommendations of FAO/WHO for riboflavin intakes for children and adolescents

Age Groups (years)	Riboflavin (mg/day)
Children	
1 – 3	0.5
4 – 6	0.6
7 – 9	0.9
Adolescents (10 – 18)	
Boys	1.3
Girls	1.0

Source: FAO/WHO (2002)

(*d*) *Adults (19 - 64 years)*

Studies on riboflavin requirements for adults have focused primarily on the occurrence of signs of clinical deficiency and on urinary excretion of riboflavin. Thus, the EAR is derived from the findings of a number of studies that addressed clinical deficiency signs and biochemical values, including EGRAC, in relation to measured dietary intake of riboflavin. Biochemical changes in riboflavin status occur well before the appearance of overt signs of deficiency. Such studies help to bracket the riboflavin requirement.

Reviewing available data, the DRI Committee noted that riboflavin intakes of less than 0.5 mg/day to 0.6 mg/day led to clinical signs of deficiency. Findings also indicated that riboflavin intakes of approximately 0.8 mg/day for men or women were minimally sufficient to avoid signs of clinical deficiency. On the basis of estimates of urinary riboflavin, it was determined that a reserve was not maintained at riboflavin intakes below 1.1 mg/day.

Based on available findings, the EAR for adults was determined to be 0.9 mg/day for women and 1.1 mg for men. To compute the RDA, a CV of 10% is assumed. Thus, the RDA for men is 1.1 + (0.2 x 1.1) = 1.3 mg/day, and the RDA for women is 0.9 + (0.2x 0.2)= 1.1 mg/day.

The DRI Committee's RDA values for riboflavin are the same as the recommended intakes of FAO/WHO (2002).

(e) *Older adults (65 years and above)*

Few additional studies estimating the riboflavin requirements for older adults have been conducted. In reviewing data from available supplementation studies, the DRI Committee concluded that the requirements of the elderly did not differ from those of young adults, although there is a decrease in energy expenditure with aging and the requirement for the older adults would be expected to decrease.

(*f*) *Pregnancy*

Few studies provide information about the riboflavin requirements of pregnant women. Pregnant women are known to have an increased EGRAC. Supplementation with riboflavin has been shown to reduce the EGRAC and a larger amount of the vitamin is required to lower this enzyme activity for pregnant women. Maternal riboflavin intake has also been observed to be positively associated with fetal growth but the data was deemed insufficient to warrant use of fetal growth as an indicator for setting the riboflavin requirement for pregnant women.

Both the DRI Committee (IOM, 1998) and FAO/WHO (FAO/WHO, 2002) had recommended an additional amount of 0.3 mg riboflavin per day for pregnant women, based on increased growth in maternal and fetal components and a small increase in energy utilization. The recommended RDA during pregnancy is thus 1.4 mg/day. This increased need is supported by the urinary excretion of less riboflavin during the progression of pregnancy and the more frequent appearance of clinical signs of ariboflavinosis in pregnant women on low intakes (less than 0.8 mg/day) than in their non-pregnant counterparts.

(*g*) *Lactation*

It is assumed that 0.3 mg of riboflavin is transferred in the milk of lactating mothers each day when their daily milk production is 0.78 litres. Assuming that the use of riboflavin for milk production by mother is CV of 70%, the additional amount required to replace the amount for lactation should be adjusted upward to 0.4 mg/day. Women who are breastfeeding older infants need slightly less, in proportion to the lower volume of milk production. For calculation, 0.4 mg/day is added to 0.9 mg/day (EAR for nonpregnant women), giving an EAR of 1.3 mg/day. Converting to RDA (120% of EAR), the amount recommended by the DRI Committee (IOM, 1998) and FAO/WHO (FAO/WHO, 2002) is 1.6 mg/day.

14.8 Current RDAs for Riboflavin in Southeast Asia

Recommended intakes for riboflavin in 6 Southeast Asian countries are summarised in Table 14.2.

Table 14.2 Comparison of current RDAs (mg/day) for riboflavin in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 - 1)	0.3 - 0.5	0.3 - 0.4	0.3 - 0.4	0.42 - 0.57	0.4[a]	0.3 - 0.5
Children (1 - 9)	0.6 - 1.0	0.5 - 0.9	0.5 - 0.7	0.69 - 1.26	0.5 - 0.6	0.8 - 1.3
Boys (10 - 18)	1.0 - 1.3	1.3	1.0 - 1.5	1.32 - 1.71	0.9 - 1.3	1.6 - 1.8
Girls (10 - 18)	1.0 - 1.2	1.0	0.9 - 1.1	1.17 - 1.29	0.9 - 1.0	1.4 - 1.5
Men (≥ 19)	1.2 - 1.5	1.3	1.3	1.47 - 1.77	1.3	1.8
Women (≥ 19)	1.0	1.1	1.1	1.20 - 1.26	1.1	1.3
Pregnancy						
1st trimester	+0.2	+0.3	+0.6	+0.15	+0.3	+0
2nd trimester	+0.2	+0.3	+0.6	+0.15	+0.3	+0.6
3rd trimester	+0.2	+0.3	+0.6	+0.15	+0.3	+0.6
Lactation						
1st 6 months	+0.4	+0.5	+0.6	+0.3	+0.5	+0.4
2nd 6 months	+0.3	+0.5	+0.6	+0.3	+0.5	+0

Notes: [a] *figures only for infants aged 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[d] *≥ 18 for Singapore*

Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

Singapore and Vietnam appear to have slightly higher recommendations for riboflavin intake for all age groups, excluding during pregnancy and lactation. Recommendations for riboflavin in the other 4 countries are rather similar.

Levels recommended for adolescent boys (1.0 mg to 1.8 mg) and adult men (1.2 mg to 1.8 mg) are marginally higher than for adolescent girls (0.9 mg to 1.5 mg) and adult women (1.0 mg to 1.3 mg). With the exception of Vietnam which only recommends additional riboflavin intake during the second and third trimesters, all other countries have recommended extra intake of the vitamin throughout pregnancy. The additional amount ranges considerably from 0.15 mg per day for Singapore to 0.6 mg per day for Philippines and Vietnam. All countries also recommend additional riboflavin intake through 12 months of lactation, except for Vietnam which recommends only additional amounts for the first 6 months. The additional amounts recommended are higher than those during pregnancy.

14.9 Recommended RDAs for Riboflavin for Southeast Asia

Upon reviewing the above information and the recommendations of the FAO/WHO (2002) and the DRI Committee (1998), the SEA-RDA Committee adopted the recommendations of the FAO/WHO as the recommended RDA for riboflavin for Southeast Asia.

Table 14.3 Recommended RDAs for Riboflavin for Southeast Asia

Age Groups	Riboflavin RDA (mg/day)
Infants (months)	
0 – 5	0.3
6 – 11	0.4
Children (years)	
1 – 3	0.5
4 – 6	0.6
7 – 9	0.9
Boys (10 – 18 years)	1.3
Girls (10 – 18 years)	1.0
Men (years)	
19 – 65	1.3
> 65	1.3
Women (years)	
19 – 65	1.1
> 65	1.1
Pregnancy	1.4
Lactation	1.6

These recommendations are also the same as the RDAs proposed by the DRI Committee. Compared with the current RDAs in selected countries, the SEA-RDAs are, generally, marginally lower for all the age groups.

Bibliography

FAO/WHO (2002). Thiamin, riboflavin, niacin, vitamin B6, pantothenic acid and biotin. In: *Human Vitamin and Mineral Requirements*. Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; Chapter 3, pp 27-51.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines*. 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

IOM (1998). Riboflavin. In: *Dietary References Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline*. Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; chapter 5, pp 87-122.

MPH (2003). *Dietary Reference Intake for Thais*. Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia*. A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Rivlin RS (1996). Riboflavin. In: *Present Knowledge in Nutrition*. 7th edition. Ziegler EE and Filer LJ Jr, (eds). International Life Sciences Institute Press, Washington, DC, pp 167-173.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

15 NIACIN (Vitamin B_3)

15.1 Introduction

Niacin, also known as vitamin B_3, was recognized as a vitamin in 1937 by C.A. Elvehjem and associates, who demonstrated that the deficiency disease black tongue in dogs could be cured by this substance. Niacin is the common name for the chemical compound nicotinic acid, which contains carbon, hydrogen, oxygen, and nitrogen. The term niacin refers to nicotinamide (nicotinic acid amide), nicotinic acid (pyridine-3-carboxylic acid), and derivatives that exhibit the biological activity of nicotinamide. The amino acid tryptophan is converted in part to nicotinamide and thus can contribute to meeting the requirement for niacin.

15.2 Characteristics and Functions

Niacin is water-soluble, more stable than thiamin (vitamin B_1) and riboflavin (vitamin B_2) and is remarkably resistant to heat, light, air, acids and alkalis. As a co-enzyme, niacin assists enzymes in the breakdown and utilization of proteins, fats and carbohydrates. Niacin is effective in improving circulation and reducing blood cholesterol level. It is vital to the proper activity of the nervous system and for the formation and maintenance of healthy skin, tongue, and digestive system tissues. Niacin is also necessary for the synthesis of sex hormones.

Niacin is essential in the formation of pyridine nucleotide coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), in which the nicotinamide moiety acts as a hydride ion acceptor or donor in many biological redox reactions. NAD has been shown to be required for important non-redox adenosine diphosphate (ADP)-ribose transfer reactions involved in DNA repair and calcium mobilization.

15.3 Absorption, Utilization and Excretion

Nicotinic acid and nicotinamide are rapidly absorbed by the stomach and the intestines. At low concentrations, absorption occurs as sodium ion-dependent facilitated diffusion. At higher concentrations, passive diffusion predominates and 3 g to 4 g of niacin can be almost completely absorbed. Nicotinamide is released from NAD in the liver and intestines by glycohydrolases and transported to tissues to be used in the synthesis of NAD when needed.

The niacin co-enzymes NAD and NADP are synthesised in all tissues of the body from nicotinic acid and nicotinamide. Tissue concentrations of NAD appear to be regulated by the concentration of extra-cellular nicotinamide, which in turn is under hepatic control and is hormonally influenced. In the liver, some excess plasma nicotinamide is converted to stored NAD (i.e., NAD not bound to enzymes). Tryptophan and nicotinic acid also contribute to stored NAD.

The two major excretion products are N'-methyl-nicotinamide and 2-pyridone; other excretion products include niacin or niacin oxide and hydroxyl forms. The proportions differ somewhat depending on the amount and form of niacin ingested and the niacin status of the individual.

15.4 Effects of Deficiency and Excess

15.4.1 Deficiency

Pellagra is the classic manifestation of a severe niacin deficiency. Common pellagra symptoms are changes in the skin; mucosa of the mouth, tongue, stomach, intestinal tract and the nervous system. The symptoms associated with the skin are most characteristic. A pigmented rash develops symmetrically in areas exposed to sunlight and is similar to a sunburn, although in chronic cases a darker colour may develop. Symptoms in relation the digestive tract are associated with vomiting, constipation or diarrhea, and the tongue becomes bright red. Neurological symptoms include depression, apathy, headache, fatigue and loss of memory.

Pellagra was first observed in the mid-18th century in Spain and described more fully a few years later by physicians in northern Italy who used the term "pellagra", meaning "raw skin", for the first time. Pellagra was common in the United States and parts of Europe in the early twentieth century in areas in which corn or maize (which is low in both niacin and tryptophan) was the dietary staple. Nowadays, pellagra has virtually disappeared from industrialized countries except for its occurrence in some alcoholics and in individuals with conditions that disrupt tryptophan pathways. It still appears in India and parts of China and Africa.

15.4.2 Excessive Intake

Upon reviewing available data, the DRI Committee concluded that there is no evidence of adverse effects from the consumption of naturally occurring niacin in foods (IOM, 1998). Concern is therefore turned to intake of niacin as a supplement, food fortificant, or pharmacological agent. With regards to the last named, Altschul *et al.* (1955) first reported that large doses of nicotinic acid can reduce serum cholesterol concentrations in human subjects. The administration of nicotinic acid in the Coronary Drug Project was associated with a reduction in recurrent myocardial infarctions and in the long-term total mortality (Canner *et al.*, 1986). The lipid-lowering effect of nicotinic acid has been extensively studied but the mechanism of action is not known. It does not appear related to any vitamin co-enzyme function because nicotinamide does not have a similar effect.

A condition called "flushing" occurs in many patients treated therapeutically with nicotinic acid. The term "flushing" covers a burning, tingling and itching sensation as well as a reddened flush primarily on the face, arms, and chest. It is often accompanied by pruritus, headaches, and increased intracranial blood flow. Other side effects reported include hepatic and ocular abnormalities and occasional hyperglycemia.

Adverse effects such as nausea, vomiting, and signs and symptoms of liver toxicity have been observed at nicotinamide intakes of 3,000 mg/day compared with intakes of nicotinic acid of 1,500 mg/day.

15.4.3 Guidance on High Intake

Tolerable upper intake levels (ULs) of niacin for different age groups, as proposed by the DRI Committee (IOM, 1998), are set out in Table 15.1.

Table 15.1 UL of niacin for various age groups

Age Groups (years)	Niacin UL (mg/day)
Infants	Not possible to establish; source of intake should be formula and food only
Children	
1 – 3	10
4 – 8	15
9 – 13	20
Adolescents (14 – 18)	30
Adults (≥ 19)	35
Pregnancy (years)	
14 – 18	30
≥ 19	35
Lactation (years)	
14 – 18	30
≥ 19	35

Source: IOM (1998)

15.5 Food Sources

Niacin is widely distributed in foods, with yeast being the richest source. Meat, poultry and fish are also rich sources, especially the organ tissues. Foods that are rich sources of thiamin and riboflavin such as liver, whole grains, nuts and legumes are also good sources of niacin. Milk, green leafy vegetables and fish also contain appreciable amounts. Many cereals and breads are enriched with niacin.

Niacin is unique among the vitamins in that an amino acid, tryptophan, is a precursor that can contribute substantially to niacin nutriture by its conversion to a niacin derivative in mammalian liver tissue.

15.6 Factors Affecting Requirement

15.6.1 Bioavailability

Niacin in mature cereal grains is largely bound and has a bioavailability of only about 30%. However, alkali treatment of the grain increases the percentage of niacin absorption. Niacin in the co-enzymes NAD/NADPH formed in meat appear have higher bioavailability, while niacin in free form (e.g. in beans and liver) has high bioavailability. Niacin added during enrichment or fortification is in the free form.

Estimations of niacin requirements are complicated by the fact that some tryptophan (an essential amino acid) is converted to niacin in the human body. The allowance recognizes that the body converts tryptophan into the vitamin, with 60 mg of tryptophan being equivalent to 1 mg niacin, sometimes called a "niacin equivalent" (NE).

60 mg of tryptophan = 1 mg of niacin = 1 mg of NE.

Although a 60-to-1 conversion factor represents the average for human utilization of trytophan as NEs, there are substantial individual differences. The conversion efficiency also varies depending on a number of dietary and metabolic factors.

15.6.2 Nutrient-nutrient interactions

Interactions occur between riboflavin and vitamin B_6 metabolism in which flavin mononucleotide (FMN) is required for the oxidase that forms the co-enzyme pyridoxal 5'-phosphate which is required for conversion of tryptophan to niacin. Inadequate iron, riboflavin, or vitamin B_6 status also decreases the conversion of tryptophan to niacin.

15.6.3 Energy intake and expenditure

Despite the lack of experimental data, the known biochemical function of niacin in the oxidation of fuel molecules suggests at least a small (10%) adjustment to reflect differences in the average energy utilization and size of men and women, a 10% increase in the requirement to cover increased energy use during pregnancy, and a small increase in the requirement to account for the efficiency of niacin used in milk production during lactation.

15.7 Estimating Requirements and Recommended Intakes

15.7.1 Indicators for estimating requirement for niacin

Niacin status and dietary requirement could be estimated using biochemical or clinical endpoints of niacin deficiency. Biochemical changes occur well before the appearance of overt signs of deficiency. Biochemical assessments can be carried out as summarized in the following paragraphs.

(*a*) *Urinary excretion*

The most reliable and sensitive measures of niacin status are urinary excretion of the two major methylated metabolites, N'methyl-nicotinamide and its 2-pyridone derivative (N'methyl 2-pyridone-5-carboxamide). The ratio of the 2 pyridone to N' methyl nicotinamide, although independent of age and creatinine excretion, is a measure of protein adequacy rather than niacin status. The indicator is however rather insensitive to a marginal niacin intake of 10 mg niacin equivalent per day.

(*b*) *Plasma concentrations*

In plasma, the 2-pyridone derivative drops below detection limits after a low niacin intake. With an oral niacin load (20 mg of nicotinamide/70 kg body weight), postdose changes in 2 pyridone were more responsive to niacin status than were changes in N'-methyl-nicotinamide, in both plasma and urine. Plasma concentrations of other niacin metabolites and of niacin are not useful markers of niacin status.

(*c*) *Red cell pyridine nucleotides*

Erythrocyte NAD concentration appears to be a sensitive indicator of niacin depletion. The indicator has been reported to be equally sensitive and reliable as urine metabolite excretions.

(*d*) *Transfer of ADP Ribose*

A possible functional measure for niacin status could be poly-adenosine diphosphate (ADP) ribosylation, since ADP-ribosylation may contribute to gene stability (Poly-ADP-ribose-polymerase in the nucleus) and may function in DNA replication and repair. However, the assays have not been developed or sufficiently refined to assess niacin status.

15.7.2 Recommendations for niacin intake by life stages

The main references used in arriving at recommendations for niacin intake for the Southeast Asia region were the 2002 FAO/WHO report on vitamins and mineral requirements and the recommendations of the DRI Committee of the IOM (1998). The rationale and steps taken in setting requirements and the levels recommended by these organizations were considered. Existing RDAs of countries in the region were additional references. There were no studies of niacin requirements in the region.

(*a*) *Infants (0 - 5 months)*

The adequate intake level of niacin for infants was set based on the observed mean intake of infants fed principally with breast milk. The FAO/WHO estimated niacin content of breast milk to be approximately 1.5 mg/L or a total of 1.1 mg in 0.75 litre of milk produced in a day (FAO/WHO, 2002). The tryptophan content of breast milk is approximately 210 mg/L. Due to the high rate of protein turnover and net positive nitrogen retention in infancy, the DRI Committee felt that it is likely that the standard method for estimating NEs would overestimate the contribution from tryptophan (IOM, 1998). Thus, the AI for niacin for infants is given in milligrams of preformed niacin only. The AI is 2 mg/day after rounding up, and the FAO/WHO has recommended the same level (FAO/WHO, 2002).

(*b*) *Infants (6 - 11 months)*

No difference in breast milk composition was noted between the first and second 6 months of lactation. If the reference body weight ratio method, used by the DRI Committee to extrapolate from the AI for niacin for infants ages 0 through 6 months, was used, the AI for preformed niacin for the older infants is 2 mg/day after rounding. The DRI Committee also considered extrapolating from the EAR for adults and adjusting for the expected variance to estimate a recommended intake, giving an AI of 4.1 mg of NEs. Thus, the AI for niacin for infants aged 7 through 12 months was set at 4 mg/day of NEs.

(c) *Children (1 - 8 years) and Adolescents (9 - 18 years)*

No data were found on which an EAR for niacin for children could be based. Thus, EARs and RDAs for children have been estimated by the DRI Committee using extrapolation from adult values. A CV of 15% was used to set the RDAs. The RDA for children aged 1 to 3 years and 4 to 8 years were thus set at 130% EAR or 6 mg of NE/day and 8 mg of NE/ day respectively.

Similarly, for adolescent boys aged 9 to 13 years and aged 14 to 18 years, the RDAs were extrapolated from adult values to be 12 mg/day and 16 mg/day respectively. RDAs for adolescent girls aged 9 to 13 years and aged 14 to18 years were computed to be 12 mg/day and 14 mg/day respectively.

The same RDAs have also been recommended by FAO/WHO (2002) for children and adolescents, although the age groupings used are slightly different from those of the DRI Committee.

(d) *Adults (≥ 19 years)*

The best biochemical measure for estimating the average requirement was thought to be niacin metabolite excretion data, namely N'-methyl-nicotinamide and its 2-pyridone. Niacin metabolites are not excreted until adequate tryptophan is available to meet needs for the synthesis of protein, NAD and NADP. Metabolite excretion measures are more sensitive to niacin depletion than other biochemical measures such as blood pyridine nucleotides or tryptophan. Excretion of N'-methyl-nicotinamide rather than the 2-pyriodone is preferred as the target measure for estimating the niacin requirement because this metabolite provides better differentiation between marginal and adequate niacin intakes. Furthermore, there are interpretive guidelines and more data relating to this metabolite.

An average niacin requirement can be estimated as the niacin intake corresponding to an excretion of N'-methyl-nicotinamide that is above the minimal excretion at which pellagra symptoms occur. Reviewing data from various available studies, a urinary excretion value for N'-methyl-nicotinamide of 1 mg/day has been chosen by the DRI Committee as an interpolated level of niacin excretion; it reflects a niacin intake that is above that which results in clinical niacin deficiency and thus is minimal or barely adequate (IOM, 1998). Examining findings from several feeding studies, it was concluded that the overall average intake equivalent to the excretion of 1 mg/day of N'-methyl-nicotinamide was 11.6±3.04, with a CV of 34%. The DRI Committee also decided that women have a slightly lower requirement than men based on their size and average energy utilization.

Therefore, the DRI Committee estimated the EAR to be 12 mg of NE/day for men and 11 mg of NE/day for women. The wide variation in the efficiency of converting tryptophan to niacin was thought to have contributed to the larger apparent variation observed for niacin requirement. Thus, a CV of 15% was assumed for the computation of RDA, ie 130% of the EAR. The computed RDA was 16 mg NE/day for men and 14 mg NE/day for women. These values are also recommended by FAO/WHO (2002). Both organizations did not provide for separate intakes for older adults.

(e) *Pregnancy*

The DRI Committee felt that there is direct evidence that would suggest an increased requirement of niacin during pregnancy. The DRI Committee estimated that during pregnancy, the need for niacin increases by 3 mg/day of NE to cover increased energy utilization and growth in maternal and fetal compartments, especially during the second and third trimesters. EAR during pregnancy is therefore 14 mg NE/ day; and 130% of EAR provides a RDA of 18 mg NE/day.

(f) *Lactation*

During lactation, an estimated 1.4 mg of preformed niacin is secreted in breast milk daily, and 1 mg is required to cover energy expenditure for milk production. The addition of 2.4 mg to 11 mg (the EAR for non-pregnant and non-lactating women) will provide an estimated EAR of 13 mg of NE/day (after rounding up) for a lactating mother. Based on 130% of EAR, the DRI Committee proposed a RDA of 17 mg of NE/day (IOM, 1998).

15.8 Current RDAs for Niacin in Southeast Asia

Current RDAs for niacin in selected Southeast Asian countries are tabulated in Table 15.2.

Table 15.2 Comparison of current RDAs (mg NE/day) for niacin in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)	Vietnam (1996)
Infants (0 - 1)	2 - 4	2 - 4	1.5 - 4.0	4.6 - 6.3	4[a]	5 - 5.4
Children (1 - 9)	6 - 12	6 - 12	6 - 9	7.6 - 13.9	6 - 8	9.0 - 14.5
Boys (10 - 18)	16	16	12 - 16	14.5 - 18.8	12 - 16	17.2 - 20.3
Girls (10 - 18)	14	16	12 - 14	12.9 - 14.2	12 - 14	15.2 - 16.4
Men (≥ 19)	16	16	16	16.2 - 19.5	16	19.8
Women (≥ 19)	14	14	14	13.2 - 14.2	14	14.5
Pregnancy						
1st trimester	+4	+4	+4	+1.3 - 1.9	+4	+0
2nd trimester	+4	+4	+4	+1.3 - 1.9	+4	+2.3
3rd trimester	+4	+4	+4	+1.3 - 1.9	+4	+2.3
Lactation						
1st 6 months	+3	+3	+3	+ 3.3	+3	+ 3.7
2nd 6 months	+3	+3	+3	+ 3.3	+3	+0

Notes: [a] *figures only for infants aged 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[d] *≥ 18 for Singapore*

Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

The recommended intakes for niacin for Singapore and Vietnam are slightly higher than those recommended by the other 4 countries. This is true for most of the age groups, except for pregnant or lactating women. The RDAs for niacin for Indonesia, Malaysia, Philippines and Thailand are rather similar for all age groups, including additional amounts recommended for pregnancy and lactation. With the exception of Malaysia, where both sexes are given the same recommendations, the RDAs for adolescent boys are marginally higher than those for girls. In all the 6 countries, the RDA for men are generally higher than those for women.

Recommended intakes pregnant women for Indonesia, Malaysia, Philippines and Thailand are the same, where an additional amount of 4 mg NE/day is recommended throughout pregnancy. Singapore has recommended a much lower additional amount of 1.3-1.9 mg NE/day throughout pregnancy whereas an addition of 2.3 mg NE/day is recommended by Vietnam for the later part of the pregnancy. The additional amount recommended by the 6 countries for lactating women are rather similar, i.e., an additional 3 mg NE/day for the duration of the lactation except for Vietnam which only recommends additional intake during the first 6 months of lactation.

15.9 Recommended RDAs for Niacin for Southeast Asia

Upon reviewing the recommendations for niacin of the FAO/WHO and the DRI Committee, it was found that the recommendation levels are the same, although the FAO/WHO used different age groupings. The SEA-RDA Committee has proposed that the recommendations of the FAO/WHO (2002) on the requirements for niacin be adopted as the SEA-RDAs.

Table 15.3 Recommended RDAs for Niacin for Southeast Asia

Age Groups	Niacin RDA (mg NE/day)
Infants (months)	
0 – 5	2*
6 – 11	4
Children (years)	
1 – 3	6
4 – 6	8
7 – 9	12
Boys (years)	
10 – 18	16
Girls (years)	
10 – 18	16
Men (years)	
19 – 65	16
> 65	16
Women (years)	
19 –65	14
> 65	14
Pregnancy	18
Lactation	17

*Note: * preformed niacin*

The proposed regional RDAs are generally lower than the existing recommended intakes for selected countries in the region (Table 15.2). This is true for all age groups, except for women where the regional RDA is very similar to current recommended intakes. For pregnant and lactating women, the revised RDA has recommended additional daily intakes of 4 mg and 3 mg of NE respectively. These recommendations are similar to the current recommendations in Indonesia and the Philippines, but higher than those of the other countries.

Bibliography

Altschul Z, Hoffer A & Stephen JD (1955). Influence of nicotinic acid on serum cholesterol in man. *Arch Biochem Biophys* 54: 558-559.

Canner PL, Berge KG, Wenger NK, et al. (1986). Fifteen-year mortality in Coronary Drug Project patients: long-term benefit with niacin. *J Am Coll Cardiol* 8:1245-1255.

DOH Thailand (1989). *Recommended daily dietary allowances and dietary guidelines for Thais.* Department of Health, Ministry of Public Health, Bangkok, Thailand.

FAO/WHO (2002). Thiamin, riboflavin, niacin, vitamin B6, pantothenic acid and biotin. In: *Human Vitamin and Mineral Requirements.* Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; Chapter 3, pp 27-51.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

IOM (1998). Niacin. In: *Dietary References Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; chapter 6, pp 123-149

MOH Indonesia (1994). *Recommended daily dietary allowances for Indonesians.* Ministry of Health Indonesia, Jakarta.

MOH Singapore (1988). *Recommended daily dietary allowances for normal healthy persons in Singapore.* Food and Nutrition Department, Ministry of Health Singapore.

MOH Vietnam (1996). Vietnamese recommended dietary allowances. Ministry of Health Vietnam, Hanoi, Vietnam.

MPH (2003). *Dietary Reference Intake for Thais.* Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia.* A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Teoh ST (1975). Recommended daily intakes for Peninsular Malaysia. *Med J Mal* 30:38-42.

16 FOLATE

16.1 Introduction

In 1937, Lucy Wills of the Royal Free Hospital in London had described a "new haemopietic factor" in yeast that cured tropical macrocytic anaemia in India. This unknown substance was referred to as "Wills factor" and was found to be in a different fraction of liver extract from that which was curative for pernicious anaemia. This "Wills factor" was subsequently found to be folic acid, with its isolation in 1943 by EL Robert Stokstad and identification and synthesis of pteroylglutamic acid in 1945. The term "folic acid" was coined in 1941 by Mitchell *et al.* because they found this in a leafy vegetable (spinach) (Selhub and Rosenberg, 1996).

16.2 Characteristics and Functions

Folate is a generic term for a water-soluble B-complex vitamin which functions in single-carbon transfer reactions and exists in many chemical forms. Folic acid (pteroylmonoglutamic acid), which is the most oxidized and stable form of folate, occurs rarely in food but is the form used in vitamin supplements and in fortified food products. Folic acid consists of a *p*-aminobenzoic acid molecule linked at one end to a pteridine ring and at the other end to one glutamic acid molecule. Most naturally occurring folates, called food folate, are pteroylpolyglutamates (tetrahydrofolate, 5-methyltetrahydrofolate, and 5-formyl tetrahydrofolate), which contain 1 to 6 additional glutamate molecules joined in a peptide linkage to the γ-carboxyl of glutamate.

All naturally occurring folates are unstable to varying degrees, due to endogenous pteroylpolyglutamyl hydrolase, which removes glutamic acid residue but leaves an active compound. Heat, oxidation and ultraviolet light cleave the folate molecule, rendering it inactive. Consequently, naturally occurring folates are labile and are lost in storage and cooking. The order of stability of these derivatives is 5-formyl-THF >5-methyl-THF>10-formyl-THF>THF.

Crystalline folic acid is a yellow (molecular weight, 441.4 g/mol). The free acid is almost insoluble in cold water; the disodium salt is more soluble – about 1.5 g/dL. Folic acid is destroyed at a pH below 4 but is relatively stable above pH 5, with no destruction in 1 hour at 100°C. The molecule usually splits into pteridine and *p*-aminobenzyol glutamate.

Folate functions as a coenzyme in single-carbon transfers in the metabolism of nucleic and amino acids. The folate coenzymes are involved in numerous reactions that involve the following:

(a) Deoxyribonucleic acid (DNA) synthesis, which depends on a folate coenzyme for pyrimidine nucleotide biosynthesis (methylation of deoxyuridylic acid to thymidylic acid) and thus is required for normal cell division.

(b) Purine synthesis, which is the formation of glycinamide ribonucleotide and 5-amino-4-imidazole carboxamide ribonucleotide.
(c) Generation of formate into the formate pool, and utilization of formate.
(d) Amino acid interconversions, including the catabolism of histidine to glutamic acid, interconversion of serine and glycine, and conversion of homocysteine to methionine.

Folate-mediated transfer of single-carbon units from serine provides a major source of substrate in single-carbon metabolism. The conversion of homocysteine to methionine serves as a major source of methionine for the synthesis of S-adenosyl-methionine, an important *in vivo* methylating agent.

16.3 Absorption, Utilization and Excretion

Food folates (polyglutamate derivatives) are hydrolysed to monoglutamate forms in the gut prior to absorption across the intestinal mucosa. The monoglutamate form of folate is actively transported across the proximal small intestine by a saturable pH-dependent process. Monoglutamates, mainly 5-methyl-tetrahydrofolate, are present in the portal circulation. Much of this folate can be taken up by the liver, where it is metabolized to polyglutamate derivatives and retained or released into the blood or bile. Approximately two-thirds of the folate in plasma is protein bound. Cellular transport of folate is mediated by a number of different folate transport systems, which can be characterized as either (a) membranes carriers or (b) folate-binding, protein-mediated systems.

Folate concentrations of 4.5 μg/g to 10 μg/g of liver have been reported following liver biopsies. Since the adult male liver weighs approximately 1,400 g, the total quantity of folate in the liver would be approximately 6 mg to 14 mg. Assuming that the liver contains 50% of the body stores of folate, the estimated total body folate store would be 12 mg to 28 mg.

Prior to tissue storage or use as a coenzyme, folate monoglutamate is converted to the polyglutamate form by the enzyme folypolyglutamate synthetase. When released from tissues into circulation, folate polyglutamates are reconverted to the monoglutamate form by γ-glutamylhydrolase.

The metabolic interrelationship between folate and vitamin B_{12} may explain why a single deficiency of either vitamin leads to the same hematological changes. Both folate and vitamin B_{12} are required for the formation of 5,10-methylenetetrahydrofolate and involved in thymidylate synthesis by way of a vitamin B_{12}-containing enzyme. In either a folate or vitamin B_{12} deficiency, the megaloblastic changes occurring in the bone marrow and other replicating cells result from lack of adequate 5,10-methylene-tetrahydrofolate.

Folate is freely filtered at the glomerulus and is reabsorbed in the proximal renal tubule. The net effect is that most of the secreted folate is reabsorbed. The bulk of the excretion products in humans are folate cleavage products. Intact urinary folate represents only a very small percentage of dietary folate. Biliary excretion of folate has been estimated to be as high as 100 μg/day; however, much of this is reabsorbed by the small intestine. Fecal folate losses occur, but it is difficult to distinguish actual losses from folate synthesized by the intestinal microflora.

16.4 Effects of Deficiency and Excess

16.4.1 Deficiency

Folate deficiency can arise in a variety of settings, including alcoholism, low dietary intake, or malabsorption. It is observed under conditions of increased cellular turnover such as pregnancy, cancer and hemolytic anaemia.

Inadequate folate intake first leads to a decrease in serum folate concentration, then to a decrease in red cell folate concentration, a rise in homocysteine concentration, and ultimately to megaloblastic changes in the bone marrow and other rapidly dividing cells. Advanced folate deficiency characteristically causes macrocytic or megaloblastic anaemia, with abnormalities on peripheral blood smear and bone marrow examinations. Clinical manifestations of folate deficiency often resemble the hematologic features of vitamin B_{12} deficiency. Status of both folate and B_{12} should be evaluated in suspected cases.

When macrocytic anemia develops, this is evidenced by a depression of the red blood cell count. Eventually, all 3 measures of anemia (hematocrit, hemoglobin concentration, and red cell concentration) are depressed. At this point, macroovalocytes and macrocytes are usually detectable in the peripheral blood, and hypersegmentation is more impressive.

Symptoms of weakness, fatigue, difficulty in concentrating, irritability, headaches, palpitations, and shortness of breath typically appear at an advanced stage of anemia. They may be seen in milder degrees of anemia in some patients, especially the elderly.

16.4.2 Excess intake

No adverse effects have been associated with the consumption of folate normally found in fortified foods. Concern for excessive intake of folate is mainly related to ingestion of supplemented folate. One of these effects studied is neurological effects especially seen in individuals with vitamin B_{12} deficiency. Vitamin B_{12} deficiency is often undiagnosed but may affect a substantial percentage of the population, especially older adults. Evidences have shown that excess folic acid intake may precipitate or exacerbate the neurologic damage of B_{12} deficiency.

For many years, it has been recognized that excessive intake of folic acid may obscure or "mask" and potentially delay the diagnosis of vitamin B_{12} deficiency. Delayed diagnosis can result in an increased risk of progressive, unrecognized neurological damage.

There have been other studies of adverse effects, but findings on these have not been conclusive. In one non-blinded, uncontrolled trial, oral doses of 15 mg of folic acid per day were given for 1 month, and mental changes, sleep disturbances and gastrointestinal effects were reported. Individual cases of hypersensitive reactions to oral and parenteral folate administration have been reported, but they were deemed to be rare. Many studies have evaluated the periconceptional use of

supplemental folate (in doses of approximately 0.4 to 5.0 mg) to prevent neural tube defects. No adverse effects have been demonstrated, but the studies were not specifically designed to assess adverse effects. No reports were found of adverse effects attributable to folate in long-term folate supplement users or in infants born each year to mothers who take supplements, but this has not been investigated systematically.

16.4.3 Guidance on high intake

Upon reviewing available findings, the DRI Committee has proposed upper tolerable intake levels (ULs) for folate and they are set out in Table 16.1 (IOM, 1998).

Table 16.1 ULs of folate for various age groups

Age Group (years)	Folate UL (μg/day)*
Infants	Not possible to establish for supplemental folate
Children	
1 – 3	300
4 – 8	400
9 – 13	600
Adolescents (14 – 18)	800
Adults (≥ 19)	1,000
Pregnancy (years)	
14 – 18	800
≥ 19	1,000
Lactation (years)	
14 – 18	800
≥ 19	1,000

Note: * *From fortified foods or supplements*
Source: *IOM (1998)*

16.5 Food Sources

Currently, there is limited data on total folates in foods in the Southeast Asian region. Microbiological assay is by far the most commonly used method for quantitative analysis of folate in foods. Table 16.2 (Refer to page 213) shows the folate content of some common food items analyzed using this method.

In order to obtain the maximum content of folates for reliable food composition data, tri-enzyme treatment (protease, amylase, and conjugase) of the samples prior to folate measurement is strongly recommended. More reliable methods for folate analysis of foods are being developed in many countries. The folate level in some food commodities in existing food composition tables may have to be re-analysed. Moreover, when the data on folate in foods is used to recommend dietary folate intake, its bioavailability must also be considered.

Table 16.2 Folate content of selected foods

Food Group/Item	Folate Content (μg/100 g)
Liver	
Chicken	600 - 1,000
Beef	250 - 400
Pork	150
Vegetables, raw	
Chive (flowers & leaves), young seeds (Parkia speciosa), water lily stem, spinach horse-tamarind (young leaves & stems), mint collard, ivy gourd (young leaves & stems), Brussel sprouts, cauliflower, sesbania (flowers), yard long beans	100 - 300
Dry legume seeds	
Peanuts	140
Mungbeans, rice, soybeans	300 - 340
Fruits	
Durian, guava, manila tamarind, banana	60 – 110
Grape, papaya, rose apple, rambutan, tangerine	20 – 30
Eggs	
Duck egg	75
Chicken egg	50
Other vegetables	
Bean sprouts, bell pepper, pumpkin, broccoli, eggplant	30 – 60
Grains and Grain Products	
Rice, whole-wheat bread	29 – 38
Meat and Dairy Products	
Breast milk, cow's milk, fish, beef chicken, pork	< 10

Source: Lavansiri (1978); Paul and Southgate (1978); Tamura (1998); Witthoft et al. (1999)

16.6 Factors Affecting Requirement

16.6.1 Bioavailability

When consumed under fasting conditions, synthetic folic acid is nearly 100% bioavailable. Based on some available data, the bioavailability of synthetic folic acid consumed with food was estimated to be 85%. Studies have not been conducted to define the bioavailability of folic acid consumed with entire meals. It is assumed that the bioavailability would be somewhat lower than that observed with folic acid alone or folic acid with a small portion of food.

With the approval of fortification of breads and grains with folic acid, there has been increased interest in the bioavailability of folic acid provided in this form. High bioavailability of folate in the form of added folic acid has been reported. On the other hand, studies of bioavailability of food folate have shown that it was no more than 50% that of folic acid.

16.6.2 Bioavailability estimates and assumptions

Many controlled studies on folate requirements have used a defined diet (food folate) supplemented with synthetic folic acid. Since folic acid taken with food is 85% bioavailable, and food folate is only about 50% bioavailable, folic acid taken with food is 85/50 (i.e., 1.7) times more available. Thus, on the basis that a mixture of synthetic folic acid plus food folate is provided, dietary folate equivalents (DFE) are calculated as follows to determine the EAR:

$$\mu g \text{ of DFE provided} = \mu g \text{ of food folate} + (1.7 \times \mu g \text{ synthetic folic acid})$$

Expressed in a different way, only half as much folic acid as compared to food folate is needed if taken on an empty stomach:

1μg DFE = 1μg food folate = 0.5 μg folic acid taken on an empty stomach = 0.6 μg folic acid with meals

When food folate was the sole source of folate in studies, no corrections were applied to convert to DFE. Adjustments made for DFE are indicated, if applicable. Adjustments cannot be made for epidemiologic studies if data are lacking on the folate sources.

16.6.3 Interactions with nutrients and other food components

No reports were found that demonstrate that the intake of other nutrients increases or decreases the requirement for folate. However, coexisting iron or vitamin B_{12} deficiency may interfere with the diagnosis of folate deficiency. In contrast to folate deficiency, iron deficiency leads to a decrease in mean cell volume (MCV). In the combined deficiency, interpretation of hematologic changes may be unclear. A vitamin B_{12} deficiency results in the same hematologic changes that occur with folate deficiency since the B_{12} deficiency results in a secondary folate deficiency.

Experimental data do not support the hypothesis that dietary fiber, per se, reduces folate bioavailability. Human studies confirmed the negative findings of both rat and chick bioassays to identify an inhibitory action of various dietary fiber sources. Certain forms of fiber (e.g. wheat bran) may decrease the bioavailability of certain forms of folate under some conditions, but many forms of fiber had no adverse effects.

Survey data of chronic alcoholics suggest that inadequate intake is a major cause of the folate deficiency that has often been observed in chronic alcohol users. Ethanol intake may aggravate folate deficiency by impairing intestinal folate absorption and hepatobiliary metabolism and by increasing renal folate excretion.

16.7 Estimating Requirements and Recommended Intakes

16.7.1 Indicators for estimating requirement for folate

The primary indicator selected to determine folate adequacy is erythrocyte folate, which reflects tissue folate stores. For some life stage or gender groups, this is used in conjunction with plasma homocysteine (which reflects the extent of the conversion of homocysteine to methionine) and plasma or serum folate.

Because folate is taken up only by the developing erythrocyte in the bone marrow and not by the circulating mature erythrocyte during its 120-day lifespan, erythrocyte folate concentration is an indicator of long-term status. In other words, erythrocyte folate concentration does not reflect recent or transient changes in dietary folate intake. A value of 305 nmol/L (140 ng/mL) of folate has been chosen as the cut-off point for adequate folate status (IOM, 1998). Erythrocyte folate concentration has also been shown to be related to tissue stores by its correlation with liver folate concentration.

Plasma homocysteine concentration increases when inadequate quantities of folate are available to donate the methyl group that is required to convert homocysteine to methionine. Controlled metabolic and epidemiological studies provide evidence that plasma homocysteine rises with reductions in blood folate indices including serum folate, plasma folate, or erythrocyte folate. Different cut-off values have been used by various investigators to define elevated homocysteine concentrations. According to the DRI Committee, the cut-off value for plasma homocysteine cited most often is greater than 16 μmol/L. There is thus evidence supporting the use of homocysteine as an ancillary indicator of folate status.

The DRI Committee recognized that plasma homocysteine is not a highly specific indicator of folate status as it can be influenced by vitamin B_{12} status, vitamin B_6 status, age, gender, race, some genetic abnormalities and renal insufficiency. Thus, plasma homocysteine alone is not an acceptable indicator on which to base the folate requirement. The DRI Committee also felt that knowledge of the relationships between folate, homocysteine, and risk of vascular disease was too weak to use as the basis for deriving the estimated average requirement for folate.

A serum folate concentration of less than 7 nmol/L (3 ng/mL) indicates negative folate balance at the time the blood sample was drawn. Serum folate concentration may be a worthwhile diagnostic test if used and interpreted correctly in conjunction with other folate status indices. In population surveys, it is generally assumed that measuring serum folate alone does not differentiate between what may be a transitory reduction in folate intake or chronic folate deficiency accompanied by depleted folate stores and functional changes. Serum or plasma folate is, however, considered a sensitive indicator of dietary folate intake.

16.7.2 Recommendations for folate intake by life stages

The DRI Committee has provided details of estimating requirements and recommended intakes of folate for all age groups (IOM, 1998). The FAO/WHO reviewed the evidences presented and agreed with the approaches and recommendations (FAO/WHO, 2002).

The SEA-RDA Committee reviewed the above reports and recommendations of the DRI Committee and the FAO/WHO, and studied the rationale and steps taken in setting requirements and the levels recommended by these organizations. Existing RDAs of countries in the Southeast Asian region were additional references; there were no studies of folate requirements in the region. After its review, the SEA-RDA Committee decided to base their recommended intakes for folate on the recommendations of FAO/WHO (2002).

(*a*) *Infants (0 - 12 months)*

The DRI Committee had used AI as the goal for folate intake by infants. The AI reflects the observed mean folate intake of infants who are exclusively breast-fed. Based on an average volume of 0.78 litres per day of milk for the infants aged 0 to 6 months and the average folate concentration of breast milk after 1 month of lactation to be 85 μg/litre, the amount of folate is taken to be 65 μg DFE per day, after rounding up.

For older infants, the DRI Committee used the reference body weight ratio method to extrapolate from the AI for folate for infants aged 0 to 6 months, and obtained an AI for folate for the older infants of 80 μg DFE per day, after rounding. The DRI Committee also used the method of extrapolating from the EAR of adults and adjusting for the expected variance; the same AI was obtained.

The DRI Committee also reviewed several controlled studies that measured folate intake and assessed the infants' status based on indicators such as serum and erythrocyte concentrations and plasma homocysteine level. The investigators included studies in which infants were fed either breast milk or formula. Data from the research studies supported the AI of 65 μg/day of DFE for young infants and of 80 μg/day DFE for older infants. FAO/WHO made slightly different recommendations of 80 μg/day DFE for infants of both age groups (FAO/WHO, 2002).

(*b*) *Children (1 - 8 years)*

No data were found on which to base an EAR for children. In the absence of additional information, the DRI Committee estimated the EARs for children aged 1 to 8 years by extrapolating from adult values. The resulting EARs are 120 μg/day and 160 μg/day of DFEs for children aged 1 to 3 years and 4 to 8 years respectively.

Assuming a coefficient of variation of 10%, RDA was calculated based on 120% of EAR. The results were recommended intakes of 150 μg/day and 200 μg/day DFE for children 1-3 years and 4-8 years respectively. Slightly different intakes were recommended in the FAO/WHO (2002) reports, namely 160 μg/day and 200 μg/day DFE for children 1-3 years and 4-6 years respectively.

(*c*) *Adolescents (9 - 18 years)*

EARs and RDAs for adolescents have also been extrapolated from adult values (IOM, 1998). Although body size varies due to gender differences, no conclusive data indicating a difference in requirements for adults were determined, thus no difference based on gender is proposed for these age groups. Hence, the EARs recommended by the DRI Committee were 250 μg/day and 330 μg/day of DFEs for adolescents aged 9 to 13 years and 14 to 18 years respectively.

Assuming a CV of 10%, RDA was calculated based on 120% of EAR. The resulting RDAs were 300 μg/day and 400 μg/day of DFEs for adolescents aged 9 to 13 years and 14 to 18 years respectively. The FAO/WHO used different age groupings and its recommended intakes are 300 μg/day and 400 μg/day of DFEs for adolescents aged 7 to 9 years and 10 to 18 years respectively. No distinctions based on gender were made.

(*d*) *Adults (19 – 50 years)*

The main approach to determining the EAR for adults uses a combination of erythrocyte folate, plasma homocysteine, and plasma or serum folate. The DRI Committee reviewed various studies on requirements of folate. The focus was on studies examining the adequacy of specific quantities of folate consumed under controlled metabolic conditions to maintain normal blood concentrations of these indicators. Cut-off points for the normal range were based on the occurrence of documented biochemical abnormalities.

In addition to data on maintenance or restoration of folate status, several other types of experimental data were examined. These included kinetic estimates of body pool size and daily turnover, quantification of urinary folate catabolites as an index of folate turnover, and repletion of severe clinical folate deficiency.

With greatest weight given to the metabolic maintenance studies, the DRI Committee concluded that the data supported an EAR of approximately 320 μg/day of DFEs for the adults aged 19 to 50 years. Based on a CV of 10%, RDA for adults was calculated as 120% of EAR or 400 μg/day of DFEs for men and women. The 2002 FAO/WHO Report has recommended the same amount as the RNI for adults.

(e) *Adults (51 years and above)*

The DRI Committee reviewed various studies to determine the EAR for the older adults, namely metabolic (depletion-repletion) studies, observational folate status assessment of specific subgroups, and epidemiological studies. Available data showed that the EAR for this age group is expected to be the same as that for younger age groups. Aging process does not appear to impair folate absorption or utilization nor do studies make a distinction between adults over the age of 70 from those aged between 51 to 70 years.

Therefore, the DRI Committee proposed a RDA of 400 μg/day of DFEs for older adults above 50 years. FAO/WHO has also recommended the same intake for folate for this age group.

(f) *Pregnancy*

Folate requirements increase substantially during pregnancy because of the marked acceleration in single-carbon transfer reactions, including those required for nucleotide synthesis and thus cell division. During pregnancy, cells multiply in association with uterine enlargement, placental development, expansion of maternal erythrocyte number, and fetal growth. Additionally, folate is actively transferred to the fetus as indicated by elevated folate concentrations in cord blood relative to that of maternal blood. When folate intake is inadequate, maternal serum and erythrocyte folate concentrations decrease and megaloblastic marrow changes may occur. If inadequate intake continues, megaloblastic anemia may develop.

Various studies were reviewed by the DRI Committee, including a number of population-based studies and several metabolic (supplementation) studies. From available data, it was concluded that low dietary folate intake plus 100 μg of supplemental folate (equivalent to approximately 200 μg/day of DFEs) is inadequate to maintain normal folate status in a significant percentage of population groups assessed. The EAR therefore was derived by adding this quantity in DFEs (200 μg/day) to the EAR for non-pregnant women (320 μg/day) to provide an EAR of 520 μg/day of DFEs.

Assuming a CV of 10%, RDA was computed as 120% EAR or 600 μg/day of DFEs. Data from the controlled metabolic study support an RDA of 600 μg/day of DFEs based on maintenance of normal erythrocyte folate concentrations and agree with the findings from the series of population studies that 600 μg/day of DFEs is adequate to maintain normal folate status in groups of pregnant women. A same amount was recommended by FAO/WHO for pregnant women (FAO/WHO, 2002).

(*g*) *Lactation*

The requirement for lactating women is estimated to be the folate intake necessary to replace the folate secreted daily in breast milk plus the amount required by the non-lactating woman to maintain folate status. The average daily amount of folate secreted in breast milk is estimated to be 85 μg/litre, as previously described in the section on requirements of infants aged 0 to 12 months. The dietary intake needed to provide this amount must account for the estimated 50% bioavailability of food folate.

The extra amount of folate needed to cover lactation is thus calculated as follows:

0.78 L (milk volume) x 85 μg/L (folate concentration) x 2 (bioavailability correction factor) = 133 μg/day.

When this quantity is added to the EAR for non-lactating and non-pregnant women (320 μg/day), the result is rounded down, giving an EAR of 450 μg/day of DFEs. Women who are only partially breast-feeding would need less.

Assuming a CV of 10%, the RDA, calculated as 120% EAR, is 500 μg/day of DFEs. The same amount has been recommended by FAO/WHO (2002).

16.8 Current RDAs for Folate in Southeast Asia

A comparison of the current RDAs in 5 countries in the Southeast Asian region is given in Table 16.3. According to the review of Tee (1998), Vietnamese RDA of 1996 did not tabulate recommended intake for folate.

Table 16.3 Comparison of current RDAs (μg/day) for folate in selected Southeast Asian countries

Age Groups (years)	Indonesia (1994)	Malaysia (2005)	Philippines (2002)	Singapore (1988)	Thailand (2003)
Infants (0 – 1)	22 – 32	80	65 – 80	60	80[a]
Children (1 – 9)	40 – 80	160 – 300	160 – 300	100	150 – 200
Boys (10 – 18)	90 – 165	400	400	100 – 200	300 – 400
Girls (10 – 18)	100 – 160	400	400	100 – 200	300 – 400
Men (≥ 19)	190	400	400	200	400
Women (≥ 19)	150 – 160	400	400	200	400
Pregnancy					
1st trimester	+150	+200	+200	+200	+200
2nd trimester	+150	+200	+200	+200	+200
3rd trimester	+150	+200	+200	+200	+200
Lactation					
1st 6 months	+50	+100	+100	+100	+100
2nd 6 months	+40	+100	+100	+100	+100

Notes: [a] *figures only for infants aged 6 - 11 months*
[b] *1 - 8 years for Thailand*
[c] *10 - 17 years for Singapore; 9 - 18 years for Thailand*
[d] *≥ 18 for Singapore*

Source: Indonesia, Singapore, Vietnam: Tee (1998); Philippines: FNRI (2002); Malaysia: NCCFN (2005); Thailand: MPH (2003)

Recommendations for daily folate intake for Malaysia, Philippines and Thailand, all updated after 2002, are much higher than the RDAs of Indonesia and Singapore, published in the 1990s. Indeed, folate RDA for Indonesia are the lowest. This is true for all age groups, except during pregnancy and lactation. For all the country RDAs reviewed, except for Indonesia, there is no difference in recommended intakes for the sexes. In the case of Indonesia, boys are recommended slightly higher intakes compared with girls and men higher than those recommended for women.

All the 5 countries reviewed recommended additional folate intake during pregnancy and lactation. Except for Indonesia, all the 4 countries recommended exactly the same amounts, i.e., an addition of 200 μg per day throughout pregnancy. These countries also recommended an addition of 100 μg per day throughout the lactation period. As for all other age groups, the additional amounts of folate recommended in the Indonesian RDA for pregnant and lactating women are much lower than those of the other countries.

16.9 Recommended RDAs for Folate for Southeast Asia

Upon reviewing available current information, the SEA-RDA Committee proposed that the FAO/WHO (2002) recommendations for folate intake be adopted for the SEA-RDAs (Table 16.4).

Table 16.4 Recommended RDAs for Folate for Southeast Asia

Age Groups	Folate RDA (μg/day)
Infants (months)	
0 – 5	80
6 - 11	80
Children (years)	
1 – 3	160
4 – 6	200
7 – 9	300
Boys (10 – 18 years)	400
Girls (10 – 18 years)	400
Men (years)	
19 – 65	400
> 65	400
Women (years)	
19 – 65	400
> 65	400
Pregnancy	600
Lactation	500

The SEA-RDAs for folate are much higher than the current RDAs for countries in the region. For all age groups, the amount recommended has been revised upwards, mostly up to almost twice the current levels. It was felt that this was appropriate as new evidence on the role of folate in health and disease justified a higher level of recommended intake. The additional amount for pregnancy and lactation remains the same, but the absolute amount for these 2 groups have also increased significantly as the amount recommended for non-pregnant women is now much higher.

Bibliography

FAO/WHO (2002). Folate and folic acid. In: *Human Vitamin and Mineral Requirements.* Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; Chapter 4, pp 53-63.

FNRI (2002). *Recommended Energy and Nutrient Intakes Philippines.* 2002 edition. Food and Nutrition Research Institute, Department of Science and Technology, Manila, Philippines.

IOM (1998). Folate. In: *Dietary References Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline.* Food and Nutrition Board, Institute of Medicine. National Academy Press, Washington, DC.; chapter 8, pp 196-305.

Lavansiri, D (1978). Determination of folates in Thai foods. MSc Thesis (Tropical Medicine), Mahidol University.

MPH (2003). *Dietary Reference Intake for Thais.* Nutrition Division, Department of Health, Ministry of Public Health, Thailand.

NCCFN (2005). *Recommended Nutrient Intakes for Malaysia.* A report of the Technical Working Group on Nutritional Guidelines. National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia, Putrajaya.

Pual AA & Southgate DAT (1978). *McCance and Widdowson's The composition of Foods.* Her Majesty's Stationary Office, London.

Selhub J & Rosenberg IH (1996). Folate. In: *Present Knowledge in Nutrition.* 7th edition. Ziegler EE and Filer LJ Jr, (eds). International Life Sciences Institute Press, Washington, DC, pp 206-219.

Tamura T (1998). Methods of nutritional biochemistry. Determination of food folate. *Nutr Biochem* 9:285-93.

Tee ES (1998). Current status of recommended dietary allowances in Southeast Asia: a regional overview. *Nutr Rev* 56 (4): S10-S18.

Witthöft CM, Forssen K, Johannesson L & Jagerstad M (1999). Folates - food sources, analyses, retention and bioavailability. *Scand J Nutr* 43:138-46.

17 CONCLUSIONS AND RECOMMENDATIONS

17.1 Recommended RDAs for Southeast Asia

The recommended RDAs for energy, protein, 7 vitamins and 5 minerals for the Southeast Asian population are summarized in the Appendix. The Southeast Asian countries that participated in these RDA harmonization efforts have agreed that the SEA-RDAs would form the basis or serve as reference for the review and revision of RDAs in their respective countries.

17.2 Research Agenda for SEA-RDAs

The SEA-RDA Committee members agreed that there is a need for each country to continue generating as much local data as possible on the requirements of the various nutrients. The generation of primary data applicable to the region, for example, a nutrient or RDA component influenced by ethnicity and body size, should be given priority. Country representatives on the SEA-RDA Committee identified the following priority research areas:

- Energy expenditure, physical activity levels, body composition and their impact on long-term health, and comparison of methodologies for energy expenditure measurements
- Requirements for other micronutrients, such as panthothenic acid, biotin, manganese and vitamin E, among the Asian population
- Factors and variables that affect requirements of nutrients
- Determining the bioavailability of nutrients, such as iron, zinc and folate, from traditional diets by using modern techniques
- Improvement and expansion of existing food analysis databases by increasing the types of food and nutrients analyzed in the databases. Analysis of nutrients such as folate, selenium, zinc and amino acids in foods, as well as important non-nutrients such as dietary fiber, should be commenced
- Effects of food preparation and cooking on the nutrient content of foods
- Gathering of data, through surveys, on the intake of nutrients among Southeast Asian populations
- Factors contributing to the low intake of nutrients among Southeast Asian populations, and strategies that would improve nutrient intakes

- Carefully-controlled human trials to determine the relationship between nutrients/other food components and health, including associations with risk of major chronic diseases prevailing in the region such as hypertension, diabetes, cancers, osteoporosis etc

- Extent and adverse effects of excessive intake of specific nutrients

- Identification of appropriate markers for assessing status of specific nutrients and obtaining nationally-representative data on the status of these nutrients among the population

17.3 Future Collaborations

The SEA-RDA Committee proposed that efforts towards harmonization be continued under the leadership of ILSI SEA Region and FAO (Bangkok), and that FNRI (the Philippines) continue to serve as Chair of the proposed Regional Committee. The following specific collaborations are proposed:

- Upgrade or improve capability to conduct research related to development of RDAs, including procurement of state-of-the-art equipment and training of researchers

- Facilitate staff training programs including allowing study visits and participation in research projects

- Conduct joint research projects and develop appropriate methodologies for deriving various data, including nutrient status of individuals

- Sharing of data on nutrient requirements

Country representatives on the SEA-RDA Committee felt that continued networking is necessary to enable member countries to be updated on activities related to development of RDAs in the region, as well as the sharing of experiences in these activities. Countries are urged to each establish a focal point to coordinate national activities on RDA. This would also facilitate cooperation between countries in the region. It is hoped that with these collaborative efforts, national RDAs can be effectively updated in line with advancements in scientific knowledge and techniques.

APPENDIX

RECOMMENDED DIETARY ALLOWANCES FOR SOUTHEAST ASIA (SEA-RDAs)

Population Groups	Weight (kg)	ENERGY (kcal/day)	PROTEIN (g/day)			CALCIUM (mg/day)	IRON (mg/day)		ZINC (mg/day)
			High Quality Protein Diet	Adjusted for 80% Protein Quality	Adjusted for 70% Protein Quality		7.5% Bioavailability	10% Bioavailability	Moderate Bioavailability
Infants (months)									
0 - 5	6	555	11	-	-	300 [a], 400 [b]	0.93 [a]	0.93 [a]	1.1 [a], 2.9 [b]
6 - 11	9	710	14	-	-	400	12.4	9.3	4.2
Children (years)									
1 - 3	14	1,180	16	20	23	500	7.7	5.8	4.8
4 - 6	20	1,470	21	26	29	600	8.4	6.3	5.7
7 - 9	27	1,825	27	34	39	700	11.9	8.9	6.0
Boys (years)									
10 - 12	34	2,110	34	42	48	1,000	19.5	14.6	6.8
13 - 14	47	2,650	45	56	64	1,000	19.5	14.6	8.9
15	47	2,650	45	56	64	1,000	25.1	18.8	8.9
16 - 18	56	2,980	49	62	71	1,000	25.1	18.8	8.6
Girls (years)									
10 - 12	36	2,010	35	44	50	1,000	18.7 [c], 43.6 [d]	14.0 [c], 32.7 [d]	6.1
13 - 14	45	2,205	41	51	58	1,000	18.7 [c], 43.6 [d]	14.0 [c], 32.7 [d]	7.2
15	45	2,205	41	51	58	1,000	41.3	31.0	7.2
16 - 18	49	2,240	40	50	57	1,000	41.3	31.0	6.8
Men (years)									
19 - 29	60	2,635	48	60	68	700	18.3	13.7	6.5
30 - 49	60	2,525	48	60	68	700	18.3	13.7	6.5
50 - 59	60	2,525	48	60	68	1,000	18.3	13.7	6.5
60 - 65	60	2,240	48	60	68	1,000	18.3	13.7	6.5
> 65	60	2,240	48	60	68	1,000	18.3	13.7	6.5
Women (years)									
19 - 29	50	2,115	40	50	57	700	39.2	29.4	4.4
30 - 49	50	2,065	40	50	57	700	39.2	29.4	4.4
50 - 59	50	2,065	40	50	57	1,000	15.1	11.3	4.4
60 - 65	50	1,720	40	50	57	1,000	15.1	11.3	4.4
> 65	50	1,720	40	50	57	1,000	15.1	11.3	4.4
Pregnancy									
1st trimester	-	-	+ 6	+ 7.5	+ 9	1,000	*	*	5.5
2nd trimester	-	+ 360	+ 6	+ 7.5	+ 9	1,000	*	*	7.0
3rd trimester	-	+ 475	+ 6	+ 7.5	+ 9	1,000	*	*	10.0
Lactation (months)									
1st 6	-	+ 505	+ 16	+ 20	+ 23	1,000	20.0	15.0	9.5 [e], 8.8 [f]
2nd 6	-	+ 675	+ 12	+ 15	+ 17	1,000	20.0	15.0	7.2 [g]

Population Groups	Weight (kg)	IODINE (µg/day)	SELENIUM (mg/day)	VITAMIN A (µg/day)	VITAMIN D (µg/day)	VITAMIN C (mg/day)	THIAMIN (mg/day)	RIBOFLAVIN (mg/day)	NIACIN (mg/day)	FOLATE (µg/day)
Infants (months)										
0 - 5	6	90	6	375	5	25	0.2	0.3	2	80
6 - 11	9	90	10	400	5	35	0.3	0.4	4	80
Children (years)										
1 - 3	14	90	17	400	5	30	0.5	0.5	6	160
4 - 6	20	90	22	450	5	30	0.6	0.6	8	200
7 - 9	27	120	21	500	5	35	0.9	0.9	12	300
Boys (years)										
10 - 12	34	120	32	600	5	65	1.2	1.3	16	400
13 - 15	47	150	32	600	5	65	1.2	1.3	16	400
16 - 18	56	150	32	600	5	65	1.2	1.3	16	400
Girls (years)										
10 - 12	36	120	26	600	5	65	1.1	1.0	16	400
13 - 15	45	150	26	600	5	65	1.1	1.0	16	400
16 - 18	49	150	26	600	5	65	1.1	1.0	16	400
Men (years)										
19 - 49	60	150	34	600	5	70	1.2	1.3	16	400
50 - 65	60	150	34	600	10	70	1.2	1.3	16	400
> 65	60	150	33	600	15	70	1.2	1.3	16	400
Women (years)										
19 - 49	50	150	26	500	5	70	1.1	1.1	14	400
50 - 65	50	150	26	500	10	70	1.1	1.1	14	400
> 65	50	150	25	600	15	70	1.1	1.1	14	400
Pregnancy										
1st trimester		200	26	800	5	80	1.4	1.4	18	600
2nd trimester		200	28	800	5	80	1.4	1.4	18	600
3rd trimester		200	30	800	5	80	1.4	1.4	18	600
Lactation (months)										
1st 6		200	35	850	5	95	1.5	1.6	17	500
2nd 6		200	42	850	5	95	1.5	1.6	17	500

Notes: [a] *Breast-fed*
[b] *Formula-fed*
[c] *Non-menstruating*
[d] *Menstruating*
[e] *0 - 3 months*
[f] *4 - 6 months*
[g] *7 - 12 months*
* *Iron supplements in tablet form recommended for all pregnant women. In non-anaemic pregnant women, daily supplements of 100 mg of iron (e.g., as ferrous sulphate) given during the second half of pregnancy are adequate. In anaemic women, higher doses are usually required.*

EDITORS

E-SIONG TEE, PhD

TES NutriHealth Strategic Consultancy

Dr E-Siong Tee was Head of the Cardiovascular, Diabetes and Nutrition Research Center of the Institute for Medical Research (IMR) in Kuala Lumpur, Malaysia. He served the IMR for 30 years until his retirement in February 2002. His main areas of interest and research were in community nutrition, nutritional value of foods and food regulatory affairs. He has published over 150 scientific articles and papers, and made over 170 presentations on various aspects of food and nutrition in seminars and conferences within and outside Malaysia. Dr Tee is also the chair person of the following technical committees of the Ministry of Health Malaysia : (1) National Codex Sub-committee on Nutrition and Foods for Special Dietary Uses: (2) Working Group on Nutrition, Health Claims and Advertisement; and (3) Working Group on Food/Drug Interface products. He is also a member of the National Technical Advisory Committee on Food Regulations and a member of the National Coordinating Committee on Food and Nutrition. Dr Tee serves as Scientific Director on the Board of ILSI Southeast Region. He is also President of the Nutrition Society of Malaysia.

RODOLFO F. FLORENTINO, MD, PhD

Nutrition Foundation of the Philippines

Dr Rodolfo F. Florentino is currently the Chairman-President of the Nutrition Foundation of the Philippines, an Advisor to the Philippines Association of Nutrition and a Scientific Director of ILSI Southeast Region. He is a Fellow of the International Union of Nutrition Science and a member of the WHO Expert Advisory Panel on Nutrition. He is a Fellow of the Philippine Association for the Advancement of Science, and member of the National Research Council of the Philippines. Dr Florentino is also a member of the Board of Directors of the Philippine Association for the Study of Overweight, Obesity (PASOO) and the Osteoporosis Society of the Philippines (OSPFI). He has been in the nutrition field since 1958. From 1983 to 1997, he served as Director of the Food and Nutrition Research Institute (FNRI), Philippines.

For more information on ILSI Southeast Asia Region, its activities
and a listing of its publications, contact:

ILSI SOUTHEAST ASIA REGION
Regional Office
One Newton Road, Goldhill Plaza, Podium Block #03-45, Singapore 308899
Tel: (65) 6352 5220 Fax: (65) 6352 5536 Email: ilsisea@singnet.com.sg

website: www.ilsi.org