Ministry of Health Malaysia

RNI

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
2017

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A Report of the Technical Working Group on Nutritional Guidelines

National Coordinating Committee on Food and Nutrition
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Ministry of Health Malaysia

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Foreword

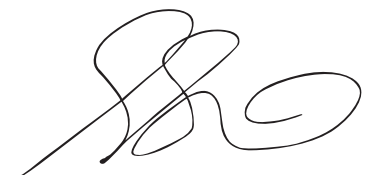
Minister of Health Malaysia

Malaysia still faces the double burden of malnutrition. The burden of NCDs continue to rise in Malaysia and unhealthy diet is one of the major risk factors for NCD. Malaysian nowadays are living in an obesogenic environment that leads to sedentary lifestyle and unhealthy eating habits.

The Ministry of Health Malaysia strives to assist Malaysians in achieving, sustaining and maintaining a certain level of health status and to facilitate them in leading a productive lifestyle. This can be better materialised by providing and strengthening the health promotion and preventive approaches. Prevention of diet-related diseases relies upon a comprehensive approach which includes a combination of targeted nutrition intervention programmes and a wide range of nutrition education for the public. Thus, the Recommended Nutrient Intakes (RNI) are crucial to these efforts as they form the basis in setting up or establishing nutrition requirement in any nutritional guidelines.

Thus, I would also like to commend the Technical Working Group (TWG) on Nutritional Guidelines established under the National Coordinating Committee on Food and Nutrition (NCCFN) for their diligent efforts in successfully updating and revising the RNI (2005) edition.

It is my fervent hope that all the relevant ministries and agencies, academia, health-related professional organisations and industries will use the RNI 2017 accordingly in their planning, monitoring and evaluation of nutrition programmes and policies.



YB Datuk Seri Dr. S Subramaniam
Minister of Health Malaysia

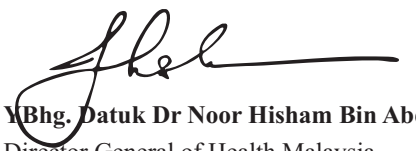
Foreword

Director General of Health Malaysia

One of the major challenges in evidence-informed policy making is the lack of timely and comprehensive data needed by the relevant policy makers. With limited resources at various levels, activities need to be prioritised that would bring the greatest impact on the quality of health and nutrition of the nation.

The need to assess energy and nutrient requirement is a never-ending task, given that Malaysia has experienced major transition in disease patterns due to rapid changes in lifestyle and social demography. In this regard, the revised edition of the RNI (2017) for Malaysia plays an important role in providing updated scientific knowledge and practices on the recommended nutrient intake for Malaysian. This RNI is developed through commendable efforts from experts of various ministries, universities, agencies, institutions and professional organisations.

I wish to congratulate the Technical Working Group on Nutritional Guidelines and the various sub-committees who have assisted in producing this document. I am confident that this revised RNI will be a valuable document for all of us.



YBhg. Datuk Dr Noor Hisham Bin Abdullah
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Foreword

Deputy Director General of Health (Public Health) Malaysia

Revising RNI is one of the main activities under the Facilitating Strategy in National Plan of Action for Nutrition of Malaysia (NPANM) III, 2016 – 2025, which is to provide standard nutrition guidelines for various target groups. Recommended Nutrient Intakes (RNI) are the level of intake of essential nutrients that, on the basis of scientific knowledge, are judged to meet the known nutrient needs of practically all healthy person. The revision of RNI 2005, will provide latest recommendation on dietary intake for the maintenance of good health. With close collaboration and valuable inputs by experts from various agencies and organizations in the country, the revision has been done based on the latest available evidence and finally agreed upon in a Consensus Meeting.

As Chairman of the National Coordinating Committee on Food and Nutrition (NCCFN), I would like to thank the Technical Working Group on Nutritional Guidelines and its three sub-committees for their great effort in updating and revising the RNI 2005.

It is my hope that this revised RNI 2017 would be greatly beneficial in helping to address malnutrition and other diet-related diseases by all the relevant stakeholders. It will also be a valuable source of reference for future nutrition guidelines.

YBhg Datuk Dr. Lokman Hakim Bin Sulaiman
Deputy Director General of Health (Public Health)
Ministry of Health Malaysia

Preface

The need for a guide to serve as a goal for good nutrition has long been well recognized. Recommended Nutrients Intakes (RNIs) are nutrient standards that may be used to plan and assess dietary nutrient intakes. The first edition of the Recommended Nutrient Intakes (RNI) Malaysia was published by the NCCFN (2005). The TWG Nutritional Guidelines under the auspices of the National Coordinating Committee on Food and Nutrition (NCCFN) proposed a revision of RNI (2005) in 2015. The Technical Working Group maintained the three Technical Sub Committees (TSC) of RNI 2005, namely, Energy and Macronutrients, Vitamins, and Minerals and Trace Elements, and its activities were presented to and endorsed by the NCCFN on 9 May 2016.

The Technical Working Group had agreed on the common approach towards deciding on the values to be adopted and the format of presenting the Malaysian RNI 2017. A total of 49 writers from the three TSCs were involved in the review exercise and the draft were presented in a Consensus Workshop on 10-11 January 2017 attended by 82 participants from 25 stakeholders, namely, MOH (5), Academia (10), Research Institutes (4), Professional Organizations (3), Federation of Malaysian Manufacturers (FMM), Federation of Malaysian Consumers Associations (FOMCA) and Prime Minister's department. This final document takes into consideration all comments and suggestions to improve each chapter and will now replace the RNI (2005) as the main reference for the Recommended Nutrient Intakes for Malaysia.

The following sections highlight the TSC recommendations for the four main chapters of the document presenting what is new and also the differences between RNI (2005) and the revised edition (RNI, 2017), the details of which could be found in the respective section.

Introduction

- The nutrient recommendations have taken into consideration relevant publications after 2005. The nomenclature and interpretation largely remain unchanged with the exception of Figure 1.3 adopted from FAO/WHO/UNU (2004) and Figure 1.4 IOM (2005).
- All age groups remain similar as RNI (2005) except for infant, which was divided into four quarterly groups.
- Reference heights for various age groups were derived from the National Health Morbidity Survey (IKU, 2015)
- Reference weight for children 0-9 years were based on weight-for-age median (WHO, 2006), adolescents 10-18 years BMI-for-age median (WHO, 2007), while for adults 19 years and above, was derived as mass equivalent to (desired) BMI 22.0 based on NHMS 2015 median height.
- RNI (2017) recommended 13 new nutrients, making a total of 30 nutrients as compared to the 17 nutrients in RNI (2005).

Energy and Macronutrients Recommendations

- The proposed recommendation for energy maintained the factorial method of the FAO/WHO/UNU (2004) report.
- Energy values were proposed for four levels of physical activity, namely low active (PAL 1.4), moderately active (PAL 1.6), active (PAL 1.8) and very active (PAL 2.0). Energy recommendation for individuals should be based on their physical activity level, but for population groups, the recommendation of PAL 1.6 can be adopted.
- Four recommendations of energy for infants were made for each 3-monthly age group as compared to RNI (2005).
- Energy recommendations are now proposed for the first trimester of pregnancy, and not just the second and third trimesters.
- The revision for protein was done based on recent consensus statements that suggested protein intake should be between 1.0 and 1.5 g/kg/day.
- Overall, the protein recommendations for infants and children are lower than the previous RNI 2005 due to a technical error in the FAO/WHO/UNU (1985) report.
- Protein recommendation for pregnancy was also revised based on a slightly higher total gestational weight gain and is available for the second and third trimesters.
- There are no specific recommendations for fats and carbohydrates but only as a percentage of contributions towards total daily energy intake (TEI).
- The TSC reviewed current evidence and recommends that macronutrient contribution towards TEI for Malaysian adults should be as follows: carbohydrates 50 - 65% TEI, fat 25 - 30% TEI, and protein 10 - 20%TEI.

Vitamins Recommendations

- Recognizing the continued relevance, all eight vitamins in RNI (2005) were reviewed and retained except for vitamin D, the TSC decided to retain the original values, i.e. adapting the values from WHO/FAO (2004).
- For vitamin D, the TSC decided to adapt the values from IOM (2011) in view of several recent reports on the unsatisfactory status of this vitamin among some population groups. These new values on vitamin D are generally 2-3 times higher than the 2005 values.
- For three of the four new vitamins, the TSC felt that the WHO/FAO (2004) values recommended for vitamin K, pyridoxine and pantothenic acid are appropriate to be adapted for use in RNI (2017).
- For vitamin B12, the TSC adapted the EFSA (2015) values derived mainly based on appropriate biomarkers. These values are higher for all age groups compared with the WHO/FAO (2004) and IOM 1998 values which were based on dietary intake levels. Although FAO/WHO did not have a revised RNI in recent years, the TSC decided that the RNI (2004) of these organizations remained as the main source of reference.
- Publications of the Institute of Medicine (IOM) and the European Food Safety Authority (EFSA) were also used in this update.

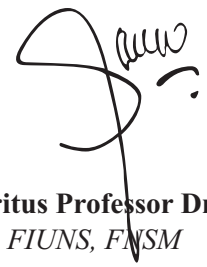
Minerals and trace elements Recommendations

- The Technical Sub-Committee (TSC) on Minerals and Trace Elements agreed to adopt the recommendations of FAO/WHO as a priority.
- However, for minerals and trace elements that the FAO/WHO did not have available guidelines, the recommendations of IOM were used instead.
- After reviewing recent dietary intake recommendations of FAO/WHO, IOM and ESFA, as well as those of countries in the region, the TSC made the decision to include besides calcium (RNI, 2005), four other minerals namely, phosphorus, sodium, potassium and magnesium for the (RNI, 2017)
- From the nine trace elements, four (iron, iodine, zinc and selenium, RNI 2005) were retained and five new elements, chromium, copper, manganese, molybdenum and fluoride were included using the most recent recommendations of IOM (various years), ESFA (2010), Australia (2006), Japan (2015) and the Philippines (2015).

Revision of RNI is an ongoing activity and nutrient recommendations will continue to evolve and be updated as new research findings using new methods of analysis are reported. These recommendations (RNI, 2017), part of an effort to update the original RNI (2005) are for healthy people and may not meet the specific nutritional requirements of individuals with various diseases or conditions, pre-term infants, or people with specific genetic profiles.

The TWG are confident that RNI (2017) would be the main source of reference to the Government to better plan, evaluate and monitor nutrition programmes, to assist public health nutritionists and other health related professionals in assessing the dietary requirements of individuals and groups and to help food legislators and the food industry for dietary modelling and/or food labelling and food formulation.

I wish to extend my personal gratitude to the members of the TWG for their continued support, the Chairmen and members of the various Technical Sub-Committees (TSCs) for their contribution in reviewing and updating the RNI (2005), the Consensus Workshop participants for their constructive comments and suggestions to further improve this document and the Secretariat (Nutrition Division, MOH) for their assistance to complete the task.



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Introduction to RNI 2017

A nutrient is a component in foods that an organism uses to survive and grow. Macronutrients provide the bulk energy an organism's metabolic system needs to function while micronutrients provide the necessary cofactors for metabolism to be carried out. Both types of nutrients can be acquired from the environment. Micronutrients are used to build and repair tissues and to regulate body processes while macronutrients are converted to, and used for energy. Nutrient recommendations differ with age, sex and physiological condition.

1.1 Evolution of Nutrient Recommendations

The first recommendation was reported by Dr. E Smith (1862) during the "Cotton Famine" to determine the least cost for which sufficient food could be purchased to prevent starvation and associated diseases in economically depressed and unemployed populations in the United Kingdom (UK). During the First World War (1914-1919), a recommendation was set for energy (Lusk) and to feed the army and nation in the UK. The British Medical Association was among the early authorities to formulate recommendations "to maintain health and working capacity" during the economic depression. In 1936, the Technical Commission of the Health Committee of the League of Nations (which became the United Nations in 1946 published its first nutrient-based dietary standards which sought to answer the following questions: What are the nutritional needs of human beings? How can they be recognized? How can it be determined that they are being satisfied? (TCHC-LON, 1936). In 1941, the US National Research Council proposed a recommendation with the aim of "building up of our people to a level of health and vigour never before attained or dreamed of."(NRC, 1941).

The first edition was published in 1943 to provide "standards to serve as a goal for good nutrition." Because RDA are intended to reflect the best scientific judgment on nutrient allowances for the maintenance of good health and to serve as the basis for evaluating the adequacy of diets of groups of people, the initial publication has been revised periodically to incorporate new scientific knowledge and interpretations. The RDAs were then replaced by Dietary Reference Intakes (DRIs) which refers to a set of 4 nutrient-based reference values Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI) and Tolerable Upper Intake Level (UL). The first set of DRI for calcium and related nutrients was published by IOM in 1997 followed by sets covering other nutrients (IOM, 1997, 1998, 2000, 2001 and 2002). Subsequently, IOM published DRI updates for water, potassium, sodium, chloride and sulphate (2004), for energy, carbohydrates, fibre, fat, fatty acids, cholesterol, protein and amino acids (2002/2005) and calcium and vitamin D (2011). By the 1940s, a number of countries including India (1944) and the Philippines (1941) had established their first national Recommended Daily Allowances.

Since 1949, FAO joined by WHO in the early 1950s and later by UNU in 1981 have convened groups of experts to evaluate scientific knowledge in order to define the energy requirements and proposed dietary energy recommendations for populations published by WHO (1985). The Commission of the European Communities Office in Luxemburg published the nutrient and energy intake for the European Communities (CEC, 1993). Subsequently, an expert consultation took place in Rome from 17-24 October 2001 with a mandate to revise and update the 1985 report, which was published by FAO/WHO/UNU in 2004.

The FAO/WHO/UNU have played major roles in consultative efforts to define human nutrient requirements. Arising from these consultations were several landmark reports including those that addressed the requirements for minerals (Appendix 1). The 2002 FAO/WHO Joint Report became a definitive reference resource on recommended intake of vitamins and minerals for countries worldwide. The recommendations included 13 vitamins and 6 minerals, the latter being calcium, iodine, iron, magnesium, selenium and zinc. The individual nutrients in the FAO/WHO 2002 Report made considerable reference to the Dietary Reference Intakes (DRIs) of the Institute of Medicine (IOM), Food and Nutrition Board, United States. The DRIs were and are still deemed as the most current scientific knowledge on nutrient needs of healthy populations built upon experimental evidence and extensive literature reviews. Appendix 2 shows the IOM reports that were cited in FAO/WHO (2002). Also, during the past decade, several relevant updates on dietary intake recommendations were published (Appendix 3). More recently, the European Food Safety Authority (EFSA) have published recommendation for energy (2013), folate (2014) and cobalamine (2015)

In Malaysia, a Technical Sub-Committee was formed in 1969 with representatives from the Institute for Medical Research and Public Health Institute of the Ministry of Health, WHO and University of Malaya "to provide advice on dietary aspects of the then proposed Applied Nutrition Pilot Project". This group prepared a new dietary standard based on various WHO recommendations published between 1964 and 1973 (Teoh, 1975), which was subsequently published as the Malaysian RDI in 1975. The task of reviewing and revising the 1975 RDA was assigned to the Technical Working Group (TWG) on Nutritional Guidelines established under the National Coordinating Committee on Food and Nutrition (NCCFN), Ministry of Health Malaysia. The first meeting of the Technical Committee was held on the 20th September 2002 and the revised dietary recommendation, RNI for Malaysia, was published in 2005. Advances in scientific knowledge in the last decade coupled with the deteriorating state of health of the nation prompted the Technical Working Group in a meeting held on 13 February 2015 to review and revise the RNI 2005.

1.2 Nomenclature and interpretations

A variety of terms are commonly used with reference to dietary recommendations, including terms such as recommended dietary allowances, recommended daily allowances, recommended daily amounts, recommended nutrient intakes, among others. In this review, the term Recommended Nutrient Intake (RNI) is maintained.

The Food and Nutrition Board that devised the RDAs in the USA defined Recommended Daily Allowance as "the level of intake of essential nutrients that, on the basis of scientific knowledge, are judged by the Food and Nutrition Board to be adequate to meet the known nutrient needs of practically all healthy persons" (NRC, 1980). Although new information is available as a result of the development of more precise techniques of determining human nutritional requirements, the definition has remained essentially unchanged since the 8th edition (NRC, 1974).

Estimates of energy requirements are derived from measurements of individuals. Measurements of a collection of individuals of the same sex and similar age, body size and physical activity are grouped together to give the average energy requirement - or recommended level of dietary intake - for a class of people or a population group. These requirements are then

used to predict the requirements and recommended levels of energy intake for other individuals with similar characteristics, but on whom measurements have not been made. Although individuals in a given class have been matched for characteristics that may affect requirements, such as sex, age, body size, body composition and lifestyle, there remain unknown factors that produce variations among individuals. Consequently, there is a distribution of requirements within the class or population group (WHO, 1985) (Figure 1.1)

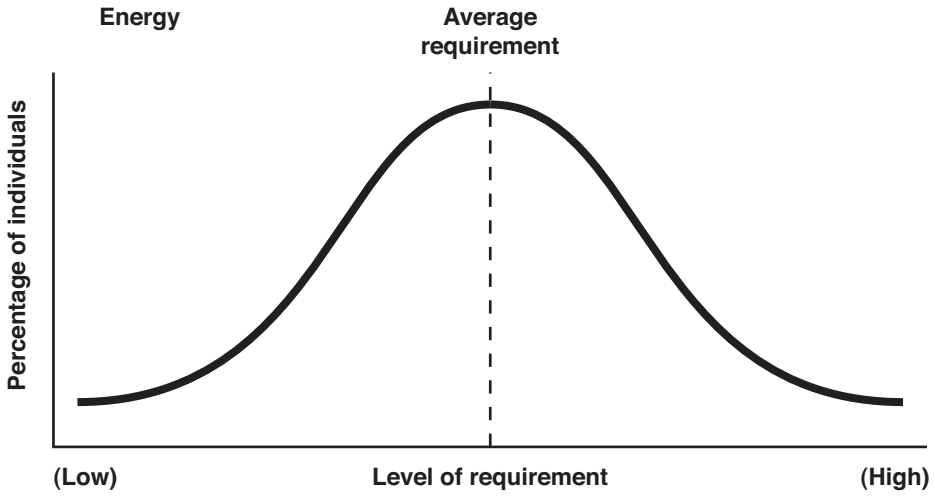

Figure 1.1 Distribution of energy requirements of a population group or class of individuals*

*It is assumed that individual requirements are randomly distributed about the mean requirements for the class of individuals, and that the distribution is Gaussian.
Source: WHO, 1985.

For most specific nutrients, a certain excess of intake will not be harmful. Thus, when dietary recommendations are calculated for these nutrients, the variation among individuals in a class or population group is taken into account, and the recommended level of intake is an amount that will meet or exceed the requirements of practically all individuals in the group. For example, the recommended safe level of intake for proteins is the average requirement of the population group, plus 2 standard deviations. This approach cannot be applied to dietary energy recommendations, because intakes that exceed requirements will produce a positive balance, which may lead to overweight and obesity in the long term. A high level of energy intake that assures a low probability of energy deficiency for most people (e.g. the average requirement plus 2 standard deviations) also implies a high probability of obesity for most people owing to a dietary energy excess (Figure 1.2). Therefore, in agreement with earlier reports, this expert consultation concluded that the descriptor of the dietary energy intake that could be safely recommended for a population group is the estimated average energy requirement of that group.

The dietary energy sources are mainly from food carbohydrates, fats and proteins. Hence, the proportion of macronutrients contributing towards energy intake may contribute risk of chronic disease. As such the Acceptable Macronutrient Distribution Ranges (AMDRs) are recommended macronutrient intakes that are associated with reduced risk of chronic disease. AMDRs apply for carbohydrates, proteins and fats; and are expressed in percent of calories from total daily energy (%TEI). WHO/FAO (2003) set population goals for macronutrient intakes as a percentage of energy intakes. Although the values are similar to those specified by US/Canadian adequate macronutrient distribution ranges (AMDRs), they are interpreted differently. The US/Canadian standards refer to adequate ranges for usual intakes of individuals, whereas the WHO standards refer to mean intake goals for populations or large groups.

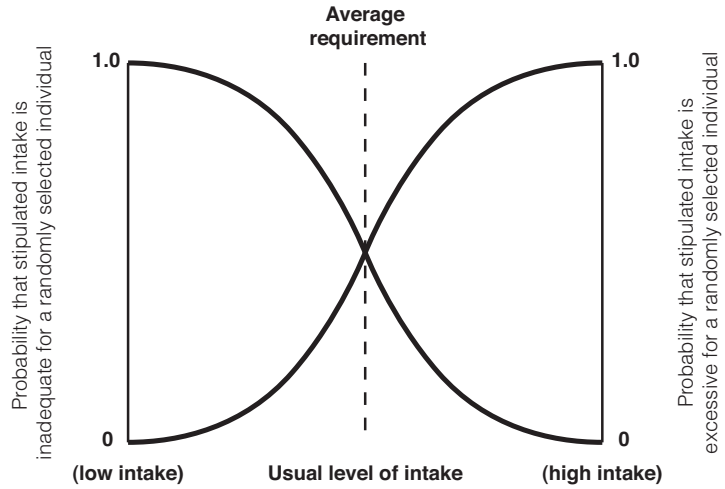

Figure 1.2 Probability that a particular energy intake is adequate or excessive for an individual*

* Individuals are randomly selected among a class of people or a population group. The two probability curves overlap, so the level of energy intake that assures a low probability of dietary energy deficiency is the same level that implies a high probability of obesity owing to dietary energy excess.
Source: WHO, 1985.

The dietary requirement for a micronutrient is defined as an intake level, which meets specified criteria for adequacy, thereby minimizing risk of nutrient deficit or excess. Functional assays are presently the most relevant indices of subclinical conditions related to vitamin and mineral intakes. The choice of criteria used to define requirements is of critical importance, since the recommended nutrient intake to meet the defined requirement will clearly vary, depending, among other factors, on the criterion used to define nutrient adequacy. Unfortunately, the information base to scientifically support the definition of nutritional needs across age ranges, sex and physiologic states is limited for many nutrients. Where relevant and possible, requirement estimates presented here include an allowance for variations in micronutrient bioavailability and utilization.

Recommended nutrient intake (RNI) is the daily intake, set at estimated average requirement (EAR) plus 2 standard deviations (SD), which meets the nutrient requirements of almost all apparently healthy individuals in an age- and sex-specific population group. If the distribution of requirement values is not known, a Gaussian or normal distribution can be assumed, and from this it is expected that the mean requirement plus 2 SD will cover the nutrient needs of 97.5% of the population. If the SD is not known, a value based on each nutrient's physiology can be used and in most cases a variation in the range of 10-12.5% can be assumed (exceptions are noted within relevant chapters). Because of the considerable daily variation in micronutrient intake, daily requirement refers to the average intake over a period of time. The cumulative risk function for deficiency and toxicity is defined in Figure 1.3, which illustrates that as nutrient intake increases the risk of deficit drops and at higher intakes the risk of toxicity increases. The definition of RNI used in this report is equivalent to that of the recommended dietary allowance (RDA) as used by the Food and Nutrition Board of the United States National Academy of Sciences (FNB, 2001).

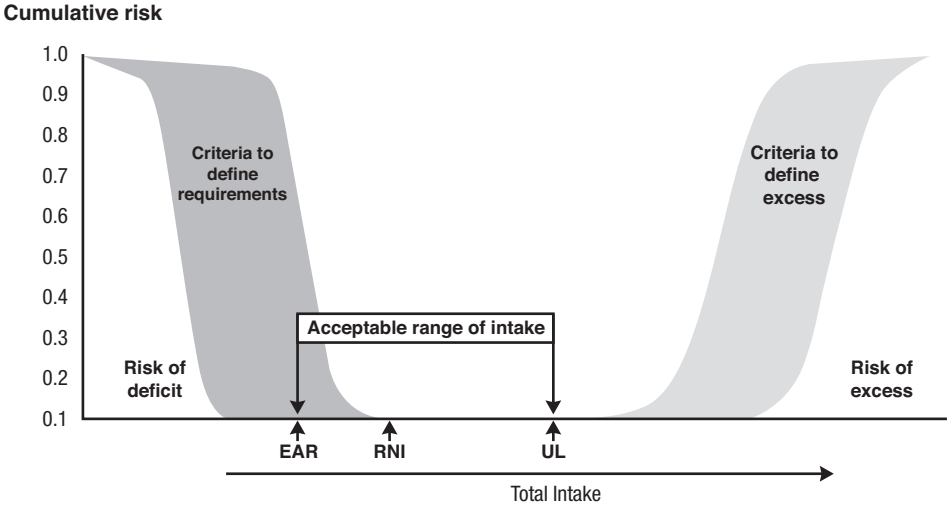

Figure 1.3 Risk function of deficiency and excess for individuals in a population related to food intake, assuming a Gaussian distribution of requirements to prevent deficit and avoid excess

The shaded ranges correspond to different approaches to defining requirements to prevent deficit and excess, respectively. The estimated average requirement (EAR) is the average daily intake required to prevent deficit in half of the population. The recommend nutrient intake (RNI) is the amount necessary to meet the needs of most (97.5%) of the population, set as the EAR plus 2 standard deviations. The tolerable upper intake level (UL) is the level at which no evidence of toxicity is demonstrable.
Source: WHO/FAO (2004)

To address the issues resulting from the application of a single value, the RDA, to a variety of uses and to incorporate the concept of reduction of risk to chronic degenerative diseases, the US Food and Nutrition Board (FNB), in collaboration with Health Canada (HC) and the Canadian National Institute of Nutrition (CNIN), developed four types of nutrient-based reference values, each for a particular use or uses, and collectively called "Dietary Reference Intakes" or DRIs (IOM, 2000). The DRIs refer to the complete set of reference intakes, including the RDA (recommended dietary allowance), AI (adequate intake), UL (tolerable upper intake level), and EAR (estimated average requirement). DRIs are expressed as intakes per day but are meant to represent average intakes of individuals over time. It is thought that the nutrient intake can vary substantially from day to day without ill effects. Each DRI expression (RDA, AI, UL, and EAR) has specific uses for planning and assessing diets or for applications to nutrition policy and education. The RNI 2017 for Malaysia adapted the concept of IOM but replaces the term 'RDA' with "RNI" (Figure 1.4).

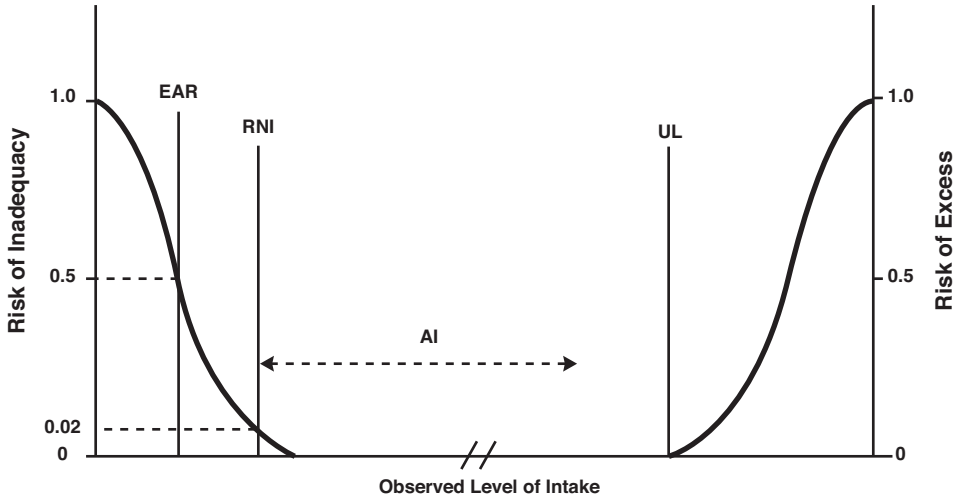

Figure 1.4 Conceptual framework for IOM/FNB's DRIs
Source: IOM (2005)

The estimated average requirement (EAR) is recommended by IOM (2005) for assessing diets for individuals and groups. For the individual, the EAR is used to examine the probability of inadequacy (or adequacy) of reported usual intake of the individual. Hence the probability of his/her intake is <0.5 when intake is below the EAR, as defined by the specified indicator of adequacy. For the group, the EAR is used to estimate the prevalence of inadequacy (or adequacy) of usual intake within a group.

The estimated average requirement (EAR) is the median intake value that is estimated to meet the requirement, as defined by specified indicator of adequacy, in half of the individuals in a life-stage or sex group. At this level of intake, the other half of a specified group will not have its nutritional needs met. The EAR is used to calculate the RDA.

The recommended dietary allowance (RDA) Recommended Nutrient Intake (RNI) is the average daily dietary intake level that is sufficient to meet the requirement of nearly all healthy individuals in a particular life stage and gender group. If the distribution of requirement of the

group is assumed to be normal, then the RDA RNI is the value computed as EAR + 2SD) to cover that exceeds requirement of almost all individuals in the population (97 to 98 percent) of the individuals in the group. The RDA is intended for use primarily as a goal for usual intake of individuals.

If sufficient scientific evidence is not available to establish an EAR and set a RDA, RNI an adequate intake (AI) is derived instead. The AI is based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group (or groups) of apparently healthy people who are maintaining a defined nutritional state or criterion of adequacy.

The tolerable upper intake level (UL) is the highest level of continuing daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals in the specified life stage group. As intake increases above the UL, the potential risk of diverse effect increases. The term tolerable intake was chosen to avoid implying a possible beneficial effect. The UL is not intended to be a recommended level of intake.

1.3 Age-categories and reference weight

In developing the recommendations, it was necessary to standardize body weights for the various age/gender groups. The age categories adopted for the RNI are as shown in Table 1.1. All age groups remain similar to the 2005 RNI except for infancy, which was divided into four quarterly groups. The reference heights for the various age categories were derived from the National Health and Morbidity Survey (NHMS) 2015 dataset (IPH, 2015). Reference weights for children aged 9 years and below were derived from weight-for-age medians based on the WHO 2006 growth standards and WHO 2007 growth reference. For adolescents aged 10 - 18 years, reference weight was derived as mass equivalent to the WHO 2007 BMI-for-age median based on the reference height, while in adults aged 19 years and above, reference weight was derived as mass equivalent to BMI 22.0 based on reference height. The reference weights and heights according to age groupings adopted for use in the proposed Malaysian RNI 2017 are as shown in Table 1.1.

Table 1.1 Reference body weights and heights for the Malaysian population

Age group[1]	Body weight (kg)[2]		Length/Height (cm)[3]	
	Males	Females	Males	Females
0 – 2.9 months	4.5	4.2	54.7	53.7
3 – 5.9 months	7.0	6.4	63.9	62.1
6 – 8.9 months	8.3	7.6	69.2	67.3
9 – 11.9 months	9.2	8.5	73.3	71.5
1 – 3 years	12.2	11.5	88.6	86.3
4 – 6 years	18.3	18.2	108.9	108.5
7 – 9 years	25.4	25.0	125.5	123.4
10 – 12 years	33.4	35.4	140.5	143.5
13 – 15 years	49.6	46.5	161.5	154.0
16 – 18 years	59.2	50.3	167.5	154.8
19 – 29 years	61.4	52.9	167.0	155.0
30 – 59 years	60.6	52.2	166.0	154.1
60 – 64 years	58.5	50.2	163.0	151.0
≥ 65 years	57.7	48.8	162.0	149.0

[1] For all age categories, the ending age extends till just before the beginning age of the subsequent category. For example, for the category 0-5 months, 5 months include up to 5.9 months.
[2] Children 0-9 years: WHO weight-for-age median; Adolescents 10-18 years: mass equivalent to WHO BMI-for-age median based on NHMS 2015 median heights; Adults: mass equivalent to BMI 22.0 based on NHMS 2015 median heights.
[3] NHMS 2015 database, median heights.

1.4 The nutrients reviewed

Having considered emerging evidence on the connections between diet and health and the recent recommendations from FAO/WHO/UNU, IOM, EFSA, the Technical Working Groups identified 13 new nutrients *(in italics)* making a total of 30 nutrients listed below, as compared to 17 nutrients (RNI, 2005).

Energy/Macronutrients	Vitamins	Minerals & Trace Elements
1. Energy	5. Thiamin (Vitamin B1)	17. Calcium
2. Protein	6. Riboflavin (Vitamin B2)	18. Iron
3. Fat	7. Niacin (Vitamin B3)	19. Iodine
4. Carbohydrate	*8. Pantothenic Acid (Vitamin B5)*	20. Zinc
	9. Pyridoxine (Vitamin B6)	21. Selenium
	10. Folate (Vitamin B9)	*22. Phosphorus*
	11. Cobalamin (Vitamin B12)	*23. Sodium*
	12. Ascorbic Acid (Vitamin C)	*24. Potassium*
	13. Vitamin A	*25. Magnesium*
	14. Vitamin D	*26. Chromium*
	15. Vitamin E	*27. Copper*
	16. Vitamin K	*28. Manganese*
		29. Molybdenum
		30. Fluoride

1.5 What is new in this Report

The Executive Summary have presented in detail this updated version (RNI, 2017) and among others;

- Reference body weights for adults are calculated based on desired BMI of 22.0 and average height of the Malaysian population
- Estimated energy requirements are based on physical activity levels (PALs)
- For energy requirements (infants) 4 age-categories, 0-2, 3-5, 6-8 and 9-11 months
- Nine (9) new minerals and 4 new vitamins are added to this report
- Age categories differs slightly for the minerals group when compared to the energy and macronutrient and vitamin groups.

1.6 Uses of RNI

The uses of recommended nutrient intakes (RNI) include the following:

Policy Maker
- Planning and monitoring of the national food supplies and stockpile of food for emergencies.
- As a basis for development of the National Dietary Guidelines.
- As a reference for development of relevant policies (e.g. Agriculture policy).

Program Planner
- Evaluation of dietary intakes for the population/ identified groups.
- Ensuring adequacy nutrients in food intervention programme.
- As reference in the development of education materials for nutrition promotion.
- Identifying risk of inadequate nutrient intakes for certain groups.

Food Industry and Marketing
- Reference for nutrition labelling of foods and supplements
- Guide for nutrient claims on food products
- Food product innovation and food fortification

Clinical Practitioner
- Assist in development of therapeutic diet manuals.
- Assessment of individual diets.
- Plan modified diets.
- Planning procurement of food supplies and menus for hospital/ institution.

Researcher
- As a main reference for dietary analysis for epidemiological studies.
- Evaluation of nutritional quality of foods by calculating the nutrient density index.

Recommended Nutrient Intake (RNI) and Chronic Disease

The RNI can also be used as a guide to reduce risk of chronic diseases. The replacement of nutrient-poor, energy-dense foods and drinks with generous amount of vegetables, fruits and wholegrain cereals can help prevent chronic diseases. The suggested target of nutrients intake in order to reduce risk of chronic diseases is equivalent to the 90th centile of population recommended nutrient intake. However, in order to achieve the target, it is recommended that the intake should be from natural food sources rather than commercially available dietary supplements.

In relation to the increasing prevalence of obesity and NCDs in the country, the Technical Working Group in Nutritional Guidelines has agreed to estimate the energy requirement for all groups using reference body weight calculated based on desired BMI of 22.0. The revised energy requirements are generally lower than RNI (2005) by about 8 - 18%, mainly for the adolescents and adults age groups.

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Appendix 3

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Summary

Energy and Macronutrients Recommendations

The Technical Sub-Committee (TSC) on Energy and Macronutrients reviewed the recommendations by referencing the latest available publications of the FAO/WHO/UNU (2004), Institute of Medicine (IOM, 2002) and the European Food Safety Authority (EFSA, 2013) reports.

The proposed recommendation for energy is still based on the factorial method of the FAO/WHO/UNU 2004 report; however, energy values were proposed for four levels of physical activity, namely low active (PAL 1.4), moderately active (PAL 1.6), active (PAL 1.8) and very active (PAL 2.0). Energy recommendation for individuals should be based on their actual physical activity level, but for population groups, the recommendation of PAL 1.6 can be adopted. Four recommendations of energy for infants were made for each 3-monthly age group as compared to RNI 2005 where only two recommendations were made for 6-monthly age groups. Energy recommendations are now available for the first trimester of pregnancy as well as the second and third trimester recommendations previously available in RNI 2005.

The revision for protein was done based on recent consensus statements that suggested protein intake between 1.0 and 1.5 g/kg/day could confers health benefits beyond those afforded by simply meeting the current requirement. Overall, the protein recommendations for infants and children are lower than the previous RNI 2005 due to a technical error in the FAO/WHO/UNU 1985 report that overestimated protein requirement considerably for this age group. Protein recommendation for pregnancy was also revised based on a slightly higher total gestational weight gain and is available for the second and third trimesters.

There are no specific recommendations for fats and carbohydrates but only as a percentage of contributions towards total daily energy intake (TEI). The TSC reviewed current evidence and recommends that macronutrient contribution towards TEI for Malaysian adults should be as follows: carbohydrates 50 – 65%TEI, fat 25 – 30%TEI, and protein 10 – 20%TEI. Of these, intake of free sugar should be 10%TEI or less, whereas intake of dietary fibre should be between 20 to 30 grams daily, for the prevention of diet-related chronic diseases. Recommendations for n-3 and n-6 polyunsaturated, monounsaturated, saturated as well as trans fatty acids are also provided based on %TEI.

1 • Energy

1.1 Introduction

An important goal in human nutrition is to ensure that the energy ingested in food is adequate to meet energy demands. The body needs energy for maintaining body temperature, metabolic activity, supporting growth and for physical work. It is also important, particularly in affluent societies, to minimize excess energy intake over expenditure in order to prevent obesity and its complications.

In addition, conceptually, the prevalence of food deprivation, which is termed undernourishment, is based on comparison of usual food consumption expressed in terms of dietary energy (kcal) with a minimum requirement level. The population group with food consumption below the minimum required level is considered undernourished. The focus on dietary energy in assessing food insufficiency or deprivation is justified from two 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, brings with it more protein and other nutrients as well, while raising intakes of the latter nutrients without ensuring a minimum level of dietary energy is unlikely to be of much benefit in terms of improving nutritional status.

The first important principle is that energy requirement must be estimated on the basis of energy expenditure and not of energy intake. It is based on the recognition that it is energy expenditure that drives energy needs rather than intake, which does not necessarily reflect energy needs and may vary independently.

A joint FAO/WHO/UNU Expert Consultation on Energy in Human Nutrition met in October 2001 to review the state of the art of the scientific literature since the 1985 report and to arrive at recommendations for energy requirement throughout the life cycle (FAO/WHO/UNU 2004). The report published in 2004 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 deposition of tissues during pregnancy and for secretion of milk during lactation consistent with good health of the mother and child.

The estimation of energy requirement is based on the factorial approach, which 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 for easier and more meaningful comparisons among diverse population groups. It is, 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 expenses, such as the costs of diverse types of physical activity, the extra energy allocated for pregnancy and lactation and the energy cost of growth.

1.2 Principles of energy balance and energy requirements

Principles of energy balance

The standard unit of energy is the joule and human energetics is usually expressed in terms of kilojoules (i.e. joules x 1000). A megajoule (MJ) is 1000 kJ. One kilocalorie or Calorie = 4.184 kJ. It is a fundamental principle of thermodynamics that energy cannot 'disappear'. Food energy eaten has to be either excreted in the faeces 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 fat or as 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, digest food and maintain body posture, and also provides energy for physical activity (James & Schofield, 1990).

Principles of energy requirements

Energy needs are determined by energy expenditure. Therefore, in principle, estimates of requirements should be based on measurements of energy expenditure. Components of energy expenditure include basal metabolic rate, physical activity, metabolic cost of food and metabolic cost of growth.

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 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 or can simply be defined as the minimal rate of energy expenditure compatible with life.

Since basal metabolic rate (BMR) is the largest component of energy expenditure, it has been adopted by the FAO/WHO/UNU Expert Committee 1981 as the basis for calculating all components of total energy expenditure. To obtain the total requirement, the estimate of BMR is multiplied by a factor 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).

The FAO/WHO/UNU (2004) report adopted the equations for predicting BMR from body weight derived by Schofield (1985), presented in Appendix 2.1. For the Malaysian population, Ismail *et al.,* (1998) had reported predictive equations for adults (Appendix 2.2) and Poh *et al.,* (1999; 2004) for adolescents aged 10 to 18 years old (Appendix 2.3 & 2.4). More recently, Henry (2005) produced the Oxford equations for predicting BMR (Appendix 2.5).

Physical activity

The level of physical activity must be considered in detail when assessing energy needs. Energy needs may be calculated based on the amount of time spent and the energy cost of various activities. To facilitate the calculations, daily activities are divided into two 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, which includes recreational activities. Apart from that, the routines of daily living (travel to and from places, household and other chores) are other domains of activities (Samitz, Egger and Zwahlen 2011). 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.

The FAO/WHO/UNU (2004) consultation endorsed the proposition that recommendations for dietary energy intake must be accompanied by recommendations for an appropriate level of habitual physical activity. The Malaysian Dietary Guidelines (2010) recommend that all individuals should be active every day and adults should be involved in moderate to vigorous physical activity at least 30 minutes per day for five to six days a week. Flexibility, strength and endurance activities should also be incorporated into our activities two to three times a week. Minimized inactivity and sedentary habits are important to lower risks associated with physical inactivity and sedentariness.

In proposing the Dietary Reference Values for Energy, European Food Safety Authority (EFSA) proposed adopting a physical activity level (PAL) of 1.4 to reflect low active (sedentary), PAL 1.6 moderately active, PAL 1.8 active and PAL 2.0 very active lifestyles (EFSA, 2013). A low active or sedentary lifestyle is defined as the type of day-to-day lifestyle with minimum amount of physical activity; for example, a person who performs almost all activities at work, transportation and leisure time in light activities.

Examples of lifestyles equivalent to the four levels of PAL are described in Table 1.1. The examples given are based on a 30-year-old man with body weight of 60.6 kg and BMI 22 kg/m^2. Appendix 1.6 provides some examples of activities based on metabolic equivalent (MET) values for sedentary or inactive (METs <3), moderately active (METs 3-6) and vigorous intensity activities (METs >6).

Table 1.1 : Examples of 24 hour activities equivalent to low active (sedentary), moderately active, active and very active lifestyles

	PAL 1.4 (Low active/ sedentary)	PAL 1.6 (Moderately active)	PAL 1.8 (Active)	PAL 2.0 (Very active)
Activities	**Time spent (hours)**			
Sleeping (MET=1.0)	8.0	8.0	8.0	8.0
Self care - showering, toweling off, standing, grooming, shaving, brushing teeth, putting on make-up (MET=2.0)	0.5	0.5	0.5	0.5
Lying down and watching television (MET=1.0)	1.0	0.5	0.5	2.5
Office work- sitting, writing, desk work, typing (MET=1.3)	8.0	8.0	8.0	0
Farming, driving harvester, cutting hay, irrigation work (MET=4.8)	0	0	0	4.0
Walking & commuting from/to the car, in the house/ office (MET=2.5)	1.0	1.0	1.0	1.0
Home/ household activities/ task-light effort (MET=2.8)	0.0	1.5	2.0	1.0
Sitting - home activities/ watching television, typing (MET=1.3)	5.5	4.0	3.0	7.0
Brisk walking (MET=4.3)	0	0.5	0	0
Jogging (MET=7.0)	0	0	1.0	0

MET = Metabolic equivalent values based on Ainsworth *et al.,* (2011)

Metabolic response to food

The increased oxygen uptake after a meal depends on the nutrient composition of food consumed and the amount of energy ingested. The measurement of energy cost of digesting, absorbing and storing ingested nutrients is not easy. It is difficult to separate the energy expended in excess of the basal rate after eating a meal, from the energy cost of physical activity involved in sitting, eating and digesting (FAO/WHO/UNU, 1985). Thus, it is often quantified at about 10% of total energy expenditure. These metabolic processes are also known by terms such as dietary-induced thermogenesis, specific dynamic action of food and thermic effect of feeding (FAO/WHO/UNU, 2004).

Growth

The energy cost of growth includes two components: the energy value of the tissue or product formed and the energy cost of synthesizing it. Growth is a component that is often included in total energy expenditure among children to account for the small increment of stored cell energy, which is about 2 kcal per gram of weight gain (FAO/WHO/UNU, 2004) required for growth and development (< 2% of total energy requirement) (Davies, 1992). Table 1.2 shows the protein and fat gains as well as the energy deposited in infants aged below 1 year old.

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. 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).

Table 1.2: Energy content of tissue deposition of infants[1] below 1 year old

Age interval (months)	Protein gain (g/day)	Fat mass gain (g/day)	Energy deposited in growing tissues (kJ/g)
Boys			
0-3 months	2.6	19.6	25.1
3-6 months	2.3	3.9	11.6
6-9 months	2.3	0.5	6.2
9-12 months	1.6	1.7	11.4
Girls			
0-3 months	2.2	19.7	26.2
3-6 months	1.9	5.8	15.6
6-9 months	2.0	0.8	7.4
9-12 months	1.8	1.1	9.8

Gross energy equivalents: 1g protein = 23.6kJ (5.65 kcal); 1g fat = 38.7kJ (9.25kcal)
Source: [1]Butte (2005)

Pregnancy and lactation

During pregnancy, extra energy is needed for the growth of the foetus, placenta and various maternal tissues, such as in the uterus, breasts and fat stores, as well as for changes in maternal metabolism and the increase in maternal effort at rest and during physical activity (FAO/WHO/UNU, 2004).

The energy cost of lactation has two components: 1) the energy content of human milk secreted and 2) the energy required to produce milk. Well-nourished lactating women can derive part of this additional requirement from body fat stores accumulated during pregnancy (FAO/WHO/UNU, 2004).

1.3 Energy deficiencies and excesses

Inadequate energy intake

By comparing the distribution of dietary energy supply with per caput energy requirements in different countries, two types of food inadequacy measures are provided, 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. whose access falls short of a specified cut-off point; while the estimates of intensity, is to assess by how far access to food falls short of requirement (FAO, 1996).

Energy deficiency can be acute or chronic. Acute energy deficiency is by nature "episodic", and 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 ultimately leading to death.

On the other hand, chronic energy deficiency (CED) is a "steady state", due to 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 environment, such as the menstrual cycle and seasons. A high incidence of LBW babies 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 behavioural consequences. From a population viewpoint, it is CED that is important to prevent and address.

Many factors impact significantly on energy intake and food availability. Environmental factors, such as water resources, arable lands and climate change; government policy, economy, as well as social culture may influence food intake (Turral, Burke and Faurès, 2011). Individual factors, such as sociodemographic characteristics, acculturation, knowledge and skills, may also be significantly associated with energy intake and food availability of individual (FNB, 2013).

Excess energy intake

Excessive energy intake and positive energy balance are conditioned by adequate availability of food energy and a sedentary lifestyle, accompanied by marketing strategies which stimulate over-consumption of highly palatable energy dense foods. 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, media or advertising, access to information and cultural beliefs, biological factors associated to a genetic predisposition and metabolic changes associated to diet and physical activity are the main conditioning factors linked to the rising prevalence of positive energy balance and excessive energy stores. Environmental factors, such as physical environment, including the workplace, school, housing area; safety, such as lighting, maintenance of sports facilities, and perception about criminal rate; as well as availability of and accessibility to facilities, such as gym, sports equipment or field, or the lack thereof, may also be associated to positive energy balance and excessive energy stores (King & Sallis, 2009).

The non-fatal but debilitating health problems associated with chronic energy excess and obesity include respiratory difficulties, chronic muscle-skeletal problems, skin problems and infertility. The more life-threatening, chronic health problems fall into four main areas: (a) condition associated with insulin resistance, namely diabetes mellitus type 2, (b) cardiovascular problems including hypertension, stroke and coronary heart disease, (c) certain types of cancers mainly the hormonal-related and large bowel cancers, and (d) gallbladder disease.

1.4 Dietary sources of energy

Energy for metabolic and physiological functions of humans is derived from the chemical energy bound in food carbohydrates, fats, proteins and alcohol, which act as substrates or fuels. 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 macronutrients in their natural forms are well established. The sources of energy is carbohydrates, fat and protein with physiological fuel values of 4, 9, 4 kcal/g (16.7, 37.7, 16.7 kJ/g), respectively. Ethanol has a caloric value of 7 kcal/g (29.3 kJ/g). The energy value of a food or diet is calculated by applying these factors to the amount of substrates determined by chemical analysis, or estimated from appropriate food composition tables (FAO/WHO/UNU, 2004).

The Joint WHO/FAO Expert Consultation on diet, nutrition and the prevention of chronic diseases (WHO, 2003) recommends that contribution of macronutrients to total daily energy intake (TEI) should be within these ranges: total carbohydrate 55 – 75%, total fat 15 – 30% and protein 10 – 15%. On the other hand, the IOM (2002) calculated an acceptable macronutrient distribution range (AMDR) for carbohydrate, fat and protein to be 45 – 65%, 20 – 35%, and 10 – 35% of energy, respectively. In 2005, the Technical Subcommittee on Energy and Macronutrients had adopted the WHO (2003) recommendation with slight modifications for the RNI Malaysia 2005, i.e. total carbohydrate 55 – 70%TEI, total fat 20 – 30 %TEI and protein 10 – 15%TEI. However, the TSC has reviewed more current evidence and recommends that contribution of macronutrients towards total daily energy intake of the Malaysian adult population should be as follows: carbohydrates 50 – 65%TEI, fat 25 – 30%TEI, and protein 10 – 20%TEI.

1.5 Factors affecting energy requirement

In view of the fact that energy requirement is determined from energy expenditure, it is therefore affected by the factors that affect basal metabolic rate and physical activity, which are the major components of energy expenditure. The FAO/WHO/UNU (1985) report has provided details of these factors as discussed below. Some other factors affecting basal metabolic rate are ethnicity, body composition, body size, the presence of disease, climate/temperature and altitude, dietary composition, pregnancy, menstrual cycle, emotion, hormone, nutritional status, drugs/medication and stimulants.

Age

The most important component of energy expenditure, the basal metabolic rate, depends on the mass of metabolically active tissue in the body, the proportion of each tissue in the body, and the contribution of each tissue to energy metabolism of the whole body. The changes in body composition with age, therefore, markedly affect energy requirements, since some organs of the body are much more metabolically active than others. Fat-free mass is known to be the most variable factor that influences basal metabolic rate, accounting for approximately 50 - 70% of variation within an individual. These differences in body composition in children and adults have to be taken into account when calculating the energy requirement of a particular section of the population. Basal metabolic rate declines by approximately 1 - 2% per decade after the third decade of life (>30 years old), and declines even when body weight remains stable. There are also altered activity patterns 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.

Sex

Men have a relatively greater muscle mass than women, which would tend 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 is made on boys and girls, their energy requirements are considered separately.

Individual variations

In any assessments 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 suggested that within individual variations in intakes are more important than between-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.

Population variations

The differences in BMR between populations of the world are equivocal. Some studies showed 8-10% lower in the tropics while others suggested no difference in BMR between Indians and Europeans provided the subjects were well nourished. Other evidence suggests 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 temperature changes.

1.6 Setting requirements and recommended intake of energy

The proposed recommended energy intake for Malaysia is calculated based on the factorial method suggested by FAO/WHO/UNU (2004). Although the basic principles set forth in the 1985 report have withstood the test of time, several modifications were proposed in the FAO/WHO/UNU, (2004) report. The IOM (2002/2005) report on Dietary Reference Intakes for Energy was also used as a reference by the Technical Sub-Committee (TSC) on Energy and Macronutrients.

The proposed recommendations also adopted EFSA's basis of using physical activity levels of 1.4, 1.6, 1.8 and 2.0 to reflect low active (sedentary), moderately active, active and very active lifestyles (EFSA, 2013); while BMR was calculated using the formulas shown in Table 1.3.

Table 1.3: BMR formulas used in calculating Total Energy Expenditure (TEE)

Age group	Males	Females	Reference
1 - 3 years	0.249 W – 0.127	0.244 W – 0.130	Schofield (1985)
3 - 9 years	0.095 W + 2.110	0.085 W + 2.033	Schofield (1985)
10 - 12 years	0.0558 W + 3.187[a]	0.05444 W + 2.781[b]	[a]Poh *et al.* (2004); [b]Poh *et al.* (1999)
13 - 18 years	0.0558 W + 3.187	0.0534 W + 2.182	Poh *et al.* (2004)
19 - 29 years	0.0550 W + 2.480	0.0535 W + 1.994	Ismail *et al.* (1998)
30 - 60 years	0.0432 W + 3.112	0.0539 W + 2.147	Ismail *et al.* (1998)
> 60 years	0.049 W + 2.459	0.038 W + 2.755	Schofield (1985)

BMR is expressed in MJ/day, W= body weight in kg.

Infants

Whitehead, Paul and Cole (1981) compiled energy intakes of infants from the literature between 1940 up to 1980. These data were later used by the FAO/WHO/UNU 1985 consultation to estimate energy requirement of infant set at 5% higher than observed intakes to compensate for underestimation of intake.

Since the 1980's, even though information on BMR of infants were available, to estimate requirements from multiples of BMR was not appropriate because reasonable allowance for physical activity were undefined. The FAO/WHO/UNU (1985) recommendations were 9-39% higher than those reported by Butte (1996). These discrepancies are not trivial and could lead to overfeeding of infants. The current recommendations therefore adopted the FAO/WHO/UNU (2004) principles.

The principle of calculating energy requirements from total energy expenditure (TEE) plus the energy needs for growth applies to infants and children of all ages. TEE had been shown to have good linear relationship with body weight (Butte *et al.,* 2000). The TEE predictive equation for breast-fed infants by Butte (2005) is as follows:

$$TEE\ (MJ/d) = -0.635 + 0.388\ W\ (kg)$$

This TEE formula is adopted for both breast-fed and formula-fed infants, as the equation for formula-fed infants is considered to be no longer appropriate due to recent significant changes in the composition of infant formula, whereby the protein to energy ratio is closer to human milk (EFSA, 2013).

Energy needs for growth comprises of two components; namely (i) the energy used to synthesize growing tissues, and (ii) the energy deposited in those tissues. Hence, energy requirements proposed for infant can be calculated by adding the energy deposited in growing tissues (as shown in Table 1.2) to TEE.

Energy requirement for infants

Boys	**0 - 2 months**	**470 kcal/ day**	or	1.97 MJ/day
	3 - 5 months	**540 kcal/ day**	or	2.28 MJ/day
	6 - 8 months	**630 kcal/ day**	or	2.65 MJ/day
	9 - 11 months	**720 kcal/ day**	or	3.02 MJ/day
Girls	**0 - 2 months**	**420 kcal/ day**	or	1.75 MJ/day
	3 - 5 months	**500 kcal/ day**	or	2.11 MJ/day
	6 - 8 months	**570 kcal/ day**	or	2.39 MJ/day
	9 - 11 months	**660 kcal/ day**	or	2.74 MJ/day

Children and adolescents

There was very little information available in 1981 on total energy expenditure (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 1-10 years old were derived from a review of published dietary intake data involving some 6,500 children, mostly from developed countries (Ferro-Luzzi & Durnin 1981). The FAO/WHO/UNU (1985) 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 is based on energy expenditure expressed as multiples of BMR rather than energy intake data (FAO/WHO/UNU, 2004). BMR for boys and girls of a given age and weight were predicted with the mathematical equations derived by Schofield (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).

According to the FAO/WHO/UNU (2004) method of estimating energy requirements for children and adolescents, energy needs of children and adolescents were also calculated from measurements of energy expenditure and the energy needs of growth. Torun (2001) analysed a large number of studies on TEE, growth and habitual activity pattern of children and adolescents in different parts of the world for the FAO/WHO/UNU expert consultation. Studies using either doubly-labelled water (DLW) or heart-rate monitoring (HRM) were included in the evaluation.

As mentioned earlier, the energy needs for growth comprises that used to synthesize growing tissues and energy deposited in those tissues. The energy spent in tissue synthesis is part of TEE measured with either DLW or HRM. Hence, only the energy deposited in growing tissues was added to TEE in order to calculate energy requirements (FAO/WHO/UNU, 2004).

The EFSA (2013) Scientific Opinion on Dietary Reference Values for Energy adopted the equation of Henry (2005) for estimation of resting energy expenditure (REE) and the following PAL values: 1.4 for the 1-3 years age group; 1.4, 1.6 and 1.8 for >3-<10 years; and 1.4, 1.6, 1.8 and 2.0 for 10-18 years. Energy expenditure for growth is accounted for by a 1% increase in PAL values for each age group.

The proposed recommendations adopted the FAO/WHO/UNU (2004) method for estimating energy requirements, and employed the PAL values suggested by EFSA (2013) in its calculations. For children aged 1-9 years, BMR was calculated based on Schofield (1985). For adolescents aged 10 - 18 years, the BMR values was calculated from Poh *et al.* (2004), and Poh *et al.* (1999) for girls aged 10 - 12 years. Body weights used for calculation of BMR was from WHO (2006; 2007) median weight-for-age for children up to 10 years, and weight equivalent to WHO (2007) median BMI-for-age calculated based on median height from the National Health and Morbidity Survey (NHMS) 2015 for adolescents aged 10 years and above. The energy intakes recommended by the TSC for each group are shown by age groups in each subtopic below and summarized in Table 1.4.

Table 1.4: Energy Requirements for Children and Adolescents in kcal/day (MJ/d)

	Boys				Girls			
	Low Active	Moderately Active	Active	Very Active	Low Active	Moderately Active	Active	Very Active
	PAL 1.4	PAL 1.6	PAL 1.8	PAL 2.0	PAL 1.4	PAL 1.6	PAL 1.8	PAL 2.0
Children								
1 - 3 years	980 (4.12)				900 (3.78)			
4 - 6 years	1300 (5.44)	1490 (6.22)	1670 (7.00)		1210 (5.06)	1380 (5.79)	1560 (6.51)	
7 - 9 years	1530 (6.40)	1750 (7.31)	1970 (8.22)		1410 (5.88)	1610 (6.72)	1810 (7.56)	
Adolescents								
10 - 12 years	1690 (7.08)	1930 (8.09)	2170 (9.10)	2420 (10.11)	1500 (6.26)	1710 (7.15)	1920 (8.05)	2140 (8.94)
13 - 15years	1930 (8.08)	2210 (9.24)	2480 (10.39)	2760 (11.55)	1580 (6.62)	1810 (7.57)	2040 (8.52)	2260 (9.46)
16 - 18 years	2050 (8.58)	2340 (9.81)	2640 (11.04)	2930 (12.26)	1660 (6.94)	1890 (7.93)	2130 (8.92)	2370 (9.91)

Note: For children aged 4 – 6 years, similar to those aged 1 – 3 years, PAL 1.4 is recommended for the general population. For children aged 7 years and above, PAL of 1.6 (i.e. moderately active) is recommended for the general population. For individuals, energy recommendation should be based on individual PAL.

Adults and elderly

The FAO/WHO/UNU Expert Consultation (1985) 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 total energy expenditure (TEE) is the BMR, which can be measured with accuracy under standardised conditions, the 1985 Report adopted in principle for the sake of simplicity, all components of TEE as multiples of BMR also known as PAL approach. 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.

The FAO/WHO/UNU (2004) report maintained the 1985 Expert Consultation's principle of using estimates of energy expenditure to estimate the energy requirements of adults. The use of techniques such as DLW and HRM confirmed the large discrepancy of TEE among adults and hence of energy requirements, that was previously reported by time-motion studies. Growth is no longer an energy-demanding factor in adulthood, and BMR is relatively constant among population groups of a given age and gender. Consequently, habitual physical activity and body weight are the main determinants for the diversity in energy requirements of adult populations with different lifestyles.

TEE was estimated though factorial estimation that combined the time allocated to habitual activities, and the energy cost of those activities. To account for differences in body size and composition, the energy cost of activities was calculated as a multiple of BMR per minute, or physical activity ratio (PAR), and the 24-hour requirement was expressed as a multiple of BMR per 24 hours, by using the physical activity level (PAL) value. Energy requirements are calculated by multiplying the PAL value by the energy equivalent of the corresponding BMR.

The EFSA, (2013) Scientific Opinion on Dietary Reference Values for Energy adopted the equation of Henry (2005) for estimation of resting energy expenditure (REE) of adults and the PAL values of 1.4 1.6, 1.8 and 2.0 to reflect low active (sedentary), moderately active, active and very active lifestyles, respectively. The REE was calculated based on individual body heights measured in nationally representative surveys in 13 EU countries, and corresponding individual body masses calculated to yield a BMI of 22 kg/m^2.

The TSC recommendation for energy requirements for adults and elderly are based on PAL values of 1.4, 1.6, 1.8 and 2.0 and the body weight equivalent to BMI 22.0 calculated based on NHMS 2015 median height. The BMR for adult Malaysians (19-59 years) is derived from local studies (Ismail *et al.,* 1998); while for the elderly ≥60 years, the Schofield (1985) equations were used (Table 1.5).

Table 1.5: Energy Requirements for Adults and Elderly in kcal/day (MJ/d)

	Low Active	Moderately Active	Active	Very Active
	PAL 1.4	PAL 1.6	PAL 1.8	PAL 2.0
Males				
19 - 29 years	1960 (8.20)	2240 (9.37)	2520 (10.54)	2800 (11.71)
30 - 59 years	1920 (8.02)	2190 (9.17)	2470 (10.31)	2740 (11.46)
≥ 60 years	1780 (7.43)	2030 (8.49)	2280 (9.55)	2540 (10.61)
Females				
19 - 29 years	1610 (6.75)	1840 (7.72)	2080 (8.68)	2310 (9.65)
30 - 59 years	1660 (6.94)	1900 (7.94)	2130 (8.93)	2370 (9.92)
≥ 60 years	1550 (6.49)	1770 (7.42)	1990 (8.34)	2220 (9.27)

Note: For adult and elderly age groups, PAL of 1.6 (i.e. moderately active) is recommended for the general population. For individuals, energy recommendation should be based on individual PAL.

The requirements for groups with different body weights and level of physical activity are shown in Appendix 1.1 - 1.4. It must however be emphasized that these values are intended to be general guidelines. It may be useful to make adjustments according to the characteristics of the population concerned.

Pregnancy

The FAO/WHO/UNU (1985) recommendations for pregnancy were based on a general acceptance that total energy needs of pregnancy were estimated at 335MJ (80,000 kcal) or about 1.2 MJ or 285 kcal/day. Most reports published after 1985 have recommended lower increments at 0.84 MJ/day or 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. The ideal situation is that women enter pregnancy with a healthy body weight (within the normal BMI range) and good nutritional 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, 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 as they are at risk of poor maternal and fetal outcomes (Han *et al.* 2011; McDonald *et al.* 2010).

Additional energy requirements for pregnancy arises from increases in maternal and feto-placental tissue mass, the rise in energy expenditure attributable to increased BMR and changes in the energy cost of physical activity. Gestational weight gain is the major determinant of

incremental energy needs during pregnancy (SACN, 2011). 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), with the assumption that pre-pregnancy BMI is within the healthy range. Presently, there are an increasing proportion of women entering pregnancy at a weight exceeding healthy range; and for those who are obese, gestational weight gain must be closely monitored and their additional energy requirement during pregnancy should be modified accordingly.

The proposed recommendation adopts FAO/WHO/UNU (2004) recommendations with slight adjustment, and is similar also to EFSA (2013) recommendations.

Additional energy requirements during pregnancy

1st trimester	**+ 80 kcal/ day**	or + 0.33 MJ/day
2nd trimester	**+ 280 kcal/ day**	or + 1.17 MJ/day
3rd trimester	**+ 470 kcal/ day**	or + 1.97 MJ/day

Lactation

The FAO/WHO/UNU (1985) recommendation 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 women 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. To operationalize 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 three months, and generally greater among women who practice exclusive breastfeeding, but the extent to which energy is 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 six months of life, the mean energy cost over the six month period is 2.8 MJ/day (675 kcal/day) calculated based on mean milk production of 807g milk/ day x energy density of milk of 2.8 kJ/day at 0.80 energetic efficiency of milk. Fat stores accumulated during pregnancy may cover part of the additional energy needed 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 = 21.8 MJ/month, or 0.72 MJ/day (170 kcal/day) from body energy stores (Butte & King 2005). This amount of energy can be deducted from the 2.8 MJ (675 kcal) per day needed during the first six months of lactation, thus reducing the additional energy requirement during lactation to 2.1 MJ/day (500 kcal/day). However, this will vary depending on the amount of fat deposited during pregnancy, as well as the lactation pattern and duration.

From the age of six month onwards, when infants are partially breast-fed and milk production is on average 550 g/day; hence, the energy cost imposed by lactation is 1.925MJ/day (460 kcal/day). Volumes of breast milk secreted during this stage are highly variable as they depend on the rates of milk production, which varies among women and populations (FAO/WHO/UNU, 2004) as well as the infant's energy intake from complementary foods (EFSA, 2013). Thus, recommendation on additional energy intake for women lactating beyond the first six months after birth is not proposed here. Energy intake required to support breastfeeding during the second six months will be modified by maternal body composition and the breast milk intake of the infant.

The proposed recommendation adopts the FAO/WHO/UNU (2004) recommendation, which is the same as EFSA (2013) recommendation.

Additional energy requirements during lactation

First 6 months **+ 500 kcal/ day** or + 2.09 MJ/day

Discussions on Revised Energy Requirements for Malaysia

The recommendations of the TSC on Energy and Macronutrients for energy requirements for Malaysians according to life stages are shown in Table 2.6. The requirements were derived based on the principles suggested in the FAO/WHO/UNU (2004) report using reliable measurements of total energy expenditure obtained from various age-groups as well as in special physiological status such as pregnancy and lactation. To derive requirements, the body weights were obtained from WHO references based on local median height data and the physical activity level (PAL) values adopted from the EFSA (2013) recommendation for energy. With the exception of adolescents and adults for which local data are available, all BMR values were adopted from the Schofield (1985) as per recommended by the FAO/WHO/UNU report.

The recommended energy requirements for Malaysia (2017) are compared to the previous energy recommendations for Malaysia (NCCFN 2005), as well as the reports of IOM (2002/2005) and FAO/WHO/UNU (2004) (Appendix 2.10). For infants, the revised energy requirements has been done for four 3-monthly age groups instead of two 6-monthly age groups; and as such were not directly comparable to the 2005 recommendations. For toddlers aged 1 - 3 years, the revised energy recommendations are very similar to RNI 2005.

For children and older age groups, the main difference with the 2005 energy recommendations is that the current recommendations are made for various levels of physical activity, namely low active (PAL 1.4), moderately active (PAL 1.6), active (PAL 1.8) and very active (PAL 2.0). For the purpose of comparison, moderate level of activity (PAL 1.6) was selected. The revised energy requirements are generally lower than RNI 2005 by about 8 - 18%, and this is especially obvious for the adolescents and adults age groups. In children and elderly, the differences are much smaller at between 1 - 10%.

For pregnancy and lactation, the current energy recommendations are made in line with the FAO/WHO/UNU (2004) report and EFSA (2013); which is very similar to the 2005 recommendations. However, the current recommendations also cover additional energy requirements for women in their first trimester of pregnancy, whereas the 2005 RNI did not have any energy recommendations for this group of women.

Several studies have revealed that most Malaysians maintained energy balance on a low intake while leading a sedentary lifestyle (Ismail *et al*., 2002; Poh *et al.,* 2010). The increasing trend in overweight and obesity in urban and rural areas is an indication that it is critical to revisit the 2005 energy recommendation that was made based on PAL 1.75. The current recommendations provide for people with various levels of physical activity, from low to very active.

Table 1.6: Recommendations for energy requirements by life stages and physical activity level

Age	Males					Females				
	Reference body weight[1] (kg)	Estimated Energy Requirements[2] kcal/day (MJ/d)				Reference body weight[1] (kg)	Estimated Energy Requirements[2] kcal/day (MJ/d)			
Infants										
0 - 2 months	4.5	470 (1.97)				4.2	420 (1.75)			
3 - 5 months	7.0	540 (2.28)				6.4	500 (2.11)			
6 - 8 months	8.3	630 (2.65)				7.6	570 (2.39)			
9 - 11 months	9.2	720 (3.02)				8.5	660 (2.74)			
		PAL 1.4	**PAL 1.6**	**PAL 1.8**	**PAL 2.0**		**PAL 1.4**	**PAL 1.6**	**PAL 1.8**	**PAL 2.0**
Children										
1 - 3 years	12.2	980 (4.12)				11.5	900 (3.78)			
4 - 6 years	18.3	1300 (5.44)	1490 (6.22)	1670 (7.00)		18.2	1210 (5.06)	1380 (5.79)	1560 (6.51)	
7 - 9 years	25.4	1530 (6.40)	1750 (7.31)	1970 (8.22)		25.0	1410 (5.88)	1610 (6.72)	1810 (7.56)	
Adolescents										
10 - 12 years	33.4	1690 (7.08)	1930 (8.09)	2170 (9.10)	2420 (10.11)	35.4	1500 (6.26)	1710 (7.15)	1920 (8.05)	2140 (8.94)
13 - 15years	49.6	1930 (8.08)	2210 (9.24)	2480 (10.39)	2760 (11.55)	46.5	1580 (6.62)	1810 (7.57)	2040 (8.52)	2260 (9.46)
16 - 18 years	59.2	2050 (8.58)	2340 (9.81)	2640 (11.04)	2930 (12.26)	50.3	1660 (6.94)	1890 (7.93)	2130 (8.92)	2370 (9.91)
Adults										
19 - 29 years	61.4	1960 (8.20)	2240 (9.37)	2520 (10.54)	2800 (11.71)	52.9	1610 (6.75)	1840 (7.72)	2080 (8.68)	2310 (9.65)
30 - 59 years	60.6	1920 (8.02)	2190 (9.17)	2470 (10.31)	2740 (11.46)	52.2	1660 (6.94)	1900 (7.94)	2130 (8.93)	2370 (9.92)
≥ 60 years	58.1	1780 (7.43)	2030 (8.49)	2280 (9.55)	2540 (10.61)	49.5	1550 (6.49)	1770 (7.42)	1990 (8.34)	2220 (9.27)
Pregnancy										
1st trimester							+80 (+0.33)			
2nd trimester							+280 (+1.17)			
3rd trimester							+470 (+1.97)			
Lactation										
1st six months							+ 500 (+2.09)			

[1] Children 0-9 years: WHO weight-for-age median; Adolescents 10-18 years: mass equivalent to WHO BMI-for-age median based on NHMS 2015 median heights; Adults: mass equivalent to BMI 22.0 based on NHMS 2015 median heights.

[2] For children aged 4 – 6 years, PAL 1.4 is recommended for the general population. For children above 7 years, adolescents and adults, PAL of 1.6 (i.e. moderately active) is recommended for the general population. For individuals, energy recommendation should be based on individual PAL.

1.7 Research recommendations

The following priority areas of research are recommended:

- Data on physical activity levels of different activities (duration, intensity and frequency) in all age groups.
- More basal metabolic rate measurements using strict criteria in order to generate predictive equations in all age groups, particularly in children under 10 years and in the elderly above 60 years of age.
- To determine whether ethnicity or habitation in a tropical environment influences BMR.
- Critical re-assessment of available data, particularly on the extent of intra- and inter-individual variability.
- Use doubly-labelled water method to validate other conventional techniques in estimating energy expenditure, particularly in infant, children and adolescents.
- There is a need to update and expand data bank on the energy cost of a range of activities undertaken in real-life conditions by children and adults.

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Appendix 1.1 Schofield equations for predicting basal metabolic rate from body weight[1]

Age range (years)	No.	kcal/ day	s.e.e.[a]	MJ/ day	s.e.e.[a]
Males					
0 - 3	162	59.512 W – 30.4	70	0.249 W – 0.127	0.292
3 - 10	338	22.706 W + 504.3	67	0.095 W + 2.110	0.280
10 - 18	734	17.686 W + 658.2	105	0.074 W + 2.754	0.411
18 - 30	2879	15.057 W + 692.2	153	0.063 W + 2.896	0.641
30 - 60	646	11.472 W + 873.1	167	0.048 W + 3.653	0.700
> 60	50	11.711W + 587.7	164	0.049 W + 2.459	0.686
Females					
0 - 3	137	58.317 W – 31.1	59	0.244 W – 0.130	0.246
3 - 10	413	20.315 W + 485.9	70	0.085 W + 2.033	0.292
10 - 18	575	13.384 W + 692.6	111	0.056 W + 2.898	0.466
18 - 30	829	14.818 W + 486.6	119	0.062 W + 2.036	0.497
30 - 60	372	8.126 W + 845.6	111	0.034 W + 3.538	0.465
> 60	38	9.082 W + 658.5	108	0.038 W + 2.755	0.451

W= body weight in kg
[1] FAO/WHO/UNU (2004); Schofield (1985)
[a] standard error of estimate

Appendix 1.2 BMR predictive equations for adult Malaysians[1]

Age group (years)	Formula	r	SE Mean
Male			
18 - 30	0.0550 W + 2.480	0.644	0.0363
30 - 60	0.0432 W + 3.112	0.501	0.0189
Female			
18 - 30	0.0535 W + 1.994	0.511	0.0263
30 - 60	0.0539 W + 2.147	0.519	0.0200

BMR is expressed in MJ/day, W= body weight in kg.
[1]Ismail *et al.,* (1998)

Appendix 1.3 BMR predictive equations for Malaysian adolescents aged 10 – 15 years[1]

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

BMR is expressed in kJ/day, W = body weight in kg
[1] Poh *et al.* (1999)
[a] standard error

Appendix 1.4 BMR predictive equations for Malaysian adolescents aged 12 – 18 years[1]

Groups	Regression equations	No. of data points	r^2	s.e.e.[a]
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

BMR is expressed in kJ/day, W = body weight in kg, S = sex: where 1 = female, 2 = male
[1] Poh *et al.* (2004)
[a] standard error of estimate

Appendix 1.5 Oxford equations for predicting BMR[1]

Age range (years)	MJ/ day	kcal/ day	SE[a]	n	r
Males					
0 - 3	0.255 W – 0.141	61.0 W – 33.7	0.255	277	0.954
3 - 10	0.0937 W + 2.15	23.3 W + 514	0.328	289	0.827
10 - 18	0.0769 W + 2.43	18.4 W + 581	0.566	863	0.861
18 - 30	0.0669 W + 2.28	16.0 W + 545	0.652	2821	0.760
30 - 60	0.0592 W + 2.48	14.2 W + 593	0.693	1010	0.742
> 60	0.0563 W + 2.15	13.5 W + 514	0.685	534	0.776
Females					
0 - 3	0.246 W – 0.0965	58.9 W + 23.1	0.242	215	0.960
3 - 10	0.0842 W + 2.12	20.1 W + 507	0.360	403	0.820
10 - 18	0.0465 W + 3.18	11.1 W + 761	0.525	1063	0.752
18 - 30	0.0546 W + 2.33	13.1 W + 558	0.564	1664	0.700
30 - 60	0.0407 W + 2.90	9.74 W + 694	0.581	1023	0.690
> 60	0.0424 W + 2.38	10.1 W + 569	0.485	334	0.786

W= body weight in kg
[1] Henry (2005)
[a] standard error

Appendix 1.6 Examples of various activities based on MET values

Activities	METs
Sleeping	1.0
Sitting quietly and watching television	1.3
Sitting at a desk	1.3
Standing quietly, standing in a line	1.3
Sitting, listening to music or watching a movie in a theatre	1.5
Sitting, in class-including note-taking or class discussion	1.8
Standing, reading	1.8
Fishing from boat or canoe - sitting	2.0
Upper body exercise- arm ergometer	2.8
Therapeutic exercise ball - Fitball exercise	2.8
Cooking, washing dishes, cleaning up - moderate effort	3.3
Walking, for pleasure, work break	3.5
Bicycling - leisure, 5.5 mph	3.5
Cleaning, sweeping - slow, moderate effort	3.8
Lawn and garden picking fruit off trees, gleaning fruits, picking fruits/ vegetables	4.5
Rock climbing, rappelling	5.0
Softball or baseball, fast or slow pitch, general	5.0

Activities	METs
Tennis, hitting balls, non-game play, moderate effort	5.0
Volleyball, competitive, in gymnasium	6.0
Resistance training (weight lifting, free weight, nautilus or universal), power lifting or body building, vigorous effort	6.0
Track and field (e.g., high jump, long jump)	6.0
Teaching exercise class (e.g., aerobic, water)	6.8
Dancing aerobic	7.3
Bicycling leisure, 9.4 mph	8
Running, 5 mph (12 min/ mile)	8.3

Source: Ainsworth *et al.,* (2011)

Appendix 1.7 Energy requirements of adults aged 19 – 29 years in populations with different body weight and four levels of habitual physical activity

Age	Low Active (PAL 1.40)		Moderately Active (PAL 1.60)		Active (PAL 1.80)		Very Active (PAL 2.00)		Height (m) for BMI values of [1]		
	kcal/ day	MJ/ day	kcal/ day	MJ/ day	kcal/ day	MJ/ day	kcal/ day	MJ/ day	24.9	21.0	18.5
Men (19 - 29 years)											
40 kg	1570	6.55	1790	7.49	2010	8.42	2240	9.36	1.27	1.38	1.47
45 kg	1660	6.94	1890	7.93	2130	8.92	2370	9.91	1.34	1.46	1.56
50 kg	1840	7.71	2110	8.81	2370	9.91	2630	11.01	1.42	1.54	1.64
55 kg	1840	7.71	2110	8.81	2370	9.91	2630	11.01	1.49	1.62	1.72
60 kg	1930	8.09	2210	9.25	2490	10.40	2760	11.56	1.55	1.69	1.80
65 kg	2030	8.48	2320	9.69	2600	10.90	2890	12.11	1.62	1.76	1.87
70 kg	2120	8.86	2420	10.13	2720	11.39	3030	12.66	1.68	1.83	1.95
75 kg	2210	9.25	2530	10.57	2840	11.89	3160	13.21	1.74	1.89	2.01
80 kg	2300	9.63	2630	11.01	2960	12.38	3290	13.76	1.79	1.95	2.08
85 kg	2390	10.02	2740	11.45	3080	12.88	3420	14.31	1.85	2.01	2.14
90 kg	2490	10.40	2840	11.89	3200	13.37	3550	14.86	1.90	2.07	2.21
Women (19 - 29 years)											
40 kg	1380	5.79	1580	6.61	1780	7.44	1980	8.27	1.27	1.38	1.47
45 kg	1470	6.16	1680	7.04	1890	7.92	2100	8.80	1.34	1.46	1.56
50 kg	1560	6.54	1790	7.47	2010	8.40	2230	9.34	1.42	1.54	1.64
55 kg	1650	6.91	1890	7.90	2120	8.89	2360	9.87	1.49	1.62	1.72
60 kg	1740	7.29	1990	8.33	2240	9.37	2490	10.41	1.55	1.69	1.80
65 kg	1830	7.66	2090	8.75	2350	9.85	2620	10.94	1.62	1.76	1.87
70 kg	1920	8.03	2190	9.18	2470	10.33	2740	11.48	1.68	1.83	1.95
75 kg	2010	8.41	2300	9.61	2580	10.81	2870	12.01	1.74	1.89	2.01
80 kg	2100	8.78	2400	10.04	2700	11.29	3000	12.55	1.79	1.95	2.08
85 kg	2190	9.16	2500	10.47	2810	11.77	3130	13.08	1.85	2.01	2.14
90 kg	2280	9.53	2600	10.89	2930	12.26	3250	13.62	1.90	2.07	2.21

[1] Height ranges are presented for each mean weight for ease of making dietary energy recommendations to maintain an adequate BMI based on a population's mean height and PAL. For example, the recommended mean energy intake for a male population of this age group with a mean height of 1.70m and a lifestyle with a mean PAL of 1.60, is around 9.25 MJ (2,210 kcal) per day, to maintain an optimum population median BMI of 21.0, with an individual range of about 8.81 – 10.13 MJ (2,110 – 2,420 kcal) per day, to maintain the individual BMI limits of 18.5 – 24.9.

Appendix 1.8 Energy requirements of adults aged 30 – 59 years in populations with different body weight and four levels of habitual physical activity

Body weight (kg)	Low Active (PAL 1.40)		Moderately Active (PAL 1.60)		Active (PAL 1.80)		Very Active (PAL 2.00)		Height (m) for BMI values of [1]		
	kcal/ day	MJ/ day	kcal/ day	MJ/ day	kcal/ day	MJ/ day	kcal/ day	MJ/ day	24.9	21.0	18.5
Men (30 - 59 years)											
40 kg	1620	6.78	1850	7.74	2080	8.71	2310	9.68	1.27	1.38	1.47
45 kg	1690	7.08	1930	8.09	2180	9.10	2420	10.11	1.34	1.46	1.56
50 kg	1760	7.38	2020	8.44	2270	9.49	2520	10.54	1.42	1.54	1.64
55 kg	1840	7.68	2100	8.78	2360	9.88	2620	10.98	1.49	1.62	1.72
60 kg	1910	7.99	2180	9.13	2450	10.27	2730	11.41	1.55	1.69	1.80
65 kg	1980	8.29	2260	9.47	2550	10.66	2830	11.84	1.62	1.76	1.87
70 kg	2050	8.59	2350	9.82	2640	11.04	2930	12.27	1.68	1.83	1.95
75 kg	2130	8.89	2430	10.16	2730	11.43	3040	12.70	1.74	1.89	2.01
80 kg	2200	9.20	2510	10.51	2830	11.82	3140	13.14	1.79	1.95	2.08
85 kg	2270	9.50	2590	10.85	2920	12.21	3240	13.57	1.85	2.01	2.14
90 kg	2340	9.80	2680	11.20	3010	12.60	3350	14.00	1.90	2.07	2.21
Women (30 - 59 years)											
40 kg	1440	6.02	1650	6.88	1850	7.75	2060	8.61	1.27	1.38	1.47
45 kg	1530	6.40	1750	7.32	1970	8.23	2190	9.15	1.34	1.46	1.56
50 kg	1620	6.78	1850	7.75	2080	8.72	2310	9.68	1.42	1.54	1.64
55 kg	1710	7.16	1950	8.18	2200	9.20	2440	10.22	1.49	1.62	1.72
60 kg	1800	7.53	2060	8.61	2310	9.69	2570	10.76	1.55	1.69	1.80
65 kg	1890	7.91	2160	9.04	2430	10.17	2700	11.30	1.62	1.76	1.87
70 kg	1980	8.29	2260	9.47	2550	10.66	2830	11.84	1.68	1.83	1.95
75 kg	2070	8.67	2370	9.90	2660	11.14	2960	12.38	1.74	1.89	2.01
80 kg	2130	8.93	2440	10.21	2740	11.48	3050	12.76	1.79	1.95	2.08
85 kg	2250	9.42	2570	10.77	2890	12.11	3220	13.46	1.85	2.01	2.14
90 kg	2340	9.80	2680	11.20	3010	12.60	3350	2340	1.90	2.07	2.21

[1] Height ranges are presented for each mean weight for ease of making dietary energy recommendations to maintain an adequate BMI based on a population's mean height and PAL. For example, the recommended mean energy intake for a male population of this age group with a mean height of 1.70m and a lifestyle with a mean PAL of 1.60, is around 9.13 MJ (2,180 kcal) per day, to maintain an optimum population median BMI of 21.0, with an individual range of about 8.78 – 9.82 MJ (2,100 – 2,350 kcal) per day, to maintain the individual BMI limits of 18.5 – 24.9.

Appendix 1.9 Energy requirements of elderly aged 60 years and above in populations with different body weight and four levels of habitual physical activity

Body weight (kg)	Low Active (PAL 1.40)		Moderately Active (PAL 1.60)		Active (PAL 1.80)		Very Active (PAL 2.00)		Height (m) for BMI values of [1]		
	kcal/ day	MJ/ day	kcal/ day	MJ/ day	kcal/ day	MJ/ day	kcal/ day	MJ/ day	24.9	21.0	18.5
Men (≥ 60 years)											
40 kg	1480	6.19	1690	7.07	1900	7.95	2110	8.84	1.27	1.38	1.47
45 kg	1560	6.53	1780	7.46	2010	8.40	2230	9.33	1.34	1.46	1.56
50 kg	1640	6.87	1880	7.85	2110	8.84	2350	9.82	1630	1.54	1.64
55 kg	1720	7.22	1970	8.25	2220	9.28	2460	10.31	1.49	1.62	1.72
60 kg	1810	7.56	2060	8.64	2320	9.72	2580	10.80	1.55	1.69	1.80
65 kg	1890	7.90	2160	9.03	2430	10.16	2700	11.29	1.62	1.76	1.87
70 kg	1970	8.24	2250	9.42	2530	10.60	2820	11.78	1.68	1.83	1.95
75 kg	2050	8.59	2350	9.81	2640	11.04	2930	12.27	1.74	1.89	2.01
80 kg	2130	8.93	2440	10.21	2740	11.48	3050	12.76	1.79	1.95	2.08
85 kg	2220	9.27	2530	10.60	2850	11.92	3170	13.25	1.85	2.01	2.14
90 kg	2300	9.62	2630	10.99	2960	12.36	3280	13.74	1.90	2.07	2.21
Women (≥ 60 years)											
40 kg	1430	5.99	1630	6.84	1840	7.70	2040	8.55	1.27	1.38	1.47
45 kg	1490	6.25	1710	7.14	1920	8.04	2130	8.93	1.34	1.46	1.56
50 kg	1560	6.52	1780	7.45	2000	8.38	2230	9.31	1.42	1.54	1.64
55 kg	1620	6.78	1850	7.75	2080	8.72	2320	9.69	1.49	1.62	1.72
60 kg	1680	7.05	1930	8.06	2170	9.06	2410	10.07	1.55	1.69	1.80
65 kg	1750	7.32	2000	8.36	2250	9.41	2500	10.45	1.62	1.76	1.87
70 kg	1810	7.58	2070	8.66	2330	9.75	2590	10.83	1.68	1.83	1.95
75 kg	1880	7.85	2140	8.97	2410	10.09	2680	11.21	1.74	1.89	2.01
80 kg	1940	8.11	2220	9.27	2490	10.43	2770	11.59	1.79	1.95	2.08
85 kg	2000	8.38	2290	9.58	2570	10.77	2860	11.97	1.85	2.01	2.14
90 kg	2070	8.65	2360	9.88	2660	11.12	2950	12.35	1.90	2.07	2.21

[1] Height ranges are presented for each mean weight for ease of making dietary energy recommendations to maintain an adequate BMI based on a population's mean height and PAL. For example, the recommended mean energy intake for a male population of this age group with a mean height of 1.70m and a lifestyle with a mean PAL of 1.60, is around 8.64 MJ (2,060 kcal) per day, to maintain an optimum population median BMI of 21.0, with an individual range of about 8.25 – 9.42 MJ (1,970 – 2,250 kcal) per day, to maintain the individual BMI limits of 18.5 – 24.9.

Appendix 1.10 Comparison of recommended Energy requirement: Malaysia (2017), Malaysia (2005), FAO/WHO/UNU (2004) and IOM (2002/2005)

Malaysia (2017)		Malaysia (2005)		FAO/WHO/UNU (2004)		IOM (2002/2005)	
Age group	**kcal/day**	**Age group**	**kcal/day**	**Age group**	**kcal/day**	**Age group**	**kcal/day**
Infants (boys)		**Infants (boys)**		**Infants (boys)**		**Infants (boys)**	
0 - 2 months	470	0 - 5 months	560	0 - 5 months	580	0 - 5 months	550
3 - 5 months	540	6 - 11 months	640	6 - 11 months	720	6 - 11 months	730
6 - 8 months	630						
9 - 11 months	720						
Infants (girls)		**Infants (girls)**		**Infants (girls)**		**Infants (girls)**	
0 - 2 months	420	0 - 5 months	550	0 - 5 months	540	0 - 5 months	500
3 - 5 months	500	6 - 11 months	630	6 - 11 months	660	6 - 11 months	660
6 - 8 months	570						
9 - 11 months	660						
Children (boys)		**Children (boys)**		**Children (boys)**		**Children (boys)**	
1 - 3 years	980	1 - 3 years	980	1 - 3 years	1110	1 - 2 years	1060
4 - 6 years	1300	4 - 6 years	1340	4 - 6 years	1470	3 - 8 years	1700
7 - 9 years	1750	7 - 9 years	1780	7 - 9 years	1830		
Children (girls)		**Children (girls)**		**Children (girls)**		**Children (girls)**	
1 - 3 years	900	1 - 3 years	910	1 - 3 years	1020	1 - 2 years	1000
4 - 6 years	1210	4 - 6 years	1290	4 - 6 years	1330	3 - 8 years	1600
7 - 9 years	1610	7 - 9 years	1590	7 - 9 years	1700		
Boys		**Boys**		**Boys**		**Boys**	
10 - 12 years	1930	10 - 12 years	2180	10 - 12 years	2350	9 - 13 years	2300
13 - 15 years	2210	13 - 15 years	2690	13 - 15 years	2980	14 - 18 years	3100
16 - 18 years	2340	16 - 18 years	2840	16 - 17 years	3370		
Girls		**Girls**		**Girls**		**Girls**	
10 - 12 years	1710	10 - 12 years	1990	10 - 12 years	2140	9 - 13 years	2080
13 - 15 years	1810	13 - 15 years	2180	13 - 15 years	2440	14 - 18 years	2350
16 - 18 years	1890	16 - 18 years	2050	16 - 17 years	2500		

Malaysia (2017)		Malaysia (2005)		FAO/WHO/UNU (2004)		IOM (2002/2005)	
Age group	**kcal/day**	**Age group**	**kcal/day**	**Age group**	**kcal/day**	**Age group**	**kcal/day**
Men		**Men**		**Men**		**Men[1]**	
19 - 29years	2240	19 - 29years	2440	18 - 29 years	2800	19 - 30 years	3050
30 - 59 years	2190	30 - 59 years	2460	30 - 59 years	2850	31 - 50 years	2880
≥ 60 years	2030	≥ 60 years	2010	≥ 60 years	1950	51 - 70 years	2630
						> 70 years	2110
Women		**Women**		**Women**		**Women[1]**	
19 - 29years	1840	19 - 29years	2000	18 - 29 years	2150	19 - 30 years	2450
30 - 59 years	1900	30 - 59 years	2180	30 - 59 years	2250	31 - 50 years	2330
≥ 60 years	1770	≥ 60 years	1780	≥ 60 years	1800	51 - 70 years	2130
						> 70 years	1680
Pregnancy		**Pregnancy**		**Pregnancy**		**Pregnancy**	
1st trimester	+ 80	1st trimester	+ 0	1st trimester	+ 0	1st trimester	
2nd trimester	+ 280	2nd trimester	+ 360	2nd trimester	+ 360	2nd trimester	+ 180
3rd trimester	+ 470	3rd trimester	+ 470	3rd trimester	+ 475	3rd trimester	+ 180
Lactation		**Lactation**		**Lactation**		**Lactation**	
1st 6 months	+ 500	1st 6 months	+ 500	Well-nourished	+ 505	1st 6 months	+ 500
				Undernourished (up to 6 months)	+ 675	2nd 6 months	+ 400

[1] Calculated based on reference weight and height of Malaysian men and women (page 8); and moderately active PAL for adults and low active PAL for elderly.

2 • Protein

2.1 Introduction

Protein is one of the major components of body tissues and an essential nutrient for growth. Aside from water, proteins form a major part of lean body tissues, constituting about 17% of the body weight. Amino acids are the building blocks for proteins, joined together by peptide bonds between the carboxyl and the amino group of the next amino acid in line (EFSA 2015). Protein in the diet and the body are associated with a number of vitamins and minerals and are more complex and variable than other energy sources such as fat and carbohydrate. Protein is available from a variety of foods and is ample in the Malaysian diet.

The body's fluids are contained within cells (intracellular) and outside the cells (extracellular). Extracellular fluids are found either in the spaces between cells (interstitial) or within blood vessels (intravascular). Wherever proteins are, they attract water and this helps to maintain the fluid balance in their various compartments.

There are two kinds of amino acids; essential and non-essential amino acids. Essential amino acids are defined as those that the body is unable to synthesize from simple molecules. They include histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Cysteine and tyrosine can partly replace methionine and phenylalanine, respectively. Under certain extreme physiological conditions, such as in prematurity or during some catabolic illnesses, the non-essential amino acids arginine, cysteine, glutamine, glycine, proline and tyrosine may be required in the diet. Under normal conditions, glutamine, glutamate or aspartate can supply arginine; methionine and serine can be converted to cysteine; glutamic acid and ammonia can be converted to glutamine; serine or choline can supply glycine; glutamate can provide proline; and phenyl-alanine can be converted to tyrosine. These amino acids are sometimes termed conditionally essential. Alanine, aspartic acid, asparagine, glutamic acid and serine are non-essential amino acids. Amino acids act as precursors for many coenzymes, hormones, nucleic acids and other molecules (Wu, 2016).

After ingestion, proteins are denatured by acid in the stomach and cleaved to smaller peptides. A number of gut enzymes including trypsin, chymotrypsin, elastase and carboxy-peptidase complete the process. The free amino acids and small peptides that result are absorbed into the mucosa by a specific carrier system. After intracellular hydrolysis of absorbed peptides, free amino acids are secreted to the portal blood where some of the amino acids are taken up and the remainder pass into systemic circulation for delivery to and to be used by peripheral tissues.

2.2 Functions

The primary roles for proteins in the body include being structural proteins, enzymes, hormones, transport proteins and immunoproteins. The maintenance of body tissues is essential because the body is constantly undergoing wear and tear, and proteins and amino acids provide continuous repairs. Proteins are important for the formation of regulatory compounds. Some hormones, all enzymes, and most other regulatory materials in the body are protein substances. Proteins defend the body against diseases. When the body detects invading antigens, it manufactures antibodies, which are large protein molecules designed specifically to combat them. The antibodies work so swiftly and efficiently that in normal, healthy

individuals, most diseases never have a chance to get started. In addition, proteins help maintain the balance between acids and bases within the body fluids by accepting and releasing hydrogen ions. Even though proteins are needed for growth, maintenance and repair, they will be used to provide glucose when the need arises (Stephenson & Schiff, 2016).

2.3 Metabolism

Protein metabolism comprises the processes that regulate protein digestion, amino acid metabolism and body protein turnover. These processes include the absorption and supply of both essential amino acids and non-essential amino acids, de novo synthesis of essential amino acids, protein hydrolysis, protein synthesis, and amino acid utilisation in catabolic pathways or as precursors for nitrogenous compounds (EFSA, 2015). The main pathway of amino acid metabolism is protein synthesis.

Digestion and Absorption

Digestion begins in the mouth with chewing, leading to food disruption and hydration/ solubilisation of the proteins. Once swallowed, the protein digestion process starts in the stomach through the action of the enzyme pepsin, which is in acidic medium, cleaves proteins into smaller peptides. The proteins then enter the small intestine and are further hydrolysed by pancreatic and intestinal enzymes. Lastly, non-digested proteins from dietary or endogenous origins reach the large intestine where they undergo important hydrolysis by microflora, which releases the amino acids, peptides and metabolites. Two major mechanisms are involved in the absorption of the luminal products of protein digestion: (1) transport of liberated free amino acids by group specific active amino acid transport systems, and (2) uptake of unhydrolysed peptides by mechanisms independent of the specific amino acid entry mechanisms (Silk, Grimble & Rees, 1985).

Storage and excretion of protein

Excessive amounts of amino acid in the body will be converted into ammonia, a highly toxic compound, through the deamination process, which mainly occurs in the liver. This is because there is no specific storage of amino acids, the metabolites of proteins, in the body, either in the muscle or other tissues.

In the liver, ammonia is converted into urea, a metabolic waste product, and released into the bloodstream. The kidneys pick up the waste products of amino acids, which include urea, a small amount of ammonia and creatinine. These products are eliminated from the body through urine excretion. The remaining carbon skeleton of amino acids after the deamination process is used for energy or converted to other compounds, such as glucose.

Nitrogen loss from the body is mainly through urine excretion. It can also be removed by the body through removal of nails, hair or dead skin. Besides, amino acids can be excreted in the faeces, at approximately 25% to 30% of total amino acid losses, or by metabolic oxidation, which is about 70% to 75% of total amino acid losses or through breastfeeding (EFSA, 2015).

To some extent, the liver or kidneys may be able to use the excess amino acids to produce glucose through the process of gluconeogenesis. The glucose product of gluconeogenesis will be synthesized into glycogen, a multi-branched polysaccharide of glucose that serves as a form of energy storage. Glycogen can be broken down into glucose through the process of glycogenolysis, and the resulting glucose molecules are then released into the bloodstream for other cells to use. However, some excessive amounts of amino acids, such as leucine and tryptophan, are in the ketogenic form, and cannot be converted directly into glucose. Ketogenic amino acids have carbon skeletons that can be converted into acetyl-coA. If the acetyl-coA molecules derived from ketogenic amino acids do not enter the citric acid cycle, they will be used for the fatty acid synthesis (Stephenson & Schiff, 2016).

Nutrient interactions with protein

Most of the proteins like enzymes, which are produced in the liver, remain in the liver. However, some of the proteins are released into the plasma. The proteins found in plasma include primarily glycoproteins, simple proteins and lipoproteins. Albumin is the most abundant of the plasma proteins. A healthy person normally produces approximately 9 to 12 g albumin daily. The main roles of plasma albumin are to maintain intravascular oncotic (colloid osmotic) pressure and facilitate transportation of substances. These substances include fatty acids, tryptophan, zinc, calcium, and copper, vitamins such as vitamin B6, lipid-soluble hormones and some drugs like warfarin, phenobutazone and clofibrate (Hankins, 2006).

Meanwhile, globulins, a family of globular proteins, act as protein transporters and antibodies for nutrient transport and blood clotting. Some of the globulins are produced in the liver, while some are produced by the immune system. Globulins exist in several classes, including α-1-globulins (such as glycoproteins and high-density lipoprotein), α-2-globulins (glycoproteins, haptoglobin, ceruloplasmin, prothrombin and very-low-density lipoproteins), β-globulins (transferrin and low-density lipoproteins), and γ-globulins (immunoglobulins or antibodies) (Gropper & Smith, 2013). Different classes of lipoproteins play important roles in transporting cholesterol and triglycerides in the blood stream, while haptoglobin is used for free hemoglobin transport, ceruloplasmin for copper transport and oxidase activity, and transferrin for iron and other mineral transport.

In addition, there are two other protein transporters synthesized by the liver and released into plasma namely, transthyretin (TTR, also called prealbumin) and retinol-binding protein (RBP). Transthyretin (TTR) is a transport protein in the serum and cerebrospinal fluid that carries the thyroid hormone thyroxine (T4). The RBP enables retinol to enter and leave the liver for several times per day in a process of retinol recycling. The RBP is useful for retinol circulation and protects cells from the damaging effects of free retinol or retinoic acid.

Some vitamins and minerals, such as vitamin B6, folate, and phosphorus are involved in amino acid metabolism. For instance, vitamin B6 (as part of pyridoxal phosphate, PLP), is involved in the conversion of the amino acid tryptophan to niacin and the transamination reactions that form nonessential amino acids, such as aspartate, glutamate and alanine. Glycine is formed from serine in the presence of two cofactors from vitamin B6 (as PLP) and folate (as part of tetrahydrofolate, THF) (Gropper & Smith, 2013).

The body is able to utilise amino acids to form nitrogen-containing compounds known as amino acid derivatives e.g. carnitine. Carnitine is important in transporting fatty acids, especially long-chain fatty acids, across the inner mitochondrial membrane for oxidation process.

2.4 Food Sources

Proteins in human diet are derived from two main sources, namely animal proteins (e.g. fish, poultry, meat, egg and milk) and plant proteins (e.g. pulses, cereals, nuts, beans and soy products). List of protein food sources by weight is shown in Table 2.1. Animal proteins are more "biologically complete" than vegetable proteins with regards to their amino acid composition. The term "complete proteins" refers to foods that contain all the essential amino acids needed by the body, whereas, incomplete proteins refers to foods lacking in one or more essential amino acids. However, an incomplete protein can be converted into a complete protein if two incomplete proteins are added together by employing what is called "complementarity of proteins". Two plant proteins, such as legumes and grains, or legumes and nuts/seeds, can be mixed to produce a complete protein from two incomplete ones.

Animal proteins

Meat, poultry and seafood are the main types of complete proteins with an almost similar amount of protein content among them. Milk is not only a valuable source of protein, but also a rich source of calcium and vitamins. A glass (250 ml) of fresh whole milk contains about 8.5 g of protein. In addition, non-fat dry milk or fortified skim milk contains equivalent amount of proteins and other nutrients to whole milk. Dairy products, such as cheese and ice cream, can provide generous amounts of protein in the diet. Breast milk is not only a complete protein, but also a complete food for infants up to 6 months of age. Egg is a complete protein with excellent quality; one egg will give 6 g of protein. The egg yolk contains protein, fat and cholesterol, while egg white contains mostly protein with no fat or cholesterol.

Plant proteins

Most proteins from plant sources are incomplete proteins and contain smaller amounts of proteins than animal sources. However, legumes are an exception: peas, lentils, beans, chickpeas, lima beans, soybeans and peanuts have large amounts of proteins in their seeds. Although proteins from legumes are not equal in quality with animal proteins, they can be an adequate substitute if they are eaten in combination with other foods.

Table 2.1: Protein contents of foods

	Foods	Protein (g/100g)
a.	**Legumes and seeds**	
	Chickpea, cooked	20.4
	Yellow dhal, cooked	19.2
	Soyabean cake, fermented	15.9
	Soyabean curd, Tau-kua	10.9
	Soyabean curd, Tau-hoo	7.2
	Soyabean milk, unsweetened	3.7
b.	**Meat and poultry**	
	Liver, Gizzard (chicken)	25.0
	Beef (lean) and beef burger patty	22.6
	Liver (ox)	21.0
	Goat (lean)	20.8
	Mutton (lean)	20.1
	Chicken frankfurter	18.5
	Chicken, breast	18.3
	Beef frankfurter	18.2
	Chicken burger patty	18.0
	Pork (lean)	16.5
	Lung (ox)	15.7
	Chicken, thigh	13.3
	Duck egg	12.9
	Duck, breast	11.4
	Hen egg	11.1
	Quail egg	10.3
	Chicken, wing	7.6
c.	**Fish and seafood**	
	Anchovy, dried, whole	50.0
	Travelly, yellow-banded	15.3
	Mackerel, Spanish	15.2
	Cuttlefish, fresh	14.5
	Fish balls	12.7
	Fish crackers, fried	12.4
	Scad, hairtail	12.1
	Prawn, pink	11.4
	Mackerel, Indian	11.3
	Sardine	10.6
	Bream, African	9.6
	Cockles, boiled	8.5

	Foods	Protein (g/100g)
d.	**Milk and milk products**	
	Milk, powder (Instant, full cream and skim	25.7
	Cheese, processed, cheddar	21.7
	Milk, sweetened condensed	8.4
	Milk, evaporated	7.7
	Milk, UHT, low fat, recombined (g/100 ml)	4.1
	Cow's milk, fresh (g/100 ml)	3.2
	Yogurt, apricot flavor	3.1

Source: Tee *et al.,* (1997).

2.5 Deficiencies and Excesses

Protein deficiency

Protein deficiency is usually accompanied by a deficiency of calories and other nutrients. The effects of protein loss during illness, injury and intense physical training can result in negative nitrogen balance, which can increase protein metabolism, and lead to muscle wasting, anemia and retarded recovery. A lowering of serum protein level and hormonal changes may result in oedema, and reduced production of antibodies leading to increased susceptible to infections.

Protein-deficient diets are in general nutrient-poor diets, deficient to varying degrees in a range of other nutrients, and also often associated with other environmental factors that can adversely influence health (WHO/FAO/UNU, 2007).

Malnutrition remains one of the most devastating problems faced by the majority of the poor and needy countries (WHO, 2000). The World Health Organization (WHO, 1993) defines malnutrition as "the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions." The term protein-energy malnutrition (PEM) applies to a group of related disorders that include marasmus, kwashiorkor and intermediate states of marasmus-kwashiorkor. Marasmus involves inadequate intake of protein, calories and other nutrients for a prolonged period whilst kwashiorkor refers to inadequate protein and energy intake with oedema. This condition is common among vulnerable young children.

The elderly is a vulnerable group undergoing physiological changes, and may be experiencing dental problems and difficulty in swallowing, which can lead to eating problems and malnutrition (Hickson, 2006). Protein intake ratio to total energy for the elderly is higher compared to adults, placing the elderly at risk of protein deficiency.

People who are most at risk of inadequate protein intake include those on strict vegan diets, with multiple food allergies, and those with limited e access to food.

Protein Excesses

The WHO/ FAO/ UNU (2007) Expert Consultation mentioned several potential adverse effects of overconsumption of proteins and amino acids. It is prudent for adults to avoid protein intakes in excess of more than twice the recommended amount. Such excessive intakes by physically active individuals consuming protein enriched diets and protein and amino acid supplements. Individuals who intend to lose substantial body weight may rely on a low carbohydrate and high protein diet, which can lead to excessive intake of protein. Although high protein diets have beneficial effects on satiety and weight control, there are some caveats, such as increased acid load to the kidneys or high fat content of animal proteins (Pesta & Samuel, 2014).

Diets containing high protein have been well documented to result in an increase in urinary calcium excretion, amounting to a 50% increase in urinary calcium for a doubling of protein intake (Lemann, 1999). This has two potential detrimental consequences; loss of bone calcium and increased risk of renal calcium stone formation. Although a high protein intake might increase the risk of kidney stones and bone resorption, as yet no clear conclusions can be drawn since dietary effects are apparent only in studies with very large differences in protein intakes (i.e. >185 g/day compared with 80 g/day) (WHO/FAO/UNU, 2007). However, acute adverse effects have been reported for protein intakes that exceed 45% of the total energy (IOM 2002/2005).

Current knowledge of the relationship between high protein intake and health is insufficient to enable clear recommendations about either optimal intakes for long-term health or to define a safe upper limit (WHO/FAO/UNU, 2007).

2.6 Factors affecting protein requirements

The availability of proteins from dietary sources is influenced by several factors summarised in the following paragraphs.

a) Protein contents of foods

The protein amounts reported in food composition tables are assessed by determining. Total nitrogen in the food, usually by the Kjeldhal method, whereby the result is multiplied by a specific factor to calculate the protein content of the food. As most proteins contain about 16% nitrogen, the total dietary nitrogen multiplied by 6.25 gives an estimate of "crude protein" content. The nitrogen content in protein differs in different categories of foods and the conversion factor to use is provided in FAO/WHO (1973).

b) Protein quality

Protein quality refers to how well or poorly a given protein can be absorbed from a diet and utilised by the body. Specifically, it refers to how well the essential amino acid profile of a protein satisfies their functions in the body, as well as the digestibility of the protein and bioavailability of the amino acids. The common methods of evaluating protein quality

include biological value, protein efficiency ratio, chemical score of protein, protein digestibility, protein digestibility corrected amino acid score (PDCASS) and digestible indispensable amino acid score (DIAAS).

i. Biological Value

Biological value (BV) of protein is a measure on how efficiently food protein, once absorbed from the gastrointestinal tract, can be turned into body tissues. Biological value can be calculated by dividing the amount of nitrogen retained for the body's use by the nitrogen absorbed from food (WHO, 2007). This product is multiplied by 100, expressed as a percentage of nitrogen utilized.

If a food possesses enough of all nine essential amino acids, it should allow a person to efficiently incorporate the food protein into body proteins. The biological value of a food depends on how closely its amino acid pattern reflects the amino acid pattern in the body tissues. For example, egg-white protein has a biological value of 100, the highest biological value of any single food protein. In other words, essentially all nitrogen that is absorbed from egg protein can be retained. Milk and meat proteins also have high biological values (Hoffman & Falvo, 2004).

If the amino acid pattern in a food is not similar with tissue amino acid patterns, many amino acids in the food will not become body protein but they simply become "leftovers" and excreted in the urine as urea. For instance, plant amino acid patterns differ greatly from those of humans. Corn has only a moderate biological value of 70, which is high enough to support body maintenance, but not for growth. Peanuts consumed as the only source of protein show a low biological value of 40.

ii. Protein Efficiency Ratio

Protein efficiency ratio (PER) is another means of measuring a food's protein quality. The PER of a food reflects its biological value, since both basically measure protein retention by body tissues. Plant proteins, because of their incomplete nature, generally yield lower PER values, whereas the values for animal proteins are higher, often above 2.0.

iii. Chemical Score of Protein

Chemical score estimates the protein quality of a food. The amount of each essential amino acid provided by a gram of the food protein is divided by an "ideal" amount for that amino acid per gram of food protein. The "ideal" protein pattern is based on the minimal amount (in milligrams) of each of the essential amino acids that is needed per gram of food protein. The lowest amino acid ratio calculated for any essential amino acid is the chemical score. Scores vary from 0 to 1.0.

iv. Protein Digestibility

The degree to which a protein is digested influences its nutritional value. Animal proteins are digested more efficiently than plant proteins (Hoffman & Falvo, 2004). This is because digestive enzymes have greater difficulty entering plant cells, which are surrounded by cellulose and woody substances. The method of cooking also affects digestibility. Heat alters the structure but not the amino acid content of protein molecules. Over-heating, however, may destroy some amino acids or may cause the formation of products resistant to digestive enzymes. Cooking with water improves the digestibility of wheat and rice proteins.

The digestibility of protein is normally expressed in relation to that of egg, milk, meat or fish, which are used as reference proteins (digestibility = 100) (WHO, 2007). Differences in digestibility result from intrinsic differences in the nature of food protein and the nature of the cell wall, from the presence of other dietary factors that modify digestion (e.g. dietary fiber, polyphenols such as tannins and enzyme inhibitors) and from chemical reactions (e.g. binding of the amino groups of lysine and cross linkages), which may affect the release of amino acids by enzymatic processes (FAO, 2013). There are few data on digestibility of specific amino acids in food proteins.

v. Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The most widely used measure of protein quality is the Protein Digestibility Corrected Amino Acid Score (PDCAAS). This is used in place of Protein Efficiency Ratio (PER) evaluations for foods intended for children over 1 year of age and for non-pregnant adults. To calculate the PDCAAS of a protein, its chemical score is determined. For example wheat has a chemical score of 0.47. The score is then multiplied by the digestibility of the protein (generally, 0.9 to 1.0), in turn yielding the PDCAAS. The maximum PDCAAS value is 1.0, which is the value of milk, eggs, and soy protein. A protein totally lacking any of the nine essential amino acids has a PDCAAS of 0, since its chemical score is 0 (FAO, 2013).

vi. Digestible Indispensable Amino Acid Score (DIAAS)

The use of a single value of crude protein digestibility to correct the amount of each individual dietary indispensable amino acid for its digestibility is considered to be a shortcoming as there are quantitative differences in digestibility between crude protein and individual dietary indispensable and dispensable amino acids. In addition, the PDCAAS approach is based on an estimate of crude protein digestibility, which is determined over the total digestive tract (i.e. faecal digestibility) in the correction for digestibility. This may lead to overestimation of the amount of amino acids absorbed. Due to these limitations, FAO has recommended a revised protein quality measure, the Digestible Indispensable Amino Acid Score (DIAAS) to replace PDCAAS (FAO, 2013).

DIAAS determines amino acid digestibility at the end of the small intestine, which provides a more precise estimate of the amounts of amino acids absorbed by the body and the contribution of protein to human amino acid and nitrogen requirements. DIAAS can be calculated as:

DIAAS % = 100 x [(mg of digestible dietary indispensable amino acid in 1 g of the dietary protein) / (mg of the same dietary indispensable amino acid in 1g of the reference protein)].

DIAAS can be used to estimate available protein intake when evaluating the protein quality in mixed dishes or in sole source foods (e.g., infant formulas) and to adjust dietary protein intakes to meet requirements. DIAAS can be used to define protein equivalent intake (protein adequacy), when it is multiplied by the actual protein content or intake (i.e. measured protein intake times DIAAS) (FAO, 2013).

The DIAAS is also used to determine the quality of a single ingredient or individual food for the consideration of complementing other protein foods (FAO, 2013). A DIAAS more than 100 demonstrates potential to complement protein of lower quality in order to maintain a suitable total N intake.

c) Biological factors

Age

Protein requirement depends on age due its demand for growth and ageing Protein requirements are the highest after birth because muscles and tissues grow at a rapid pace. Protein needs during adolescence are influenced by the amount of protein required for maintenance of existing lean body mass and to accrue additional body mass during the growth spurt. Therefore, requirements based on developmental age are more accurate in estimating protein requirement as compared to chronological age. Insufficient protein intake will result in delayed or stunted increases in height and weight as well as weight loss and lean body mass loss that can subsequently alters body composition (Stephenson & Schiff, 2016).

The protein needs of older adults are higher than that of adults due to the ageing process. Protein synthesis and whole body proteolysis in response to an anabolic stimulus is low as compared to younger adults. The greater protein requirement is thought to be related to the enhanced protein synthesis necessary to assist in the repair and remodeling process of damaged skeletal muscle fibers (Hoffman *et al.,* 2006). Therefore, incorporating a small increase in protein intake is also helpful to ensure nitrogen balance in older adults.

Sex

For infants and children, the protein requirements for both males and females are similar due to similarity of growth and development rates. In adolescence, pubertal development incurs differences in protein requirements between adolescent boys and girls. A greater muscle mass in males places a higher requirement for protein, compared to females.

Physiological state

Physiological state such as infections, worm infestations, injury, emotional disturbances and stress may affect an individual's protein requirement. A negative nitrogen balance after injury tends to be higher in muscular well-nourished individuals than in malnourished individuals (Kurpad, 2006). Injuries or infections lead to an increased nitrogen loss from the body that subsequently increases the risk of malnutrition. Severe critical conditions such as sepsis and trauma can result in significant protein loss. Individuals suffering from protein loss should increase their protein intake, particularly during the recovery phase. However, the body may react slowly to increased protein intake due to increased insulin resistance, thus limiting the usefulness of an enhanced protein intake (Simsek, Simsek & Cantürk, 2014).

Pregnancy

Additional protein is required during pregnancy to provide support for the synthesis of maternal and fetal tissues. Maternal protein requirement increases from early gestation period and reaches its maximum level during the third trimester.

As for adolescent pregnancy, as the adolescent herself is undergoing rapid growth and development, she will have a higher protein needs compared to a pregnant adult. Pre-pregnancy weight and weight gain during pregnancy are correlated with birth weight of the infant. The WHO/FAO/UNU (2007) Expert Consultation reported that, an average pre-pregnancy weight of a pregnant adolescent is about 55 kg, and estimated that an average weight gain throughout adolescent pregnancy is 12.5 kg. Therefore, the requirement for protein intake is 1.5 g/kg pregnant weight/day.

Lactation

Mean production rates of milk produced by well-nourished women exclusively breastfeeding their infants during the first 6 months postpartum and partially breastfeeding in the second 6 months postpartum were used together with the mean concentrations of protein and non-protein nitrogen in human milk to calculate mean equivalent milk protein output (WHO/FAO/UNU, 2007).

d) Other considerations

Vegetarians

Vegetarianism is increasingly popular in Malaysia. This dietary practice, which focuses on plant-based food sources may affect the quality and quantity of protein consumed by vegetarians. For instance, ingestion of soy protein was found to result in lower postprandial muscle protein synthesis rates both at rest and during recovery from exercise , compared to ingestion of beef, whey, or milk, (Tang *et al.* 2009; van Vliet, Burd & van Loon, 2015; Wilkinson et al. 2007). Diets that are solely based on cereals, root crops, vegetables, and legumes may not provide adequate amounts of indispensable amino acids, especially for children undergoing development stage. IOM (2005) concluded that available evidence does not support recommending a separate protein requirement for vegetarians, who consume a complementary mixtures of plant proteins.

Athletes

The rationale for a higher protein requirement for athletes is to repair and replace damaged proteins, remodel protein within muscle, bone, tendon, and ligaments; maintain optimal functions of all metabolic pathways that use amino acids; support increments of lean mass; support an optimal functioning immune system; support the optimal rate of production of plasma proteins and support other acid amino requiring processes functioning at rates higher than non-athletes (IOM, 2002/2005). Based on Institute of Medicine (IOM, 2002/2005), the proportion of protein as a percentage of total energy that is considered sufficient for endurance athlete is 10-20% and 20-40% for strength athletes. In order to optimize the ratio of fat-to-lean tissue mass loss during hypo-energetic periods, athletes are advised to ensure that they increase their protein intake to 20–30% of their energy intake or 1.8–2.7 g/kg/day (Phillips & van Loon 2011). Athletes are advised to consume protein food immediately after resistance exercise, particularly high-quality milk protein, to maximize exercise-induced increases in muscle mass.

IOM (2005) concluded that no additional dietary protein is suggested for healthy adults undertaking resistance or endurance exercise.

Twin Pregnancy

In the WHO/FAO/UNU (2007) Expert Consultation report, women with twin pregnancy have higher protein needs than women having singleton births. Results from nutritional intervention by Montreal Diet Dispensary shows that an additional 50 g of protein daily can improve twin pregnancy outcomes, whereby low birth weight rate are decreased by 25% and very low birth weight by 50%, and preterm delivery reduced by 30%. An additional 50 g daily is needed from the 20th week of pregnancy, which is double the pregnancy allowance for women with singleton pregnancies.

2.7 Setting requirements and recommended intake of protein

The RNI 2005 (NCCFN, 2005) had recommended 10-15% for protein contribution to total energy intake (TEI) based on WHO (2003). This TEI was also aligned with the finding of the Malaysian Adults Nutrition Survey (MANS, 2003), which reported the median protein intake of Malaysian adults of 55.3g/day, which amounted to 14.3% of the TEI.

For the 2017 RNI, the upper limit of protein contribution to TEI has been set higher at 20% TEI, as compared to the 15% TEI in the 2005 RNI. Protein intake of Malaysian is higher than in 2003, as reported in MANS (2014), which recorded protein intake of 56.7g/day contributing to 16% of TEI. The increased upper limit of protein intake is in line with the recommendations of other countries namely, the Nutrient Reference Values for Australia and New Zealand (2005) 15-25%TEI, IOM (2006) 10-35%TEI, and Japan DRI (2015) 13-20%TEI.

According to the members of the Consultation of WHO/FAO/UNU (2007), the FAO/WHO/UNU (1985) had over-estimated the protein requirement for infants, children and adolescent. Hence, the protein recommendation for these age groups in the 2017 RNI is lower compared to the 2005 RNI. According to WHO/FAO/UNU (2007), calculation of protein requirements, except for pregnant and lactating women, should be made in two steps: first, the requirement per kg should be obtained according to the age range; and second, this should be multiplied either by the actual weight or by the median weight for age to obtain the total requirement.

The WHO/FAO/UNU (2007) has been used as a reference in setting requirements for protein intake for infants, children and adolescents (Table 2.2). A safe level was calculated as average plus 2SD, assuming a coefficient of variation derived from the coefficients of variation for growth and maintenance, which fell from 16% at 6 months to 12% at 2 years of age. If actual weights are not available, the median weight at the actual age from the WHO weight-for-age growth charts is recommended (WHO 1994).

The main references used by the Technical Sub-Committee (TSC) on Energy and Macronutrients in making recommendations for protein intake for the revised RNI are based on the report of DRI committee of Institute of Medicine IOM (2002/2005), the Report of a joint WHO/FAO/UNU (2007) Expert Consultation and the Scientific report of European Food Safety Authority (EFSA, 2012).

Table 2.2 Safe level of protein intake for infants, children and adolescent boys and girls

Age (years)	Boys			Girls		
	Weight (kg)	Safe level of protein intake (g/kg/day)	Safe level of protein intake (g/day)	Weight (kg)	Safe level of protein intake (g/kg/day)	Safe level of protein intake (g/day)
0.5	7.8	1.31	10.2	7.2	1.31	9.4
1	10.2	1.14	11.6	9.5	1.14	10.8
1.5	11.5	1.03	11.8	10.8	1.03	11.1
2	12.3	0.97	11.9	11.8	0.97	11.4
3	14.6	0.90	13.1	14.1	0.90	12.7
4-6	19.7	0.87	17.1	18.6	0.87	16.2
7-10	28.1	0.92	25.9	28.5	0.92	26.2
11-14	45.0	0.90	40.5	46.1	0.89	41.0
15-18	66.5	0.87	57.9	56.4	0.84	47.4

Source: WHO/FAO/UNU (2007)

General considerations

The methods used as basis for estimating protein requirements are the factorial method and the nitrogen (N) balance method which takes into consideration protein required for maintenance and growth (maintenance of 0.66 g/ kg body weight/day and a protein efficiency utilisation of 58%). However, for young infants, estimations of protein requirements are based on human milk intake (WHO/FAO/UNU, 2007).

The nitrogen-balance technique involves the determination of the difference between the intake of nitrogen and the amount excreted in urine, faeces, and sweat, together with minor losses by other routes. In a healthy adult who is in energy balance, the protein requirement (maintenance requirement) is defined as that amount of dietary protein sufficient to achieve zero nitrogen balance. The requirement for dietary protein is considered to be the amount needed to replace obligatory nitrogen loses, after adjustment for the efficiency of dietary protein utilisation and the quality of the dietary protein.

In positive nitrogen balance, more nitrogen is taken in than is lost. Positive nitrogen 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. Negative nitrogen balance is seen when a person had an infection or traumatic injury. More nitrogen is excreted than ingested. Negative nitrogen balance also happens in under-nutrition when protein intake is too low or is of poor quality. In this case, body protein is broken down to supply energy and for recovery.

The factorial method is used to calculate protein requirements for physiological condition such as growth, pregnancy or lactation, in which nitrogen is not only needed for maintenance but also for the deposition of protein in newly formed tissue or secretions (milk).

In the RNI 2005, protein quality of 80% was assumed in the recommendations for protein requirements for ages above 6 months. This level of protein quality is maintained by the TSC on Energy and Macronutrients for 2017 RNI based on two considerations. First, the total daily protein intake and TEI values for MANS (2003) and MANs (2014) were approximately similar as explained in Item 2.7 above. Besides that, the proportion of protein from animal products available to Malaysians in 2003 and 2013 were also quite similar, 55.1% and 55.7% respectively (FAOSTAT, various years). This data is based on Malaysia Food Balance Sheets since MANS surveys did not differentiate the sources of protein consumed between animal and plant food products.

The TSC for Energy and Macronutrients decided to adopt WHO/FAO/UNU (2007) in estimating protein requirements for all age groups.

Recommended intakes by age groups

The recommended protein intake for the revised RNI for the various groups is given in the following sections in bold and summarised in Appendix 2.1.

Infants, 0 – 5 months

Estimations of protein requirements for infants aged 0 - 5 months are based on human milk intake (WHO/FAO/UNU, 2007). The assumption is made that for the first 6 months of life, human milk from a healthy well-nourished mother can be regarded as providing an optimal intake of protein for the infant. The average protein requirement for the 3-4 month old infant (1.47 g protein/kg body weight/day) derived from the factorial method is very similar to the average human milk protein intake values (1.49 g protein/kg body weight/ day) for this age group, with protein intakes of breastfed infants of healthy mothers assumed to provide adequately for the infants' protein needs. Protein intake per kg body weight is 55-80% higher in formula than in breast fed infants and it has been found that high early protein intakes in excess of metabolic requirements enhance weight gain in infancy and increase later obesity risk (Alexy *et al.,* 1999). Thus, breast milk should be used as the gold standard in recommending protein intake for infants 0-5 months (Koletzko *et al.,* 2009).

The TSC on Energy and Macronutrients recommended to adopt the WHO/FAO/UNU (2007) recommended intake of 1.31 g protein/kg body weight/day (Appendix 2.1) and to use the reference body weight for the Malaysian population of 5.6 kg for infants 0-5 months.

RNI for infants

0 – 5 months	**8 g/day**

Infants, 6-11 Months

The period from 6 to under 12 months is clearly the most critical, because of rapid growth during this time and because the child increasingly relies on complementary foods. The average protein requirements for infants greater than 6 months of aged was estimated based on the average level plus 1.96 SD.

For infants aged from 6 to under 12 months, the maintenance requirement was estimated at 0.56g/ kg body weight/day from nitrogen balance studies.The WHO/FAO/UNU (2007) recommended protein intake of 1.14g/ kg body weight/day (10g/day) of high quality protein for infants aged 6 to under 12 months.

The TSC on Energy and Macronutrients recommended adopting the WHO/FAO/UNU (2007) recommended intake of 1.14g protein/kg body weight/day (Appendix 2.1) and to use the reference body weight for the Malaysian population of 8.6 kg for infants 6-11 months.

RNI for infants

6 - 11 months	**10 g/day**

Children and adolescents

In the WHO/FAO/UNU (2007) Expert Consultation report, maintenance requirement for children and adolescent was estimated at 0.63 g/ kg body weight/day and total requirement, allowing for decreasing requirement for growth with age, was estimated to range from 0.63-0.67 g/kg body weight/day. An additional 30% allowance was made to take into account for inter-individual variability in protein utilisation and digestibility. The established the recommended intake for child and adolescent groups in four categories, which are children aged 1 to under 4 years (1.0g/ kg body weight/ day) and 4 to under 15 years, and for boys aged 15 to under 19 years (0.9g/ kg body weight/ day) and girls aged 15 to under 19 years (0.8g/ kg body weight/ day).

The safe level of protein intake for children of various ages was referred to the Table 5.2. The TSC on Energy and Macronutrients recommended adopting the recommendations of this report, which the values are 1.01, 0.87 and 0.92 g/kg body weight/day for children ages 1-3 years, 4-6 years and 7-9 years (Appendix 2.1), respectively. The corresponding reference weights appropriate for Malaysian children used are 12 kg, 18 kg and 25 kg, respectively.

For adolescent boys, the recommended protein intake in g/kg body weight/day, are 0.90, 0.90 and 0.87 for ages 10-12 years, 13-15 years and 16-18 years, respectively (Appendix 2.1). The corresponding recommended protein intake for adolescent girls is 0.89, 0.89 and 0.84 g/kg body weight/day, respectively. The reference weights for adolescent Malaysian boys for the three age groups are 33 kg, 50 kg and 59 kg, respectively. The corresponding weights for girls are 35 kg, 47 kg and 50 kg, respectively.

Based on these data, the RNI for protein have been calculated and summarised below.

RNI for children

1 - 3 years	**12 g/day**
4 - 6 years	**16 g/day**
7 - 9 years	**23 g/day**

RNI for adolescents

Boys	**10 - 12 years**	**30 g/day**
	13 - 15 years	**45 g/day**
	16 - 18 years	**51 g/day**
Girls	**10 - 12 years**	**31 g/day**
	13 - 15 years	**42 g/day**
	16 - 18 years	**42 g/day**

Adults

For adults, the accepted value for the safe level of protein intake is 0.83 g/kg body weight/day with a protein digestibility-corrected amino acid score value of 1.0 (WHO/FAO/UNU 2007). There is no safe upper limit has been identified. Any intakes of twice from the safe level are associated with any risk. However, caution is advised to those contemplating the very high intakes of 3–4 times the safe intake, since such intakes approach the tolerable upper limit and cannot be assumed to be risk-free.

There is also a broad agreement that the requirement for protein at 0.8 g protein/kg body weight/day, although sufficient to prevent deficiency, is insufficient to promote optimal health, particularly in populations exposed to catabolic stressors such as illness, physical inactivity, injury, or advanced age. Several recent consensus statements have suggested that a protein intake between 1.0 and 1.5 g protein/kg body weight/day may confer health benefits beyond those afforded by simply meeting the current requirement.

The revised RNI for protein for adults, are based on the recommendations from both WHO/FAO/UNU (2007) and EFSA (2012), which is at 1.00 g protein/kg body weight/day after taking into consideration with the studies mentioned above. The reference weights for Malaysian male adults for the two age groups are 61.4 kg and 60.6 kg, respectively. The reference weights for Malaysian female adults are 52.9 kg and 52.2 kg, respectively.

RNI for adults

Men	**19 - 29 years**	**62 g/day**
	30 - 59 years	**61 g/day**
Women	**19 - 29 years**	**53 g/day**
	30 - 59 years	**52 g/day**

Elderly

Although WHO/FAO/UNU (2007) and EFSA (2012) have estimated that protein requirements do not change with age during adult life, recent evidence have shown that the current recommended intake for protein, while fulfilling the criteria as the 'minimal daily average dietary intake level that meets the nutritional requirements of nearly all healthy individuals', does not promote optimal health or protect the elderly from sarcopenic muscle loss. By doubling the recommended intake of protein from 0.8 g/kg body weight/day to 1.5 to 1.6 g/kg body weight/day, it may result in better muscle and bone health in elderly individuals. The doubled recommended intake is considered within the acceptable range of intake (10–35% of total calories). In addition, the recommended intake of 1.0 to 1.2 g protein/kg body weight/day for elderly may represent a compromise while longer term protein supplement trials are still pending.

The revised RNI for protein for elderly are based on the recommendations from WHO/FAO/UNU (2007) and EFSA (2012), which is at 1.00 g protein/kg body weight/day after taking into consideration with the studies mentioned above. The reference weights for elderly Malaysian for male and female at the age of > 60 years are 58.1 kg and 49.5 kg, respectively.

RNI for elderly

Men	**≥ 60 years**	**58 g/day**
Women	**≥ 60 years**	**50 g/day**

Pregnancy

In the 2005 RNI, a single value for extra protein was recommended throughout pregnancy (+7.5g/day). However, in the proposed RNI (2017), the recommendation is based on WHO/FAO/UNU (2007). It is suggested additional protein intake during pregnancy is needed for newly deposited protein and the maintenance costs associated with increased body weight. Mean protein deposition has been estimated from total body potassium (TBK) accretion in well-nourished women with a mean gestational weight gain of 13.8 kg. Recommended additional protein intake during pregnancy is shown in Table 2.3.

More recent body-composition measurements do not show any maternal storage in early pregnancy, thus increasing amounts are recommended for each trimester. The efficiency of protein utilisation was taken to be 42%. The maintenance costs were based upon the mid-trimester increase in maternal body weight and the adult maintenance value of 0.66 g/kg per day. It is recommended that a higher intake of protein during pregnancy should consist of normal food, rather than commercially prepared high protein supplements. The safe level was derived from the average requirement, assuming a coefficient of variation of 12%. Based on an efficiency of protein utilisation of 42%, the recommended additional protein intake for pregnant women as shown below:

Table 2.3: Recommended additional protein intake during pregnancy

Trimester	Mid-trimester weight gain (kg)	Additional protein maintenance (g/day)	Protein deposition (g/day)	Protein deposition, adjusted efficiency (g/day)	Additional protein requirement (g/day)	Additional safe intake (g/day)
1	0.8	0.5	0.0	0.0	0.5	0.7
2	3.2	3.2	1.9	4.5	7.7	9.6
3	7.3	7.3	7.4	17.7	24.9	31.2

Source: WHO/FAO/UNU Expert Consultation report (2007)

The TSC for Energy and Macronutrients decided to recommend an additional 8 and 25 g/day protein in the second and third trimesters based on the WHO/FAO/UNU (2007) Expert Consultation recommendation (Appendix 2.1).

RNI for Pregnancy

1st Trimester **+0.5 g/day**
2nd Trimester **+8 g/day**
3rd Trimester **+25 g/day**

Lactation

Based on WHO/FAO/UNU (2007) Expert Consultation, protein requirements during lactation was derived using a factorial approach which requires assessing milk volumes produced and the content of both protein nitrogen and non-protein nitrogen, as well as calculating the amount of dietary protein needed for milk protein production. As the efficiency of protein utilisation for milk protein production is unknown, the efficiency associated with the production of milk protein was taken to be the same as for protein deposition in the non-lactating adult (47%) was assumed. Thus, the additional dietary protein requirement during lactation will be an amount of digestible protein equal to milk protein, divided by an efficiency of 0.47. The safe protein intake was calculated as mean +1.96SD with 1SD calculated on the basis of a coefficient of variation of 12%. The additional safe protein intakes during the first 6 months of lactation ranged from 19 to 20 g protein/day reduced to 13 g protein/day after 6 months.

The TSC for Energy and Macronutrients proposed to recommend an additional 19 g for the first 6 months of lactation and an additional 13 g protein per day for second 6 months of lactation based on WHO/FAO/UNU (2007) Expert Consultation.

RNI for lactation

1st 6 months	**+19 g/day**
2nd 6 months	**+13 g/day**

2.8 Discussion on Revised RNI for Malaysia

Comparing the RNI (2005) and RNI (2017) recommended intakes of protein for the latter are lower in the case of infants, children and adolescents, while the recommended intakes are higher for adults and the elderly. In the revised RNI (2017), the additional amounts recommended for pregnancy at 2nd and 3rd trimesters are higher, while the additional amounts of lactation at 1st and 2nd 6 months are lower, as compared to the RNI (2005) These changes in the recommended values are mainly based on the adoption of the WHO/FAO/UNU (2007) and changes in the reference body weight for Malaysians (Appendix 2.1).

2.9 Research Recommendations

There is a need to improve our understanding of the relationship between protein intakes and overall health. This is a particularly important area for future research.

The following priority areas of research are recommended:

- Conducting periodic national nutrition surveys to obtain updates on the intake of protein and amino acids, especially among vulnerable groups.
- Generate data on protein and amino acid compositions of Malaysian foods.
- Assessment of body protein homeostasis and balance.
- Evaluation of the impact of high protein diets in weight reduction regiments.
- Evaluation of vegetarian diets and determination of ways to maximise protein and amino acid contents in these diets.
- Reevaluation of protein deficiency in relation to energy and micronutrient deficiencies among malnourished children and elderly.

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Appendix 2.1 Comparison of recommended intake for protein: RNI Malaysia (2017), RNI Malaysia (2005), WHO/FAO/UNU (2007) and IOM (2002/2005)

Malaysia (2017)		Malaysia (2005)		WHO/FAO/UNU (2007)		IOM (2002/2005)	
Age group	**RNI (g/day)**	**Age group**	**RNI (g/day)**	**Age group**	**Safe intake (g/kg/day)**	**Age group**	**AI (g/kg/day)**
Infants		**Infants**		**Infants**		**Infants**	
0 - 5 months	8	0 - 5 months	11	0 - 5 months	1.31	0 - 6 months	1.52
6 - 11 months	10	6 - 11 months	12	6 - 11 months	1.14	7 - 12 months	
Children		**Children**		**Children**		**Children**	
1 - 3 years	12	1 - 3 years	17	1 - 3 years	1.01	1 - 3 years	1.1
4 - 6 years	16	4 - 6 years	23	4 - 6 years	0.87	4 - 8 years	0.95
7 - 9 years	23	7 - 9 years	32	7 - 9 years	0.92		
Boys		**Boys**		**Boys**		**Boys**	
10 - 12 years	30	10 - 12 years	45	10 - 12 years	0.90	9 - 13 years	0.95
13 - 15 years	45	13 - 15 years	63	13 - 15 years	0.90	14 - 18 years	0.85
16 - 19 years	51	16 - 19 years	65	16 - 19 years	0.87		
Girls		**Girls**		**Girls**		**Girls**	
10 - 12 years	31	10 - 12 years	46	10 - 12 years	0.89	9 - 13 years	0.85
13 - 15 years	42	13 - 15 years	55	13 - 15 years	0.89	14 - 18 years	0.85
16 - 19 years	42	16 - 19 years	54	16 - 19 years	0.84		
Men		**Men**		**Men**		**Men**	
19 - 29 years	62	19 - 59 years	62	19 - 64 years	1.00	19 - 70 years	0.80
30 - 59 years	61			≥70 years	0.80		

Malaysia (2017)		Malaysia (2005)		WHO/FAO/UNU (2007)		IOM (2002/2005)	
Age group	**RNI (g/day)**	**Age group**	**RNI (g/day)**	**Age group**	**Safe intake (g/kg/day)**	**Age group**	**AI (g/kg/day)**
Women		**Women**		**Women**		**Women**	
19 - 29 years	53	19 - 59 years	55	19 - 64 years	1.00	19 - 70 years	0.80
30 - 59 years	52			≥70 years	0.80		
Elderly		**Elderly**		**Elderly**			
Men ≥60 years	58	Men ≥60 years	59	Men ≥60 years	1.00		
Women ≥60 years	50	Women ≥60 years	49	Women ≥60 years	1.00		
Pregnancy		**Pregnancy**	**+7.5**	**Pregnancy**		**Pregnancy**	**1.1**
1st trimester	+0.5			1st trimester	+1 g/day		
2nd trimester	+8			2nd trimester	+9 g/day		
3rd trimester	+25			3rd trimester	+31 g/day		
Lactation		**Lactation**		**Lactation**		**Lactation**	**1.1**
1st 6 months	+19	1st 6 months	+20	1st 6 months	+19 g/day		
2nd 6 months	+13	2nd 6 months	+15	2nd 6 months	+13 g/day		

3 • Fats

3.1 Introduction

Between 2000 and 2012, 73% of total deaths (n=146,000 cases) in Malaysia were attributed to non-communicable diseases (NCDs) with a proportional mortality of 36% alone contributed by cardiovascular disease (CVD) (WHO, 2014). *The National Health and Morbidity Survey (NHMS)* 2011 (n= 28,650) and *The Malaysian Cohort* (n=106,527) have reported 35.1-44.9% of Malaysian adults were hypercholesterolemic (IPH, 2011; Rahman *et al.* 2014). Further, metabolic syndrome (MS), which is a precluding factor in the development of NCDs, has an overall prevalence rate of 27.5% in Malaysia and hypertriglyceridemia, a MS diagnostic criteria, is reported to be 29.3% (Rampal *et al.* 2012).

According to the NHMS 2015, obese Malaysians make up 17.7% of the population while those categorised as overweight make up 30% (IPH, 2015). Despite the overwhelming tendency to associate obesity with higher fat intake, conclusive association between obesity and fat intake in the Malaysian population is not evident. In fact, the mean fat intake of the Malaysian population is reported to be <30% total energy intake (TEI) (Mirnalini *et al.,* 2008).

Incidence data for CVD keeps increasing in Malaysia along with burden of treatment. In the first decade of the 21st century, the most influential United States dietary guidelines (USDA 2010) focused on reducing total fat intake to <30%TEI, specifically saturated fats (SFA) to <10% TEI and dietary cholesterol to <300mg, in order to reduce the risk of cardiovascular disease (CVD). In contrast, the FAO (2010) and IOM (Otten *et al.* 2006) considered a relatively higher fat intake of 35%TEI as acceptable for a normal active person. With newer findings in recent years, the US dietary guidelines (USDA, 2015) revised the upper limit for total fat intake to 35%TEI, limiting SFA intake between 5 to 10%TEI and eliminated the limitation for dietary cholesterol.

The *Malaysian Dietary Guidelines* 2010 for a healthy population were developed to restrict dietary fat <30%TEI with a lower limit of 20%TEI and limiting the use of SFA to less than 10%TEI as well as dietary cholesterol to 300mg; and this view supports current public health strategies to combat the rising NCDs in Malaysia (NCCFN, 2010). The Ministry of Health (MOH), Malaysia, launched a medium term strategic plan in 2010, namely the *National Strategic Plan for Non-Communicable Disease* (NSP-NCD) with the aim to strengthen the CVD and diabetes prevention and control programmes in Malaysia (NSP-NCD MOH 2010).

This Technical Sub-Committee (TSC) presents a rationale for the revision of recommended fat intake for Malaysians based on current available evidence.

3.2 Functions

Fat is essential in the human diet because of its energy density as well as its essentiality for physiological function, growth and development. In addition, dietary fat aids the digestion, absorption and transport of fat-soluble vitamins and fat-soluble phytochemicals, such as carotenoids and lycopenes. During digestion, dietary fat depresses gastric secretions, slows gastric emptying and stimulates biliary and pancreatic secretions, thereby aiding the digestive process. Excess fat is stored as adipose tissue, which enables human survival during limited food availability. Fat also functions structurally to support organs in position and insulate nerves, protect the body from mechanical pressure and insulate the body to preserve body heat and temperature.

Chemically, dietary fats are triacylglycerol molecules with biological function and utilization related to the type of fatty acids esterified to a glycerol backbone. Structures of these fatty acids are built on carbon chains of varying length with either shared bonding (unsaturation) or single-bonding (saturation). The diversity of fats therefore depends on fatty acids that are either saturated or unsaturated and this nature together with carbon chain length determines health risk. Saturated fatty acids (SFA) are hypercholesterolaemic (Hegsted *et al.* 1965; Katan *et al.* 1994), while longer chain fatty acids such as n-6 polyunsaturated fatty acids (n-6 PUFA), mainly linoleic acid (LA) are hypocholesterolaemic (Karupaiah *et al.* 2005). The intake of SFA for our population is expected to be high from palm oil consumption. It is estimated that human consumption of LA is insufficient (Jakobsen *et al.* 2009) and therefore the public health approach is to encourage increased consumption (USDA 2015; NCCFN 2010). The n-3 PUFA family have a hypotriglyceridemic effect which is useful to offset dyslipidemia which is common in a MS-prone population.

3.3 Metabolism

Specific essential fatty acids (EFAs) metabolically act as precursors for longer chain fatty acids (LCFAs) through chain elongation, which in turn are required for both formation of cell membranes as well as become precursors for the synthesis of eicosanoids (Gropper & Smith 2012). Eicosanoids exert 'hormone-like effects moderating a variety of metabolic -physiological actions including blood pressure lowering, diuresis, inhibit blood clotting, reduce inflammation, moderate immune function, vasoconstriction and other vital functions.

Human digestion does not differentiate between animal or vegetable sources of dietary fat but instead the chain length of fatty acids in the food matrix determines the route of absorption (Karupaiah & Sundram 2007). All dietary fats, predominantly triglycerides, undergo enzymatic hydrolysis in the stomach initiated by the gastric lipases, which release fatty acid esters. Fatty acids of short (C4-C6) and medium- (C8-C10) chain fatty acids (SCFAs and MCFAs) solubilize in intestinal fluids and are absorbed directly into the portal system. Once in portal circulation the SCFAs and MCFAs will form complexes with albumin and are carried to the liver for oxidation. The major activity of digestion largely occurs in the duodenum for fatty acids of chain lengths greater than 12 carbon atoms and is carried out by the pancreatic lipases. Released long-chain fatty acids (LCFAs) undergo a protein-mediated absorption pathway. Ultimately post-assimilation, new triacylyglycerol (TAG) structures are re-assembled via the phosphatidic acid pathway for the LCFAs before incorporation into chylomicrons. Following fat absorption, chylomicrons are released into intestinal lymphatics, enter circulation via the thoracic duct and move to capillary surfaces of target organs (liver, heart, etc) or tissue (adipose, skeletal) where TAG disassembly takes place. The norm after a meal is that chylomicron breakdown will be directed towards adipose tissue through the activation of adipose tissue lipoprotein lipase, which is triggered by the elevation of plasma insulin levels concurrent to a meal. In the fasting state, the hormonal balance favours skeletal tissues by the activation of muscle lipoprotein lipase. LCFAs from the diet undergo metabolic conversion with desaturases and elongases for conversion into the n-9, n-6 and n-3 families of fatty acids. The n-6 and n-3 fatty acids in the membrane phospholipids exert metabolic control through their role as precursors of eicosanoids.

Aside from dietary sources of fat, endogenous synthesis of triglycerides (TG) can also occur with excess intake of carbohydrate, as observed in individuals with hypertriglyceridemia. Those fatty acids that are not used for synthesis of eicosanoids or incorporated into tissues are oxidized for energy (Gropper & Smith 2012). Fatty acids yield energy by beta-oxidation in the mitochondria of all cells, except those in the brain and kidney.

3.4 Food sources

Dietary fats can be divided into two categories: visible and invisible fats. Visible fats come from cooking oils that are plant-based (vegetable oils) and table spreads, which may be either plant-based (margarines) or animal fats (butter). Invisible fats are natural constituents of edible biological material ranging from cereals, vegetables, fruits, pulses, nuts and oilseeds, dairy products, meat, eggs or seafood (Gunstone *et al.* 2007).

Palm oil represents the main cooking oil for most Malaysians, as this country is a primary producer of palm oil. Current consumption is 6.6kg per capita/year or 17.8g/capita/day (FAO 2011). Palm oil or palm olein has an almost equal amount of saturated and unsaturated fatty acids as indicated by 40% of palmitic acid (C16:0), 4% of stearic acid (C18:0) and 43% of oleic acid (C18:1). Coconut oil is one of the major sources of SFA in the Malaysian diet as *santan* is commonly used in preparing meals. For instance, *nasi lemak,* curry and *cendol* are traditionally consumed foods that use *santan*. *Santan* contains 92% of SFA with the major fatty acids being lauric acid and myristic acid. The fatty acid composition of various dietary fats and oils is provided in Appendix 3.1. Significant fat content distribution of various food categories in terms of total fat and fatty acid classes are provided in Appendices 3.2a and 3.2b. Vegetable oils such as soybean and corn oils are the mains sources of the EFAs linoleic acid (LA) and α-linolenic acid (ALA), which are classified as n-6 PUFAs, whereas the long chain fatty acids (LCFAs) such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, classified as n-3 PUFAs, are mainly found in fish. The types of fish rich in LCFAs commonly consumed by Malaysians are Indian mackerel *(kembong),* anchovies, yellow-tail and yellow-stripe scards, tuna, sardines, torpedo scads, Indian and short-fin scads, pomfret, red snapper, king mackerel, marine catfish and stingray (Ahmad *et al.* 2016). Appendix 3.3 provides the overall varying distribution of total fat in local fish, examples of cold water fish as well as the n-6 and n-3 fatty acid distribution in Malaysian foods.

3.5 Deficiencies and Excesses

Fat deficiency is rare and usually occurs in individuals with malabsorption related to inhibition of bile salts necessary for fat digestion such as liver disease, and adjunct deficiency of the fat-soluble vitamins (A, D, E and K) would also be adversely affected (Jeppesen *et al.,* 2000). Fat deficiency may also occur in malnutrition related to chronic disease conditions where blood levels of EFAs, mainly LA and ALA, may be below normal as in chronic kidney disease patients (Jeejeebhoy *et al.,* 1990). Clinical symptoms of fat deficiency (LA and ALA) include growth retardation, skin lesions, reproductive failure, and fatty liver. Fat-free diets are noted to lead to clinical EFA deficiency symptoms such as marked weight loss, dryness of the skin and atopic eczema and eventually death (Guarnieri & Johnson, 1970; Holman, 1971).

Although ALA is considered an essential n-3 fatty acid because it cannot be synthesized by humans, its conversion to LCFAs is less efficient. Less than 4% of ALA is metabolised to DHA in young healthy men (Burdge *et al.* 2002) but conversion is more than two-folds higher in woman (Burdge & Wootton 2002; Burdge 2004; Giltay *et al.* 2004).

Arachidonic acid (AA) is approximately three times as effective as LA in promoting growth (Holman 1971), but is not an essential nutrient since LA can be converted to AA in the body (Barr *et al.* 1981). In recent years, there is an inclination to categorise AA and DHA as EFAs, mainly in relation to their role in neurological and cognitive development in young children and infants. Although, AA and DHA can be synthesised in the body, daily requirement in certain groups of people may exceed body capacity to produce them. AA and DHA are highly metabolised to numerous important functional metabolites (Flock *et al.* 2013; Serhan & Chiang 2013) as well as crucial components of the cell membranes particularly in the brain, nervous system and important organs such as heart and lung (Harris *et al.* 2004; San Giovanni & Chew 2005; Innis 2008; Calder 2013).

In the past decades, fat deficiency has never been an issue in the Malaysian population. Instead, with the abundance of cooking oil available for local consumption, the concern is tipping toward excess intake. Affordability and increased access to high-fat foods predisposes the population to increased consumption of energy-dense foods which coupled together with the sedentary living would create a risk for obesity. According to the National Health and Morbidity Survey of 2015, obese Malaysians make up 17.7% of the population while those who are categorised as overweight make up 30%. If added both together, almost half the population of Malaysia are either overweight or obese (IPH, 2015). Despite the overwhelming tendency to associate obesity with the percentage of fat intake, many long term epidemiological studies fail to provide causal relationships between the two. Fat alone cannot explain obesity as higher carbohydrate intakes also contribute to the high calorie matrix of diets (Astrup *et al.,* 2000; Nordman *et al.,* 2006).

3.6 Factors affecting requirements

Fat provides 9.0kcal/g compared with the much lower 4.0 kcal/g for carbohydrate and protein. As such, people consuming high-fat diets (>35% energy) coupled with a sedentary lifestyle, would likely become overweight or obese as their energy intake exceeds energy expenditure (Astrup *et al.,* 2000).

Current median total fat intake as reported in the Malaysian Adult Nutrition Survey (MANS) 2014 is estimated as 46g/day or 29%TEI. This intake varies between urban (median = 49g/day) and rural (median = 44g/day) populations as well as between ethnic groups, with Chinese (median= 53g/day) having the highest fat intake compared to Malays (median = 45g/day) and Indians (median = 47g/day) (IPH 2014). Previously, the MANS 2003 reported that fat intake was also different geographically, with the lowest intakes reported for Northern Peninsular of Malaysia (median=44g/day), followed by Central and Southern Peninsular of Malaysia (both median = 45g/day), East Coast Peninsular of Malaysia (median 46g/day), while the highest fat intakes were reported in Sabah (median = 47g/day) and Sarawak (median = 49g/day) (Mirnalini *et al.,* 2008). In terms of fat contribution to daily total energy intakes (TEI), reports for smaller populations ranges between 30 - 33 %TEI with 32.8%TEI reported for an urban Malay population (Eng & Moy, 2011), while 30%TEI of fat intake was reported in Peninsular Malaysia (Chee *et al.,* 1997).

The MANS 2014 also reports a decreasing trend in total fat consumption across age groups. Young adults (below 40 years, median = 47g/day) consumed more fat than those aged more than 50 years (median = 44g/day) (IPH 2014). This is not surprising as apart from physiological aging and cultural preferences, food intake is also influenced by socio-economic status and temporal society transitions (Popkin 2006; Manderson & Naemiratch, 2010).

Physiological condition(s) such as pregnancy or lactation do not affect daily total fat percentage of energy requirements but infants and children up to 3 years are recognised to have higher percentage of energy requirements for total fat. Conditions of CVD, liver disease and diabetes will also affect fat requirements in order to fit therapeutic medical nutrition therapy goals (Simopoulos 1999). This shall be discussed later with regard to the requirements for the n-6 LA. As implied earlier, reducing excess fat consumption would also reduce total calorie intake, which would be beneficial for individuals with excess body weight (Astrup *et al.,* 2000; *Bray et al.,* 2002).

3.7 Setting requirements and recommended intakes for fats

3.7a Current opinion on Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and trans fatty acids (TFA)

SFA

Based on the findings from three meta-analyses, a higher SFA intake is not found to be significantly associated with increased CVD risk (Mente *et al.* 2009; Siri-Tarino *et al.,* 2010; Chowdhury *et al.* 2014). Only Mente *et al.* (2009), who included 146 sub-cohorts, found a marginally significant association between SFA intake and CVD risk when including both genders in the analysis. However, when the analysis separated effect of gender, no association was found in either men or women. Chowdhury *et al.,* (2014) in pooling 32 observational studies (n=544,727 subjects) to compare the extreme tertiles of SFA consumption, also found no association between SFA intake and CVD risk.

MUFA

The meta-analysis by Chowdhury *et al.* (2014) also showed that MUFA intake did not affect CVD outcomes. In contrast, a meta-analysis by Mente *et al.,* (2009) reported a significant inverse association between MUFA intake and CVD risk in men but not in women. A systematic review and meta-analysis of 32 cohort studies (n=841,211 subjects) (Schwingshackl & Hoffmann, 2014) comparing the highest to the lowest tertiles of MUFA consumption from olive oil, with oleic acid as well as MUFA:SFA ratio determined, concluded that increased MUFA intake resulted in a significant risk reduction for CVD mortality, CVD events and stroke. Subgroup analyses showed that only olive oil had a significant inverse correlation with CVD events and stroke whereas oleic acid or increased MUFA:SFA ratio had no observed effect on CVD events and stroke.

PUFA

The meta-analysis of 18 prospective cohorts by Mente *et al.* (2009) showed either higher n-3 PUFA intakes or total PUFA intake for 6 cohorts reduced risk of CVD in women but not in men or when both genders were combined in the analyses. However, Chowdhury *et al.* (2012) in their meta-analyses of 14 prospective cohorts found no association between higher intakes of long-chain n-3 PUFA with CVD risk. But with the inclusion of 32 observational studies, Chowdhury *et al.* (2014) reported a significant inverse association between long-chain n-3 PUFA (n=16 cohorts) and CVD risk whereas total n-6 intake (n=8 cohorts) was not associated with CVD risk.

A higher ALA (α-linolenic acid, C18:3n-3) intake was not significantly associated with CVD risk in both genders (Chowdhury *et al.* 2014; Mente *et al.* 2009). However, with the inclusion of 27 prospective and retrospective cohorts, Pan *et al.* (2012) found that higher ALA intake reduced CVD risk and coronary heart disease (CHD) mortality whereas there was no significant effect on total CHD and stroke events.

Trans-fatty acids (TFA)

A meta-analysis of 4 large-scaled cohorts, which followed 139,836 subjects (Mozaffarian & Clarke 2009), found that an additional 2%TEI derived from TFA resulted in a 23% increase in CVD risk. The meta-analysis by Chowdhury *et al.,* (2014), which included 5 observational studies in their pooled analysis of the effect of TFA on CVD risk, showed that total TFA intake was positively correlated with increased risk of CHD, and resulted in a 16% increased CVD risk.

Replacing SFA with other fatty acids and carbohydrates

A meta-analysis of 11 cohorts, involving 344,696 subjects with 4 to 10 years of follow-up, found that replacing dietary SFA with PUFA reduced the risk of CVD by 13% and coronary deaths by 26%. However, replacing SFA with carbohydrate resulted in a 7% increase in CVD events without any effect on CVD mortality (Jakobsen *et al.,* 2009).

A review by Volek and Feinmann (2005) compiled 15 intervention studies which substituted dietary carbohydrate with fat, and reported that when high-fat low-carbohydrate diets were introduced, body weight, insulin and TG levels had a greater reduction while HDL-C levels were higher compared to low-fat high-carbohydrate diets. Another intervention study comparing low-fat high-carbohydrate diet (28%TEI from fat and 58%TEI from carbohydrate) with high-fat low-carbohydrate diet (40%TEI from fat and 46%TEI from carbohydrate) after a 6-week intervention, had reported a significant reduction in diastolic blood pressure and TG:HDL-C ratio without affecting insulin and LDL-C levels.

n-6:n-3 fatty acid balance

The n-6 FA in the diet consists of mainly LA and its metabolite AA, while the n-3 FA comprises ALA, EPA and DHA. Currently, the n-6:n-3 FA ratio of a chemically analysed typical Malaysian diet is about 24.2±9.6 (~8.45±0.64/0.45±0.56g) (Karupaiah *et al.,* 2016) compared to a calculated ratio of 10 (3.5/0.35) (Ng 1995). FAO/WHO (1994) recommends an n-6:n-3 FA ratio of 5-10. Both the absolute amounts of n-6 and n-3 FA, as well as their ratio are important nutritional considerations. Increasing the intake of n-3 poses a serious challenge, and any excess intake of LA (>7%TEI) would enable difficulty in achieving the recommended n-6:n-3 FA ratio.

A meta-analysis on RCTs showed that supplementing with ALA, total LC-n-3 and total n-6 did not reduce the relative risk for CVD events (Chowdhury *et al.,* 2014). Another meta-analysis also concurred that n-3 supplementation did not result in a significant reduction in overall CVD events, all-cause mortality and CVD death (Kwak *et al.,* 2012).

A review by Simopoulos (2008) discussed the effect of n-6:n-3 ratio on secondary CVD prevention, indicating that a ratio lower than 4:1 is associated with a 70% decrease in total mortality. However, n-3 is not common in the daily diet as indicated in urban India (n-6:n-3 ratio=38-50) (Pella *et al.,* 2003), the United States (n-6:n-3 ratio=16.74) (Eaton *et al.,* 1998), UK and Northern Europe (n-6:n-3 ratio=15.0) (Sanders, 2000) while only Japan where fish consumption is very high had the lowest ratio (n-6:n-3 ratio=4.0) (Sugano & Hirahara, 2000) Therefore, it is clear that a supplementary dose of n-3 fatty acids is required for therapeutic effect on cardiometabolic risk lowering. LA can be found in a large quantity in vegetable oils compared to n-3 PUFA, which mainly comes from fish in the Malaysian diet (Appendices 3.2a, 3.2b and 3.3).

In the Malaysian context, n-6 PUFA (LA) intakes are recommended at 3 to 7%TEI, while n-3 PUFA at 0.3-1.2%TEI. This range n-3 FA intakes is recommended with due consideration to the present n-3 content of habitual Malaysian diets and the practicability of increasing the intake which requires substantial changes in dietary habits.

The n-3 fatty acids intake of the average Malaysian remains low and there are several ways that we can remedy this, namely: [1] consuming more pulses (eg. beans, dhal), tofu and fishes, [2] use a cooking oil that is a blend of palm olein + n-3 rich vegetable oil (eg. canola, soybean), and [3] to include n-3 (ALA or EPA + DHA)- rich novel foods as shown in Appendix 3.3 in the household food basket.

Dietary cholesterol

Dietary guidelines in the past focused on the reduction of dietary cholesterol intake from the diet as a strategy in reducing CVD risk, until 2013 (NCEP Expert Panel 2002; USDA 2010). These guidelines were based on a positive correlation between high plasma total cholesterol (TC) and increased CVD risk, which formed the basis to recommend reduced dietary cholesterol consumption; and became subsequently a highly advocated strategy in lowering the morbidity and mortality of CVD (Goodman 1991). Nevertheless, the *Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study* reported that a higher dietary cholesterol intake did not

correlate with higher rates of CHD events and CHD mortality when comparing the highest (median=768 mg) with the lowest (median=390 mg) quintiles of cholesterol intake (Pietinen *et al.,* 1997). Previously, the AHA in the ATP III Guidelines recommended not exceeding a daily consumption of 300 mg of dietary cholesterol by healthy people whilst ≤200 mg was recommended for individuals with hypercholesterolemia (Frolkis *et al.,* 2002) as well as those with MS (Grundy *et al.,* 2004).

When the earlier dietary recommendation was to reduce dietary cholesterol consumption, egg consumption was often perceived as a health risk (NCEP Expert Panel 2002; NCCFN 2010) based on a greater consumption of eggs been linked to higher CVD risk (Mann *et al.,* 1997; Nakamura *et al.,* 2004). A recent meta-analysis of 22 prospective cohorts (n=558,700 subjects) followed between 6 to 20 years (Shin *et al.* 2013), did not establish any association between egg consumption and CVD in a healthy population. Consumption was assessed in terms of ≥1 egg per day compared to <1 egg per week. However, a significant effect was noted in studies involving diabetic patients consuming ≥ 1 egg per day with increased risk of CVD compared to those consuming <1 egg per week (HR=1.69, 95% CI: 1.09, 2.62). Another meta-analysis associated consumption of ≥ 3 eggs per week with a significantly higher risk for Type-2 diabetes in US studies but this effect was not evident for non-US studies (Djousse *et al.* 2016).

The *Malaysian Dietary Guidelines,* which was published in 2010, still recommends a restricted dietary cholesterol intake of <300 mg per day as a goal in plasma cholesterol reduction (NCCFN, 2010). Due to the lack of evidence from clinical studies showing a benefit from dietary cholesterol reduction, the recent Adult Treatment Panel (ATP) IV guidelines of the American Heart Association (Eckel *et al.* 2013) only focused on reducing SFA (5 to 6 %TEI) and avoiding trans-fat. USDA 2015 has also removed the cholesterol intake recommendation from the list of 'nutrients of concern'. However, it is important to highlight that this guideline is meant for a healthy population.

The TSC recommends removing the restriction on dietary cholesterol intake for a healthy population. However, it must be cautioned that dietary cholesterol-rich foods such as beef, pork and shellfish also carry significant content of SFA, which are known to increase TC and LDL-C levels (Fattore *et al.* 2014; Sun *et al.* 2015).

3.7b Dietary fats requirements and recommended intakes

The previous RNI 2005 recommended dietary macronutrient distribution to be 55-70%TEI from carbohydrate, 20-30%TEI from fat and 10-15%TEI from protein (NCCFN, 2005). The setting for 20-30%TEI for fat originates from MDG 1999 (NCCFN, 1999). These dietary fat levels are within the range of 15-30% energy recommended by several WHO/FAO Expert Consultations over the years. This Technical Sub-Committee (TSC) has reviewed the prevailing global recommendations (USDA 2015; PHE, 2016; PHC, 2016) as well as the chronic disease burden from NCDs, which originate from the twin burdens of insulin resistance and atherogenic risk (IPH, 2015) thus implicating not just fats but also carbohydrates (PHC, 2016). At the high end, the TSC notes that the FAO (2010) reports consider a relatively high fat intake of 35%TEI as acceptable for an active person. Given the upper limit for safety for fat consumption is 35%TEI (FAO, 2010) and limits below 25%TEI which would increase the carbohydrate load (Siri-Tarino *et al.,* 2010; Appel *et al.,* 2005), the recommended range for fat in terms of the total

daily diet consumed should fall within these safety limits for health for a normal population. However, the overall recommendation for adults will be restricted to a conservative range of 25 to 30%TEI from fats with an upper limit of 35%TEI from fats for active persons. For children and adolescent (up to age of 18 years) the recommended range for fat intake will be broader between 25 to 35%TEI.

Palm oil is extensively used in locally processed foods as well as commercial and home food preparations. By consuming palm olein alone, at 30%TEI, an individual ingests sufficient LA (3-4%TEI EFA), 12%TEI saturated and 14%TEI monounsaturated fatty acids as well as other micronutrients such as vitamin A and E. Therefore, the predisposition for a high saturated intake (>10%TEI) will need to be addressed for individuals who are hypercholesterolemic.

The requirement to increase n-6 PUFA is addressed separately below in this section.

The RNI 2005 recommended 0.3-1.2 %TEI of n-3 PUFA (NCCFN, 2005) whereas WHO recommends 0.5-2.0 %TEI of n-3 PUFA for healthy adults (FAO, 2010). Sufficient n-3 PUFA is possible with regular consumption of fish more than 3 times a week, which roughly provides at least 0.3%TEI as observed in a human trial (Karupaiah *et al.,* 2016). A range in n-3 PUFA intakes is recommended with due consideration to the present n-3 content of habitual Malaysian diets, and the practicability of increasing the intake which requires substantial changes in dietary habits. Appendices 3.2a, 3.2b and 3.3 provide the distribution of the different fatty acid classes in foods commonly consumed by Malaysians.

Fat is an essential nutrient and its dietary level should not fall too low, otherwise the diet prepared becomes monotonous, has low palatability, low energy density, and the amount of EFA or LA can become limiting in the overall diet.

Infants

Breast milk is the preferred source of nutrition for infants. Human milk provides 50-60% of its energy as fat with 5% energy as EFA (LA + ALA) and 1% energy as LCFAs. During lactation in well-nourished mothers, milk fat contribution increases from 40-50g/litre at three weeks to 60-70g/litre by 4-6 months (FAO/WHO, 1994).

There is convincing evidence that during the first six months of life, total dietary fat should contribute 40-60 %TEI to cover the energy needed for growth and the fat required for tissue deposition (FAO, 2010). There is convincing evidence that from age 6-24 months, fat intake should be reduced gradually, depending on the physical activity of the child, to ~35%TEI in line with the upper limit for adult recommendations (FAO, 2010).

The absolute amount of dietary fat recommended per day by the TSC for the 0 to 5 months age group are calculated based on the current proposed Malaysian RNI for energy (470 kcal for boys aged 0 to 2 months; 540 kcal for boys aged 3 to 5 months; 420 kcal for girls aged 0 to 2 months; 500 kcal for girls aged 3 to 5 months) with 40 - 60 %TEI contributed by fat calories. During weaning, the fat component should provide ~35% of the energy (FAO, 2010). This means that complementary foods used during the weaning period should include adequate amounts of fats and oils as the breast milk component of the diet declines. Therefore, the

absolute amounts of dietary fat recommended per day by the TSC for the 6 to 11 months age group are calculated based on the current proposed Malaysian RNI for energy (630 kcal for boys aged 6 to 8 months; 720 kcal for boys aged 9 to 11 months; 570 kcal for girls aged 6 to 8 months; 660kcal for girls aged 9 to 11 months) with 30 - 40 %TEI as fat calories.

RNI for infants

Boys	
0 - 2 months	**21 - 31 g/day**
3 - 5 months	**24 - 36 g/day**
6 - 8 months	**21 - 28 g/day**
9 - 11 months	**24 - 32 g/day**
Girls	
0 - 2 months	**19 - 28 g/day**
3 - 5 months	**22 - 33 g/day**
6 - 8 months	**19 - 25 g/day**
9 - 11 months	**22 - 29 g/day**

Children and adolescents

The range of 25 to 35 %TEI as fat calories for these age groups are calculated from the current proposed energy requirements for younger age groups (980 kcal for boys aged 1 to 3 years; 1300 kcal for boys aged 4 to 6 years; 1750 kcal for boys aged 7 to 9 years; 900 kcal for girls aged 1 to 3 years; 1210 kcal for girls aged 4 to 6 years; 1610 kcal for girls aged 7 to 9 years). The new recommendation adopted from FAO (2010) coupled with epidemiological evidence of a significant progression of the obesity epidemic in young children by Broyles *et al.* (2015) supports the need to restrict the percentage of fat intake to <35%TEI to facilitate energy balance without undue increase in body fat.

RNI for children

Boys	**1 - 3 years**	**27 - 38 g/day**
	4 - 6 years	**36 - 51 g/day**
	7 - 9 years	**49 - 68 g/day**
Girls	**1 - 3 years**	**25 - 35 g/day**
	4 - 6 years	**34 - 47 g/day**
	7 - 9 years	**45 - 63 g/day**

For adolescent boys and girls (10 to 18 years), who are more active than adults, 25 - 35% of the unweighted means for daily energy requirements (1930 kcal for boys aged 10 to 12 years; 2210 kcal for boys aged 13 to 15 years; 2340 kcal for boys aged 16 to 18 years; 1710 kcal for girls aged 10 to 12 years; 1810 kcal for girls aged 13 to 15 years; 1890 kcal for girls aged 16 to 18 years) were used to calculate the absolute amounts in grams of fat required per day.

RNI for adolescents

Boys	**10 - 12 years**	**54 - 75 g/day**
	13 - 15 years	**61 - 86 g/day**
	16 - 18 years	**65 - 91 g/day**
Girls	**10 - 12 years**	**48 - 67 g/day**
	13 - 15 years	**50 - 70 g/day**
	16 - 18 years	**53 - 74 g/day**

Adults and elderly

Dietary intervention studies have shown low fat diets (<25%TEI) would lower serum HDL-C and increase TG levels, as well as lower insulin sensitivity compared to extremely high fat diets (>55%TEI) (Volek *et al.,* 2009). A meta-analyses of edible vegetable oils in human consumption inclusive of palm oil concludes intervention studies providing <35%TEI as fats did not significantly increase blood LDL-C levels regardless of fats been saturated or unsaturated (Fattore *et al.,* (2014). However, the overall fat recommendation is restricted to a conservative range of 25 to 30 %TEI with an upper limit of 35%TEI for active persons.

For adults 19 to 59 years of age, 25 to 30 % of the unweighted means for daily energy requirements (2240 kcal for men aged 19 to 29 years; 2190 kcal for men aged 30 to 59 years; 1840 kcal for women aged 19 to 29 years; 1900 kcal for women aged 30 to 59 years) were used to calculate the recommended daily amounts of dietary fat. For the elderly, the recommended fat intake was computed based on energy requirements of 2030 kcal for men and 1770 kcal for women.

RNI for adults

Men	**19 - 29 years**	**62 - 75 g/day**
	30 - 59 years	**61 - 73 g/day**
Women	**19 - 29 years**	**51 - 61 g/day**
	30 - 59 years	**53 - 63 g/day**

RNI for elderly

Men	**≥ 60 years**	**56 - 68 g/day**
Women	**≥ 60 years**	**49 - 59 g/day**

Pregnancy and lactation

Fat is used for energy as well as critical building material for membranes (FAO, 2010). During pregnancy, there is an additional requirement for dietary fat to provide for maternal fat storage during the early trimester, and subsequent uterine growth, preparative development of the mammary glands, the expansion of blood volume, and placental and foetal growth in the second and third trimesters (FAO/WHO,1994). However, there is no indication that recommendations for dietary total fat intake, expressed as a percentage of energy intake, need to differ in pregnancy and lactation from those for non-pregnant, non-lactating women (Koletzko *et al.,* 2007). Although the recommended range of dietary fat remains at 25 - 30 % energy during pregnancy, the increased dietary fat needs are reflected in the higher daily energy requirements currently proposed for the first (+80 kcal), second (+280 kcal) and third (+470 kcal) trimesters (see Chapter 2 on Energy). Similarly, the increased dietary fat needs correspond to the proposed addition of 500 kcal per day for lactation during the first 6 months.

RNI for

Pregnancy, 1st trimester	**54 - 65 g/day**
Pregnancy, 2nd trimester	**60 - 71 g/day**
Pregnancy, 3rd trimester	**65 - 78 g/day**
Lactation, 1st 6 months	**66 - 79 g/day**

Recommendation for Linoleic Acid (LA) intake of infants (0 to 24 months)

The essential fatty acids, LA (18:2, n-6) and ALA (18:3, n-3) which cannot be physiologically-synthesized are specifically critical in the diet of infants from birth to 2 years (refer to section 3.5). Further DHA, which can be synthesized from ALA, is also considered conditionally essential for the first six months as it plays a critical role in normal retinal and brain development (FAO 2010). The total diet should provide infants with at least 3.0 to 4.5 %TEI from LA and 0.4 to 0.6 %TEI from ALA to meet essential fatty acids requirement.

0 to 6 months

Since the primary food source for this age group is human milk, it is conventional to base the recommendation on the fatty acid composition of human milk (Appendix 3.4). There is convincing evidence that adequate intake for DHA is 0.1 to 0.18 %TEI and for both AA and ALA to be 0.2 to 0.3 %TEI (FAO 2010). Optimal maternal milk feeding is sufficient to meet these requirements.

6 to 24 months

There is convincing evidence that the adequate intake for EFAs for optimal growth and development of this age group to be 3.0 to 4.5 % of TEI for LA and 0.4 to 0.6%TEI for ALA (FAO 2010). The upper acceptable macronutrient distribution range suggested by FAO (2010) for LA is <10%TEI and for ALA is <3%TEI at a probable level of evidence. The adequate intake for DHA is 10-12 mg/kg at a probable level of evidence.

Recommendation for children 2 to 18 years as regards to other fatty acids

FAO (2010) suggests that there is probable evidence to recommend an adequate intake range of EPA + DHA intake targeted at preventing chronic disease (adjusted for age) of:

100 - 150 mg for 2 to 4 years
150 - 200 mg for 4 to 6 years
200 - 300 mg for 6 to 10 years

However, the currently available evidence does not permit defining an age-specific quantitative estimate of recommended nutrient intake for EPA + DHA for children aged 2-18 years. There is currently insufficient evidence to link increased intake levels of DHA and/or EPA to improved physical or mental development or specific functional benefits in children 2-18 years of age (FAO, 2010)

Recommendation for Linoleic Acid (LA) intake for adults

The minimum requirement for EFA in humans to prevent biochemical and clinical evidence of EFA deficiency is 1-2%TEI. For infants and adults, a dietary intake of at least 3%TEI of LA is considered adequate (FAO/WHO, 1977).

During pregnancy, the additional demand for uterine, placental and foetal growth, together with the increased maternal blood volume and mammary gland development, raises the EFA requirement by 1.5%TEI in the maternal diet, adding up to a total of at least 4.5%TEI EFA.

Perinatal health effects of LCFAs have been most closely associated with improvement of infant visual and cognitive function, treatment and prevention of maternal depression, and slight increases in gestational length to reduce the prevalence of prematurity (FAO, 2010). Long-term consequences of the impact of preformed DHA and AA intake for mothers and infants have also been found to be either showing neutral or positive effects on health outcome. In addition, there is compelling evidence showing intake of DHA and EPA and of AA combined with DHA are not associated with toxicity for mothers, infants or children. Therefore, average nutrient requirement for DHA is suggested to be 200mg per day with an upper nutrient limit of 1.0g per day based on no observed adverse effect level in randomised controlled trials. Further, based on minimum adult acceptable macronutrient distribution range and an increment for energy demands of pregnancy and lactation, a combined intake of DHA + EPA of 300 mg per day is recommended by FAO (2010) with an upper nutrient limit of 2.7 g per day. The upper nutrient limit for AA is suggested to be 800 mg day while trans-fat should be kept as low as practical.

As for other fatty acids, there is no compelling evidence showing energy requirements from saturated, monounsaturated or total polyunsaturated is different in pregnancy and lactation. Therefore, no changes are recommended on acceptable macronutrient distributions for these nutrients (FAO, 2010).

In summary, this RNI for dietary fat intake for Malaysians Recommendations, as provided in Appendix 3.5:

- Revised the minimum recommended intake for fat from 20 to 25%TEI with an upper limit of 30%TEI and introduced an upper safe limit of 35%TEI for active adults.
- Proposed that for children and teenagers under 18 years the range has been set as 25 to 35%TEI.
- Maintained the recommended distribution of saturated fatty acids to be less than 10%TEI, monounsaturated fatty acids to be between 12 to 15%TEI, n-6 polyunsaturated fatty acids to be between 3 to 7%TEI and n-3 polyunsaturated fatty acids to be between 0.3 to 1.2%TEI.
- Specifically offers no recommendation for limits on dietary cholesterol intake for a healthy population, with caution that dietary cholesterol-rich foods such as beef, pork and shellfish also carry significant content of SFA which are known to increase TC and LDL-C levels.

3.8 Research recommendations

The lack of local data linking the effect of dietary fatty acids intake with obesity, cardiovascular disease and diabetes mellitus are challenges in determining the appropriate recommendations for dietary intake for Malaysians. Currently, NHMS only reports the health status and prevalence of pre-disease stage, such as hypercholesterolemia, whereas MANS only reported the dietary intake patterns of healthy Malaysian population. No large-scale population studies in Malaysia have correlated the effects of dietary intake patterns with health outcomes. Below is the direction of future research required in Malaysia to make appropriate recommendations for fat intake.

1. Population-based prospective cohorts to evaluate the effect of dietary fat intake, specifically SFA and the type of SFA, MUFA and PUFA on CVD, obesity and diabetes mellitus.
2. RCTs to compare the effect of substituting dietary fat with carbohydrate or protein in iso-caloric diets.
3. Retrospective studies to investigate the dietary fat intake among CVD patients.

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Appendix 3.1 Fatty acid composition of selected dietary fats and oils

Type of fats and oils	SFA	MUFA	PUFA	P/S ratio	<12:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	Others
Coconut oil	91.9	6.5	1.5	0.02	14.9	48.5	17.6	8.4	-	2.5	6.5	1.5	-	0.1
Palm kernel oil	84.2	13.7	2.0	0.02	8.2	49.6	16.0	8.0	-	2.4	13.7	2.0	-	0.1
Cocoa butter	60.4	35.6	2.9	0.05	-	-	0.1	25.8	0.3	34.5	35.3	2.9	-	1.1
Beef fat	50.6	42.1	2.8	0.06	0.1	0.1	3.3	25.5	3.4	21.6	38.7	2.2	0.6	4.6
Shea butter	46.0	48.0	5.1	0.11	-	-	-	5.0	-	41.0	48.0	5.1	-	0.9
Palm oil	44.9	43.4	10.8	0.24	-	0.3	0.8	39.5	0.3	4.3	43.1	10.5	0.3	0.5
Palm olein	42.4	44.0	11.8	0.28	-	0.2	0.8	37.2	0.4	4.2	43.6	11.5	0.3	0.3
Lard	38.7	48.2	11.0	0.28	0.1	0.1	1.4	24.8	3.1	12.3	45.1	9.9	1.1	3.0
Olive oil	18.8	68.2	14.6	0.78	-	-	-	16.5	1.8	2.3	66.4	13.0	1.6	0
Groundnut oil	9.6	71.2	18.2	1.89	-	-	0.04	7.5	0.1	2.1	71.1	18.2	-	0.9
Corn oil	14.2	27.8	57.1	4.02	-	-	-	12.3	0.1	1.9	27.7	56.1	1.0	0.9
Soybean oil	14.8	24.1	59.9	4.05	-	-	0.1	10.8	0.2	3.9	23.9	52.1	7.8	1.2
Canola oil	7.4	56.0	35.6	4.81	-	-	-	5.6	-	1.8	56.0	25.8	9.8	1.0
Sunflower oil	9.1	28.1	62.4	6.85	-	0.02	0.09	6.2	0.12	2.8	28.0	62.2	0.16	0.4
Safflower oil	9.2	11.6	79.2	8.60	-	-	0.1	6.7	0.1	2.4	11.5	79.0	0.15	0.1

Notes: values represent %/100g edible fat.
Sources: Dubois *et al.* (2007), Grundy & Denke (1990), Kris-Etherton *et al.* (1988), Orsavova et al. (2015), Gunstone *et al.* (2007), and Karupaiah *et al.* (2005)

Appendix 3.2a Malaysian foods with significant content of dietary fat (g/100g)

Food	Total fat	SFA	MUFA	PUFA	TFA*
Fishes					
Black Pomfret (Bawal Hitam)	2.3	0.94	0.14	0.71	N/A
Giant Seaperch (Siakap)	2.7	0.13	0.23	0.93	N/A
Golden Snapper (Jenahak)	1.3	0.42	0.94	0.51	N/A
Indian Mackerel (Kembong)	1.8	0.59	0.3	0.19	N/A
Silver Pomfret (Bawal Putih)	2.1	0.88	0.15	0.57	N/A
Yellowstripe scad (Selar Kuning)	2.1	0.83	0.29	0.14	N/A
Shellfish					
Cockles (Kerang)	1.9	0.64	0.40	0.61	N/A
Cuttlefish (Sotong)	1.4	0.57	0.11	0.50	N/A
Oyster (Tiram)	1.2	0.56	0.82	0.34	N/A
Prawn (Udang)	1.1	0.31	0.11	0.46	N/A
Nuts and Seeds					
Almond	49.4	3.7	30.9	12.1	-
Hazelnut	62.4	4.5	46.6	8.5	-
Peanut	49.7	6.9	24.6	15.7	-
Walnut	59.0	3.4	15.0	35.1	-
Confectionary					
Chocolate wafer	27.3	62.3	27.9	6.4	2.72
Cooking chocolate	33.1	80.7	15.64	2.0	1.27
Fats, oils, spreads, dressing					
Butter	80.6	57.8	31.7	5.9	1.32
Fat spread	73.4	36.3	39.4	23.2	0.22
Ghee	99.8	61.5	29.7	3.3	1.04
Margarine	77	46.5	36.3	16.8	0.36
Peanut butter	42	20.3	48.6	26.9	0.52
Salad dressing	45	14.5	22.7	61	0.18
Shortening	99.8	57	33.6	8.8	0.2
Vanaspati	99.8	50.6	37.9	10.7	0.43
Dairy-based products					
Adult milk powder	25.6	58.9	30.8	5.2	1.65
Cheese	21.5	59.8	31.6	4.6	0.78
Children's milk > 3 years	17.8	44.7	36.8	16.4	0.93
Children's milk < 1 years	27.4	39.6	40.7	18.3	0.14
Ice cream	11.0	68.3	23.4	4.8	2.09

Source: Tee *et al.* (1997), Karupaiah *et al.* (2014), Abd. Aziz *et al.* (2013)
*relates to total TFA content as a sum of 18:1 n9t; 18:2 n6t; cis-9 t-12; t-9, cis-12; 18:3t1; 18:3t2; 18:3t4; and 18:3t5 excluding natural isomers of conjugated linoleic acid (cis-9,t-11).
N/A=not available

Appendix 3.2b Malaysian foods with significant content of dietary fat (g/100g)

Food	Total fat	SFA	MUFA	PUFA	TFA*
Soups					
Soup, canned	45.8	10.7	54.6	32.3	0.09
Soup, concentrates	17.0	52.0	36.3	9.0	1.94
Snacks					
French fries	2.55	51.3	36.9	10.8	0.26
Frozen Chappati/paratha	9.1	52.1	34.3	12.2	0.64
Frozen dough	5.5	48.9	37.8	12.2	0.28
Potato chips	32.7	38.3	45.3	15.1	0.24
Meat & products					
Beef lean	1.1	0.6	0.4	0	N/A
Burger patties	13	40.9	43.4	12.3	0.08
Chicken thigh, farm with skin	3.7	1.1	1.8	0.8	N/A
Chicken thigh, farm, without skin	0.5	0.1	0.2	0.1	N/A
Hen egg	8.1	2.6	4.7	0.8	N/A
Mutton	4.6	2	2.4	0.2	N/A
Nuggets	15	43.5	42.1	13.1	0.18
Pork fat	89.3	37.8	45.9	5.5	N/A
Pork lean	21	7.9	11	2.1	N/A
Prawn	0.3	0.1	0.1	0.1	N/A
Sausages	13.8	31.1	45.9	21.3	0.15
Popular Street Foods					
Char Siew Pau	15.4	7.2	7	1.2	N/A
Chicken rice	4.6	1.8	2.1	0.7	N/A
Curry laksa	6.4	4.4	1.4	0.6	N/A
Dosai	0.7	0.4	0.2	0	N/A
Fried Kueh Tiau	9.7	3.9	4.5	1.2	N/A
Fried mee - Hokkien	6.6	2.7	3	0.9	N/A
Fried mee - Indian style	9	5.6	2.3	1.1	N/A
Lor Mai Kai	5	1.9	2.4	0.7	N/A
Nasi goreng cina	13.2	5.3	6.5	1.4	N/A
Nasi lemak	3.6	2	1.1	0.5	N/A
Satay	10.8	3.6	4.6	2.6	N/A

Source: Tee *et al.* (1997), Karupaiah *et al.* (2014)
*relates to total TFA content as a sum of 18:1 n9t; 18:2 n6t; cis-9 t-12; t-9, cis-12; 18:3t1; 18:3t2; 18:3t4; and 18:3t5 excluding natural isomers of conjugated linoleic acid (cis-9,trans-11).
N/A=not available

Appendix 3.3 Local fish choices with varying fat content, Cold water fish and Overall rich sources of fatty acids content

Food group	Sources
Low fat fish (<1g/100g)	Kikek, bulus-bulus, toman, stingray, grouper, seluang, nyior-nyior, baung.
Moderate fat fish (1-3g/100g)	Dory, cencaru, black pomfret, sepat, ikan parang, tenggiri, gelama, jenahak, pelata, kerisi, ikan merah, selar, tilapia, belida.
High fat fish (>3g/100g)	Siakap, keli, patin, senangin, kembong, white pomfret.
Deep sea cold fish	Tuna, sardines, cod fish, salmon.
SFA	Coconut oil, santan, palm kernel oil, palm oil, beef, pork, milk, yogurt, cheese
MUFA	Olive oil, Canola oil, peanut oil, almond, peanut, hazelnut, palm oil
n-6 PUFA	Soybean oil, sunflower oil, corn oil, tofu, tempeh, walnut
n-3 PUFA	Soybean oil, Canola oil, fish, walnut

Appendix 3.4 Composition of human breast milk in comparison to cow's milk

Nutrients	Human breast milk Concentration (g/L)	Cow's milk Concentration (g/L)
Protein		
Total whey protein	67.3	6.3
Immunoglobulins	1.3	0.7
Lactoferrin	1.5	0.1
α-lactalbumin	1.9	1.2
Total caseins	2.7	26
Carbohydrate		
Lactose	67	53
Oligosaccharides	0.05-0.20	-
Fat	**32-36**	**33**
Triglycerides	97-98%	97%
AA	0.35-0.70 % total fat	N/A
DHA	0.17-1.0% total fat	N/A

Source: Nguyen *et al.* (2015), Koletzko *et al.* (2008)

Appendix 3.5 Comparison of recommended intake of fat and its components: RNI Malaysia (2017), RNI Malaysia (2005), FAO (2010) and IOM (2006)

Nutrient	Age /Life stage group	Malaysia (2017)		Malaysia (2005)[a]	FAO (2010)	IOM (2006)[b]
		g/day	% TEI	% TEI	% TEI	% TEI
Total fat	**Infants (Boys)**					
	0 – 2 months	21 – 31	40 – 60	50 – 60	40 – 60	30 – 40
	3 – 5 months	24 – 36	40 – 60	50 – 60	40 – 60	30 – 40
	6 – 8 months	21 – 28	30 – 40	30 – 40	~ 35	30 – 40
	9 – 11 months	24 – 32	30 – 40	30 – 40	~ 35	30 – 40
	Infants (Girls)					
	0 – 2 months	19 – 28	40 – 60	50 – 60	40 – 60	30 – 40
	3 – 5 months	22 – 33	40 – 60	50 – 60	40 – 60	30 – 40
	6 – 8 months	19 – 25	30 – 40	30 – 40	~ 35	30 – 40
	9 – 11 months	22 – 29	30 – 40	30 – 40	~ 35	30 – 40
	Children (Boys)					
	1 – 3 years	27 – 38	25 – 35	25 – 35	25 – 35	30 – 40
	4 – 6 years	36 – 51	25 – 35	20 – 30	25 – 35	25 – 35
	7 – 9 years	49 – 68	25 – 35	20 – 30	25 – 35	25 – 35
	Children (Girls)					
	1 – 3 years	25 – 35	25 – 35	25 – 35	25 – 35	30 – 40
	4 – 6 years	34 – 47	25 – 35	20 – 30	25 – 35	25 – 35
	7 – 9 years	45 – 63	25 – 35	20 – 30	25 – 35	25 – 35
	Adolescents (Boys)					
	10 – 12 years	54 – 75	25 – 35	20 – 30	25 – 35	25 – 35
	13 – 15 years	61 – 86	25 – 35	20 – 30	25 – 35	25 – 35
	16 – 18 years	65 – 91	25 – 35	20 – 30	25 – 35	25 – 35
	Adolescents (Girls)					
	10 – 12 years	48 – 67	25 – 35	20 – 30	25 – 35	25 – 35
	13 – 15 years	50 – 70	25 – 35	20 – 30	25 – 35	25 – 35
	16 – 18 years	53 – 74	25 – 35	20 – 30	25 – 35	25 – 35
	Adults (Men)					
	19 – 29 years	62 – 75	25 – 30 [c]	20 – 30	20 – 35	20 – 35
	30 – 59 years	61 – 73	25 – 30 [c]	20 – 30	20 – 35	20 – 35
	≥ 60 years	56 – 68	25 – 30	20 – 30	20 – 35	20 – 35

Nutrient	Age /Life stage group	Malaysia (2017)		Malaysia (2005)[a]	FAO (2010)	IOM (2006)[b]
		g/day	% TEI	% TEI	% TEI	% TEI
	Adults (Women)					
	19 – 29 years	51 – 61	25 – 30 [c]	20 – 30	20 – 35	20 – 35
	30 – 59 years	53 – 63	25 – 30 [c]	20 – 30	20 – 35	20 – 35
	≥ 60 years	49 – 59	25 – 30	20 – 30	20 – 35	20 – 35
	Pregnancy					
	1st trimester	54 – 65	25 – 30 [c]	20 – 30	20 – 35	20 – 30
	2nd trimester	60 – 71	25 – 30 [c]	20 – 30	20 – 35	20 – 35
	3rd trimester	65 – 78	25 – 30 [c]	20 – 30	20 – 35	20 – 35
	Lactation					
	1st 6 months	66 – 79	25 – 30 [c]	20 – 30	20 – 35	20 – 35
n-6 PUFA (linoleic acid)	General population	-	3.0 – 7.0	3.0 – 7.0	2.5 – 9.0	5 – 10
	Pregnancy	-	5.0 – 7.0	5.0 – 7.0	-	-
	Lactation	-	5.0 – 7.0	5.0 – 7.0	-	-
n-3 PUFA (ALA+EPA+ DHA)	General population	-	0.3 – 1.2	0.3 – 1.2	0.5 – 2.0	0.6 – 1.2
Saturated fatty acids		-	< 10	< 10	10	Low
Monounsaturated fatty acids		-	12 – 15	12 – 15	By diff.	-
Trans fatty acids		-	< 1	< 1	< 1	Low

[a] NCCFN (2005); [b] Otten *et al.* (2006); [c] for active men and women with normal BMI up to 35% TEI from fat is a safe limit

4 • Carbohydrate

4.1 Introduction

Carbohydrates may be classified according to their degree of polymerisation (DP) and may be divided into three principal groups i.e. sugars (DP 1-2; monosaccharides, disaccharides, polyols), oligosaccharides (DP 3-9; malto-oligosaccharides, other oligosaccharides) and polysaccharides (DP ≥10; starch, non-starch polysaccharides). Each of these three groups may be further subdivided on the basis of the monosaccharide composition of the individual carbohydrates. The FAO/WHO Consultation (1998) pointed out that there could be several problems associated with the use of the term "total carbohydrates" in most food composition tables. Firstly the figures given are obtained "by difference" and are therefore not very accurate. Moreover, a single global figure for carbohydrates in food was felt to be uninformative because it fails to identify the major types of carbohydrates in a food and thus to allow some understanding of the potential physiological properties of these carbohydrates.

With regard to the terms available and unavailable carbohydrates, the FAO/WHO Consultation suggested that in view of the improved understanding of carbohydrate physiology, a more appropriate substitute for these terms would be to describe carbohydrates as glycaemic (i.e. providing carbohydrate for metabolism) or non-glycaemic. The Consultation also suggested the use of the term "complex carbohydrate" to substitute starch is inappropriate and it is better to discuss carbohydrate components by using their common chemical names.

The FAO/WHO Scientific Update in 2006 still recommended the main classification based on chemistry division, whilst recognizing that the classification should also include aspects of physical effects (food matrix), functional/ physiological effects and health consequences (Cummings & Stephen, 2007). This is because chemistry divisions of carbohydrate are complex and does not provide practical basis into nutritional effects, and thus lead to numerous terms being employed.

Meanwhile, European Food Safety Authority (EFSA, 2010) and Scientific Advisory Committee on Nutrition of United Kingdom (SACN, 2015) suggested to classify carbohydrates based on their nutritional properties rather than their chemical characteristics: glycaemic carbohydrate as "carbohydrates digested and absorbed in the human small intestine".

Sugars are also part of carbohydrates. The term sugars is conventionally used to describe mono- and di-saccharides (FAO/WHO, 1998). "Free sugars" is a new term introduced by WHO and refers to all monosaccharides and disacharides added to foods and beverages by the manufacturer, cook or consumer, as well as to sugars naturally present in honey, syrups and fruit juices concentrates. However, free sugar does not include sugars naturally present in milk (lactose), whole fruit (fructose, glucose) and vegetables (fructose, sucrose) as there is no report of adverse effect (WHO, 2015). According to Van Horn *et al.* (2010), the American Heart Association (AHA) and SACN (2015) uses the term "added sugars" to refer to sugars and syrup added to foods during processing or preparation, and sugars and syrups added at the table. Therefore, based on the definitions used by WHO (2015) and by SACN (2015), free sugars and added sugars turn out to be the same terminology.

The main components of dietary fibre are derived from the cell walls of plant material in the diet and comprise cellulose, hemicellulose and pectin (the non-starch polysaccharides). Lignin, a non-carbohydrate component of the cell wall is also often included. The concept of dietary fibre has changed over the years. Fibre was originally described as plant cell wall material which simply passed through the gut unchanged and provided bulk to faeces. There is currently no consensus as to which components of carbohydrates should be included as dietary fibre. The American Association of Cereal Chemists AACC (2001) adopted the definition of dietary fibre as the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine.

Dietary fibre has been revised and defined differently by different organizations during the last decade. In 2011, AACC continued to adopt the definition of dietary fibre they had been defined in 2001. Another definition of dietary fibre is that fraction of the edible part of plants or their extracts, or synthetic analogue that (i) are resistant to the digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine; and (ii) promote one or more of the benefit physiological effects which is laxation, reduction in blood cholesterol and/or modulation of blood glucose (FSANZ, 2006).

In Malaysia, dietary fibre means carbohydrate polymers with three or more monomeric units which are not hydrolysed by the endogenous enzyme in the small intestines of human and belong to the following categories:

i) Edible carbohydrate polymers naturally occuring in the food as consumed,

ii) Carbohydrate polymer, which have been obtained from raw food material by physical, enzymatic or chemical means and which have been shown to have physiological effect that benefits health as demonstrated by generally accepted scientific evidence to competent authorities,

iii) Synthetic carbohydrate polymers which have been shown to have physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

It is to be noted that when derived from the plant origin, dietary fibre may include fraction of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for dietary fibre. However, such compounds are not included in the definition of dietary fibre if extracted and reintroduced into a food. The above definition of dietary fibre is based on that of Codex Alimentarius (2016) and steps shall be taken to amend the Malaysian Food Regulations (1985), accordingly.

4.2 Functions

Total carbohydrates

Carbohydrates are an important source of energy in human diets comprising some 40 - 80% of total energy intake (TEI). There are several reasons why it is desirable that carbohydrates should provide the main source of energy (FAO/WHO, 1998). In addition to providing easily available energy for oxidative metabolism, carbohydrate-containing foods are vehicles for important micronutrients and phytochemicals. Dietary carbohydrate is important to maintain glycaemic homeostatis and for gastrointestinal integrity and function.

It is important to note that the nature of dietary carbohydrate is more critical on health effects rather than the quantity of total energy obtained from carbohydrate intake (Mann *et al.,* 2007). There is evidence that sugar-sweetened beverages do not induce satiety to the same extent as solid forms of carbohydrate and increasing the consumption of sugar-sweetened beverages induce weight gain.

Carbohydrate-containing foods such as wholegrains, legumes, vegetables and whole fruits are recognized to be able to reduce the risk of diabetes and cardiovascular disease (Mann *et al.,* 2007). However, Scientific Advisory Committee on Nutrition (SACN, 2015) emphasized that the effects on health mainly depends on specific components of carbohydrates. For example, excess sugar-sweetened beverage is associated with greater risk of developing type-2 diabetes mellitus, weight gain and body mass index. Jakobsen *et al.,* (2010) conducted a longitudinal observational study among 5000 Danish subjects over 12 years. It was acknowledged that substituting saturated fat with carbohydrates will reduce cardiovascular risk only if these carbohydrates have low glycaemic index. This study also concluded that high glycaemic index carbohydrates remarkably increase the risk of myocardial infarction. Diet higher in glycaemic index or glycaemic load is associated with higher risk of type-2 diabetes mellitus. There is no evidence from prospective cohort studies on association between glycaemic index and cardiovascular disease or coronary heart disease. However, there is association between glycaemic load with greater risk of cardiovascular disease, but only reported in a small number of studies (SACN, 2015).

Sugars

Free sugars contribute to the overall energy density of diets, and may promote a positive energy balance. Sustaining energy balance is critical to maintaining healthy body weight and ensuring optimal nutrient intake (FAO, 2010). There is increasing concern that intake of free sugars - particularly in the form of sugar-sweetened beverages - increases overall energy intake and may reduce the intake of foods containing more nutritionally adequate calories, leading to an unhealthy diet, weight gain and increased risk of NCDs (Hauner *et al.,* 2012, Malik *et al.,* 2013). Another concern is the association between intake of free sugars and dental caries, which has received increasing interest in recent years (Sheiham & James, 2014).

Dietary fibre

Dietary fibre is not categorised as an essential nutrient. However, over the years, it has become recognised as an important component of a healthy diet and plays important roles in health and disease. Evidence from prospective cohort studies have shown that dietary fibre are asociated with lower risk of cardio-metabolic diseases and colorectal cancer (SACN, 2015).

Dietary fibre is also known to be an important moderator of digestion in the small bowel and insufficient intake from the diet can result in inadequate faecal bulk and may affect overall health. It is a major substrate for fermentation in the colon, where non-starch polysaccharides of the plant cell wall are metabolised to short-chain fatty acids. Absorption of the latter provides some energy. In addition, it has been shown that other carbohydrates are present in the diet which enter the colon and are fermented, including resistant starch and non-digestible oligosaccharides.

Recent evidence indicated that naturally-occurring dietary fibre which can be found in whole grains, legumes, vegetables and whole fruits have protective effect against cardiovascular disease and type-2 diabetes mellitus, although there were some inconsistencies in study results. The EFSA Panel noted that consumption of diets rich in fibre-containing foods greater than 25g per day give health benefits, such as reduced risk of coronary heart disease and type-2 diabetes, and weight maintenance among adults (EFSA, 2010).

The Dietary Guidelines Advisory Committee (USDA, 2015a) in their conclusion statements described the dietary patterns associated with beneficial health outcomes. They concluded that there is strong evidence for reduce cardiovascular risk with dietary patterns characterised by higher consumption of vegetables, fruits, whole grains, regular consumption of nuts and legumes and diets that are richer in fibre. There was moderate evidence for reduce risk of overweight and obesity, type-2 diabetes and cancer in dietary patterns that are higher in vegetables, fruits and whole grains. These statements about healthy dietary patterns indicate the importance of foods that are rich in dietary fibre for prevention of diet-related chronic diseases.

4.3 Metabolism

Glucose plays the main role in carbohydrate metabolism. Every body cells depends on glucose for its fuel especially the cells of the brain and the rest of the nervous system. The breakdown of glucose begins with glycolysis, a pathway that produces pyruvate. Pyruvate may be converted to lactate anaerobically or to acetyl CoA aerobically. Once the commitment to acetyl CoA is made, glucose is not retrievable. As carbohydrate is known to be a chief energy nutrient, its metabolism is described by storing glucose as glycogen, using glucose for energy, making glucose from protein which is called gluconeogenesis, making ketone bodies from fat fragments when there is inadequate supply of carbohydrate and in contrary or when carbohydrate is abundant, the body will be using glucose to make fat (Rolfes *et al.,* 2009).

4.4 Sources

Food balance sheet data (FAOSTAT 2013) over the years have shown that the major sources of carbohydrates in the human diet are cereals, root crops, sugar crops, pulses, vegetables, fruits and milk products. In the Malaysian diet, starch is naturally abundant in grains and vegetables such as rice, wheat, maize, barley, cassava, potatoes and sweet potatoes. Natural sugars are found in fruits and juices. Sources of added sugars in diets are carbonated drinks, fruit juices/drinks, desserts, cakes, biscuits and candies. Wholegrains, legumes, vegetables and whole fruits are the most appropriate sources of carbohydrates.

Carbohydrates serve as the main source of energy for many communities. However, there has been a fall in carbohydrate intake in many countries. In Malaysia, the trend for carbohydrate intake after a decade has declined from 220g in 2003 to 195g in 2014 among adults as reported by Wan Shakira *et al.* (2015). Wan Nazaimoon *et al.* (2011) reported that the prevalence of obesity in Malaysia kept increasing over the years. Despite the consumption of carbohydrate within the recommendation, the increasing prevalence of obesity may be because most of the carbohydrate food choices are high in sugar. This is supported by latest report showing the consumption of sugar/sweetened foods was quite high. From the latest Malaysian Adult Nutrition Survey (2014), the 20 to 29 years age group, Bumiputera Sarawak government and semi-government staffs have notably higher intake of carbohydrates (median = 200g, 212g, 203g, respectively) compared with other groups. From the analysis of food balance sheet data over the past three decades, the intake of carbohydrates, notably cereals, has declined from 60% in the 1960s to 40% in the late 1990s (Tee, 1999). There has also been a clear decline in the proportion of energy from carbohydrate from 72% in the 1960s to about 60% in the 1990s. Within these national averages, there is considerable variations in between rural and urban population groups.

Based on food balance sheet data (FAOSTAT 2013), per capita supply of sugar (from sugar crops comprising cane and beet sugar, and sugar and sweeteners comprising raw sugar, honey, other sweeteners) available for consumption in Malaysia increased from 297 kcal/day in 2005 to 385 kcal/day in 2009, contributing 10.5 and 13.3 percent, respectively, of total available calories for these two periods (Amarra *et al.,* 2016).

Meanwhile, Malaysian Adults Nutrition Survey 2003 (MANS), reported that sugar-containing foods that contributed most to the energy intakes of Malaysian adults were beverages to which sugar is added during preparation such as cordial syrup, tea, coffee, chocolate flavoured beverages, condensed milk added to beverages and local kuih (starchy traditional cakes). Less than 1.2% of the daily caloric intake was obtained from jam, carbonated drinks, and "ABC ice" (shaved ice topped with syrup, nuts and beans) (Amarra *et al*., 2016).

Dietary fibre is present in majority of fruits, vegetables and refined grains (IOM, 2002). Nuts, legumes and high fibre grain typically contained more than 3% of dietary fibre. About a third of the fibre in legumes, nuts, fruits and vegetables is present as hemicellulose. Approximately one-fourth of the fibre in grains and fruits and one-third in nuts and vegetables consist of cellulose. Although fruits contain the greatest amount of pectin, 15-20% of pectin is found in legumes, nuts and vegetables.

4.5 Deficiencies and Excesses

Fats and amino acids (basic components of proteins) are utilized for energy when glucose production or availability decreases below that required for the complete energy requirements of the brain,. However, in order to provide the brain with an alternative fuel, this route of energy production results in a rise of ketoacid in the liver. This process is referred to as a ketosis. Generally this occurs in a starving person only after glycogen stores in the liver are reduced to a low concentration and the contribution of hepatic glycogenolysis is greatly reduced or absent. Prolonged deprivation leads to the symptoms and diseases connected with severe carbohydrate short fall (Veech, 2004).

FAO/WHO (1998) highlighted that the minimal amount of carbohydrate in the human diet that is needed to avoid ketosis is 50g/day in adults. However, there is increasing evidence in the literature, suggesting that very-low-carbohydrate ketogenic diets (usually less than 50g/day and a relative increase in the proportions of protein and fat) could have a therapeutic role in numerous diseases. The use of very low ketogenic diet in treating epilepsy has been well-established for many decades and these ketogenic diets are commonly considered to be a useful tool for weight control and many studies suggest that they could be more efficient than low-fat diets (Dashti *et al.,* 2004). There are new and exciting scenarios about the use of ketogenic diets in cancer, type-2 diabetes, polycystic ovary syndrome (PCOS), cardiovascular and neurological diseases.

There is no adverse effect of high intakes of unrefined complex carbohydrate. However, overconsumption of added sugars has been linked to risk of obesity. It is recommended that the dietary reference value for total carbohydrate intake should be approximately 50% of total dietary energy and the average population intake of free sugar should not exceed 5% of total dietary energy for age group from two years upwords (SACN, 2005).

Total carbohydrate intake appears to be neither detrimental nor beneficial to cardio-metabolic health, colorectal health and oral health. However, there are specific components or sources of carbohydrates which are associated with other beneficial or detrimental health effects. The hypothesis that diets higher in total carbohydrate cause weight gain is not supported by existing evidence (SACN, 2015). However, studies showed that consumption of sugar-sweetened beverages, as compared with non-calorically sweetened beverages among children and adolescent results in greater weight gain and increases in body mass index. Higher consumption of sugars and sugars-containing foods and beverages is associated with a greater risk of dental caries while studies indicate that greater consumption of sugar-sweetened beverages is associated with increased risk of type-2 diabetes mellitus (SACN, 2015).

There is no association between total starch intake and incidence of coronary events or type-2 diabetes mellitus or between the intake of refined grains and risk of type-2 diabetes mellitus. Consumption of brown rice is associated with reduction in risk of type 2 diabetes melllitus, but the evidence is limited. Studies indicate an association between greater consumption of white rice and increased risk of type-2 diabetes mellitus in the Asian population, specifically in Japan and China (SACN, 2015).

Dietary fibre can have variable compositions and therefore it is difficult to link a specific source of fibre with a particular adverse effect, especially when phytate is also present in the natural fibre source. It is concluded that as part of an overall healthy diet, a high intake of dietary fibre will not produce deleterious effects in healthy individuals. While occasional adverse gastrointestinal symptoms are observed when consuming some isolated or synthetic fibres, serious chronic adverse effects have not been observed. Due to the bulky nature of fibres, excess consumption is likely to be self- limiting. Therefore, a tolerable upper intake level (UL) was not set for individual functional fibres. Strong evidence that increased intake of total dietary fibre, and particularly cereal fibre and wholegrain, are associated with lower risk of cardio-metabolic disease and colo-rectal cancer. Higher intake of oat bran and isolated β-glucans leads to lower total cholesterol, LDL cholesterol ang tricyglycerol concentration and lower blood preasure (SACN, 2015).

4.6 Factors affecting requirements of carbohydrate

The minimal amount of carbohydrate required, either from endogenous or exogenous sources, is determined by the brain's requirement for glucose (IOM, 2002). The brain is the only true carbohydrate-dependent organ in that it oxidises glucose completely to carbon dioxide and water. Normally the brain uses glucose almost exclusively for its energy needs. The endogenous glucose production rate in a post-absorptive state correlates very well with the estimated size of the brain from birth to adult life. However, not all of the glucose produced is utilised by the brain. The requirement for carbohydrate has been reported to be 130 g/day in adults (IOM, 2002/2005). However, the brain can adapt to a carbohydrate-free, energy sufficient diet, or to starvation, by utilising ketoacids for part of its fuel requirements.

Carbohydrate is a key fuel source for exercise, especially during prolonged continuous or high-intensity exercise. Carbohydrate requirements are dependent on the fuel needs of the athlete's training and competition program. It is recommended to take carbohydrate in range of 6-10g/kg of body weight depending on sex and physical fitness of the individual, total training load, energy expenditure, type of physical activity and environment (Hassapidou, 2011).

Athletes seem to benefit from 200 to 300 grams of carbohydrates consumed 3-4 hours before a sports event. During exercise, athletes should consume 30-60 grams of carbohydrates per hour (or 0.7g/kg of body weight) in order to maintain blood glucose levels. This is very important when the event lasts more than an hour and takes place in extreme environmental conditions (e.g. cold, heat or high altitude). After exercise, athletes should consume 1.0-1.5g/kg of body weight during the first half hour and again every 2 hour for 4-6 hours in order to replace liver and muscle glycogen stores (Hassapidou, 2011).

The available evidence based on majority of studies on the relationship of dietary fibre to gastrointestinal health, several chronic diseases (such as colon cancer, breast cancer), glucose tolerance, insulin response as well as weight control and maintenance, indicated that the beneficial effects of fibre in humans are most likely related to the amount of food consumed but not the individual's age or body weight (IOM, 2002).

4.7 Setting requirements and recommended intakes of carbohydrate

Total carbohydrates

Acceptable ranges of intake for each of these energy sources were set based on a growing body of evidence that shows that macronutrients play a role in the risk of chronic disease. It is defined as a range of intake for a particular energy source that is associated with reduced risk of chronic diseases, such as coronary heart disease (CHD), obesity, diabetes and/or cancer, while providing adequate intakes of essential nutrients. These ranges are also based on adequate energy intake and physical activity to maintain energy balance. For example, studies have shown a connection between low-fat and high-carbohydrate diets has decreased high density lipoprotein (HDL) cholesterol in the bloodstream, an indicator associated with increased risk of CHD. Conversely, diets too high in fat may result in increased energy and saturated fat intake, and therefore lead to increased risk of obesity and its complications, such as CHD (IOM 2006).

FAO/WHO Scientific Update (2007) acknowledged the need to re-evaluate the current carbohydrate intake range (55-75% of total energy) as the rationalization for recommended lower limit is still lacking. The Scientific Update also suggested the revision using lower limit of 50% total energy was used, similar as recommendation from Scientific Advisory Committee on Nutrition (2015). This range was based on the remaining percentages of protein energy (10-15%) and fat energy (15-30%). Carbohydrate intake, in particular, was set at 50 to 70% of total energy. A daily minimum intake of 400g of vegetables and fruits, including at least 30g of pulses, nuts and seeds, should meet this recommendation.

The IOM (2002) report indicated that the RDA for carbohydrate is based on the average minimum amount of glucose that would provide the brain with an adequate supply of glucose fuel without the requirement for additional glucose production from ingested protein or triacylglycerols, which is set at 130g/day for adults and children. The median intake of carbohydrate has been derived from data from the Continuing Food Survey of Intakes by Individuals (CSFII) in 1994-1996 and 1998 i.e. 200g to 330 g/day for men and 180g to 230 g/day for women. This represents 45% to 65% of energy sufficient diet containing an Acceptable Macronutrient Distribution Range of carbohydrate intake. Food Standard Australia New Zealand (FSANZ, 2006) has also set the range of carbohydrate to be between 45% to 65% of energy (predominantly from low energy density and/or low glycaemic index foods). The upper bound carbohydrate recommendations were set so as to accommodate the essential requirements for fat (20%) and protein (15%) and taking into account that the types of carbohydrates consumed are of paramount importance in relation to their health effects.

The Technical Sub-Committee (TSC) on Energy and Macronutrients considered these various recommendations including revising the previous RNI Malaysia (2005) carbohydrate recommendation of 55-70% TEI and adopting the term free sugars from WHO (2015). It was felt that the appropriate proportion of energy from carbohydrate should be 5% lower from the previous RNI Malaysia (2005) due to reduction of free sugars recommendation from 15% to 10% of total carbohydrate. The TSC recommended that in the revised RNI, carbohydrate should comprise 50-65% TEI. This decision also takes into consideration the proportion of energy contributed by protein and fat, described in chapters 2 and 3 of this monograph.

Sugars

The population nutrient intake goals of WHO (2003) for the prevention of diet-related chronic diseases has recommended that not more than 10% of total energy should be from free sugars. WHO (2015) and Scientific Advisory Committee on Nutrition (2015) also recommended a further reduction of free sugars to 5% as there is no harm to further limit free sugars intake. The DRI committee of IOM (2002) has recommended an upper limit of 25% of total energy for sugar intake.

Based on food balance sheet data for Malaysia, the available sugars in the country was estimated to be about 86 g/day or 13% of total energy in 1985. This was found to have increased to 104 g/day or 14% of total energy in 2002 (FAO, 1985; 2002). Based on Food Balance Sheets, it was showed that the amount of available sugar and sweeteners (kg per capita per year) has increased almost 70% (from 28.8kg to 48.7kg) between year 1967 to 2007 and Malaysia ranks second (48.7kg per capita per year) only to United States (67.6kg per capita per year) as countries with most sugar and sweeteners availability (Khor, 2012). Study conducted by Nik Shanita *et al.* (2012) concluded that mean intake of added sugar of adults in Klang Valley was approximately 9 teaspoons or 45.5±28.8 g/day. In addition, latest Malaysian Adults Nutrition Survey in 2014 deduced that sugar (white, brown and Melaka) is one of the top five foods consumed daily (55.9%), especially by adults from urban areas. The survey also concluded that the consumption of sugar and sugar-based foods contributed to at least 4 food items in a day (≈ 6.5 times/ day).

However, a review by Amarra *et al.,* (2016) showed that there is insufficient evidence to allow an estimation of intake levels of added sugar among different age groups in Malaysia, and to identify major sources of added sugar. National level data obtained from MANS in 2003 is outdated. While, data from MANS (2014) only indicated the list of commonly eaten foods and drinks containing sugar consumed by Malaysian adults (Table 4.1) but not the estimation of total sugar intake, hence another national assessment of sugar intake is needed.

Table 4.1 Some commonly eaten foods and drinks containing sugar consumed by Malaysians

Food items	(%)
Local kuih	79.9
Tea	70.4
Malted drink	59.1
Condensed milk	51.3
Carbonated drinks	45.7
Cake	38.5
Kaya	35.3
Cordial syrup	34.4
Ready-to-drink beverage	30.8
Pre-mixed drink	28.8
Ice blended	25.8
Jelly/ custard	18.4
Yoghurt drinks	14.0
Energy drinks	12.6

Source: Institute for Public Health (2014)

A high level of free sugars intake is of concern because of its association with poor dietary quality, obesity and risk of NCDs (WHO, 2014). However, WHO (2015) in their Guidelines Sugars Intake For Adults and Children, agreed that excess weight gain and dental caries should be the key outcomes of concern in relation to free sugars intake. Risk of developing type 2 diabetes and CVD is often mediated through the effects of overweight and obesity, among other risk factors. Therefore, measures aimed at reducing overweight and obesity are likely to also reduce the risk of developing type 2 diabetes and CVD, and the complications associated with those diseases (WHO, 2015).

Fructose or fruit sugar is a simple monosaccharide found in many plants and honey. It can be found in its monosaccharide form or can be bound to glucose with a disaccharide bond in sucrose. Fructose is one of the three dietary monosaccharides, along with glucose and galactose. The primary dietary sources of fructose are high-fructose corn syrup and sucrose (cane or beet sugar) because both are commonly used to sweeten beverages and processed foods. Fructose is sugar that is naturally present in fruits and vegetables (Mintz, 1985)

Tappy & Lê (2015) in their review of health effect of fructose and FCCS, concluded that a high-fructose diet can increase blood triglyceride, alter hepatic glucose output and increase uric acid concentratrations. Whether these effects are associated with increase risk of metabolic or cardiovascular disease independently of an increase in body fat mass remains debatable. Tappy & Lê (2015) also highlighted that epidemiological prospective studies show a strong association between fructose-containing caloric sweeteners (FCCS) intake and body weight

gain. Furthermore, FCCS consumption is also associated with the incidence of dyslipidemia, insulin resistance, and type-2 diabetes, incidence or risk factors for cardiovascular diseases, cardiovascular mortality, chronic kidney diseases, hyperuricemia and gout. In human studies, fructose is associated with increasing hepatic fat, inflammation, and possibly fibrosis (Vos & Lavine, 2013).

High fructose intake has been suggested to be a key factor that induces non-alcoholic fatty liver disease (NAFLD), but the evidence from large epidemiologic studies is lacking. However, the results of a cross-sectional study among older Finnish adults showed that high intake of fructose is not associated with a higher prevalence of NAFLD as assessed by using the Fatty Liver Index and NAFLD liver fat score (Kanerva *et al.,* 2014).

In both adults and children, WHO (2015) strongly recommended reducing the intake of free sugars to less than 10% of total energy intake. These recommendations were based on the totality of evidence reviewed regarding the relationship between free sugars intake with body weight and dental caries. WHO suggested a conditional recommendation for a further reduction of the intake of free sugars to below 5% of total energy intake. However, based on limited studies involving relatively small samples, the intake of added sugar of Malaysian adults and children appears to exceed 10% of total calories. This exceeds the 2015 sugar intake recommendation by WHO, which indeed is advocating for a further reduction in the intake of free sugars to below 5% of total energy intake (Amarra *et al.,* 2016).

The TSC on Energy and Macronutrients considered the recommendations of WHO and IOM and the local dietary pattern and recommends that intake of free sugar should be less than 10% of total energy intake. This is felt to be a realistic figure and appropriate advice for the local population based on limited data on sugar intake of the population.

Glycaemic response, glycaemic index and glycaemic load

Carbohydrate-containing foods have a wide range of effects on blood glucose concentration during the course of digestion (glycaemic response). A significant body of data suggests that more slowly absorbed starchy foods that are less processed, may have health advantages over those that are rapidly digested and absorbed (IOM, 2006).

Glycaemic index (GI) has been proposed as a method to classify foods based on their blood glucose-raising potential. It is defined as the incremental area under the blood glucose response curve of a 50g of carbohydrate portion of a test food expressed as a percentage of the response to the same amount of carbohydrate from a standard or reference food (white bread or glucose) taken by the same subject (FAO/WHO, 1998).

Atkinson *et al.,* (2008) have compiled the average GI of 62 common foods derived from multiple studies. Soy beans have the lowest value of GI (16±1) and rice crackers/ crisp has the highest value (87±2). The GI of the standard food is expressed as 100. According to International Standard ISO 26642:2010 (2010), GI can be sorted into three groups: a GI value below 55 is defined as low, 56 to 69 as moderate and 70 and above as high. The Consultation report also explained how GI can also be applied to mixed meals or whole diets by calculating the weighted GI value of the meal or diet.

The concept of glycaemic load (GL) stresses the fact that the amount of carbohydrate in a food is important in determining fasting triglyceride and the post-prandial plasma glucose response (Table 2). The GL, the product of the food carbohydrate content by its GI, divided by 100 (GL=GI/100 x carbohydrate content) is a measure that incorporates both the amount and quality of dietary carbohydrate. According to Venn & Green (2007), GL value can be classified as low (≤10), moderate (10-19) and high (≥20). The GL of a specific food serves as a basis to evaluate the total GL of the diet. Hence, a food with very high GI but with only a small amount of carbohydrate will have a small GL. Dietary GI and GL have relatively predictable effects on circulating glucose, hemoglobin A1c, insulin, triacylglycerol, high density lipoprotein (HDL) cholesterol, and urinary C-peptide concentrations. As such, it is theoretically plausible to expect a low GI diet to reduce risk of type-2 diabetes and cardiovascular disease. However, the sufficient evidence needed to recommend substantial dietary changes based on GI is not available (IOM, 2006).

Table 4.2 Food factors influencing glycaemic responses

- **Amount of carbohydrate**
- **Nature of monosaccharide components**
  - Glucose
  - Fructose
  - Galactose
- **Nature of starch**
  - Amylose
  - Amylopectin
  - Starch-nutrient interaction
  - Resistant starch
- **Cooking/ food processing**
  - Degree of starch gelatinization
  - Particle size
  - Food form
  - Cellular structure
- **Other food components**
  - Fat and protein
  - Dietary fibre
  - Antinutrients
  - Organic acids

Source: FAO/WHO (1998)

In Malaysia, research into glycaemic response of foods is still at its infancy. However, interest in the subject has been increasing. Some data on GI values of frequently consumed Malaysian foods are given in Appendix 4.1.

There are a number of longer-term implications of altering the rate of absorption, or GI, of dietary carbohydrate (FAO/WHO, 1998). Reducing diet GI, for example, has been shown to improve overall blood glucose control in subjects with diabetes and reduce serum triglycerides in subjects with hypertriglyceridemia. There is some evidence that the GI is relevant for sports nutrition and appetite regulation.

Used in conjunction with information about food composition, GI has thus been proposed to guide consumers in food choices. The practical application of GI has however been the subject of much controversy. Its practical use worldwide has also been limited to a few countries. The practical use of GI of single food items has been particularly doubtful because glycaemic responses to foods are influenced by many factors including its carbohydrate content and the other food components present and even cooking or food processing methods (FAO/WHO, 1998) (Table 4.2). Some low GI foods may not always be a good choice because they are high in fat. Conversely, some high GI foods may be a good choice because of convenience or because they have low energy and high nutrient content. It is not necessary or desirable to exclude or avoid all high GI foods.

The TSC on Energy and Macronutrients, therefore, has no definite recommendations on the use of GI or GL at this time. It, however, recommends research and practitioner groups in the country to continue to monitor global developments on the matter and to actively research the subject. Therefore, the FAO/WHO Scientific Update (2006) recommended caution regarding the use of GI as only basis in choosing carbohydrate-containing foods.

Dietary fibre

There is no biochemical assay that reflects the dietary fibre status of an individual. Clearly, one cannot measure blood fibre concentration since, by definition, fibre is not absorbed. Hence, the DRI Committee of IOM (2002) had reviewed the potential health benefits of fibre consumption, which may be compromised by lack of fibre in the diet. These include a number of epidemiological studies conducted to evaluate the relationship between fibre intake and risk of chronic diseases. The Committee recommended an adequate intake ranging from 19-25g/day of total fibre for young children whereas intakes for adolescents range from 26-38g/day, the lower figures being for girls. Adult intakes are recommended to be 25g/day for women and 38g/day for men. Intakes for adults more than 51 years are 20% lower whilst for pregnant and lactating women, 12% higher.

The American Dietetic Association (ADA, 2002) has recommended intakes that are slightly lower than those of IOM, i.e. 20-35g dietary fibre/day or 10-13g per 1,000 kcal for adults. Although recognising the lack of clinical data, the ADA recommends that for children older than 2 years, the dietary fibre intake should be equal to or greater than their age plus 5 g/day.

The nutrient intake goals recommended by WHO (2003) for the prevention of diet-related chronic diseases has indicated a total dietary fibre intake of >25g per day whereas non-starch polysaccharides (NSP) intake is recommended to be >20g per day. The report further recommends that whole grain cereals, fruits and vegetables are the preferred sources of NSP.

Upon reviewing all available information, the TSC for Energy and Macronutrients decided to maintain the RNI (2005) recommendation of 20-30 g of dietary fibre per day for all age groups. This is largely based on the range recommended by ADA (2002) 20-35g and WHO (2003) >25g per day. Greater efforts have to be made to encourage Malaysians to consume a wide variety of plant foods in order to meet the recommendations.

Discussion on revised RNI for Malaysia

The recommended intake of total carbohydrate, sugars and dietary fibre for Malaysia are compared with that of the Malaysian RNI (2005), WHO (2015) and SACN (2015) in Appendix 4.1

The revised recommendations for total carbohydrate and free sugars are lower by 5% compared to the Malaysian RNI (2005). This is justifiable as new evidence has surfaced that a high level of sugar intake is associated with poor dietary quality, obesity and risk of NCDs. Recommendation for dietary fibre remains the same as the Malaysian RNI (2005).

4.8 Research recommendations

The following priority areas of research are recommended:

- Improved methodologies for the measurement of carbohydrates and their components in foods.
- Improved national food composition table with data on types of carbohydrates (starch, total and individual sugars).
- Studies related to glycaemic response (including glycaemic index and glycaemic load) of carbohydrate-containing food based on local foods.
- Data on the content of dietary fibre in local foods should be obtained for inclusion in the national food composition database.
- Systematic reviews and meta-analyses relating free sugars intake to blood lipid levels, blood pressure and diabetes-related outcomes (i.e. glucose, insulin, metabolic syndrome, prediabetes and insulin resistance).
- Evaluation of different behavioural-change approaches to promote the reduction of free sugars intake; in particular, the intake of sugar-sweetened beverages, which is identified as a behavioural risk factor contributing to calorie over-consumption, especially among children.

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Appendix 4.1 Comparison of recommended intake of carbohydrate and its component: RNI Malaysia (2017), RNI Malaysia (2005), WHO (2015) and SACN (2015)

Food items	Malaysia (2017)	Malaysia (2005)	WHO (2015)	SACN (2015)
	% of total energy intake	% of total energy intake		
Total carbohydrate	50 - 65	55 - 70	55 - 75	50
Sugars	< 10[#]	< 15	< 10[#]	< 5 *
Dietary fibre	20 -30 g	20 -30 g	25 g	15 - 30 g

[#] Free sugars; *Added sugars

Appendix 4.2: Glycaemic index value of selected Malaysian foods

Food/ Drink	GI	GI Indicator
Fried meehoon*	99	High
Fragrant rice*	97	
Rice *(beras kilang)*+	90	
Noodle rice *(kueh-teow)* +	85	
Wholemeal bread*	85	
White bread*	83	
Pineapple*	82	
Fragrant rice*	80	
Pau kari ayam*	80	
Teh tarik *	78	
Sweet potato +	77	
Fried macaroni*	74	
Sardine sandwich*	73	
Roti canai & dhal*	71	
Nasi lemak*	66	Moderate
Herba ponni rice*	65	
White bread with margarine & sugar spread*	64	
Brown rice*	60	

Food/ Drink	GI	GI Indicator
Fried rice*	59	
Papaya*	58	
Doughnut*	57	
Multi-grains breads*	56	
Pau biji lotus	55	Low
White bread with pineapple jam*	55	
Banana (pisang berangan) +	55	
Watermelon*	55	
Currypuff*	54	
White bread with peanut butter spread*	54	
Pau kacang merah*	51	
Durian*	49	

*glucose was used as the reference food; + White bread was used as the reference food
(Source: Nik Shanita, 2004; Lee MC, 2005; Lee SY, 2005; Nor Muaiza, 2005; Barakatun Nisak *et al.,* 2005; Robert *et al.,* 2008; Barakatun Nisak *et al.,* 2009) GI Indicator: Brand-Miller JB, Foster-Powell K & Colagiuri S (1996)

Appendix 4.3 : Types of food sources with fibre content per 100 grams

Food	Dietary Fibre per 100 grams (g)
High fibre bran (ready-to-eat cereal)	29.3-47.5
French beans (cooked)	9.4
Chick peas (canned)	6.4
Lentils (cooked)	7.9
Mung beans (cooked)	7.6
Black beans	8.7
Kidney beans, cooked	6.4
Pear, raw	3.1
Pumpkin seeds, roasted	18.4
Baked beans, canned, plain	6.0
Soybean	6.0
Avocado	6.7
Apple with skin	2.4

Food	Dietary Fibre per 100 grams (g)
Green peas, cooked	4.1-5.5
Prune (stewed)	3.1
Sweet potato, baked with skin	3.3
Figs (dried)	9.8
Potato (baked with skin)	2.1
Almonds	12.5
Whole wheat spaghetti, cooked	4.5
Sunflower seeds kernel, dry roasted	11.1
Orange	2.2
Banana	2.6
Guava	5.4
Pearly barley, cooked	3.6
Winter squash (pumpkin), cooked	2.8
Dates	8.0
Pistachios, dry roasted	9.5
Peanuts, oil roasted	9.4
Whole wheat parata bread	9.5

Source: USDA, 2015b

Summary

Vitamins Recommendations

The Technical Sub-Committee (TSC) on Vitamins reviewed all the eight vitamins in RNI 2005 and agreed that they should be retained in the updated RNI, recognising their continued relevance. Several other vitamins were considered for addition to the list and four were agreed to be of importance to be included in the updated RNI, namely Vitamin K, Cobalamin, Pyridoxine and Panthothenic Acid. These are felt to be relevant and important for the Malaysian population. A total of 12 vitamins are therefore included in Malaysian RNI (2017).

The vitamins were assigned to members of the TSC to prepare write-ups. For the eight vitamins in RNI (2005), the writers reviewed the original write-up and determined if changes to the write-up are required or if new information need to be added. For the four additional vitamins, members were assigned to prepare new write-ups. For the preparation of these write-ups the TSC referred to several recent publications published after 2005 by reputable national and regional research organisations that generate primary data on recommended nutrient intakes. These include publications of the Institute of Medicine (IOM) and the European Food Safety Authority (EFSA). FAO/WHO did not have a revised RNI in recent years, but the TSC decided that the RNI (2004) of WHO/FAO remained as another main source of reference since it was prepared by an international group of experts appointed by these United Nations agencies.

Members of the TSC had agreed on the common approach towards deciding on the values to be adopted as Malaysian RNI (2017). For each vitamin, the rationale and the value recommended intake in the new references were considered. These were compared with the RNI 2005 values, which were adapted from WHO/FAO (2004). If the references provided more recent and better approaches to establishing recommended intake, especially those based on biomarkers, their recommended values were considered. The appropriateness of the recommended values in relation to the local situation were also considered in deciding if a recommended value needed to be amended. There was also agreement to have a uniform format for the write up for each of the vitamins, including the sub-headings, the depth and length of the write up.

In the final version of RNI (2017), for all the 8 vitamins in the RNI (2005), except for vitamin D, the TSC decided to retain the original values, ie adapting the values from WHO/FAO (2004). For vitamin D, the Committee decided to adapt the values from IOM (2011) in view of several recent reports on the unsatisfactory status of this vitamin among some population groups. These new values on vitamin D are generally 2-3 times higher than the 2005 values. For the three of the four new vitamins, the TSC felt that the WHO/FAO (2004) values recommended for vitamin K, pyridoxine and pantothenic acid are appropriate to be adapted for use in RNI (2017). For vitamin B12, the TSC adapted the EFSA (2015) values derived mainly based on appropriate biomarkers. These values are higher for all age groups compared with the WHO/FAO (2004) and IOM (1998) values which were based on dietary intake levels.

5 Thiamin (Vitamin B1)

5.1 Introduction

Thiamine or thiamin, also known as vitamin B1 or aneurin, is a colourless compound with the chemical formula C12H17N4OS. About 80% of the approximately 25-30 mg of thiamin in the adult human body is in the form of thiamin diphosphate (TDP; also, known as thiamin pyrophosphate-TPP), the main metabolically active form of thiamin. It is soluble in water and insoluble in alcohol. Thiamin decomposes if heated. Thiamin was first discovered by Umetaro Suzuki in Japan in 1910 when researching how rice bran cured patients of beri-beri. It was the first nutrient deficiency studied in Malaya in the beginning of the 20th century.

5.2 Functions

Thiamin functions as the co-enzyme thiamin pyrophosphate (TPP) in the metabolism of carbohydrates and branched-chain amino acids. TPP, coordinated through magnesium (Mg++), participates in two main types of metabolic reactions: (a) the formation of (α–ketols (e.g. among hexose and pentose phosphates) as catalysed by transketolase; and (b) in the oxidation of (α–keto acids (eg pyruvate, (αketoglutarate and branched-chain (αketo acids) by dehydrogenase complexes. Hence, thiamin deficiency will result in overall decrease in carbohydrate metabolism and its inter-connection with amino acid metabolism (via α–keto acids). Severe consequences can arise such as a decrease in the formation of acetylcholine for neural function. Thiamin is also essential for normal growth and development and helps to maintain proper functioning of the heart and the nervous and digestive systems. Thiamin cannot be stored in the body; however, once absorbed, the vitamin is concentrated in muscle tissue.

5.3 Metabolism

Thiamin is released by the action of phosphatase and pyrophosphatase in the upper small intestine. At low concentrations, the process is carrier-mediated, and, at higher concentrations, absorption occurs via passive diffusion. Active transport is greatest in the jejunum and ileum (it is inhibited by alcohol consumption and by folic deficiency). Decline in thiamin absorption occurs at intakes above 5 mg/day. The cells of the intestinal mucosa have thiamin pyrophosphokinase activity, but it is unclear as to whether the enzyme is linked to active absorption. The majority of thiamin present in the intestine is in the pyrophosphorylated form thiamin diphosphate (TDP), but when thiamin arrives on the serosal side of the intestine it is often in the free form. The uptake of thiamin by the mucosal cell is likely coupled in some way to its phosphorylation/dephosphorylation. On the serosal side of the intestine, evidence has shown that discharge of the vitamin by those cells is dependent on Na+ dependent ATPase.

Uptake of thiamin by cells of the blood and other tissues occurs via active transport and passive diffusion. The brain requires a much greater amount of thiamin than in other cells of the body. Much of ingested thiamin never reaches the brain because of passive diffusion and the blood brain barrier. About 80% of intracellular thiamin is phosphorylated and most is bound to proteins. In some tissues, thiamin uptake and secretion appears to be mediated by a soluble thiamin transporter that is dependent on Na+ and a transcellular proton gradient. Thiamin and its acid metabolites (2-methyl-4-amino-5-pyrimidine carboxylic acid, 4-methyl-thiazole-5-acetic acid, and thiamin acetic acid) are excreted principally in the urine.

5.4 Sources

Thiamin is found in a wide variety of foods of plant and animal origin. However, only a few foods, including yeast, lean pork and legumes can be considered as good sources of the vitamin (Tee *et al.,* 1997). Yeast contains an extraordinary amount of the vitamin; a commercial yeast extract has a content of up to 0.6 mg/100 g. Pork and pork products example lean pork, ham and sausage contain high concentrations of thiamin of close to 0.9 mg/100 g. Other meat products such as beef, chicken and duck have much lower amounts of the vitamin, generally about 0.1 mg/100 g. A commercial brand of beef extract has an exceptionally high level of thiamin (1.6 mg/100 g), but this is usually taken in small amounts. Fish and shell fish contain even less thiamin. Various types of legumes, example chickpea, dhal, green gram and red gram and soya bean contain thiamin ranging from 0.45mg to 1.7 mg/100 g bean (Table 5.1).

There are also several processed products in the market, especially bread, cereal products and biscuits, that are fortified or enriched with thiamin and several other B vitamins and can become important sources of the vitamin. Fruits are poor sources of thiamin, containing in general not more than 0.03 mg/100 g. Vegetables contain slightly more thiamin but are generally less than 0.1 mg/100 g and are therefore not good sources as well.

Table 5.1: Thiamin (Vitamin B1) content of foods

Food	mg/100 g
Cereals and cereal products and tubers	
Wheat germ	1.86
Cereals RTE (commercial brand)*	1.33
Cereals RTE rice (commercial brand)*	1.25
Wheatflour, wholemeal	0.75
Oat, rolled	0.46
Bread, white	0.42
Bread. Wholemeal	0.40
Maize	0.22

Food	mg/100 g
Fish, poultry and meat	
Beef extract	1.60
Pork, lean	0.85
Bacon	0.84
Anchovy, dried	0.17
Chicken, matured, dressed carcass	0.15
Beef, lean	0.10
Legumes, nuts and seeds	
Sunflower seeds	1.48
Dhal, Australian (yellow)	1.40
Peanuts	1.00
Soya beans, white	0.87
Mung beans	0.72
Lotus seeds	0.72
Cashew nuts	0.67
Chickpeas	0.45
Milk and milk products	
Milk, filled	0.72
Milk, powder, infant formula	0.74
Milk, powder, instant, full cream	0.70
Others	
Yeast, dried, brewers	0.75
Yeast extract	0.60

Source: Tee *et al.,* 1997; USDA Food Composition Database, 2016*

5.5 Deficiencies

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 human-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 foods containing 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, oedema occurs while in dry beri-beri, muscle wasting is obvious. In infants, cardiac failure may occur rather suddenly. In relatively industrialised nations, the neurologic reflections of Wernicke-Korsakoff syndrome are frequently associated with chronic alcoholism with limited food consumption.

Clinically manifest thiamin deficiency is rare today, although some segments of the populations 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.

In Malaysia, in the early part of the 20th century, beri-beri was found to be prevalent amongst migrant workers working in camps, consuming primarily a polished rice diet. The Institute for Medical Research (IMR) embarked on a series of intensive studies into the cause of the disease, beginning 1900. It was also the first nutrient deficiency studied in the country. Various hypotheses on the etiology of the disease were proposed and actively investigated. Although the IMR researchers were not the first to discover that thiamin deficiency was the cause of the disease, they contributed significantly to the prevention and cure of the disease. Their efforts in distributing methods for the preparation of extracts of rice polishings to hospitals and dispensaries for the treatment of beri-beri patients and the prohibition of the use of white polished rice helped to control the disease (Tee *et al.,* 2002).

The most widely used biomarkers for estimating thiamin status by measuring the transketolase activity in erythrocytes which require TDP as a coenzyme. The TDP effects of >25% are defined as deficiency and effects between 15% and 25% as marginal deficiency (Bemeur and Butterworth, 2014). Recently, the Nutrition Societies of Germany, Austria and Switzerland (D-A-CH) (Strohm *et al.,* 2016) derived the reference values for thiamin intake based on studies investigating the transketolase activity in erythrocytes and also the excretion of thiamin in the urine. A fall in TDP levels in erythrocytes below 120 nmol/l indicates deficiency (Sauberlich, 1999; Finglas, 1993), while excretion levels between 27 µg and 65 µg are defined as marginal deficiency and of 27 µg as deficiency (Finglas, 1993).

Since the 1950s, there have been no further reports of clinically manifest vitamin B1 deficiency in the country. Very few reports of subclinical thiamin deficiency in any age group have been documented. Indeed, very few biochemical studies on the status of thiamin have been undertaken, due to lack of laboratory facilities for the required analyses. A major study of the nutritional status of various communities in poverty villages in Peninsular Malaysia included the determination of urinary excretion of thiamin in 1170 subjects. The prevalence of "low" excretors varied with different age groups, with most groups having a prevalence of about 25%, indicating the need to improve vitamin B1 intake in these groups (Chong *et al.,* 1984).

In Malaysia, thiamin deficiency seems to have been practically eliminated over the years, although it cannot be ruled out that certain segments of the community could have marginal and sub-marginal deficiencies of the vitamin. For example, in 2004 a possible outbreak of beri-beri was detected in a drug detention and rehabilitation centre in Perlis. It was reported that 74% of the sample studied (n=154 inmates) had thiamin deficiency due to poor dietary intake and coupled with possible intake of certain thiamin antagonists in their diet (Fozi *et al.,* 2006).

Another beri-beri outbreak occurred in the LG Detention Camp in Negeri Sembilan in 2014. A total of 1.9% (n=19) had bilateral leg oedema with symptoms of paraesthesia (52.6%), fatigue (36.8%), difficulty breathing (36.8%), poor appetite (21.1%), and abdominal pain (33.3%). The only risk factor identified was alcohol intake (Noor Aizam *et al.,* 2015).

5.6 Factors affecting thiamin requirements

There are no studies that have examined the effect of energy intake on thiamin requirement. There is also no agreement as to whether expressing thiamin requirements in absolute terms is more useful for predicting biochemical thiamin status than expressing it in relation to energy intake. Despite the lack of direct experimental data, the known biochemical function of thiamin as thiamin pyrophosphate (TPP) in the metabolism of carbohydrate suggests that at least a small (10%) adjustment to the estimated requirement to reflect differences in the average energy utilisation and size of men and women, a 10% increase in the requirement to cover increased energy utilisation during pregnancy, and a small increase to cover the energy cost of milk production during lactation appears to be necessary (IOM, 1998).

Heavy exercise under certain conditions may increase the requirement for thiamin as well as other vitamins. However, the observations on the effects of physical activity on thiamin requirement have been inconsistent, the effects are minor and the experimental conditions highly variable. It was thus concluded 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 (IOM, 1998).

There are no studies that directly compare the thiamin requirements of males and females. A small (10%) difference in the average thiamin requirements of men and women is assumed on the basis of mean differences in body size and energy utilization.

5.7 Setting requirements and recommended intake of thiamin

There are no known local studies on thiamin requirements of communities that the Technical Sub-Committee (TSC) on Vitamins could use as a reference when considering RNI for this vitamin. The two main references used by the TSC when establishing thiamin requirement in the previous RNI (NCCFN, 2005) were WHO/FAO (2004) consultation report and the IOM (1998) DRI recommendations. The rationale and steps taken in setting requirements and the levels recommended by these organisations as well as available reports of thiamin status of communities in the country were considered in setting thiamin requirement for RNI Malaysia 2005. There have been no updated recommendations by WHO/FAO (2004), IOM or other international scientific organisations. There are also very few recent reports of the biochemical status of the vitamin amongst the local population groups. Therefore, in this revision of RNI (2017) for Malaysia, the TSC on Vitamins decided to retain the WHO/FAO (2004) values. These recommendations, which remain the same as in RNI (2005), are given in bold in the following paragraphs according to age groups and summarised in Appendix 5.1.

Infants

The recommended intake for young infants is based on observed mean intake data from infants fed human milk exclusively during their first 6 months as well as the thiamin concentration of milk produced by well-nourished mothers. The FAO/WHO Consultation estimated that the mean thiamin content of human milk is 0.21 mg/l which corresponds to 0.16 mg thiamin per 0.75 L of secreted milk per day. The Consultation rounded the figure and set the requirement at 0.2 mg/day for infants 0-6 months (WHO/FAO, 2004).

For the group 6-11 months, in addition to thiamin from breast milk, the intake of solid food has also to be taken into account. Thus the average requirement was calculated to be 0.3 mg/day.

RNI for infants

0 - 5 months	**0.2 mg/day**
6 - 11 months	**0.3 mg/day**

Children 1 - 9 years

There appears to be no direct data on which to base the estimated average requirement for children 1-9 years. The RDA for these age groups have thus been determined by IOM (1998) by extrapolating downwards from the average requirement of young adults by adjusting for metabolic body size and growth and adding a factor for variability. The RDA for thiamin is set by assuming a coefficient of variation (CV) of 10% because information is not available on the standard deviation of the requirement for thiamin. As RDA is defined as equal to the estimated average requirement (EAR) plus twice the CV to cover the needs of 97 to 98% of the individuals in the group, therefore, the RDA is 120 % of the EAR.

The WHO/FAO (2004) consultation did not provide details on how the recommended intakes were arrived at, but they were similar to those of the IOM (1998).

RNI for children

1 - 3 years	**0.5 mg/day**
4 - 6 years	**0.6 mg/day**
7 - 9 years	**0.9 mg/day**

Adolescents

The IOM Dietary Reference Intakes (DRI) Standing Committee reviewed several studies amongst adolescents in attempting to obtain data to estimate the requirements of thiamin for this age group (IOM, 1998). These included dietary intake studies, status of thiamin, and a controlled-diet dose-response experiment. In the absence of additional definitive information, requirements for these groups were extrapolated from adult values as described above for young children.

Similar to what has been outlined for the recommended intake for children, the FAO/WHO Consultation did not provide details on how the recommended intakes for adolescents were arrived at, but they were similar to those of the IOM (1998).

RNI for adolescents

Boys 10 - 18 years	**1.2 mg/day**
Girls 10 - 18 years	**1.1 mg/day**

Adults and elderly

The same recommendations were made for the intakes of adults by the WHO/FAO (2004) Consultation as well as the IOM (1998). No details for the recommendations were given by the former group. The IOM publication referred to several studies that were reviewed, especially a study by Sauberlich *et al.* (1979), who reported a carefully controlled, thiamin depletion-repletion experiment amongst 7 healthy young men. These investigators concluded that thiamin at 0.30 mg/1,000 kcal (approximately 1.0 mg per day) met the minimum requirement for young men as determined by using urinary excretion of thiamin. This value is also close to the average requirement for normal erythrocyte transketolase activity. The requirement for men was thus set at 1.0 mg/day and 0.9 mg/day for women, assuming a 10% decrease for women based on body size and energy needs.

The RDA for thiamin was thus set by assuming a coefficient of variation (CV) of 10% because information is not available on the standard deviation of the requirement for thiamin. RDA is thus defined as equal to the estimated average requirement (EAR) plus twice the CV to cover the needs of 97 to 98 of the individuals in the group, or 120% of the EAR.

In considering the requirements of the elderly, although there are some data to suggest that requirements might be somewhat higher in the elderly than in younger adults, the IOM DRI Standing Committee recognised that there is also a concomitant decreased energy utilisation that may offset this (IOM, 1998). Thus the recommended intake for older adults is the same as those for adults.

RNI for adults

Men	**19 - 65 years**	**1.2 mg/day**
Women	**19 - 65 years**	**1.1 mg/day**

RNI for elderly

Men	**> 65 years**	**1.2 mg/day**
Women	**> 65 years**	**1.1 mg/day**

Pregnancy and lactation

The WHO/FAO (2004) Consultation accepted an estimated average total energy cost of 230 MJ for pregnancy. With an intake of 0.4 mg thiamin/4184 kJ, this amounts to a total of 22 mg, or 0.12 mg/day for additional thiamin need for the second and third trimesters (180 days). Taking into account an increased growth in maternal and foetal compartments, an overall additional requirement of 0.3 mg/day was felt to be adequate.

Lactating women are estimated to transfer 0.2 mg thiamin in their milk each day, and an additional 0.2 mg is estimated as a need for the increased energy cost of lactation of about 2092 kJ/day. A total amount of 0.4 mg/day was thus added to the recommended intake for the adult women.

RNI for

Pregnancy	**1.4 mg/day**
Lactation	**1.5 mg/day**

Discussions on revised RNI for Malaysia

The RNI values for thiamin for Malaysia, adapted from WHO/FAO (2004), are also the same as those adopted by the Working Group for the Harmonization of RDAs in SEA region. (Tee & Florentino, 2005). Appendix 5.1 provides a summary of RNI, compared with the previous Malaysian RNI of 2005, the WHO/FAO (2004) recommendations and the values recommended by IOM (1998).

The revised RNI is actually similar to that of IOM (1998) but with slight differences in the groupings of children and adolescents of both sexes. The TSC discussed the need to increase the thiamin requirement for older adults, but decided to maintain the RNI to be the same as that for adults because of the generally more sedentary lifestyle and therefore lower energy expenditure of the elderly.

5.8 Tolerable upper intake levels

There does not appear to be a problem with thiamin toxicity because renal clearance of levels usually ingested is rapid. There have been no reports of adverse effects from the consumption of excess thiamin by ingestion of food and supplements. Neither the WHO/FAO (2004) nor the IOM (1998) reports provided any indications of toxicity levels. Because the data are inadequate for a quantitative risk assessment, no Tolerable Upper Intake Level (UL) could be derived for thiamin (IOM, 1998).

5.9 Research recommendations

The following priority areas of research are recommended:

- Magnitude of thiamin deficiency among high risk groups such as alcoholic individuals, elderly people and psychiatric patients.
- Efficacy of thiamin supplementation on high risk individuals or groups.
- Determine effects of food preparation and cooking methods on thiamin content of selected foods to enable establishing conversion factors for calculating thiamin losses for a wide variety of foods
- Study nutrient values (including vitamin B content) of the various types of rice available in the market and their claims of health benefits for various chronic diseases.

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Appendix 5.1: Comparison of recommended intake for Thiamin (Vitamin B1): RNI Malaysia (2017), RNI of FAO/WHO (2004) and RDA of IOM 1998)

Malaysia (2017)*		WHO/FAO (2004)		IOM (1998)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**
Infants		**Infants**		**Infants**	
0 - 5 months	0.2	0 - 6 months	0.2	0 - 6 months	0.2
6 - 11 months	0.3	7 - 12 months	0.3	7 - 12 months	0.3
					RDA (mg/day)
Children		**Children**		**Children**	
1 - 3 years	0.5	1 - 3 years	0.5	1 - 3 years	0.5
4 - 6 years	0.6	4 - 6 years	0.6	4 - 8 years	0.6
7 - 9 years	0.9	7 - 9 years	0.9		
Boys		**Boys**		**Boys**	
10 - 18 years	1.2	10 - 18 years	1.2	9 - 13 years	0.9
				14 - 18 years	1.2
Girls		**Girls**		**Girls**	
10 - 18 years	1.1	10 - 18 years	1.1	9 - 13 years	0.9
				14 - 18 years	1.0
Men		**Men**		**Men**	
19 - 65 years	1.2	19 - 65 years	1.2	19 - 30 years	1.2
> 65 years	1.2	> 65 years	1.2	31 - 50 years	1.2
				51 - 70 years	1.2
				> 70 years	1.2

Malaysia (2017)*		WHO/FAO (2004)		IOM (1998)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**
Women		**Women**		**Women**	
19 - 65 years	1.1	19 - 65 years	1.1	19 - 30 years	1.1
> 65 years	1.1	> 65 years	1.1	31 - 50 years	1.1
				51 - 70 years	1.1
				> 70 years	1.1
Pregnancy		**Pregnancy**		**Pregnancy**	
	1.4		1.4	14 - 18 years	1.4
				19 - 30 years	1.4
				31 - 50 years	1.4
Lactation		**Lactation**		**Lactation**	
	1.5		1.5	14 - 18 years	1.4
				19 - 30 years	1.4
				31 - 50 years	1.4

*Recommendations same as RNI (2005)

6 • Riboflavin (Vitamin B2)

6.1 Introduction

Riboflavin (vitamin B2) is 7,8 dimethyl-10- isoalloxazine, a free vitamin which is a weak base normally isolated and synthesised as a yellowish orange amorphous solid. It is a precursor of essential coenzymes such as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Through these two flavoco-enzymes, riboflavin functions as a catalyst for redox reactions in numerous metabolic pathways and in energy production.

Riboflavin is also important for the synthesis, conversion and recycling of niacin, folate and vitamin B6 and for the synthesis of all heme proteins, including hemoglobin, nitric oxide oxidases, P450 enzymes and proteins involved in electron transfer and oxygen and storage (Rivlin, 2007). Riboflavin derivatives also have direct antioxidant properties and increase endogenous antioxidant status essential cofactors in glutathione redox cycle (Ashoori and Saedisomeolia, 2014).

6.2 Functions

The main function of riboflavin is to help the body to convert carbohydrate to energy. Riboflavin is important for the metabolism of carbohydrate, amino acids and lipids. It carries out these functions as coenzymes flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN). These two coenzymes play major roles in energy production, cellular function, growth and development and metabolism of fat. Due to its fundamental roles in metabolism, riboflavin deficiencies are first evident in tissues such as skin and epithelia.

Riboflavin also acts as an antioxidant due to its ability to activate antioxidant enzymes. Antioxidants fight free radicals and reduce DNA damage. Riboflavin also has a role in helping the body to convert vitamin B6 to folate to a form that it can be used in the body.

6.3 Metabolism

Riboflavin in the diet is ingested as food protein. Riboflavin is converted to its coenzyme forms by ATP-dependent phosphorylation to yield riboflavin-5'phosphate or FMN. Most FMN is then converted to FAD by FAD pyrophosphorylase. In the stomach, gastric acid cleaves most of the coenzymes (FAD and FMN) from the protein. This is then hydrolysed to riboflavin by pyrophosphatase and phosphatase in the upper intestine. The primary absorption of riboflavin is via rapid, active and saturable transport system. The rate of absorption is proportional to intake. Riboflavin is transported in the blood as free riboflavin and FMN. Although small amounts of riboflavin can be found in the liver and kidney, it is not stored in any useful amount and must therefore be supplied in the diet regularly. Most excess riboflavin is excreted in the urine.

6.4 Sources

Riboflavin is widely distributed in foodstuffs and all plants and animal cells. Good sources include dairy products especially milk, leafy vegetables, legumes, kidneys and mushrooms. The food sources of riboflavin are similar to those of other B vitamins. Therefore, it is not surprising that if an individual's diet has inadequate amounts of riboflavin, it will very likely be inadequate

in other B vitamins as well. Some data from the nutrient composition of Malaysian foods are extracted to indicate the riboflavin content of various local foods (Tee *et al.,* 1997).

Various types of legumes, including chick peas, lentils, red and black gram and soya bean contain fairly high levels of riboflavin, around 0.2-0.5 mg/100 g. Various meat products have moderate amounts of the vitamin. Pork (0.3 mg/100g) may have slightly higher amounts, whilst other meats for example beef, mutton, chicken and duck contain about 0.2 mg per 100 g. Fishes, with 0.1 mg/100 g have lower amounts of riboflavin. Hen eggs are a good source of the vitamin, with about 0.6 mg/100 g, with slightly more being concentrated in the egg yolk. A food with exceptionally high riboflavin is beef extract (6.9 mg/100 g), but small amounts of this food is generally consumed.

Fruits and vegetables are poor sources of riboflavin. Recent data on riboflavin content of selected commercial rice such as fragrant rice, basmathi rice and siam rice showed that all varieties contain 0.06mg riboflavin per 100g (Mohd Fairulnizal *et al.,* 2015). There are several processed products in the market, especially bread, cereal products and biscuits, malted drinks that are fortified or enriched with riboflavin and several other B vitamins, thus can become important sources of these vitamins. To ensure a riboflavin-rich diet, milk and dairy products as well as whole grain products should be consumed daily. Meat in moderation and fish contribute to an adequate supply (Strohm *et al.,* 2016). Examples of food sources of riboflavin (Tee *et al.,* 1997) are shown in Table 6.1.

Table 6.1 Riboflavin (Vitamin B2) content of foods

Food	mg/100g
Legumes and nuts	
Lentils	0.52
Green gram	0.45
Soya bean, white	0.34
Red gram	0.34
Black gram	0.20
Chick pea	0.20
Almond	0.52
Peanut	0.43
Meat and organ meats	
Chicken liver	1.28
Ox liver	0.58
Pork	0.30
Mutton	0.28
Duck	0.25
Chicken	0.22
Beef, lean	0.21

Food	mg/100g
Milk	
Milk powder	1.40
Milk, UHT	0.40
Cheese, processed	0.40
Milk, UHT, flavoured	0.33
Yoghurt	0.25
Vegetables	
Swamp cabbage (kangkung)	0.55
Mushroom, grey oyster	0.46
Mustard leaf (sawi)	0.27
Spinach (bayam)	0.25
Chinese cabbage (pak choy)	0.25
French bean	0.21
Long bean	0.13
Egg, hen whole	0.58
Egg yolk	0.37
Egg white	0.27

Source: Tee *et al.,* 1997

6.5 Deficiencies

The clinical features of human riboflavin deficiency do not have the specificity that may characterise deficits of some other vitamins, such as ascorbic acid. Deficiency of riboflavin may manifest itself as weakness, oral pain, tenderness or burning and itching of the eyes, dermatitis and anaemia (Rivlin,2007), cracks and sores at the corners of the mouth, inflammation of the mouth and tongue, and skin lesions. Cellular functions of B vitamins including B2 are closely inter-related. Thus vitamin B2 deficiency almost invariably occurs in combination with a deficiency of other B-complex vitamins. A growing body of research has evaluated the effects of multivitamins and minerals on brain function. Hence deficiency of riboflavin has also been associated with brain specific symptoms such as fatigue, personality change and brain dysfunction (Rivlin, 2007).

The major cause of hypo-riboflavinosis is inadequate dietary intake. Even though riboflavin deficiency levels are under researched, biochemical deficiency is potentially widespread due to the high prevalence of inherited restricted riboflavin absorption/utilization that affects 10-15% of the world population (Sinigaglia-Coimbra, Lopes and Coimbra, 2011). In recent years, a factor that continues to be associated with the deficiency levels of riboflavin is the paradoxical malnutrition associated with obesity. Incidentally in Malaysia, the prevalence of adult overweight and obesity has increased to 30% overweight and 17.7% obesity in the most recent National Health Morbidity Survey (NHMS) (IPH, 2015). This deficiency phenomenon is predicated on the basis that

obesogenic diets are typically biased towards energy rich processed foods that are high in fats and simple sugars but low in micronutrients (Kimmons *et al.,* 2006). The recent Malaysian adults nutrition survey (MANS) also reported that majority of Malaysian adults consumed more than 30% of their diet contributed from fat (IPH, 2014)

Riboflavin supply can be determined by measuring the glutathione reductase activity in the erythrocytes, for which FAD is needed as a coenzyme. The activity coefficient is calculated from the ration of enzyme activity with and without FAD addition. Activity coefficients of >1.4 indicate a riboflavin deficiency, while activity coefficients between 1.2 - 1.4 indicate marginal deficiency. Another option to determine riboflavin supply is the measurement of urinary excretion of riboflavin, which reflects the short term supply and correlates with riboflavin intake in people with body nitrogen equilibrium. 24-hour urinary excretion of riboflavin levels of riboflavin between 40 ug and 119 ug are defined as a marginal deficiency, with levels <40 ug being defined as a deficiency (Strohm *et al.,* 2016).

There are not many data reporting on riboflavin deficiencies in Malaysia. The few studies reported were among the elderly. Suzana *et al.* (1999) carried out a study among 350 elderly in the rural villages of the East Coast of Malaysia. 77% of the elderly studied were riboflavin deficient, assessed by erythrocyte gluthathione reductase activity coefficient. In another study among 820 elderly in four rural areas in Peninsular Malaysia, Suzana *et al.* (2007) reported that the most likely nutrients to be deficient among these elderly were thiamin, riboflavin and calcium, evaluated by dietary history. However in this study, almost half of the elderly were under reporting their energy intake. Thus there should be caution when interpreting the results.

In Cambodia, Whitfield *et al.* (2015) reported suboptimal riboflavin status among women of childbearing age, measured by erythrocyte gluthathione reductase activity coefficient. The prevalence of suboptimal and deficient riboflavin status was 89% among the urban women while 92% among the rural women.

6.6 Factors affecting riboflavin requirements

There are no reported studies that examined the effect of energy intake on riboflavin requirement. The biochemical function of riboflavin in the metabolism of carbohydrate suggests that at least a small (10%) adjustment to the estimated requirement to reflect differences in the average energy utilisation and size of men and women (IOM, 1998). A 10% increase in the requirement is also suggested to cover increased energy utilisation during pregnancy, and a small increase to cover the inefficiencies of milk production is needed. Despite energy expenditure is decreased with aging, a study by Boisvert *et al.* (1993) supported the use of the same requirement for the elderly as for younger adults.

Riboflavin status measurements is affected by physical activity. Exercise produces stress in the metabolic pathways that use riboflavin (Manore, 2000). Thus the requirement of riboflavin for individuals who are physically active and who exercise or who are athletes should be higher due to this physiological demands (American Dietetic Association *et al.,* 2009). Some studies have demonstrated a moderate rise in the erythrocyte glutathione activity coefficient (EGRAC) as well as a decrease in urinary riboflavin excretion with an increase in physical activity. However, there is no available data on which to quantify the adjustment that should be made.

6.7 Setting requirements and recommended intakes of riboflavin

Riboflavin is an existing vitamin in Malaysia RNI 2005. In that recommendation, the TSC had referred to the WHO/FAO (2004) consultation report and IOM (1998) DRI recommendations as the main references. The rationale and steps taken in setting the requirements and the levels recommended by these organisations as well as available reports of riboflavin status of communities in the country were considered when setting the recommendations in RNI 2005. The WHO/FAO (2004) recommendations were adopted as riboflavin requirement for RNI 2005.

In setting the requirement for this revised RNI, the Technical Sub-Committee (TSC) referred to any known local studies on riboflavin that could be used as a reference. There are very few reports of the biochemical status of the vitamin amongst the population groups. To date, there is only one study (Suzana *et al.,* 1999) which reported the biochemical status among the elderly and another study which showed dietary riboflavin intake among the elderly (Suzana *et al.,* 2007). In terms of international literature, there have been no recent updates from WHO/FAO or IOM or other international organisations on riboflavin requirement. Recognising this, the TSC on Vitamins decided to continue to adopt the WHO/FAO (2004) values as the revised RNI (2017) for Malaysia. These recommendations, which are similar to RNI 2005, are outlined below, according to age groups and summarised in Appendix 6.1

Infants

In determining riboflavin requirement, the IOM (1998) report took the same approach as that used for estimating intake of thiamin. As there was no sufficient data that reliably reflected response to dietary riboflavin intake in infants, adequate intake was estimated. This was estimated based on the mean riboflavin intake of infants fed principally with human milk.

On the basis of several available studies, a riboflavin concentration of 0.35 mg/l was used for human milk consumed by infants younger than 6 months. Using the mean value of 750 ml/day for intake of human milk, the estimated adequate intake was 0.3 mg/day, after rounding up. By extrapolation from adequate intake for younger infants, the intake for riboflavin for older infants was estimated to be 0.4 mg/day after rounding up.

The German, Austria and Switzerland Nutrition Societies also derived the reference value (RV) for riboflavin for infants 0-5 months, based on the content of the three vitamins (B1, B2 and B3) in breast milk which is considered to be optimal diet for infants (Butte *et al.,* 2002). The RV is therefore an estimated value. For infant 6-11 months, The RV is based on average requirement for adults (Strohm *et al.,* 2016).

RNI for infants

0 - 5 months	**0.3 mg/day**
6 - 11 months	**0.4 mg/day**

Children and adolescents

As there was a lack of data concerning the riboflavin requirements of children or adolescents, the requirement for these age groups have thus been determined by IOM (1998) by extrapolating downwards from the average requirement of young adults by using a metabolic body weight ratio multiplied by a growth factor. The RDA for riboflavin was next set by assuming a coefficient of variation (CV) of 10% because information is not available on the standard deviation of the requirement for riboflavin. As RDA is defined as equal to the estimated average requirement (EAR) plus twice the CV to cover the needs of 97 to 98% of the individuals in the group, therefore, the recommended intake is 120 % of the EAR.

For children and adolescents, the German, Austria and Switzerland Nutrition Societies derived the reference values for riboflavin intake based on the average requirement for adults and are calculated considering the age based guiding values for energy intake as well as assuming a coefficient of variation of 10% (Strohm *et al.,* 2016).

RNI for children

1 - 3 years	**0.5 mg/day**
4 - 6 years	**0.6 mg/day**
7 - 9 years	**0.9 mg/day**

RNI for adolescents

Boys 10 - 18 years	**1.3 mg/day**
Girls 10 - 18 years	**1.0 mg/day**

Adults and elderly

The IOM DRI Committee established estimated requirements based on findings from several studies of riboflavin requirements of adults that addressed clinical deficiency signs and biochemical values including erythrocyte glutathione reductase activity coefficient (EGRAC) in relation to measured dietary intake of riboflavin (IOM, 1998). The reviewed data showed that clinical signs of deficiency appear at intakes of less than 0.5 to 0.6 mg/day whereas most studies reported normal EGRAC values at intakes of less than 1.3 mg/day. And because there is an expected curvilinear biological increase of values from deficient to minimally adequate, the requirement for riboflavin for men was set at 1.1 mg/day and for women at 0.9 mg/day.

Recommended intake was set by assuming a coefficient of variation (CV) of 10% because information is not available on the standard deviation of the requirement for riboflavin. Since RDA is defined as equal to the estimated requirement plus twice the CV to cover the needs of 97 to 98% of the individuals in the group, the RDA for riboflavin was calculated as 120% of the estimated requirement or 120% of 1.1 for men and 120% of 0.9 for women. The recommended intake, after rounding, is thus 1.3 mg/day and 1.1mg/day respectively for men and women.

Few additional studies estimating riboflavin requirements have been conducted in the elderly. Although there is a decrease in energy expenditure with aging and the estimated requirement for older adults would be expected to decrease, the IOM (1998) report decided to use the same requirement for the elderly as for younger adults.

RNI for adults

Men	**19 - 65 years**	**1.3 mg/day**
Women	**19 - 65 years**	**1.1 mg/day**

RNI for elderly

Men	**> 65 years**	**1.3 mg/day**
Women	**> 65 years**	**1.1 mg/day**

Pregnancy and lactation

The additional requirement of 0.3 mg for pregnancy is an estimate based on increased growth in maternal and foetal compartments. For lactating women, an estimated 0.3 mg riboflavin is transferred in milk daily and, because utilisation for milk production is assumed to be 70% efficient, the value is adjusted upward to 0.4 mg/day.

There are no data to suggest that the relationship between riboflavin and energy requirement for pregnant and lactating women is any different from that for women who are not pregnant nor lactating. Due to the higher guiding value for energy intake during pregnancy (+250kcal /day second trimester and +500 kcal in third trimester) as well as increased energy during lactation and based on average requirement for adults, a higher recommended intake is derived assuming a coefficient of variation of 10%.

RNI for

pregnancy	**1.4 mg/day**
lactation	**1.6 mg/day**

Discussions on revised RNI for Malaysia

The RNI values for riboflavin for Malaysia, adopted from WHO/FAO (2004), are also the same as those adopted by the Working Group for the Harmonisation of RDAs in Southeast Asia (Tee & Florentino, 2005). Appendix 6.1 provides a summary of these revised RNI, compared with the previous Malaysian RNI of 2005, the WHO/FAO (2004) recommendations and the values recommended by IOM (1998).

For almost all the age groups, the revised RNI is the same as those recommended in the 2005 RNI. The revised recommended intakes are also similar to the IOM (1998) values. There should therefore be little controversy on these values. Roughead and McCormick (1991) found that most of a 1.7-mg dose of riboflavin given to healthy adults consuming at least this amount was largely excreted in the urine. Such findings corroborate earlier work indicating a relative saturation of tissue with intakes above 1.1 mg/day (FAO/WHO, 1998).

6.8 Tolerable upper intake levels

The absorption of riboflavin is limited when administered in high doses. Data on adverse effects from high oral riboflavin intake are not sufficient for risk assessment. Available subchronic data from human studies and pharmacokinetics do not show reported oral effects on oral toxicity of riboflavin

The apparent lack of harm resulting from high oral doses of the vitamin may be due to its limited solubility and limited capacity for absorption in the human gastrointestinal tract. No study has reported significant adverse effects in human of excess riboflavin consumption from foods or supplements. Since it is not possible, based on the present database to derive an Tolerable Upper Intake Level (UL) for riboflavin, the limited evidence available from clinical studies indicates that the current levels of intake of riboflavin from all sources do not represent a risk to human health (European Food Safety Authority, 2006).

The IOM (1998) concluded that the data on adverse effects from high riboflavin intake are not sufficient for a quantitative risk assessment, and a UL cannot be derived for the vitamin.

6.9 Research recommendations

The following priority areas of research are recommended:

- The magnitude of riboflavin deficiency among high risk groups such as alcoholic individuals, the elderly, physically active individuals, obese individuals and vegans.
- Efficacy of riboflavin supplementation on high risk individuals or groups.
- Determine effects of food preparation and cooking methods on riboflavin content of selected foods to enable establishing conversion factors for calculating riboflavin losses for a wide variety of foods
- Study nutrient values (including vitamin B content) of the various types of rice available in the market and their claims of health benefits for various chronic diseases.

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Appendix 6.1 Comparison of recommended intake for Riboflavin (Vitamin B2): RNI Malaysia (2017), RNI of WHO/FAO (2004) and RDA of IOM (1998)

Malaysia (2017)*		WHO/FAO (2004)		IOM (1998)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**
Infants		**Infants**		**Infants**	
0 - 5 months	0.3	0 - 6 months	0.3	0 - 6 months	0.3
6 - 11 months	0.4	7 - 12 months	0.4	7 - 12 months	0.4
					RDA (mg/day)
Children		**Children**		**Children**	
1 - 3 years	0.5	1 - 3 years	0.5	1 - 3 years	0.5
4 - 6 years	0.6	4 - 6 years	0.6	4 - 8 years	0.6
7 - 9 years	0.9	7 - 9 years	0.9		
Boys		**Boys**		**Boys**	
10 - 18 years	1.3	10 - 18 years	1.3	9 - 13 years	0.9
				14 - 18 years	1.3
Girls		**Girls**		**Girls**	
10 - 18 years	1.0	10 - 18 years	1.0	9 - 13 years	0.9
				14 - 18 years	1.0
Men		**Men**		**Men**	
19 - 65 years	1.3	19 - 65 years	1.3	19 - 30 years	1.3
> 65 years	1.3	> 65 years	1.3	31 - 50 years	1.3
				51 - 70 years	1.3
				> 70 years	1.3

Malaysia (2017)*		WHO/FAO (2004)		IOM (1998)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**
Women		**Women**		**Women**	
19 - 65 years	1.1	19 - 65 years	1.1	19 - 30 years	1.1
> 65 years	1.1	> 65 years	1.1	31 - 50 years	1.1
				51 - 70 years	1.1
				> 70 years	1.1
Pregnancy		**Pregnancy**		**Pregnancy**	
	1.4		1.4	14 - 18 years	1.4
				19 - 30 years	1.4
				31 - 50 years	1.4
Lactation		**Lactation**		**Lactation**	
	1.6		1.6	14 - 18 years	1.6
				19 - 30 years	1.6
				31 - 50 years	1.6

*Recommendations same as RNI (2005)

7 • Niacin (Vitamin B3)

7.1 Introduction

Niacin is a water-soluble vitamin, also known as vitamin B^3. Niacin is the generic term for nicotinic acid (pyridine 3-carboxylic acid) and nicotinamide (Pyridine-3-carboxamide) and the coenzyme forms of the vitamin. Chemical structure of nicotinic acid is $C^6H^5NO^2$ and nicotinamide is $C^6H^6N2^O$.

Nicotinamide is the active form of the vitamin, which functions as a constituent of two coenzymes, namely, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP).

7.2 Functions

In the forms of these coenzymes, niacin functions in many biological redox reactions that activate about 200 dehydrogenases essential to electron transport and other cellular respiratory reactions. NAD functions as an electron carrier for intracellular respiration as well as a co-factor for enzymes involved in the oxidation (catabolism) of fats, proteins, carbohydrates and alcohol to produce energy. NADP functions as a hydrogen donor in reductive biosynthesis (anabolism), such as in fatty acid and steroid synthesis. Like NAD, NADP is a cofactor for enzymes, such as in the oxidation of glucose-6-phosphate to ribose-5-phosphate in the pentose phosphate pathway.

In non-redox reactions, NAD is the substrate for two classes of enzymes that separate the niacin moiety from NAD and transfer ADP-ribose to proteins. A third class of enzymes catalyses the formation of cyclic ADP-ribose (Lautier *et al.,* 1993). This molecule also functions within cells to provoke the release of calcium ions from internal storage sites and may play a role in cell signaling (Kim, Jacobson & Jacobson, 1994).

7.3 Metabolism

Intestinal absorption of nicotinic acid and nicotinamide supplied from food is mediated by sodium ion-dependent, carrier-mediated diffusion, but a role for the human organic anion transporter 10 (hOAT10) and the intracellular protein-tyrosine kinase pathway has also been proposed (EFSA, 2014).

In vivo nicotinic acid is converted to nicotinamide, which is a precursor for nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which are essential to cells and involved in many biochemical reactions.

Niacin circulates in the plasma as nicotinamide and nicotinic acid. Both forms are transported to cells and tissues, which they enter by diffusion to perform the intracellular functions of niacin. Niacin is trapped within the cell as NAD or NADP.

The major pathway of catabolism of nicotinic acid and nicotinamide is by methylation in the liver to N-methyl-nicotinamide (NMN) and subsequent oxidation to N-methyl-2-pyridone-carboxamide (2-Pyr) and N-methyl-4-pyridone-carboxamide (4-Pyr).

In humans, the two major excretion products are NMN and 2-Pyr, which under normal conditions represent about 20-35% and 45-60% of niacin metabolites, respectively. The amount of niacin metabolites excreted depends on the niacin and tryptophan intake. Long-term inadequate intake of tryptophan and niacin results in reduced urinary excretion of niacin metabolites, and can lead to the development of pellagra (EFSA, 2014).

Besides dietary niacin as a source for the synthesis of NAD, it may also be synthesized in the liver from tryptophan, an essential amino acid. The synthesis of niacin from tryptophan also depends on enzymes that require vitamin B6 and riboflavin, as well as an enzyme containing heme. On average, 1 mg of niacin can be synthesized from 60 mg of tryptophan. Thus, 60 mg of tryptophan can be considered to be 1 mg of niacin equivalent (NE) (Horwitt, Harpewr & Henserson, 1981).

7.4 Deficiencies

Clinical evidence of niacin deficiency includes fatigue, poor appetite, diarrhoea, irritability, headache, emotional instability and possible memory loss. These may lead to changes in the skin, mucosa of the mouth, stomach and intestinal tract and the nervous system. These changes are called “pellagra”, which means “raw skin” and are most pronounced in the parts of the skin exposed to sunlight. Other signs and symptoms include dizziness, vomiting, constipation or diarrhoea, and inflammation of the tongue and gastric mucosa. The neurological symptoms can include fatigue, sleeplessness, depression, memory loss and visual impairment. If untreated, pellagra is ultimately fatal.

The intake of niacin correlates with the excretion of niacin metabolites in urine. Low excretion of the niacin metabolites N-methyl nicotinamide and N-methyl-2-pyridon-5-carboxamide indicates a low body store. Therefore, these can serve as markers of niacin supply (Shibata & Matsua, 1989).

Excretion of N-methyl nicotinamide and N-methyl-2-pyridon-5-carboxamide totaling less than 1.5 mg in 24 hours indicates severe niacin deficiency (Hegyi, Schwart & Hegyi, 2004). As the concentration of N-methyl-2-pyridon-5-carboxamide decline to the higher degree than that of N-methyl nicotinamide as a result of reduced niacin intake, a ratio of less than 1.0 is a further indicator for niacin deficiency (Kirkland, 2014).

At present, pellagra is rarely seen in most industrialized countries except among alcoholics and individuals with conditions that disrupt tryptophan pathways. However, major outbreaks have been described in association with humanitarian emergencies in Malawi, Mozambique, Angola, Zimbabwe and Nepal (Seal *et al.,* 2007).

7.5 Sources

Important sources of preformed niacin include beef, liver, pork, fish, anchovies, peanuts and other nuts, whole grains and whole-meal wheat flour. In general, foods rich in protein, with the exception of tryptophan-poor grains, can satisfy some of the requirement for niacin. Human milk contains a higher concentration of niacin than cow’s milk.

In plants, especially in mature cereal grains like corn and wheat, niacin may be bound to sugar molecules in the form of glycosides, thus significantly reducing niacin bioavailability.

In unprepared foods, niacin is present mainly in the form of the cellular pyridine nucleotides NAD and NADP. Enzymatic hydrolysis of the coenzymes can occur during the course of food preparation. Significant amounts of niacin can be lost if large quantities of liquid are used in preparation and cooking of food sources. Whole grains and wholegrain products, seeds and nuts are good sources of niacin for vegetarians. Table 7.1 provides examples of food with substantial amounts of niacin in 100 g portion of the food.

Table 7.1 Niacin content of foods

Food	Niacin (mg/100g)
Fish, meat and poultry	
Tuna (cooked)*	22.1
Anchovy, dried	13.2
Liver	11.5
Pork (Cooked and lean)*	10.9
Beef (Cooked lean rib)*	9.0
Beef extract	5.6
Pomfret, black (bawal hitam)	4.2
Sardine, canned	3.5
Beef, lean	3.4
Mackerel (Tenggiri batang)	3.2
Chicken, breast meat	3.1
Cereal & cereal products	
Wheat flour, whole-meal	10.2
Biscuit, peanut & coconut	11.3
Barley, pearl (beras belanda)	3.4
Glutinous rice, black	3.4
Rice, polished	3.3
Siam rice, polished#	1.1
Basmati rice, polished#	1.1
Fragrant rice, polished#	0.9
Rice, cooked	0.0
Legume & Legume products	
Peanut	11.7
Sunflower seed*	8.3
Tempeh	3.7
Soy bean, black	3.4

Food	Niacin (mg/100g)
Soy bean noodle	2.9
Dhal, Mysore (Orange)	2.4
Red Gram (kacang merah)	2.3
Green gram (Kacang hijau)	1.8
Vegetables	
Mushrooms (Portobello)*	6.3
Green peas	2.1
Avocado	1.7

Source: Tee *et al.,* (1997),*USDA Food Composition Database (2015) and #Mohd Faizulnizal *et al.,* (2015)

7.6 Factors affecting niacin requirement

Bioavailability of niacin varies depending on the food sources. From mature cereal grains, only 30% of niacin is available because it is largely bound. The bioavailability can be increased by treating the grains with alkali. For enrichment and fortification of food, free form of niacin is used, thus making it highly available. Foods that contain niacin in the free form include beans and liver.

The conversion of tryptophan to niacin may be affected by a number of factors. There are several dietary, drug and disease factors that reduce this conversion, such as use of oral contraceptives. The requirement for pre-formed niacin is increased by factors that reduce the conversion of tryptophan to niacin. It tends to be lower with higher tryptophan intakes and during pregnancy, when conversion is more efficient.

There is also an interdependence of enzymes within the tryptophan-to-niacin pathway where vitamin B6 (as pyridoxal phosphate), riboflavin (as FAD) and iron are functional. Further, riboflavin (as FMN) is required for the oxidase that forms coenzyme pyridoxal 5'-phosphate from the alcohol and amine forms of phosphorylated vitamin B6. Therefore, inadequate iron, riboflavin, or vitamin B6 status decreases the conversion of tryptophan to niacin.

Variation in the levels of energy intake may influence niacin requirement. However, there are no directly relevant data that examined this relationship. Despite this lack of direct experimental data, the known biochemical function of riboflavin in the metabolism of carbohydrate suggests that at least a small (10%) adjustment be made to the estimated requirement to reflect differences in the average energy utilization and size of men and women (IOM, 1998). A 10% increase in the requirement is also suggested to cover increased energy utilization during pregnancy, and a small increase in the requirement to account for the efficiency of niacin use during lactation.

7.7 Setting requirements and recommended intake of niacin

Niacin was included in the Malaysia RNI 2005. There were no local data available to help establish niacin requirements. The Technical Sub-Committee (TSC) on vitamins reviewed the consultation report of WHO/FAO (2004) and IOM (1998) DRI recommendations. In reviewing these reports, considerations were given to the rationale and steps for arriving at the RNI for niacin, the levels of niacin recommended for different age categories. The TSC on vitamins decided to adopt the WHO/FAO (2004) recommendations for niacin intake for Malaysia RNI 2005.

In this 2017 review of RNI for Malaysia, the TSC on Vitamins again did not have any local publications on niacin status or requirement. There were also no updated recommendations from WHO/FAO or IOM. The additional reference from an international scientific organization that was available was the report of the European Food Safety Authority (EFSA, 2014). The EFSA report noted that in the absence of new data, the recommended intakes adopted the requirement proposed by Scientific Committee for Food of 1993.

The TSC on Vitamins therefore decided to continue to adopt the WHO/FAO (2004) recommended intake for niacin for the 2017 version of Malaysia RNI. These recommendations, which are similar to RNI for Malaysia (2005), are given in bold in the following paragraphs according to age groups. The proposed RNI are summarised in Appendix 7.1.

Infants

The adequate intake for niacin for infants ages 0 through 6 months is based on the reported mean volume of milk (0.78 L/day) consumed by this age group, the estimated niacin concentration in human milk of 1.8 mg/l and the tryptophan content of human milk (210 mg/l). Thus the IOM (1998) recommended intake for niacin for infants (0-6 months) is 2 mg of preformed niacin.

One of the methods that can be used to determine adequate intake of niacin for infants 7-12 months is to use the estimated niacin content of human milk as 1.1 mg/l and a mean milk volume of 0.6L, and adding the amount of niacin provided by solid foods (8 mg). The amount of niacin equivalents (NE) thus obtained would be 9 mg/day. The IOM (1998) DRI Committee felt that this amount was too high and used the approach of extrapolating from estimated average requirement of adults to estimate adequate intake for this group of infants. Thus, IOM (1998) set the adequate intake for infants 7 through 12 months as 4 mg/day of NEs.

RNI for infants

0 - 5 months	**2 mg/day of NEs**
6 - 11 months	**4 mg/day of NEs**

Children and adolescents

Since there was no available data on which to base the requirements for children or adolescents, the DRI Committee of IOM (1998) had based the recommended intakes for these groups on extrapolation from adult values. The recommended intake was determined as 130% of the EAR.

RNI for children

1 - 3 years	**6 mg/day of NEs**
4 - 6 years	**8 mg/day of NEs**
7 - 9 years	**12 mg/day of NEs**

RNI for adolescents

Boys 10 - 18 years	**16 mg/day of NEs**
Girls 10 - 18 years	**16 mg/day of NEs**

In teenage (age of 14 - 17 years old) pregnancy and lactating, EFSA (2014) provided an additional requirement for niacin of 1 mg NE/day and 3 mg NE/day respectively.

Adults and elderly

The metabolites of niacin excretion, N1-methyl-nicotinamide and its 2-pyridone derivative are thought to be the best biochemical measure for estimating niacin requirement. An average niacin requirement can be estimated as the niacin intake corresponding to an excretion of N1-methyl-nicotinamide that is above the minimal excretion at which pellagra symptoms occur. A urinary excretion value for N1-methyl-nicotinamide of 1.0 mg/day has been chosen as the level of niacin excretion that reflects a niacin intake that is minimal or barely adequate.

Upon reviewing data from four experimental studies, the IOM (1998) DRI Committee observed that the overall average intake equivalent to the excretion of 1 mg/day of N1-methyl-nicotinamide was 11.6 (3.94, with a CV of 34%. It was also assumed that women have a slightly lower requirement than men because of their size and average energy utilization. The average requirement was estimated to be 12 mg/day NEs for men and 11 mg/day of NEs for women. There are insufficient data to determine whether the niacin requirement changes with age in adults. The FAO/WHO (2004) consultation also felt that there are insufficient data to justify changes in requirements for the elderly for most of the B vitamins, including niacin.

Taking into consideration the wide variation in the efficiency of converting tryptophan to niacin, the DRI Committee assumed a higher coefficient of variation (CV) of 15%. The daily recommended intake for adults was thus calculated as 130% of the estimated requirement or 16 mg NEs for men and 14 mg NEs for women.

RNI for adults

Men	**19 - 65 years**	**16 mg/day of NEs**
Women	**19 - 65 years**	**14 mg/day of NEs**

RNI for elderly

Men	**> 65 years**	**16 mg/day of NEs**
Women	**> 65 years**	**16 mg/day of NEs**

Pregnancy and lactation

During pregnancy, it is estimated that the need for niacin increases by 3 mg/day of NEs to cover increased energy utilization and growth in maternal and fetal compartments, especially during the second and third trimesters. Thus, the estimated niacin requirement is 14 mg/day of niacin equivalents during pregnancy with no adjustment being made for the woman's age. As has been explained for adult requirement, a CV of 15% is assumed for niacin requirement.The calculated recommended intake for niacin during pregnancy is thus 130% of the requirement or 18 mg NEs per day.

For lactating women, an estimated 1.4 mg of preformed niacin is secreted daily into breast milk. To cover the energy expenditure involved in milk production, 1 mg is further added. Therefore, for women who are exclusively breastfeeding an infant, the additional amount of niacin needed is 2.4 mg/day of NEs. Taking into consideration the CV for niacin requirement, the recommended intake during lactation is 17 mg/day of NEs.

RNI for

Pregnancy	**18 mg/day of NEs**
Lactation	**17 mg/day of NEs**

Discussions on revised RNI for Malaysia

The RNI values for niacin for Malaysia 2017, adapted from WHO/FAO (2004), are also the same as those adopted by the Working Group for the Harmonization of RDAs in Southeast Asia (Tee & Florentino 2002). Appendix 7.1 provides a summary of these revised RNI, compared with the previous RNI (2005), the recommendations of WHO/FAO (2004), IOM (1998) and EFSA (2014).

The TSC on Vitamins felt that the proposed RNI can be easily met by adhering to the recommended dietary guidelines. Moreover, deficiency of this vitamin has not been reported in the country for over 50 years.

7.8 Tolerable upper intake levels

Niacin toxicity is rarely observed at doses generally consumed and niacin from foods is not known to cause adverse effects. Nevertheless, considerations should still be given to intake of niacin as a supplement and fortified foods.

Symptoms of acute toxicity include flushing, itching of skin, nausea, vomiting and gastrointestinal disturbances. In addition, jaundice, hyperglycemia, abdominal pain, elevated serum bilirubin, alkaline phosphatase and aminotransferase levels can be seen with ingestion of high levels of nicotinic acid (generally intakes of 3,000 mg/day or more) for long periods of time.

The Tolerable Upper Intake (UL) Level of 35 mg/day as proposed by IOM (1998) was adopted by the FAO/WHO (2004) Consultation Group. This value was also set for pregnant and lactating adult women.

The UL of 35 mg/day was adjusted for children and adolescents on the basis of relative body weight and using revised reference weights. The UL is not meant to apply to individuals who are being treated with a nutrient under medical supervision, such as for lowering of cholesterol levels. The UL recommended by IOM (1998) are given in Table 7.2.

Table 7.2 Tolerable Upper Intake (UL) levels of niacin for various age groups

Age groups		mg/day niacin equivalents
Infants	0 - 12 months	Not possible to establish
Children	1 - 3 years	10
	4 - 8 years	15
	9 - 13 years	20
Adolescents, 14 - 18 years		30
Men, 19 years and older		35
Women, 19 years and older		35
Pregnant women		35
Lactating women		35

Source: IOM (1998)

7.9 Research recommendations

The following priority areas of research are recommended:

- Determination of niacin status and extent of deficiency among high risk groups
- Identification of more sensitive and specific biochemical measures of niacin status
- Identification of specific roles or functions of niacin in disease prevention
- Determine effects of food preparation and cooking methods on niacin content of selected foods to enable establishing conversion factors for calculating niacin losses for a wide variety of foods.

7.10 References

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Appendix 7.1 Comparison of recommended nutrient intake for Niacin: RNI Malaysia (2017), PRI of EFSA (2014), RNI of WHO/FAO (2004), AI and RDA of IOM (1998)

Malaysia (2017)*		EFSA (2014) #		WHO/FAO (2004)		IOM (1998)	
Age group	**RNI (mg/day)**	**Age group**	**PRI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**
Infants		**Infants**		**Infants**		**Infants**	
0 - 5 months	2	0 - 5 months	NA	0 - 6 months	2	0 - 6 months	2
6 - 11 months	4	7 - 11 months		7 - 12 months	4	7 - 12 months	4
		Boys	4.2 - 4.8				
		Girls	3.7 - 4.4				
							RDA (mg NE/day)
Children		**Children**		**Children**		**Children**	
1 - 3 years	6	1 - 3 year		1 - 3 years	6	1 - 3 years	6
		Boys	5.1 - 7.7				
		Girls	4.6 - 7.2				
4 - 6 years	8	4 - 6 years		4 - 6 years	8	4 - 8 years	8
		Boys	8.2 - 9.2				
		Girls	7.6 - 8.6				
7 - 9 years	12	7 - 9 years		7 - 9 years	12		
		Boys	9.8 - 11.0				
		Girls	9.1 - 10.2				
Boys		**Boys**		**Boys**		**Boys**	
10 - 18 years	16	10 - 18 years	12.6 - 19.2	10 - 18 years	16	9 - 13 years	12
				14 - 18 years	16		
Girls		**Girls**		**Girls**		**Girls**	
10 - 18 years	16	10 - 18 years	11.9 - 14.9	10 - 18 years	16	9 - 13 years	12
				14 - 18 years	14		

Malaysia (2017)*		EFSA (2014) #		WHO/FAO (2004)		IOM (1998)	
Age group	**RNI (mg/day)**	**Age group**	**PRI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**
Men		**Men**		**Men**		**Men**	
19 - 65 years	16	19 - 60 years	14.4 - 15.3	19 - 65 years	16	19 - 30 years	16
> 65 years	16	> 60 years	12.9 - 13.2	> 65 years	16	31 - 50 years	16
						51 - 70 years	16
						> 70 years	16
Women		**Women**		**Women**		**Women**	
19 - 65 years	14	19 - 60 years	11.6 - 12.3	19 - 65 years	14	19 - 30 years	14
> 65 years	14	> 60 years	10.5 - 10.6	> 65 years	14	31 - 50 years	14
						51 - 70 years	14
						> 70 years	14
Pregnancy		**Pregnancy**		**Pregnancy**		**Pregnancy**	
	18	1st trimester	+ 0.5	18		14 - 18 years	18
		2nd trimester	+1.7			19 - 30 years	18
		3rd trimester	+3.3			31 - 50 years	18
Lactation		**Lactation**		**Lactation**		**Lactation**	
	17	(0-6 months	+3.3		17	14 - 18 years	17
		post-partum)				19 - 30 years	17
						31 - 50 years	17

#The Average Recommendation (ARs) for niacin in mg NE/day were calculated from the ARs for niacin of 1.3 mg NE/MJ (5.6 mf NE/1000 kcal) using ARs for energy according to Scientific Opinion on Dietary Reference Values for energy (EFSA NDA Panel, 2013), and the PRIs were calculated assuming a CV of 10%.
* Recommendations are similar to RNI (2005)

8 • Pantothenic Acid (Vitamin B5)

8.1 Introduction

Pantothenic acid is a water-soluble vitamin, also known as vitamin B5. Pantothenic acid consists of pantoic acid and ß-alanine joined by the amide linkage. The chemical formula is C9H17NO5. It will be converted to form pantetheine and then converts again into the only known biologically active form which is the pantethine. Pantethine, is the active form and the stable disulphide form of pantetheine. It is the major precursor of coenzyme A (CoA) after the chemical structure of vitamin B5 is connected by two sulfur atoms that play a central role in the lipid and carbohydrate metabolism. The properties of vitamin B5 are easily destroyed when during heating and freezing. Also, it is stable when dry and in solution at a neutral pH but destroyed in acidic and alkaline solutions. Pantothenic acid is yellow, viscous, oily, and readily soluble in water, alcohol, and dioxane, but it is rarely soluble in diethyl ether and acetone. It is insoluble in benzene and chloroform (Gonzalez-Lopez, Jesus, *et al.,* 2016).

8.2 Functions

Pantothenic acid helps the body convert food (carbohydrates) into fuel (glucose), which the body uses to produce energy. It helps the body use fats and protein. In addition to playing a role in the breakdown of fats and carbohydrates for energy, pantothenic acid is critical to the manufacture of red blood cells, as well as sex and stress-related hormones produced in the adrenal glands. It is also important in maintaining a healthy digestive tract, and it helps the body use other vitamins, particularly riboflavin.

Pantothenic acid is required for other vitamin syntheses which are vitamin A and vitamin D. It also enhances the process of cell division, and protein synthesis thus promotes wound healing (Weimann & Hermann, 1999) and post-surgical therapy. Besides, it helps in alcohol detoxification which is metabolism of acetaldehyde (Chernikevich, Dorofeev & Moiseenok, 1992). Lastly, pantothenic acid also functions in immunity where it helps fight viral hepatitis (Komar, 1990).

8.3 Metabolism

Pantothenic acid is used in CoA and acyl carrier proteins (ACP), which carry and transfer acetyl and acyl groups, respectively. In vivo effects of pantothenic acid are thought to be a result of its incorporation into these molecules. CoA is an essential cofactor in fatty acid oxidation, lipid elongation, and fatty acid synthesis. It is involved in the production of many secondary metabolites such as polyisoprenoid-containing compounds, steroid molecules, acetylated compounds, acetylated neurotransmitters, prostaglandins and prostaglandin-like compounds. In vitro evidence suggests that biotin and pantothenic acid use the same sodium-dependent, specialized carrier-mediated system for uptake in colonic epithelial cells. In this rodent experiment, pantothenic acid caused a concentration-dependent competitive inhibition in biotin uptake. That means high pantothenic acid intake could inhibit the absorption of biotin in the large intestine (Said *et al.,* 1998).

Being an integral part of coenzyme-A (CoA), the pantothenic acid has critical roles in nutrient metabolism including metabolism of energy-producing nutrients such as carbohydrate, lipids, and protein. Sodium-dependent multivitamin transporter (SMVT) carrier is responsible for the

uptake of lower concentration of pantothenic acid into organs and by passive diffusion when present in high concentrations (Prasad *et al.,* 1999). The bioavailability of pantothenic acid is suggested in the range of 40%- 61% by estimated urinary pantothenic acid excretion.

Pantothenic acid is excreted in urine, after hydrolysis of CoA in a multistep reaction. In a few groups of healthy subjects, average daily urinary excretion of pantothenic acid was observed to range between about 2.0 and 3.5mg/day in children and adolescents (Schmidt, 1951; Kathman & Kies, 1984; Eissenstat, Wyse & Hansen, 1986) and between about 2.0mg and 4.0mg/day in adults (Schmidt, 1951; Fox & Linkswiler, 1961; Fry, Fox & Tao, 1976; Kathman & Kies, 1984; Song *et al.,* 1985). Urinary excretion of pantothenic acid is positively correlated with pantothenic acid intakes. CoA is hydrolysed to pantothenate, and the pantothenic acid is excreted intact in urine.

Pantothenic acid is a water-soluble vitamin, which means that it cannot be stored by the body and must be replenished every day. Supplement storage between 15° and 30°C in a cool, dry place away from direct heat, light, and moisture. Dietary CoA is hydrolysed in the intestine to dephospho-CoA, phosphopantetheine, and pantetheine. Pantetheine is further hydrolysed to pantothenic acid (Trumbo, 2014). Intestinal absorption of pantothenic acid occurs via saturable sodium-dependent carrier-mediated process (Stein & Diamond, 1989; Prasad *et al.,* 1999). In blood, pantothenic acid is transported mainly as CoA within erythrocytes (Trumbo, 2014). Pantothenic acid uptake in tissues occurs through an active sodium-dependent mechanism. Most of the pantothenic acid in tissues is present as CoA, mainly found in mitochondria, with lesser amounts present as acyl-carrier protein and free pantothenic acid.

8.4 Sources

Pantothenic acid is present in a wide variety of foods (Table 8.1). Foods rich in pantothenic acid include meat (products), eggs, nuts, avocados and cruciferous vegetables (FSA, 2002; Anses/CIQUAL, 2012). The main contributors to pantothenic acid intakes include meat products, bread, milk-based products and vegetables (Afssa, 2009; DGE, 2012). Currently, pantothenic acid (as D-pantothenate, calcium; D-pantothenate, sodium or dexpanthenol) may be added to foods and food supplements.

Table 8.1: Pantothenic acid content of foods

Food	mg per 100g
Poultry, meats, fish,	
Oily fish (Trout cooked)	2.2
Beef & Veal (Veal Shoulder, Cooked)	1.6
Lean Pork (Sirloin, Cooked)	1.6
Eggs	1.5
Chicken patty, frozen, cooked	1.1
Chicken breast tenders, breaded, cooked, microwaved	1.0
Fruits	
Avocados	1.4
Apricots, dehydrated, sulfured, uncooked	1.1
Dates, deglet noor	0.6
Vegetables	
Mushrooms (Shiitake, Cooked)	3.6
Seeweed, spirulina, dried	3.5
Sweet Potato (Baked)	0.9
Broccoli	0.4
Cereals and grains	
Rice bran, crude	7.4
Sunflower Seeds	7.1
Milk and dairy products	
Cheese (Gjetost)	3.4
Yogurt (plain, non-fat)	0.7
Milk	0.4

Source: USDA, 2015

8.5 Deficiency

Pantothenic acid is ubiquitous in foods and dietary deficiency is rare. Deficiency symptoms have been described in subjects on a pantothenic acid antagonist and/or pantothenic acid-deficient diet and include mood changes, as well as sleep, neurological, cardiac and gastrointestinal disturbances (Smith & Song, 1996; SCF, 2002; Trumbo, 2014). Based on the deficiency experiments done on the human subjects in the mid-to-late 1950's, the signs and symptoms of pantothenic deficiency are commonly fatigue, malaise, apathy, headache and muscle weakness. Other symptoms reported are gastrointestinal complaints such as nausea, vomiting, abdominal cramps, epigastric burning sensation, and increased flatulence (Sampedro *et al.,* 2015).

There are also some neurobiological effects that include numbness, paresthesia in toes and on the soles of feet, muscle cramps, and staggering gait. Other symptoms associated with a deficiency in pantothenic acid are sleep disturbances, emotional disorders, and personality changes. Deficiency of pantothenic acid will decrease the synthesis of Keratinocyte Growth Factor (KGF) and procollagen 4a2 in fibroblasts which result in the reduction of keratinocyte differentiation and proliferation (Kobayashi et. al., 2011).

Laboratory analysis to determine pantothenic acid concentration of human milk from healthy mothers has been carried out using standard microbiological methods such as microbiological assay using Lactobacillus arabinosus or Lactobacillus plantarum and radioimmunoassay (EFSA, 2014).

Pantothenic acid concentration in erythrocytes or whole blood has also been determined in laboratory analysis. In the study among adolescents by Eissenstat *et al.* (1986), positive correlations were also reported between pantothenic acid intakes and its concentrations in erythrocytes or in whole blood (mean intake of about 5 mg/day from food only). A positive correlation between pantothenic acid intakes and its concentrations in whole blood has also been observed in older adults (Srinivasan *et al.,* 1981).

Urinary excretion of pantothenic acid and, to a lesser extent, pantothenic acid concentration in whole blood or erythrocytes reflect pantothenic acid intake. Data from the general population are limited so that the variability characteristics of these biomarkers and their ability to discriminate between pantothenic acid insufficiency and adequacy are not well known. No cut-off values have been established for these biomarkers (EFSA, 2014).

8.6 Factors affecting pantothenic acid requirements

Few investigations about the effect of pantethine treatment on the platelet function have been done. It has been shown that oral treatment with pantethine helps to lower the total cholesterol and total phospholipids significantly, not only in the plasma but also in the platelet while maintaining their ratio. The pantethine may affect the fluidity of the cell membrane of platelet composition. Thus, some suggestions have been made that pantethine supplementation could prevent atherogenesis by its effectiveness on serum lipid profile and platelet aggregability in people (Horvath & Vecsei, 2009).

Specific considerations during pregnancy and lactation

Two small cohort studies in pregnant and lactating women and non-pregnant, non-lactating women provide data on pantothenic acid intakes as well as urinary pantothenic acid excretion (Song *et al.,* 1985) and whole blood pantothenic acid concentration (Song *et al.,* 1985). Mean pantothenic acid intakes were between 5.3 and 6.2 mg/day in pregnant and lactating women and between 4.8 and 5.0 mg/day in controls. In both studies, average urinary pantothenic acid excretion levels were lower than intakes in all groups of women. Results were inconsistent with respect to differences in urinary excretion of pantothenic acid between pregnant or lactating and non-pregnant, non-lactating women.

Song *et al.* (1985) observed that concentrations of pantothenic acid in whole blood were significantly lower in pregnant and lactating women than in non-pregnant, non-lactating women, and significantly lower in pregnant women than in lactating women. The ESFA Panel (2014) concludes that data on biomarkers in pregnant and lactating women are scarce and provide inconsistent results and cannot be used to infer on a difference in the pantothenic acid status of pregnant and lactating women compared with non-pregnant, non-lactating women.

Assuming an average breast milk pantothenic acid concentration of 2.5 mg/L and an average breast milk secretion of 0.8 L/day over the first six months of lactation (Butte, Lopez-Alarcon & Garza, 2002; WHO/FAO, 2004; EFSA, 2014), the Panel notes that mean pantothenic acid secretion in milk is 2 mg/day in fully breast-feeding women.

8.7 Setting requirements and recommended intakes of pantothenic acid

There were no recommendations for pantothenic acid in the previous version of RNI (2005). There are no local studies available on pantothenic acid requirements that the Technical Sub Committee (TSC) on Vitamins could use as a reference when considering RNI for the vitamin. There are also no local studies of the biochemial status of the population. The main references used by the TSC to establish intake of pantothenic acid were WHO/FAO (2004), IOM (1998) and EFSA (2014). The rationale and steps taken in setting the requirements and the levels recommended by these organisations were considered.

According to EFSA (2014), there are not many evidence and studies done on pantothenic acid. There are only a few reports of the biochemical status of the pantothenic acid among the population group. The TSC on Vitamins accepts the approach taken by WHO/FAO (2004), the main reference of the TSC and decided to adopt these values as the RNI for pantothenic acid for Malaysia 2017. As will be discussed later in this chapter, these values are generally similar to the recommendations of EFSA (2014). Hence, the TSC decided not to adopt the EFSA recommendations although this is the latest publication on recommended intake for pantothenic acid. These values are given in the bold in the following paragraphs according to age groups and summarised in Appendix 8.1.

Infants

Infants aged between 0 to 6 months usually consumed human milk as it is the optimal milk source for infants. Besides that, it is recommended to consume human milk throughout the first year of life because of its nutritional value. Human milk also contains pantothenic acid. Based on IOM (1998), the estimated concentration of pantothenic acid in human milk is between 2.2 to 2.5 ml/L. Thus, AI for pantothenic acid for infants aged between 0 to 6 months is 1.7 mg/day. While the AI for infants aged between 7 to 12 months is 1.8 mg/day as it is the mean obtained from two methods of extrapolation which is by extrapolating the AI for pantothenic acid for younger infants aged 0 to 6 months and also AI for adults to estimate a recommended intake. The values for both extrapolations are 2.2 mg/day and 1.4 mg/day respectively.

For infants over six months, an AI of 3 mg/day is proposed by extrapolating from the pantothenic acid intake of exclusively breast-fed infants aged zero to six months, using allometric scaling and reference body weight for each age group, in order to account for the role of pantothenic acid in energy metabolism (ESFA, 2014).

German Society of Nutrition - Austrian Society of Nutrition - Swiss Society of Nutrition Research - Swiss Association for Nutrition (D-A-CH) (2013), WHO/FAO (2004) and Afssa (2001) proposed AIs for infants aged 7-12 months based on extrapolation from typical pantothenic acid intakes with human milk in younger exclusively breast-fed infants. Following the same approach, IOM (1998) estimated a value of 2.2 mg/day, while a value of 1.4 mg/day was obtained by downward extrapolation of the AI for adults using allometric scaling (body weight to the power of 0.75 and reference body weights) and allowing for the needs for growth by addition of a growth factor; thus, an AI of 1.8 mg/day was set for infants aged 7-12 months, being the mean of both values.

RNI for infants

0 - 5 months	**1.7 mg/day**
6 - 11 months	**1.8 mg/day**

Children and adolescents

The same recommendation in term of adequate intake (AI) was made for the intake of children and adolescent by the FAO/WHO Consultation as well as the IOM (1998). AI are used instead as no data were found on which to base an Estimated Average Requirement (EAR) and thus a Recommended Dietary Allowance (RDA) for pantothenic acid for children or adolescents of any age group. The data for IOM are tabulated by extrapolating extrapolation downward from the EAR for the children by adjusting for metabolic body size and growth and adding a factor for variability. The same approaches are used to establish AI for the adolescent.

The AI for children and adolescents is set at 4 and 5 mg/day, respectively, based on observed intakes in the EU (EFSA, 2014). Estimates of pantothenic acid intakes in children and adolescents, adults and older adults were available from eight EU countries. In boys and girls (3-12 years), mean/median intakes of 3.0 to 5.7 mg/day were reported, while mean/median intakes of 3.0 to 7.2 mg/day were observed in adolescent boys and girls (11-19 years) (EFSA, 2014).

D-A-CH (2013) derived AIs for children by interpolation between the values for infants. IOM (1998) extrapolated the AIs for children and adolescents from the AI of adults using allometric scaling and allowing for the needs for growth by the addition of a growth factor, which resulted in values consistent with available observed intakes for these age groups and intakes associated with blood and urinary pantothenic acid concentrations considered adequate.

AI for children

1 - 3 years	**2 mg/day**
4 - 6 years	**3 mg/day**
7 - 9 years	**4 mg/day**

AI for adolescents

Boys 10-18 years	**5 mg/day**
Girls 10-18 years	**5 mg/day**

Adult and Elderly

The AI for adults is set at 5 mg/day. In adult men and women below 65 years, mean/ median intakes of 3.2 to 6.3 mg/day were reported, while mean/ median intakes were between 2.2 and 6.0 mg/day in older men and women.

Study by Tarr, Tamura and Stokstad (1981), reported that average American diet used contained 5.8 mg/day of pantothenate, as reported for small groups of U.S. adults and adolescents. As there is no evidence the range of intake is inadequate, therefore an average value of 5 mg/day is set as the AI for adults. Dietary Reference Intakes for Japanese (Ministry of Health, Labour and Welfare, 2015) as well recommended AI of 5 mg/day pantothenic acid for both adults and elderly except women adults that were recommended at 4 gm/day.

A study by Srinivasan *et al.* (1981), which involved elderly aged 65 years and older, state that pantothenic acid intakes from food averaged 2.9 mg/1,000 kcal, or 5.9 ± 0.1 mg/day (range 2.5 - 9.5 mg/day). Thus, urinary pantothenic acid excretion of unsupplemented individuals averaged 6 mg/day. These data support the adequacy of the 5.9 mg/day intake from diet alone. There is no relationship between urinary excretion and age. Thus, as there is no basis to expect increased of pantothenic acid requirements in the elderly, the same recommendation as the younger adult is set for elderly, aged 51 years and older, at 5 mg/day. Similarly, there is no basis for determining a separate recommendation based on gender, so the AIs for men and women are the same.

AI for adults

Men	**19 - 65 years**	**5 mg/day**
Women	**19 - 65 years**	**5 mg/day**

AI for elderly

Men	**> 65 years**	**5 mg/day**
Women	**> 65 years**	**5 mg/day**

Pregnancy and Lactation

D-A-CH (2013) and Afssa (2001) considered the AI set for adults to be sufficient to cover the period of pregnancy. WHO/FAO (2004) and IOM (1998) noted some evidence of lower whole blood pantothenic acid concentrations in pregnant women compared to non-pregnant women, although no differences in urinary excretion were observed and average intakes were found to exceed excretion (Song *et al.,* 1985). The IOM (1998) set an AI of 6 mg/day based on observed average intakes in pregnant women (Song *et al.,* 1985) and rounding up.

During pregnancy, there is a study done which found that the blood pantothenate concentrations in pregnant women are significantly lower, but there is no difference in urinary excretion in pregnant women compared with non-pregnant women. Hence, the AI set by the IOM (1998) is 6 mg/day since there is absence of information that is usual intakes in the United States and Canada is insufficient to support a healthy pregnancy outcome. Meanwhile, the AI for adults also applies to pregnant women (EFSA, 2014).

According to IOM (1998), there is no evidence that pantothenic acid intakes are inadequate to support function during lactation. However, consider that the loss of 1.7 mg/day through human milk and lower maternal blood concentrations corresponding to intakes of about 5 to 6 mg/day, the AI of 7 mg/day of pantothenic acid is being set. This value is the same as proposed by EFSA (2014) for lactating women, an AI of 7 mg/day is proposed, to compensate for pantothenic acid losses through breast milk. WHO/FAO (2004), Afssa (2001) and IOM (1998) proposed an AI of 7 mg/day for lactating women, to compensate for losses through breast milk. D-A-CH (2013) considered the AI set for adults to be sufficient to cover the period of lactation.

RNI for

Pregnancy	**6 mg/day**
Lactation	**7 mg/day**

Discussions on revised RNI for Malaysia

There were no recommendations for pantothenic acid in the previous version of the Malaysian RNI (2005). The proposed recommended intakes for the revised recommended intakes for Malaysia 2017 are same as those adopted by WHO/FAO (2004). The recommendation levels of pantothenic acid by WHO/FAO (2004) are also almost similar to the Adequate Intake (AI) values of IOM (1998). The recommendations of EFSA (2014), which is the latest report published, are also similar to those of these two organisations for most age groups, except for infants and young children. The recommendations in EFSA (2014) are higher for infants and young children compared with those of WHO/FAO (2004) and IOM (1998).

8.8 Tolerable upper intake level

A Tolerable Upper Intake Level (UL) for pantothenic acid could not be derived but evidence available from clinical studies using high doses of pantothenic acid (up to 2 g/day) indicates that intakes considerably in excess of observed levels of intake from all sources do not represent a health risk for the general population (SCF, 2002).

A study by General Practitioner Research Group (1980) involved patients who were treated with various dosage of calcium pantothenate for eight weeks, starting with 500 mg/day in the first two days, then 1g/day for the next three days. After that, 1.5 g/day of calcium pantothenate are given for the following four days and 2 g/day from day 10 until the end of the trial. This study reported that there is no side effect of the treatment given, and there is evidence of beneficial effect on pain and disability in rheumatoid arthritis patients.

8.9 Research recommendations

The following priority areas of research are recommended:

1. Research on pantothenic acid biomarkers that could be used to characterise the adequacy of pantothenic acid status.

2. To determine pantothenic acid content in food sources.

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Appendix 8.1 Comparison of recommended nutrient intake for Pantothenic Acid: RNI Malaysia (2017), AI of WHO/FAO (2004), AI of IOM (1998) and AI of EFSA (2014)

Malaysia (2017)		WHO/FAO (2004)		IOM (1998)		EFSA (2014)	
Age group	**AI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**
Infants							
0 - 5 months	1.7			0 - 6 months	1.7		
6 - 11 months	1.8	7 - 12 months	1.8	7 - 12 months	1.8	7 - 11 months	3
Children							
1 - 3 years	2	1 - 3 years	2	1 - 3 years	2	1 - 3 years	4
4 - 6 years	3	4 - 6 years	3	4 - 8 years	3	4 - 10 years	4
7 - 9 years	4	7 - 9 years	4				
Adolescent							
Boys		**Boys**					
10 - 18 years	5	10 -18 years	5	9 - 13 years	4	11 - 17 years	5
				14 - 18 years	5		
Girls		**Girls**					
10 - 18 years	5	10 - 18 years	5	9 - 13 years	4	> 18 years	5
				14 - 18 years	5		
Adult and elderly							
Men		**Men**		**Men**		**Men**	
19 - 65 years	5	≥ 19 years	5	19 - 30 years	5	≥ 19 years	5
> 65 years	5			31 - 50 years	5		
				51 - 70 years	5		
				>70 years	5		

Malaysia (2017)		WHO/FAO (2004)		IOM (1998)		EFSA (2014)	
Age group	**AI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**
Women		**Women**		**Women**		**Women**	
19 - 65 years	5	≥ 19 years	5	19 - 30 years	5	≥ 19 years	5
> 65 years	5			31 - 50 years	5		
				51 - 70 years	5		
				> 70 years	5		
Pregnancy							
	6		6	14 - 18 years	6		5
				19 - 30 years	6		
				31 - 50 years	6		
Lactation							
	7		7	14 - 18 years	7		7
				19 - 30 years	7		
				31 - 50 years	7		

9 • Pyridoxine (Vitamin B6)

9.1 Introduction

Vitamin B6 or pyridoxine is used to generically describe vitamers that include the alcohol pyridoxine, the aldehyde pyridoxal, the amine pyridoxamine, and their 5'-phosphates. It is a water-soluble vitamin, stable in acid and unstable in alkali. The metabolically active form is pyridoxal 5'-phosphate (PLP). Plasma PLP concentration is the biomarker of status used to derive recommendations for vitamin B6. Vitamin B6 is ingested mostly in the pyridoxine and phosphorylated forms from plant foods and in the pyridoxamine and pyridoxal forms in animal foods. Ingested phosphorylated forms are dephosphorylated in the intestinal mucosa.

9.2 Functions

Pyridoxal phosphate (PLP) is involved in amino acid metabolism. Transamination of amino acids into oxo (keto) acids requires PLP dependent enzymes to produce non-essential amino acids. PLP is also involved as a cofactor in the decarboxylation of amino acids to yield amines. Amines that are dependent on PLP include histamine, dopamine (which is then further converted into noradrenaline and adrenaline, also known as norepinephrine and epinephrine), serotonin and GABA (γ-aminobutyric acid) and phosphatidylethanolamine.

Many enzymes are PLP dependent because they are needed to transform amino acids into other resources. For example, kynureninase is a PLP dependent enzyme, which is involved in the biosynthesis of niacin (vitamin B3) from trytophan (Rios-Avila *et al.,* 2013). Another example is 5-aminolevulinate synthase, which is the precursor in haem synthesis (Ferreira & Gong, 1995). It is also a coenzyme in the synthesis of sphingoid bases, which are involved in signal transmission and cell recognition.

In the muscle, PLP functions as a coenzyme of glycogen phosphorylase. The phosphate group of PLP donates a proton to an inorganic phosphate molecule, which sets off a chain of deprotonation which catalyses the breakdown of glycogen and results in the formation of glucose-1-phosphate.

Vitamin B6 also acts as a regulator of actions of steroid hormones (including androgens, oestrogens, progesterone, glucocorticoids), calcitrol, retinol and retinoic acid, and the thyroid hormones. PLP releases the hormone-receptor-complex from tight nuclear binding, and terminates hormone actions.

9.3 Metabolism

Vitamin B6 must be obtained from the diet, as human beings cannot synthesise it. All forms of vitamers except 4-pyridoxic acid (4-PA) and pyritinol can be interconverted. Ingested Vitamin B6 is absorbed as pyridoxal (PL), pyridoxamine (PM) and pyridoxine (PN) by passive diffusion in the jejunum, and are converted into pyridoxamine 5'-phosphate (PMP) by pyridoxal kinase. PMP is further converted to pyridoxal 5'-phosphate (PLP) by pyridoxamine phosphate transaminase or pyridoxine 5'-phosphate oxidase. PLP is the metabolically active form.

Pyridoxine 5'-phosphate oxidase is dependent on riboflavin-5'-phosphate (also known as flavin mononucleotide, FMN) as a cofactor, which is produced from riboflavin (vitamin B2) (McCormick, 1989). Therefore dietary vitamin B6 is not available for absorption without vitamin B2 in this pathway.

The liver is the primary site of metabolism for the B6 vitamers. Most absorbed vitamin B6 is transported to the liver in their unphosphorylated forms. From the liver, PLP is released into the plasma, as a PLP-albumin complex. From the plasma, tissues and erythrocytes transport nonphosphorylated forms and convert them to PLP, where it could then bind with proteins. The protein binding allows for accumulation and retention in tissues, where it is found primarily in the mitochondria and cytosol. For uptake into extrahepatic tissues, the phosphate is hydrolysed by extracellular alkaline phosphotase, then retained intracellularly by phosphorylation. The muscle stores most of the body's vitamin B6.

Despite high dietary intakes of B6, PLP accumulation in tissues is limited by this capacity for protein binding. When this capacity is exceeded, free PLP is rapidly dephosphorylated. In the liver, free pyridoxal is irreversibly oxidized to 4-pyridoxic acid, which is then released to be excreted via urine. If very large doses of vitamin B6 are consumed, other forms of the vitamin may also be found excreted in the urine. Vitamin B6 is also excreted in the faeces as a product of excess B6 intake and microbial synthesis of B6 in the lower gut.

The IOM (1998) reviewed the literatures available on possible interaction between increased protein intake and reduction in vitamin B6 status as indicated by various biomarkers, and concluded that the decrease in plasma PLP resulted from induction of PLP-dependent enzymes possibly leading to retention of PLP in the tissue.

9.4 Sources

Vitamin B6 is available in a wide variety of foods particularly in meat, fish, poultry, organ meats, enriched cereal products, fortified soy-based meat substitutes, beans, lentils and bananas. Pyridoxamine and pyridoxal forms of vitamin B6 in foods of animal origin have a bioavailability of about 75% (Roth-Maier, Kettler & Kirchgessner, 2002) to almost 100% in some foods (Reynolds, 1998).

Most Malaysians consume rice and bread. It is important to note that brown rice (Lebiedzinska & Szefer, 2006) and bread made with whole-wheat flour (Batifoulier *et al.,* 2006) contain about double the pyridoxine concentration compared to polished rice and white bread. Plant foods have vitamin B6 in glycosylated form, which reduces its bioavailability (Reynolds, 1998; Hansen, Leklem & Miller, 1996). Waldmann and colleagues (2005) recommend that vegans and vegetarians should include foods with a high bioavailability of pyridoxine, such as beans, lentils and bananas.

However, Golden (2009) reported that the glycosylated form constitutes 20% of pyridoxine in rice, 28% in wheat, and 15% to 57% in beans, which if ingested could reduce the availability of free pyridoxine from other sources. A significant increase in faecal excretion of total vitamin B6 was observed in women who were prescribed a high pyridoxine glucoside diet compared to their excretion when they were receiving a low pyridoxine glucoside diet in a crossover trial

(Hansen *et al.,* 1996). The increase in faecal excretion during the high pyridoxine glucoside diet was accompanied by decreases in total plasma vitamin B6 and PLP levels, which indicates reduced absorption of vitamin B6 and/ or increased synthesis by intestinal microflora. Processing of soy-products reduced its pyridoxine concentration (Lebiedzinska & Szefer, 2006); therefore vegans and vegetarians should include fortified versions in their diet.

There is wide variation in the B vitamin content of cooked foods as such items are susceptible to cooking losses, particularly when liquids in which food is cooked are not consumed (IOM, 1998). The losses may vary from 0% to about 40% (Golden, 2009). A comparison of vitamin B6 content is given in Table 9.1.

Table 9.1: Pyridoxine (vitamin B6) content of foods

Food	mg per 100g
Poultry, meats, fish, legumes	
Vegetarian fillets*	1.5
Tuna, yellowfin, fresh, cooked	1.0
Beef, variety meats and byproducts, liver, pan fried	1.0
Tempeh, cooked	0.2
Lentils, mature seeds, boiled without salt	0.2
Egg, chicken, whole, poached	0.1
Fruits	
Banana	0.4
Jackfruit (nangka)	0.3
Durian	0.3
Raisins, seedless	0.2
Vegetables	
Shallots	0.3
Taro *(keledek),* cooked, without salt	0.3
Potatoes, without skin, boiled, without salt	0.3
Spinach, boiled, drained, without salt	0.2
Capsicum	0.2
Cereals and grains	
Oat cereal, instant, fortified, plain, prepared with water	0.3
Wheat cereal, RTE, puffed, fortified	0.2
Milk and dairy products	
Milk, whole	0.04
Yoghurt, plain, whole milk	0.03

*Vegetarian fillets are meat-free products made from soy derived textured vegetable proteins or fungi derived or other plant origin materials.
Source: USDA, 2015

9.5 Deficiencies

Deficiency is rare because the amount of vitamin B6 absorbed exceeds physiological needs, except in individuals with autoimmune disorders that cause inflammation, coeliac disease, individuals with impaired renal functions probably due to increased metabolic clearance of pyridoxal 5'-phosphate, and individuals with chronic alcohol dependency because pyridoxal 5'-phsophate loses out in competition with alcohol acetaldehydes. When it occurs, vitamin B6 deficiency is associated with microcytic hypochromic (sideroblastic) anaemia, electroencephalographic abnormalities, dermatitis with cheilosis, naso-lateral seborrhoea, glossitis, depression and confusion, weakened immune function, and peripheral neuropathy (epiletic form convulsions in infants) (FAO/WHO, 2001; IOM, 1998). Electroencephalographic abnormalities have been observed in young non-pregnant women in controlled depletion studies with experimental diets consisting less than 0.06 mg/day of vitamin B6 (Kretsch, Sauberlich & Newbrun, 1991), which was reversed with administration of 0.5 mg/day vitamin B6.

Moderate vitamin B6 deficiency results in abnormalities of amino acid metabolism because they are dependent on PLP as a coenzyme. These include impairment of conversion of tryptophan to niacin, and methionine to cysteine. The conversion of trytophan to nicotinic acid is interrupted, which leads to accumulation of hydroxykynurenine and xanthurenic acid. Xanthurenic acid is then detectable in urine after an oral trytophan load. Deprivation of vitamin B6 also deprives gluconeogenesis of its PLP-dependent transaminases and glycogen phosphorylase, which results in impaired glucose tolerance. Vitamin B6 deficiency is also associated with increased sensitivity of tissues to steroid hormone actions.

Vitamin B6 status can be determined by various markers. The most commonly measured marker is fasting plasma PLP, which is a good biomarker for PLP content in the liver (Chiang *et al.,* 2005). PLP is positively associated to vitamin B6 intake and changes in intake are reflected in the biomarker within 1 to 2 weeks (Ueland *et al.,* 2015). However other factors not related to vitamin B6 status affect plasma PLP concentration: diabetes (Ueland *et al.,* 2015), albumin concentration, alkaline phosphatase activity and alcohol consumption (Brussaard *et al.,* 1997). Another marker is activation of erythrocyte aspartate aminotransferase (α-EAST) and alanine aminotransferase (α-EALT) by PLP, which are used as a marker for long-term vitamin B6 status. Their large variation in values limits their usefulness as biomarkers of status (Raica & Sauberlich, 1964).

The prevalence of vitamin B6 deficiency in Malaysia is uncertain because its biomarker of status has not been measured. Among Malaysian women of reproductive age, median intake was 1.0 mg/d (5th to 95th percentile: 0.4 - 2.6 mg/d); biomarkers of vitamin B6 were not measured (Khor *et al.,* 2005). The lack of data on prevalence of deficiency in lactating women and therefore infants globally can also be attributable to lack of reported studies (Allen, 2012).

9.6 Factors affecting pyridoxine requirements

Factors to be considered when estimating the pyridoxine requirement include the bioavailability of vitamin B6 (Reynolds, 1998; Roth-Maier *et al.,* 2002), genetic variability of grains (Batifoulier *et al.,* 2006), nutrient - nutrient interactions (IOM, 1998), processing and cooking procedures, drug interactions (IOM, 1998), and disease state of individuals (IOM, 1998). Age is

associated with decrease in plasma concentration of vitamin B6. It is not known whether this decrease in the elderly reflects an inadequate intake, a greater requirement, or changes in tissue distribution and metabolism of the vitamin with increasing age.

9.7 Setting requirements and recommended intake of pyridoxine

There had been no recommendations for pyridoxine in the previous Malaysian RNI of 2005. In sourcing of references to establish RNI for B6 for Malaysian RNI 2017, the Technical Sub Committee (TSC) for Vitamins noted that there are no studies on the requirement for this vitamin among the local population. Biochemical studies of the status of the vitamin is also scarce in the country. The TSC reviewed the rationale and approaches adopted by three international scientific organisations, namely WHO/FAO (2004), IOM (1998) and EFSA (2016). The TSC decided to base their recommendations for Malaysian RNI 2017 for pyridoxine on WHO/FAO (2004) as those values were derived from carefully considered larger body of studies. The TSC decided not to base their recommendations according to recommendations by EFSA (2016) as they were derived from studies with small sample sizes, comprising mostly of Europeans. The TSC for Vitamins felt that small randomised controlled studies were not adequate to revise recommended values.

Infants

An adequate intake (AI) was used by the IOM DRI Committee to estimate vitamin B6 requirements for infants. The AI is based on the observed mean intake of dietary vitamin B6 for healthy exclusively breastfed infants for the first 6 months and B6 concentration of milk produced by well-nourished mothers. The AI for infants 0 through 6 months of age is derived by using 780 ml as the average volume of milk per day and 0.13mg/L (West & Kirksey, 1976) as the average B6 concentration. The requirement thus calculated is 0.1 mg/day.

For older infants, the DRI Committee used the reference body weight ratio method to extrapolate from the AI for B6 for infants ages 0 - 6 months. The AI for this group of infants was thus calculated to be 0.2 mg/day after rounding. The Committee also extrapolated from the Estimated Average Requirement (EAR) for adults and after adjusting for expected variance, gave an AI for B6 of 0.4 mg/day. The Committee also reviewed data obtained from several controlled studies that measured B6 intake and assessed infant status. These data were found to support the estimated AI of 0.3 mg/day of B6 for older infants as the mean obtained from the two methods of extrapolation (IOM, 1998).

The WHO/FAO (2004) recommends a RNI of 0.1 mg/day for infants 0 through 6 months of age, and 0.3 mg/day for older infants. The EFSA (2016) Dietetic Products, Nutrition and Allergies (NDA) Panel recommends an AI of 0.3 mg/day for infants aged 7 - 11 months.

RNI for infants

0 - 5 months	**0.1 mg/day**
6 - 11 months	**0.3 mg/day**

Formulas for full-term infants studied showed those samples provide higher levels of vitamin B6 than does human milk (Young, Eitenmiller & Soliman, 1998) and formula fed full-term infants have higher plasma pyridoxal 5'-phosphate (PLP) concentrations than full-term infants fed with human milk (Borschel *et al.,* 1986).

Children and adolescents

For older children, the recommended intakes were estimated from maintenance needs with respect to body weight and extrapolating from adult values (IOM, 1998). The AI for children was estimated to range from 0.5 - 0.6 mg per day. The AI for adolescents was estimated to range from 1.0 to 1.3 mg per day. It was assumed that total needs for males and females do not differ substantially until age 14 years, when reference weights differ. The WHO/FAO (2004) recommends the same amounts for children aged 1 to 9 years, 1.3 mg/day for adolescent boys aged 10 to 18 years, and 1.2 mg/day for adolescent girls aged 10 to 18 years. The EFSA NDA Panel (2016) sets an Average Requirement between 0.5 - 1.2 mg/day for children aged 1 - 14 years of both sexes. For adolescents aged 15 - 17 years, the EFSA NDA Panel derives their Average Requirement as similar to adults.

The IOM (1998) method of estimating EAR and RDA was used to determine EAR (RDA) for children and adolescents; boys aged 7 - 12 years: 0.84 (1.01) mg/d and girls aged 7 - 12 years: 0.75 (0.89) mg/d (Chang *et al.,* 2002); boys aged 13 - 15 years: 1.07 (1.28) mg/d and girls aged 13 - 15 years: 0.90 (1.08) mg/d (Chang, Hsiao & Hsuen, 2003). The RDA for boys aged 16 - 18 years is suggested to be 1.1 mg/d and girls 1.0 mg/d (Chang *et al.,* 2007).

When treating malnourished children, it is recommended that pyridoxine requirements be set at 1.8mg/1,000 kcal for a fortified food approach, 0.8 g/1,000 kcal for mixed diets, and 1 mg/1,000 kcal if the diet contains milled whole cereals (Golden, 2009).

RNI for children

1 - 3 years	**0.5 mg/day**
4 - 6 years	**0.6 mg/day**
7 - 9 years	**1.0 mg/day**

RNI for adolescents

Boys 10 - 18 years	**1.3 mg/day**
Girls 10 - 18 years	**1.2 mg/day**

Adults and elderly

The IOM (1998) used mostly depletion - repletion studies to estimate EAR for adults and the elderly, and assumed a coefficient of variation (CV) of 10 percent because of unavailability of standard deviation of the requirement for vitamin B6. A plasma PLP level of 20 nmol/L was used as the criteria for sufficiency for setting the EAR (Bailey *et al.,* 1999; IOM, 1998; Liu *et al.,* 1985).

The RDA is then defined as twice the CV, therefore the RDA is 120 percent of the EAR. Based on these studies, the RDAs for the elderly is higher than those for adults (Selhub *et al.,* 1993; Ribaya-Mercado *et al.,* 1991). The WHO/FAO (2004) recommends 1.3 mg/day for men and women aged 19 to 50 years, 1.7 mg/day for men above 50 years, and 1.5 mg/d for women above 50 years.

The EFSA NDA Panel (2016) considers the Average Requirements and Population Reference Intakes (PRIs) from the vitamin B6 intake required to maintain mean concentration of plasma PLP above 30 nmol/L from several intervention studies on 44 young women. The EFSA NDA Panel sets the AR for all adult women at 1.3 mg/day and PRI at 1.6 mg/day. In the absence of similar data for men, the EFSA NDA Panel sets the AR for all adult men at 1.5 mg/day and PRI at 1.7 mg/day, using allometric scaling from the AR of women and taking into account the difference in reference body weight.

Low plasma vitamin levels have been associated with cognitive decline in the elderly (van de Rest *et al.,* 2012). In the elderly, supplementation with vitamin B6, B12 and/or folic acid may normalise homocysteine levels, but have not been shown to clearly improve or slow cognitive decline (Krause & Roupas, 2015). An earlier Cochrane review of two randomised control trials concluded that vitamin B6 had no overall effects on cognition or mood (Malouf & Grimley, 2003).

The IOM (1998) noted that 1 mg/day is sufficient for most adults because clinical symptoms of B6 deficiency have been reported only in controlled studies with very low levels of B6 during the depletion experimental diet. Individuals with very high protein diets may require higher levels of vitamin B6. There is an Evidence Level IIA suggestion from a depletion - repletion study that non-pregnant young women consuming a high-protein diet (1.55g/kg body weight) should consume 1.94 mg/d of vitamin B6 (Huang *et al.,* 1998). Kretsch *et al.* (1995) suggested in a depletion study (Evidence Level IB) that a RDA of 0.020 mg/g protein for vitamin B6 be used for young non-pregnant women on a high protein diet (1.55 g /kg body weight).

There is an Evidence Level IB suggestion that the EAR should be revised to 1.2mg/d and the RDA should be 1.7 mg/day for young women (Hansen *et al.,* 2001). There is an Evidence Level III study, which suggests RDA values of 1.6 mg/d for men and 1.5 mg/d for women aged 17 - 25 years (Cho & Kim, 2004).

RNI for adults

Men	**19 - 50 years**	**1.3 mg/day**
Women	**19 - 50 years**	**1.3 mg/day**
Men	**51 - 59 years**	**1.7 mg/day**
Women	**51 - 59 years**	**1.5 mg/day**

RNI for elderly

Men	**60 - 65 years**	**1.7 mg/day**
Women	**60 - 65 years**	**1.5 mg/day**
Men	**> 65 years**	**1.7 mg/day**
Women	**> 65 years**	**1.5 mg/day**

Pregnancy and Lactation

The IOM (1998) recommends RDAs of 1.9 mg/day for pregnant women and 2.0 mg/day for lactating women. The increased recommendations are based on average estimated accretion by the placenta and foetus, and increased demands of about 0.25 mg/day WHO/FAO (2004); IOM, 1998). Consequently both the WHO/FAO and IOM recommend an increase of 0.5 mg/day of vitamin B6 to reasonably meet the need in the third trimester of pregnancy. The EFSA NDA Panel (2016) recommends an increase of 0.2mg/day for pregnant women, and sets an AR of 1.5 mg/day and PRI of 1.8 mg/day. For lactating women, the EFSA NDA Panel estimates an additional intake of 0.133 mg/day, and sets an AR of 1.4 mg/day and PRI of 1.7 mg/day. The EFSA recommendation is based on vitamin B6 bioavailability from a mixed diet (75%).

The vitamin B6 intake of mothers is a strong predictor of infant status (Allen, 2012; Kang-Yoon *et al.,* 1992). Mothers receiving higher levels of vitamin B6 supplements post-natal in the form of daily PN.HCl produced breast milk with higher levels of vitamin B6 content (Chang & Kirksey, 1990; Borschel, Kirksey & Hannerman, 1986). Chang and Kirksey (1990) reported that PLP concentrations in infant cord blood, and maternal plasma, vitamin B6 concentration in colostrum were positively correlated with vitamin B6 supplementation during pregnancy. Consequently the WHO/FAO (2004) and IOM (1998) recommend an increase of 0.6 mg/day of vitamin B6 for lactation. The IOM (1998) recommends the EAR of 1.1 mg/day to ensure that breast milk contains 0.13 mg/L of vitamin B6. A Cochrane review concluded that there were few trials and inadequate evidence to demonstrate clinical benefits of vitamin B6 supplementation during pregnancy (Salam, Zuberi & Bhutta, 2015).

RNI for

Pregnancy	**1.9 mg/day**
Lactation	**2.0 mg/day**

Discussion on revised RNI for Malaysia

The recommendations for vitamin B6 in Malaysian RNI 2017 are similar to WHO/FAO (2004) as well as those in the IOM (1998) recommendations. The recommendations for adolescents and adults are lower than values recommended by EFSA (2016). On the other hand, the recommendations during pregnancy and lactation are slightly higher than those of EFSA (2016).

9.8 Tolerable upper intake levels

The IOM (1998) reported no adverse effects from high intake of vitamin B6 from food sources. Large oral doses of supplemental pyridoxine used to treat medical conditions have been associated with sensory neuropathy and dermatological lesions (Cohen & Bendich, 1986). Schaumburg *et al.* (1983) reported sensory neuropathy at dosages of 2,000 to 6,000 mg/day of pyridoxine for 2 to 40 months. Friedman, Resnick & Baer (1986) reported dermatological lesions after consumption of 2 to 4 g/day of pyridoxine for more than 1 year. The WHO/FAO (2004) adopts a Tolerable Upper Intake Level (UL) of 100 mg/day of pyridoxine, which was proposed by the IOM (1998). The European Union adopts a UL of 25 mg/day (SCF, 2000), and the UK adopts a UL of 10 mg/day (EVM, 2003).

There are homeopathic remedies that suggest vitamin B6 intake of 100 to 500 mg/d, including being used to treat morning sickness during pregnancy (Masino & Kahle, 2002). Given the UL, such actions should be viewed with caution despite very little evidence of reported adverse side effects (Simpson *et al.* 2010; Shrim *et al.* 2006). There is inadequate evidence on adverse effects from intakes of pyridoxine of 100 to 200 mg/day and the duration of intake at these levels (Renwick, 2006). The tolerable upper intake for vitamin B6 for various age groups as proposed by IOM (1998) is given in Table 9.2.

Table 9.2: Total Upper Intake (UL) levels of vitamin B6 for various age

		mg/d of vitamin B6
Infants	0 - 5 month	ND
	6 - 11 month	ND
Children and adolescents	1 - 3 y	30
	4 - 8 y	40
	9 - 13 y	60
	M:14 - 18 y	80
	F:14 - 18 y	80
Adults	M:19 - 50 y	100
	F:19 - 50 y	100
	M:51 - 59 y	100
	F:51 - 59 y	100
Elderly	M:60 - 65 y	100
	F:60 - 65 y	100
	M:>65 y	100
	F:>65 y	100
Pregnancy		100
Lactation		100

ND: Not possible to establish
Source: IOM (1998)

9.9 Research recommendations

The following priority areas of research are recommended:

- Data on dietary intake of vitamin B6 among community groups, particularly children and women, in terms of dietary B6 and supplemental B6.
- Content of vitamin B6 and pyridoxine glycoside in a variety of foods available in the market to improve the food composition tables.
- Plasma vitamin B6, plasma pyridoxal phosphate (PLP), urinary 4-pyridoxic acid (4-PA) concentrations, erythrocyte alanine aminotransferase activity coefficient (α-EALT), aspartate aminotransferase activity coefficient (α-EAST), which are biomarkers for vitamin B6 status.
- Vitamin B6 concentration in breast milk of lactating Malaysian women and their dietary vitamin B6 intake and supplementation levels.
- Bioavailability and loss of vitamin B6 from cooking process.

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Appendix 9.1: Comparison of recommended intake for Pyridoxine (Vitamin B6): RNI Malaysia (2017), WHO/FAO (2004), IOM (1998), and EFSA (2016)

Malaysia (2017)		WHO/FAO (2004)		IOM (1998)		EFSA (2016)	
Age group	**RNI (mg/d)**	**Age group**	**RNI (mg/d)**	**Age group**	**RNI (mg/d)**	**Age group**	**RNI (mg/d)**
Infants		**Infants**		**Infants**		**Infants**	
0 - 5 months	0.1	0 - 6 months	0.1	0 - 6 months	0.1*	0 - 6 months	-
6 - 11 months	0.3	7 - 12 months	0.3	7 - 12 months	0.3*	7 - 11 months	0.3 (AI)
Children		**Children**		**Children**		**Children**	
1 - 3 years	0.5	1 - 3 years	0.5	1 - 3 years	0.5	1 - 3 years	0.6
4 - 6 years	0.6	4 - 6 years	0.6	4 - 8 years	0.6	4 - 6 years	0.7
7 - 9 years	1.0	7 - 9 years	1.0			7 - 10 years	1.0
Boys		**Boys**		**Boys**		**Boys**	
10 - 18 years	1.3	10 - 18 years	1.3	9 - 13 years	1.0	11 - 14 years	1.4
				14 - 18 years	1.3	15 - 17 years	1.7
Girls		**Girls**		**Girls**		**Girls**	
10 - 18 years	1.2	10 - 18 years	1.2	9 - 13 years	1.0	11 - 14 years	1.4
				14 - 18 years	1.2	15 - 17 years	1.6
Men		**Men**		**Men**		**Men**	
19 - 50 years	1.3	19 - 50 years	1.3	19 - 50 years	1.3	≥18 years	1.7
51 - 65 years	1.7	≥ 51 years	1.7	≥ 51 years	1.7		
> 65 years	1.7						

Malaysia (2017)		WHO/FAO (2004)		IOM (1998)		EFSA (2016)	
Age group	**RNI (mg/d)**	**Age group**	**RNI (mg/d)**	**Age group**	**RNI (mg/d)**	**Age group**	**RNI (mg/d)**
Women		**Women**		**Women**		**Women**	
19 - 50 years	1.3	19 - 50 years	1.3	19 - 50 years	1.3	≥18 years	1.6
51 - 65 years	1.5	≥ 51 years	1.5	≥ 51 years	1.5		
> 65 years	1.5						
Pregnancy	1.9	**Pregnancy**	1.9	**Pregnancy**		**Pregnancy**	1.8
				14 - 18 years	1.9		
				19 - 30 years	1.9		
				31 - 50 years	1.9		
Lactation	2.0	**Lactation**	2.0	**Lactation**		**Lactation**	1.7
				14 - 18 years	2.0		
				19 - 30 years	2.0		
				31 - 50 years	2.0		

* The IOM gives these values as Adequate Intakes (AI), which is the mean intake in healthy breastfed infants. Other values are given as RDAs. The RDA is a value for average daily dietary intake level, which is sufficient to meet the nutrient requirements of 97–98 percent of the healthy individuals in a group, which in turn is calculated from the Estimated Average Requirement (EAR). The EAR is the value for the average daily nutrient intake level estimated to meet the requirements of half of the healthy individuals in a group.

10 • Folate (Vitamin B9)

10.1 Introduction

Folate, also known as vitamin B9, consists of a 2-amino-4-hydroxy-pteridine (pterin) group conjugated by a methylene group to p-amino benzoic acid, which is linked to one or more glutamic acid residues. The active form of folate is known as 5-methyltetrahydrofolate (5-MTHF). Folic acid is a synthetic form of folate which is found in supplements and fortified food products, such as flour and breakfast cereals.

10.2 Functions

Folate plays an important role in hematopoiesis and the production of red blood cells. Folic acid functions as coenzymes in single-carbon transfer in the metabolism of nucleic and amino acids. The DNA and methylation cycles both regenerate tetrahydrofolate (one form of folate). However, there is a considerable amount of catabolism of folate and a small loss of folate occurs via excretion from the urine, skin, and bile. Therefore, there is a need to replenish the body's folate content from the diet.

10.3 Metabolism

Folate is metabolised by intestinal cells to produce 5-MTHF. This 5-MTHF is the most common form that is found in plasma and serum. Folic acid is biotransformed in the intestinal absorptive mucosa and transferred to the hepatic portal vein as 5-MTHF in the same way as dietary folates. Food folates are hydrolysed to monoglutamate forms in the gut to allow their absorption across the intestine. The monoglutamates enter the portal circulation and are metabolised to polyglutamate derivatives in the liver. They are either retained, or released to the blood as reconverted monoglutamates or to bile. The liver contains about 50% of the body stores of folate.

Intracellular pools of reduced tetrahydrofolates serve as cofactors in several metabolic processes. Folate is present in serum either free or bound to other carrier proteins like the folate-binding protein or other serum protein like albumin. Serum folates exist mostly in the monoglutamate form, which is the form that can be readily transported across cell membranes. Once outside cells, folate is converted to polyglutamate forms by addition of several glutamic acid residues by folylpolyglutamate systhetase. This leads to retention of folate pools inside the cells for subsequent metabolism. The bulk of excretion products are folate cleavage products. Intact urinary folate accounts for only a small percentage of dietary folate. Biliary excretion of folate can be as high as 100 µg/day, however much of this is reabsorbed.

10.4 Sources

Folate is available in a wide variety of foods but in relatively low concentrations. However, it is particularly abundant in legumes, while green leafy vegetables are outstanding sources. Diets that contain adequate amounts of fresh green vegetables (>3 servings per day) are good folate sources. Fortified grain products also contribute to folate intake. Meat, poultry, seafood, eggs, and dairy products have small amounts of folate. Liver is high in folate content but is

also high in cholesterol; it is often recommended to reduce its intake. Folate losses occur during harvesting, storage, distribution and cooking. Table 10.1 shows food sources of folate in Malaysian foods (Chew *et al.,* 2012).

The bioavailability of natural folate is affected by the removal of the polyglutamate chain by the intestinal conjugase. This process is apparently not complete, thereby reducing the bioavailability of natural folate by as much as 25-30 percent. In contrast, synthetic folic acid appears to have a bioavailability of close to 100 percent. The low bioavailability and, more importantly, the poor chemical stability of the natural folate have a profound influence on the development of nutrient recommendations (Gregory III, 2001). Food fortification can provide significant amounts of folic acid to the diet (Crider *et al.,* 2011). In the United States adult population from 1988 to 1994 (before cereal grains were fortified with folate), the reported median intake of folate from food was approximately 250 (g/day, but this value underestimates current intake. After the fortification of cereal grains with folate, the average intake of folate is expected to increase by about 80 to 100 (g/day for women and by more for men (IOM, 1998).

Table 10.1 Folate content of foods

Source of folate	μmg/100 g
Cereal and cereal products	
Cornflakes*	156
Breakfast cereal*	155
Rice, brown (Beras perang)	45
Rice, parboiled	26
Rice, basmathi	25
Rice, broken (Beras hancur)	18
Bread, white (Roti putih)	18
Rice, uncooked	16
Fruits	
Papaya	31
Sapodilla (Ciku)	30
Kiwi fruit	29
Orange	24
Soursop (Durian belanda)	19
Honeydew (Tembikai susu)	9
Mango	4
Legumes and legume products	
Baked bean, canned	27
Soya bean milk, unsweetened	23
Soya bean curd, sheet/film (fucok)	13
Soya bean cake, fermented (tempeh)	10

Source of folate	µg/100 g
Soya bean curd, fried	10
Vegetables	
Vietnamese mint (kesom)	10
Pumpkin	8
Sweet potato shoots (pucuk ubi keledek)	6
Tapioca shoots	6

Source: Chew *et al.* (2012);* indicates the food has been fortified with folate.

10.5 Deficiencies

Nutritional deficiency of folate is common among people consuming an inadequate diet of legumes and folate-rich vegetables and fruits. This can be exacerbated by malabsorption conditions, including celiac disease and tropical sprue. Pregnant women are at risk of folate deficiency because pregnancy significantly increases folate requirement, especially during periods of rapid foetal growth (second and third trimester). Folate deficiency during pregnancy can results in neural tube defects (NTD). Losses of folate in milk during lactation also increase the requirement for lactating mother.

Inadequate folate intake results in a decrease in serum folate concentration, then adecrease in erythrocyte folate concentration. This is followed by a rise in homocysteine concentration and megalablastic changes in the bone marrow and other tissues with rapidly dividing cells. Macrocytic anaemia finally results. If there is inadequate dietary folate, the activity of both the DNA and the methylation cycles will be reduced, thus, reducing cell division (particularly the red blood cell) and resulting in anaemia. Other cells derived from bone marrow also decrease, leading to leucopenia and thrombocytopenia. There is also a reduction in cell division in the lining in the gut that may result in an increased susceptibility to infection, a decrease in blood coagulation and secondary malabsorption. The decrease in the methylation cycle will also result in an elevation in plasma homocysteine which has been associated with the aetiology of cardiovascular disease. Vitamin B12 and vitamin B6 are also required for the methylation cycle. Interruption of the methylation cycle resulting from impaired folate status or decreased vitamin B12 or vitamin B6 may have serious long-term risk such as neuropathy. Symptoms of folate deficiency include weakness, fatigue, difficulty in concentrating, irritability, headache, palpitations, shortness of breath and atrophic glossitis.

There is increasing interest in homocysteine and folic acid. Plasma homocysteine concentration, even if only moderately elevated, is an independent risk factor for cardiovascular disease (CVD), stroke, poor cognitive function and osteoporosis. Even in populations that are apparently normal and consuming diets adequate in folate, there is a range of elevated plasma homocysteine that could be lowered by consuming an extra 100 or 200µg/day folic acid. However, large scale intervention trials regarding the significance of interrelationships among folate levels, plasma homocysteine levels with CVD and other diseases need to be carried out. The DRI Committee (IOM, 1998) felt that knowledge currently available on this relationship is too weak to be used as the basis for deriving estimated average requirement for folate.

EFSA (2014) considered that red blood cell folate is the most reliable biomarker of folate status. It reflects tissue folate status and can be used as indication for long-term dietary folate intake. Folate adequacy is characterized by serum and red blood cell folate concentration of ≥ 10 nmol/L and 340 nmol/L, respectively. Serum and red blood cell folate below 6.8 nmol/L and 317 nmol/L are catogerised as folate deficiency. Although total plasma homocysteine is a sensitive biomarker, it is not a specific biomarker of folate status and function because of the influence by various other dietary factors such as selected B-vitamins, choline, betaine and alcohol consumption.

There is only a limited number of studies on folic acid intake or deficiency in the country. In a study of nutritional anaemia among 309 pregnant women of lower socio-economic strata in the Maternity Hospital Kuala Lumpur, serum folate was one of the numerous parameters investigated. A high prevalence of 61% of low serum folate levels (<3 ng folate per ml serum) was reported and the highest prevalence was amongst Indian women (77.3%) (Tee *et al.,* 1984).

In a study conducted by Khor *et al.* (2006) involving 399 Malaysian women of childbearing age (140 Malay, 131 Chinese and 128 Indian), the median intake of folate was 202.4 µg (59.4-491.8 µg), which only achieved 50.6% of the Malaysian Recommended Nutrient Intakes level. In terms of folate deficiency, 15.1% of the Malaysian subjects showed plasma folate deficiency (<6.8 nmol/L), with the highest prevalence among Indians (21.5%) (Khor *et al.,* 2006). According to Khor *et al.* (2006), Chinese had significantly higher red blood cell folate levels than the Malays and Indians. Folate content is reduced in prolonged cooking, and the Chinese style of cooking usually entails quick stir-frying of leafy green vegetables and legumes. Malays and Indians, however, prefer their vegetables to be well-cooked.

In another study comparing red blood cell folate levels in three Asian cities, Green *et al.* (2007) found out that red blood cell folate levels were the highest in women from Jakarta (872 nmol/L), followed by Kuala Lumpur (674 nmol/L) and the lowest in Beijing (563 nmol/L). The predicted neural tube defects were the highest in Beijing (30/10000), followed by Kuala Lumpur (24/10000) and the lowest in Jakarta (15/10000). The plausible explanation for higher folate status in women in Jakarta is the implementation of mandatory folic acid fortification in 2001 of local and imported wheat (200 µg/100 g of wheat). Neither Malaysia nor China had a mandatory folic acid fortification scheme (Green *et al.* 2007).

10.6 Factors affecting folate requirements

Factors to be considered when estimating the folate requirement include the bioavailability of folic acid and food folate, nutrient-nutrient interactions, interactions with other food components, smoking, folate-drug interactions and genetic variations. These have been thoroughly reviewed in IOM (1998) and EFSA (2014).

Dietary Folate Equivalents

Folic acid recommendation is expressed as dietary folate equivalents (DFE), which are units that account for the differences in the absorption of food folate and of synthetic folic acid obtained from dietary supplements or food fortified with folic acid. When synthetic folic acid is consumed as a supplement without food, it is nearly 100% bioavailable. In contrast, when folic

acid is consumed with food, as it is always the case with fortified cereal-grain products, its absorption is reduced by a small percentage. Its estimated bioavailability is approximately 85%. Naturally occurring food folate is less well absorbed by the body than is synthetic folic acid. The best estimate of the bioavailability of food folate is provided by data from the study of Gibson (2007). Thus, folic acid taken when a person is fasting is 2 times (100/50) more bioavailable than food folate, and folic acid taken with food (which includes folic acid added to food during fortification) is 1.7 times (85/50) more bioavailable than food folate. Thus, if a mixture of synthetic folic acid plus food folate is consumed, dietary folate equivalents (DFE) to determine the EAR can be calculated as follows:

µg of DFE provided = [µg of food folate + (1.7 x µg of synthetic folic acid)]

DFE may be expressed in different ways, depending on the type of conversion needed (Suitor and Bailey 2000):

1 µg DFE =1.0 µg food folate = 0.6 (g folic acid added to foods = 0.5 µg folic acid taken without food.

1 µg folic acid as a fortificant = 1.7 µg DFE

1 µg folic acid as a supplement, fasting = 2.0 µg DFE.

Thus, 100 µg folate from a serving of cooked spinach equals to 100 µg DFE, but 100 µg folic acid from a serving of fortified ready-to-eat cereal equals 170 µg DFE, and 100 µg supplemental folic acid taken without food equals to 200 µg DFE.

Interactions with nutrients

Coexisting iron or vitamin B12 deficiency may interfere with the diagnosis of folate deficiency. Iron deficiency leads to a decrease in mean cell volume, whereas, folate deficiency results in the opposite direction. Therefore, in combined deficiency, interpretation of haematological changes may be unclear. A vitamin B12 deficiency results in the same haematological changes that occur with folate deficiency because the vitamin B12 deficiency results in a secondary folate deficiency (IOM, 1998).

Certain forms of fibre (e.g. wheat bran) may decrease the bioavailability of certain forms of folate under some conditions but many forms of fibre appear to have no adverse effects. Alcohol intake may result in folate deficiency by impairing intestinal folate absorption and hepatobiliary metabolism and by increasing renal folate excretion (Gregory III, 2001).

10.7 Setting requirements and recommended intake of folate

The Malaysian RNI 2005 had included folate in the list of recommended vitamins. In establishing folate recommendation, the main references used by the Technical Sub-Committee (TSC) on Vitamins were the WHO/FAO (2004) consultation report and the IOM (1998) DRI recommendations. The rationale and steps taken in setting requirements and the levels recommended by these organisations were considered. There were no local studies on folate requirements of communities that the TSC on Vitamins could use as a reference. There are also very few reports of the biochemical status of the vitamin amongst the population groups.

The WHO/FAO (2004) Expert Consultation took note of the work of the DRI Committee of IOM (1998) which had exhaustively reviewed the evidence of folate intake, status, and health for all age groups and also reviewed the literature on the extra requirements during pregnancy and lactation. This review led to calculations of an EAR and a subsequent estimation of RDA to the EAR plus 2 standard deviations. The Expert Consultation agreed with the approach taken and the RDAs published and adopted these recommended intakes as the WHO/FAO RNI for folate. The RNI used food folate as the source of dietary folate because most societies in developing countries consume folate from naturally occurring sources.

The TSC on Vitamins decided to adopt the WHO/FAO (2004) folate recommendations as the RNI 2005 for Malaysia.

In the current review of the Malaysian RNI 2017, the TSC on Vitamins again referred to the WHO/FAO (2004) consultation report and the IOM (1998) recommendations and noted that there were no updated recommended intakes for folate by these organisations. In searching for recommendations by other international scientific organisations, the TSC referred to the publication of EFSA (2014).

In setting up the dietary recommended values (DRV) for folate, EFSA (2014) had considered serum and red blood cell folate concentrations as suitable primary criteria for deriving the DRV for European in all age groups. An Adequate Intake (AI) for infants aged from 7 - 11 months and pregnant women, and both Average Requirement (AR) and Population Reference Intake (PRI) for aged groups from 1 to ≥ 15 years and lactation woman. AR was estimated from the folate intake required to maintain folate adequacy. PRI was calculated by assuming a coefficient of variation (CV) of 15% to account for the additional variability associated with the higher requirement for folate in individual with the methylene-tetrahydrofolate reductase (MTHFR) 677TT genotype.

The latest reference from EFSA (2014) is more related to European countries, and is not appropriate to be directly adopted for the revised RNI. RNI 2017 for folate shall be retained as RNI 2005. The reason is because based on the local study by Khor *et al.* (2006), the median intake of folate among Malaysian women achieved only 50% of the Malaysian RNI 2005. In addition, researchers have reported that excessive consumption of folate may increase the risk of cancer and cardiovascular disease (Sauer *et al.,* 2010). A study in China has shown that supplementation of folic acid in the first trimester increased the risk of gestational diabetes among pregnant women (BMI > 25 kg/m^2 before pregnant). The TSC had decided to maintain the RNI 2017 to be the same as RNI 2005.

The TSC on Vitamins decided to adapt the WHO/FAO (2004) values as the revised RNI 2017 for Malaysia. These recommendations which are therefore similar to the RNI 2005 recommendations, are given in bold in the following paragraphs according to age groups and summarised in Appendix 10.1.

Infants

An adequate intake (AI) was used by the DRI Committee to estimate folate requirements for infants. This is also the quantity of dietary folate that maintains blood folate concentration comparable with those of the infant exclusively fed human milk. The AI for infants 0 through 6 months of age is derived by using 780 ml as the average volume of milk per day and 85 µg/l as the average folate concentration. The requirement thus calculated is 65 µg per day, after rounding up. Because this is food folate, the amount is the same in dietary folate equivalents (DEF).

EFSA (2014) concluded that due to limitations of available data on folate intake and status in infants, an AI was set based on the extrapolation from the intake of folate in fully breast-fed infants (0 - 6 months) which folate deficiency has not been observed. The panel had decided not to set an AR and a PRI for folate for this age group (7 - 11 months).

For older infants, the DRI Committee used the reference body weight ratio method to extrapolate from the adequate intake for folate for infants ages 0 - 6 months. The adequate intake for this group of infants was thus calculated to be 80 µg per day. The Committee also reviewed data obtained from several controlled studies that measured folate intake and assessed the infant's status. These data were found to support the estimated AI of 65 µg per day of folate for young infants and of 80 µg per day for older infants.

The FAO/WHO Consultation made slight adjustments to these recommendations and established a RNI of 80 µg folate per day for both groups of infants.

RNI for infants

0 - 5 months	**80 µg/day DFE**
6 - 11 months	**80 µg/day DFE**

Children and adolescents

No data were found to assist in establishing estimated requirements for children. Hence the EARs for these ages have been extrapolated from adult values. The same approach was used to establish the EAR for adolescents. The RDAs were then calculated as 120% of the EAR, assuming a coefficient of variation (CV) of 10% (IOM, 1998).

EFSA (2014) found no reliable data of folate for children and adolescents to set AR for these age groups. AR for folate for adults (250 µg DFE/day) was used to set AR for children and adolescents in all age groups because no indication the requirement differs by sex and age.

Therefore, AR was calculated by extrapolation from the AR of adults. Allometric scaling was used on the assumption that folate requirement is related to metabolically active body mass:

$AR_{child} = AR_{adults} \times (Weight_{child} / Weight_{adults})^{0.75} \times (1 + Growth\ Factor)$

The IOM 1998 recommended intakes were adopted by FAO/WHO as the recommended RNI, after making some adjustments according to age groupings.

RNI for children

1 - 3 years	**160 µg/day DFE**
4 - 6 years	**200 µg/day DFE**
7 - 9 years	**300 µg/day DFE**

RNI for adolescents

Boys 10 - 18 years	**400 µg/day DFE**
Girls 10 - 18 years	**400 µg/day DFE**

Adults and elderly

The main approach to determining the EAR for adults uses a combination of erythrocyte folate, plasma homocystein and plasma or serum folate. The focus used was on the adequacy of specific quantities of folate consumed under controlled metabolic conditions to maintain normal blood concentrations of these indicators. Intakes either as food or as food plus folic acid related to these indicators were computed to derive Dietary Folate Equivalents (DFEs). Four studies were considered by the IOM DRI Committee and the reports of Sauberlich *et al.* (1987) and O'Keefe *et al.* (1995) were given the most weight. The findings indicated an EAR of 320 µg/day of DFEs. This figure is well supported by data from epidemiological studies. Assuming a CV of 10%, RDA for folate was computed as 120% of the estimated requirement or 400 µg/day of DFE. In addition, IOM (1998) also made a special recommendation for women capable of becoming pregnant to reduce risk to neural tube defect.

IOM (1998) reviewed data from metabolic folate status assessment and epidemiological studies and concluded that EAR (and hence RDA) for adult ages 51 years and older was expected to be the same for younger adults. It was felt that the aging process does not appear to impair folate absorption or utilisation.

RNI for adults

Men	**19 - 65 years**	**400 µg/day of DFE**
Women	**19 - 65 years**	**400 µg/day of DFE**

RNI for elderly

Men	**> 65 years**	**400 µg/day of DFE**
Women	**> 65 years**	**400 µg/day of DFE**

Pregnancy and lactation

Folate requirements increase during pregnancy due to the significant acceleration in single-carbon transfer reactions, including those required for synthesis of nucleotide and thus division of cells. The maintenance of erythrocyte folate, which reflects tissue stores, was selected as the primary indicator of adequacy in pregnant women. EFSA (2014) noted that there is no evidence available to assess folate requirements in pregnancy compared to non-pregnant adults. For setting up an average requirement for pregnancy is not possible. Therefore, an adequate intake for folate for pregnant women at 600 µg DFE/day is set (EFSA, 2014). IOM (1998) reviewed various available studies including population-based studies relating folic acid consumption and maintenance of normal folate concentration in erythrocytes, serum, or both; controlled supplementation studies; and controlled metabolic studies.

It was observed that low dietary folate intake plus 100 µg of supplemental folate (equivalent to approximately 200 µg/day of DFEs) was inadequate to maintain normal folate status in pregnancy. Therefore, the EAR was derived by adding 200 µg/day of DFE to the EAR for non-pregnant women (320 µg/day) to provide an EAR of 520 µg/day. RDA was next computed as 120% of the EAR or 600 µg/day of DFE.

A lactating woman would require an additional intake of folate to compensate folate losses during exclusively breast-feeding. In setting up an average requirement for folate for lactation women, EFSA (2014) has considered the volume and folate content of milk transfer over the first 6 months post partum, and the bioavailability of dietary folate. Therefore, an additional intake of 130 µg DFE/day of folate to the average requirement of non-lactation woman (250 DFE/day) is required. However, in Malaysia there is no available data of folate requirements in lactating woman.

The IOM (1998) has estimated that the EAR for lactating women (450 µg/day) is the folate intake necessary to replace the folate secreted daily in human milk (133 µg/day) plus the amount required by the non-lactating women to maintain folate status (320 µg/day). Women who are only partially breastfeeding would need less. RDA was then computed as 120% of the EAR or 500 µg/day of DFE. These RDAs were adopted by the FAO/WHO Consultation as RNI for these two groups of women.

RNI for:

Pregnancy	**600 µg/day of DFE**
Lactation	**500 µg/day of DFE**

Discussions on revised RNI for Malaysia

The RNI 2017 values for folate for Malaysia, adapted from WHO/FAO (2004), are also the same as those adopted by the Working Group for the Harmonisation of RDAs in SEA (Tee & Florentino, 2005). Appendix 9.1 provides a summary of these revised RNI, compared with the previous Malaysian RDA of 2005, the WHO/FAO (2004) recommendations, the values recommended by IOM (1998) as well as the EFSA (2014) recommendations.

10.8 Tolerable upper intake level

Excessive intake of folic acid has been associated with increased cancer risk such as colorectal cancer (Cole *et al.* 2007; Hirsch *et al.* 2009) and prostate cancer (Figueiredo *et al.,* 2009). In addition, treatment with folic acid was found to increase the cancer outcomes and all-cause mortality in patients with ischemic heart disease (Ebbing *et al.,* 2009). The presence of unmetabolised folic acid in the blood has also been reported to be associated with decreased natural killer cytotoxicity (Troen *et al.,* 2006).

According to the IOM (1998), there is no evidence that consumption of sufficient natural dietary folate may pose a risk of toxicity. However, this clearly does not apply to folic acid given in supplements or fortified foods. Individuals who are at risk of vitamin B12 deficiency (e.g., those who eat no animal foods (vegans) may be at risk of the precipitation of neurological disorders if they consume excess folate. The tolerable upper levels (UL) for various age groups as suggested by IOM (1998) are given in Table 10.2.

Table 10.2 Tolerable Upper Level for Folic Acid according to age groups

Age groups	µg/day of folate from fortified foods or supplements
Infants	Not possible to establish for supplemental folate
Children	
1 - 3 years	300
4 - 8 years	400
9 - 13 years	600
Adolescents 14 – 18 years	800
Adult women	1000
Adult men	1000
Pregnant women	
14 - 18 years	800
≥ 19 years	1000
Lactation women	
14 - 18 years	800
≥ 19 years	1000

(Source: IOM, 1998)

10.9 Research recommendations

The following priority areas of research are recommended:

- Data on content and bioavailability of natural folate in foods and diets.
- Determination of folate status and identification of population at high risk of poor folate status.
- Relationship between folate deficiency and health outcomes such as the incidence of neural tube defects, cardiovascular disease, stroke and cognitive function in high risk groups including children, adolescents, women of reproductive age and elderly people
- Effect of long term high folic acid intake on immune function and health\adverse health effect.
- Effect of long term fortified folate food intake on inflammation and cancer.

10.10 References

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Appendix 10.1 Comparison of recommended intake for Folate: RNI Malaysia (2017), EFSA (2014) RNI of FAO/WHO (2002), AI and RDA of IOM (1998)

Malaysia (2017)		EFSA (2014)			FAO/WHO (2002)		IOM (1998)	
Age group	**RNI (µg/day)**	**Age group**	**DRV (µg/day)**		**Age group**	**RNI (µg/day)**	**Age group**	**AI (µg/day)**
Infants		**AIInfants**			**Infants**			
0 - 5 months	80	7 - 11 months	80		0 - 6 months	80	0 - 6 months	65 (9.4 µg/kg)
6 - 12 months	80				7 - 12 months	80	7 - 12 months	80 (8.8 µg/kg)
Children			**AR**	**PRI**	**Children**		**Children**	**RDA (µg/day)**
1 - 3 years	160	1 - 3 years	90	120	1 - 3 years	160	1 - 3 years	150
4 - 6 years	200	4 - 6 years	110	140	4 - 6 years	200	4 - 8 years	200
7 - 9 years	300	7 - 10 years	160	200	7 - 9 years	300		
Boys					**Boys**		**Boys**	
10 - 18 years	400	11 - 14 years	210	270	10 - 18 years	400	9 - 13 years	300
		≥ 15 years	250	330	14 - 18 years	400		
Girls					**Girls**		**Girls**	
10 - 18 years	400				10 - 18 years	400	9 - 13 years	300
					14 - 18 years	400		

Malaysia (2017)		EFSA (2014)		FAO/WHO (2002)		IOM (1998)	
Age group	**RNI (µg/day)**	**Age group**	**DRV (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**AI (µg/day)**
Men				**Men**		**Men**	
19 - 65 years	400			19 - 65 years	400	19 - 30 years	400
> 65 years	400			> 65 years	400	31 - 50 years	400
						51 - 70 years	400
						> 70 years	400
Women				**Women**		**Women**	
19 - 65 years	400			19 - 65 years	400	19 - 30 years	400
> 65 years	400			> 65 years	400	31 - 50 years	400
				51 - 70 years	400		
				> 70 years	400		
Pregnancy		**Pregnancy**	**AI**	**Pregnancy**		**Pregnancy**	
	600	600		600		14 - 18 years	600
						19 - 30 years	600
						31 - 50 years	600
Lactation		**Lactation**	**AR PRI**	**Lactation**		**Lactation**	
	500		380 500	500		14 - 18 years	00
						19 - 30 years	500
						31 - 50 years	500

*Same as in RNI 2005

11 • Cobalamin (Vitamin B12)

11.1 Introduction

Cobalamin, also known as Vitamin B12, is a water-soluble vitamin that exists in several forms and contains the mineral cobalt. Cobalamin refers to a group of cobalt-containing compounds (corrinoids) that contains the sugar ribose, phosphate, and a base (5, 6-dimethyl benzimidazole) attached to the corrin ring. Cyanocobalamin is a common synthetic form of the cobalamin that is produced by chemically modifying bacterial hydroxocobalamin and used for addition to food and food supplements and drugs. In the body, cyanocobalamin is converted into the human physiological forms methylcobalamin and 5'-deoxyadenosylcobalamin, the forms of cobalamin that are active in human metabolism.

11.2 Functions

Cobalamin is required for proper red blood cell formation, neurological function, and DNA synthesis (IOM, 1998). Cobalamin plays essential roles in folate metabolism and acts as a cofactor for two enzymes, namely L-methylmalonyl-CoA mutase and methionine synthase.

L-methylmalonyl-CoA mutase requires 5'-deoxyadenosylcobalamin as a cofactor to catalyse the conversion of L-methylmalonyl-CoA to succinyl-CoA which then enters the citric acid cycle. Succinyl-CoA plays a major role in the production of energy from lipids and proteins and is also required for the synthesis of haemoglobin, the oxygen-carrying pigment in red blood cells (Shane, 2000).

Methylcobalamin is required for the function of the folate-dependent enzyme, methionine synthase. Methionine synthase is required for the synthesis of the methionine and tetrahydrofolate from homocysteine (IOM, 1998). Methionine, in turn, is required for the synthesis of S-adenosylmethionine (SAM), a methyl donor for almost 100 different substrates, including DNA, RNA, hormones, proteins, and lipids as well as detoxification reactions (Shane, 2000). Without adequate supplies of cobalamin and folate, the synthesis of methionine and its derivative SAM is disrupted, with profound effects on normal cellular function. Without methionine, myelin and neurotransmitters (serotonin, dopamine, acetylcholine and norepinephrine) cannot be produced that are needed for neurological development, maintenance and functions. Furthermore, inadequate function of methionine synthase can lead to an accumulation of homocysteine, an amino acid that is related to many neurodegenerative diseases that lead to brain damage and cognitive disturbances. Thus, the synthesis of methionine prevents the accumulation of homocysteine in the brain (de Jager, 2014).

11.3 Metabolism

Normally, cobalamin is attached to a protein either for transport or storage. In the stomach, hydrochloric acid and pepsin are secreted to degrade the cobalamin from protein (IOM, 1998). The released cobalamin then binds to another protein, R protein secreted by the salivary glands and the gastric mucosa and transports it through the stomach and into the small intestine. The stomach cells also produce a protein called intrinsic factor (IF), a glycoprotein secreted by the stomach's parietal cells which travel to the small intestine.

In the small intestine, the vitamin 12, bound to IF interacts with the protein receptor known as cubilin-IF receptor when it is transported from the duodenum to the ileum. Another protein, amnionless, facilitates the attachment of cubilin to the ileal cell membrane. The binding of the vitamin B12-IF complex to the cubilin receptor is important in order for the vitamin to be absorbed. Cobalamin is then released and degraded in lysosomes. Cobalamin is finally metabolised to its methyl and deoxyadenosyl- derivatives.

In plasma, cobalamin is bound to the cobalamin-binding proteins transcobalamin (TC) and haptocorrin. TC combines with cobalamin at the ileal cell to form holotranscobalamin (holoTC) and rapidly delivers cobalamin to tissues. The liver takes up approximately 50 percent of the cobalamin, and the remainder is transported to other tissues. The highest cobalamin losses occur through the faeces. Sources of faecal cobalamin include unabsorbed cobalamin from food or bile, desquamated cells, gastric and intestinal secretions and cobalamin synthesized by bacteria in the colon. There are no known interactions of cobalamin with other nutrients with regards to absorption or excretion.

11.4 Sources

Cobalamin is found only in animal foods. Unlike other B vitamins, cobalamin is not a normal constituent of plant foods except for certain algae. Cobalamin is not supplied by commonly eaten plant foods unless they have been exposed to bacterial action that has produced cobalamin; contaminated with soil, insects, or other substances that contain cobalamin; yogurt, tempeh, miso, kimchi, and pickles are all examples of foods sometimes made with the lactic acid bacteria that can produce vitamin B12. Examples of cobalamin content of some foods are shown in Table 11.1.

Table 11.1 Cobalamin (Vitamin B12) content of foods

Food	µg/100g
Rice, noodle, bread, cereals and cereals products and tubers	
Fortified cereals	20.0
Fish, poultry and meat	
Clams, cooked	98.9
Liver (lamb), cooked	85.7
Liver (beef) cooked	83.1
Liver (veal), cooked	72.5
Oysters, cooked	28.8
Mussels, cooked	24.0
Liver (chicken, pork), cooked	21.1
Mackerel, cooked	19.0
Tuna, bluefin, raw or cooked	10.9
Salmon, cooked	3.2
Beef	2.6
Shrimp, cooked	1.7
Pork	1.2
Egg	0.9
Duck or chicken, cooked	0.3
Milk and milk products	
Tofu	2.4
Parmesan	2.3
Mozzarella	0.9
Cheddar	0.8
Yougurt Plain (regular, low fat)	0.8
Milk, Semi-skim/skim	0.5
Milk, Whole	0.4
Others	
Seaweed	2.3
Tempeh	0.1

Source: USDA nutrient database (2012)

11.5 Deficiencies

Megaloblastic anaemia is the most frequent clinical expression of cobalamin deficiency, which affects red blood cells and all other blood cells. Most of the cobalamin deficiency occurs due to cobalamin malabsorption, rather than inadequate dietary intake. The deficiency results in 1) an autoimmune condition called pernicious anemia and 2) food-bound cobalamin malabsorption syndrome. Impairment of cobalamin absorption can cause megaloblastic anaemia and neurologic disorders in deficient individuals. Both conditions have been associated with a chronic inflammatory disease of the stomach known as atrophic gastritis. Atrophic gastritis is associated with the presence of autoantibodies directed towards stomach cells and infection by the bacteria, Helicobacter pylori (H. pylori) that damage stomach cells that make intrinsic factor, a substance the body needs to absorb B12 (Lahner, Persechino and Annibale, 2012). Insufficient dietary intake is rare in adults living in developed countries but is more often reported in vegans.

The clinical signs of deficiency include fatigue, weakness, constipation, loss of appetite, and weight loss (Kapadia, 1995). Apart from that, neurological changes can also occur in the individual with cobalamin deficiency such as numbness and tingling in the hands and feet, difficulty walking, memory loss, disorientation, and dementia (IOM, 1998). In an infant, failure to thrive, movement disorders, developmental delays, and megaloblastic anemia are signs of a cobalamin deficiency (Monsen and Ueland, 2003).

Cobalamin status is assessed via serum or plasma cobalamin levels. The plasma cobalamin levels of adults with cobalamin deficiency are approximately (120-180 picomol/L) (IOM, 1998). Another reliable indicator of cobalamin status is elevated methylmalonic acid (MMA) levels (values >0.4 micromol/L) in blood and urine, resulting from impaired metabolic cobalamin activity with no specific clinical symptoms (Andrès *et al.,* 2007). This indicator can detect a metabolic change that is highly specific to cobalamin deficiency.

In 1950s, cobalamin was found to be a major contributing factor to anaemia in pregnancy amongst Malaysians (Tasker, Richardson and Llewellyn, 1956; Tasker, 1958). The Institute for Medical Research (IMR) embarked on a series of two periods of five-year interval (1987/88 and 1992/93) intensive studies into the cause of the cobalamin deficiency anaemia from hospitals all over Malaysia (Roshidah and Khalid, 1994). The IMR researchers reported that there was increase in cobalamin deficiency anaemia over the five-year interval from 2.6% to 8.2% with most Indians having a prevalence of about 49%, indicating the need to improve cobalamin intake in this group. The problem seems to have been observed in individuals consuming vegetarian diets too since cobalamin is found only in animal products (Roshidah and Khalid, 1994). In a more recent study, Khor *et al.* (2011) reported adequate concentrations of serum cobalamin among 7-12 primary school children in Kuala Lumpur.

11.6 Factors affecting requirements

Strict vegetarians and vegans are at greater risk of developing cobalamin deficiency than lacto-ovo vegetarians since natural food sources of cobalamin are limited to animal foods (IOM, 1998).

In the general elderly population, plasma cobalamin tends to decrease and concentration of serum methylmalonic acid (MMA) tends to increase resulting in a decline in cobalamin status. Factors that may contribute to these changes include a decrease in gastric acidity and the presence of atrophic gastritis and bacterial overgrowth accompanied by food-bound cobalamin malabsorption. Older adults with atrophic gastritis are unable to absorb the vitamin B12 due to progressive reduction of the inability of the parietal cells to secrete hydrochloric acid. It has also been suggested that the decreased hydrochloric acid levels might also increase the growth of normal intestinal bacteria in the stomach and intestine that use vitamin B12, further reducing the amount of vitamin B12 available to the body. Despite the absence of this acid in preventing the release of protein-bound vitamin B12, it would not interfere with the absorption of free vitamin B12 found in fortified foods or supplements. As a result, the IOM recommends that adults older than 50 years obtain most of their cobalamin from vitamin supplements or fortified foods (e.g., fortified cereals) to prevent cobalamin deficiency (IOM, 1998). However, some elderly patients with atrophic gastritis require doses much higher than the RDA to avoid subclinical deficiency.

11.7 Setting requirements and recommended intake

There were no recommendations for vitamin K in the previous version of Malaysian RNI (2005). The main references used by the Technical Sub-Committee (TSC) on Vitamins, cobalamin were the reports from the IOM (1998), WHO/FAO (2004) and the EFSA (2015). The rationale and approaches taken by these consultations were considered. There are no known local studies on cobalamin requirements of communities that the Technical Sub-Committee (TSC) on Vitamins could use as a reference when considering RNI for the vitamin. Previous studies of the biochemical status of cobalamin among Malaysians were published over 6 decades ago and recent studies are rare.

The Food and Nutrition Board of the National Academy of Sciences (NAS) Institute of Medicine derived the recommended dietary allowances based on their review on the evidence of intake, status and health for all age groups and during pregnancy and lactation using serum or plasma vitamin B12 and MMA as biomarkers of cobalamin status. From this review, the consultation group derives the calculations of an Estimated Average Requirement (EAR). Recommended dietary allowances were estimated to be the EARs plus 2 standard deviations (SDs). WHO/FAO (2004) consultation agreed to adopt the approach of the IOM (1998) for deriving its recommended nutrient intake of cobalamin.

In the European Food Safety Authority (EFSA) 2015 consultation report, the Panel agreed that the most suitable way to derive Dietary Reference Values (DRV) for cobalamin is a combination of biomarkers of cobalamin status i.e. serum cobalamin, holoTC, MMA and plasma total homocysteine (tHcy) (EFSA, 2015). Based on the literature, the Panel considered that

serum concentrations of holoTC and cobalamin within the reference ranges for healthy adults, together with MMA and plasma tHcy concentrations below cut-off values for cobalamin insufficiency and hyperhomocysteineaemia are indicative of an adequate cobalamin status. In fact, in the IOM (1998) report, the use of holoTC and MMA as biomarkers was highlighted as the high-priority recommendations for future studies. This recommendation was adopted by EFSA (2015).

The TSC is in general agreement with the comprehensive approach of the EFSA (2015) report and decided to adopt the values proposed by this organisation. The proposed values for the RNI (2017) for Malaysia are given in bold in the following paragraphs according to age groups and summarised in Appendix 11.1

Infants

In determining cobalamin requirement, EFSA (2015) recommendation was based on the estimation of adequate intake (AI) since there is limited number of studies using an accurate method to estimate the breast milk concentration of cobalamin (EFSA, 2015). Due to uncertainties in estimating breastmilk cobalamin concentration used for upward extrapolation from the cobalamin intake in exclusively breast-fed infants aged 0-6 months, and considering the use of scaling down approach from adults as a basis, an intake consistent with biomarker data, an AI for cobalamin for infants aged 7-11 months at 1.5µg/day was set. The recommendation in term of adequate intake (AI) for infant aged 0-6 months was made using the proportion based on recommended value of 0-6 months in IOM and WHO/FAO, that is 20% lower than 7-12 months recommendation, thus an AI for cobalamin for infants aged 0-6 months was set at 1.2 µg/day.

RNI for infants

0 - 5 months 1.2 µg/day
6 - 11 months 1.5 µg/day

Children and Adolescents

The same recommendation in term of adequate intake (AI) was made for the intake of cobalamin in children and adolescents by the EFSA (2015) since there are insufficient data to derive an Average Requirement (AR) (EFSA, 2015). AIs were derived from the unrounded AIs for adults after adjustment on the basis of differences in reference body weight, and then rounded to the closest 0.5.

RNI for children

1 - 3 years	**1.5 µg/day**
4 - 6 years	**1.5 µg/day**
7- 9 years	**2.5 µg/day**

RNI for adolescents

10 - 12 years	**3.5 µg/day**
13 - 18 years	**4.0 µg/day**

Adults and elderly

The same recommendations were made for the intakes of adults by the EFSA (2015). The EFSA Panel decided to use a combination of cobalamin biomarkers of status to derive DRVs for cobalamin for adults. However, there are uncertainties with respect to cut-off values for cobalamin insufficiency of these indicators and limited data available to determine an AR.

In the absence of the required information, the cobalamin recommendation was made for the adults by the EFSA Panel based on consistent evidence from observational and intervention studies. The studies showed that intake of cobalamin at 4µg/day and greater is associated with serum concentrations of holoTC and cobalamin within the reference ranges derived from healthy subjects, together with MMA and tHcy concentrations below the cut-off values for adults that indicates an adequate cobalamin status. Therefore, the EFSA (2015) Panel sets an AI for cobalamin at 4µg/day for adults based on data on different biomarkers of cobalamin status and in consideration of observed intakes. The recommended intake for older adults is the same as those for adults.

RNI for adults

19 - 65 years	**4.0 µg/day**

RNI for elderly

> 65 years	**4.0 µg/day**

Pregnancy and Lactation

The EFSA Panel suggested that 0.2 µg/day of cobalamin is transferred to the foetus and absorption of 40%. An additional requirement of 0.5µg/day of cobalamin to the AI for non-pregnant women was felt to be adequate. This addition results in an AI of 4.5µg/day for pregnant women.

An AI for lactating women is based on the cobalamin intake that is required to compensate for the amount of cobalamin secreted in breast milk. It is estimated that a mean milk transfer of 0.8L/day in exclusively breastfeeding women during the first six months of lactation would secrete breast milk with 0.4µg cobalamin/ day. Taking into account 40% absorption, an extra amount of 1.0µg/day cobalamin is required to balance the secretion in milk. Therefore, an AI of 5µ/day is recommended for lactating women.

RNI for

Pregnancy	**4.5 µg/day**
Lactation	**5.0 µg/day**

Discussion on revised RNI for Malaysia

There were no recommendations for cobalamin in the previous version of the Malaysian RNI. The proposed recommended intakes for the revised RNI for Malaysia 2017 are adopted from the latest report by EFSA (2015). These proposed recommended intakes are higher than the recommendations by IOM (1998) and WHO/FAO (2004) across all age groups by approximately 50%. As previously mentioned in Section 15.7, the use of additional biomarkers by EFSA 2015 to derive the values is highlighted and this probably contributes to the differences in values of recommendations by IOM (1998) and WHO/FAO (2004) (refer Appendix 11.1).

11.8 Toxicity and tolerable intake levels

No toxic or adverse effects have been associated with large intakes of cobalamin from food or supplements in healthy people. Doses as high as 2mg/day by mouth or 1 mg monthly by intramuscular (IM) injection have been used to treat pernicious anaemia without significant side effects. When high doses of cobalamin are given orally, only a small percentage can be absorbed, which may explain the low toxicity (Carmel, 2008). Because of the low toxicity of cobalamin, no tolerable upper intake level (UL) has been set by the US Food and Nutrition Board.

11.9 Research recommendations

The following priority areas of research are recommended:

- Determination of cobalamin status and extent of deficiency especially among vegan.
- Identification of more sensitive and specific biochemical measures of cobalamin status.
- The contribution of fermented vegetable foods to cobalamin status of vegan communities.

11.10 References

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Appendix 11.1 Comparison of recommended intakes for Cobalamin (Vitamin B12): RNI Malaysia (2017), AI and RDA (IOM, 1998), RNI (WHO/FAO, 2004) and AI (EFSA, 2015)

Malaysia (2017)		IOM (1998)		WHO/FAO 2004 (2004)		EFSA (2015)	
Age group	**RNI (µg/day)**	**Age group**	**AI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**AI (µg/day)**
Infants (boys)		**Infants**		**Infants**		**Infants**	
0 - 5 months	1.2	0 - 6 months	0.4	0 - 6 months	0.4		
6 - 11 months	1.5	7 - 12 months	0.5	7 - 12 months	0.7	7 - 11 months	1.5
Infants (girls)							
0 - 5 months	1.2						
6 - 11 months	1.5						
			RDA (µg/day)				
Children (boys)		**Children (boys)**		**Children (boys)**		**Children**	
1 - 3 years	1.5	1 - 3 years	0.9	1 - 3 years	0.9	1 - 3 years	1.5
4 - 6 years	1.5	4 - 8 years	1.2	4 - 6 years	1.2	4 - 6 years	1.5
7 - 9 years	2.5			7 - 9 years	1.8	7 - 10 years	2.5
Children (girls)		**Children (girls)**		**Children (girls)**			
1 - 3 years	1.5	1 - 3 years	0.9	1 - 3 years	0.9		
4 - 6 years	1.5	4 - 8 years	1.2	4 - 6 years	1.2		
7 - 9 years	2.5			7 - 9 years	1.8		
Adolescent (Boys)		**Adolescent (Boys)**		**Adolescent (Boys)**		**Adolescent**	
10 - 12 years	3.5	9 - 13 years	1.8	10 - 18 years	2.4	11 - 14 years	3.5
		14 - 18 years	2.4				
13 - 14 years	4					15 - 17 years	4
15 years	4						
16 - 18 years	4						

Malaysia (2017)		IOM (1998)		WHO/FAO 2004 (2004)		EFSA (2015)	
Age group	**RNI (µg/day)**	**Age group**	**AI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**AI (µg/day)**
Adolescent (Girls)		**Adolescent (Girls)**					
10 - 12 years	3.5	9 - 13 years	1.8				
13 - 14 years	4	14 - 18 years	2.4				
15 years	4						
16 - 18 years	4						
Men		**Men**		**Adults**		**Adults**	
19 - 29 years	4	19 - 30 years	2.4	19 - 65 years	2.4	>18 years	4
30 - 50 years	4	31 - 50 years	2.4	>6.5 years	2.4		
51 - 59 years	4	51 - 70 years	2				
60 - 65 years	4	>70 years	2				
>65 years	4						
Women		**Women**					
19 - 29 years	4	19 - 30 years	2.4				
30 - 50 years	4	31 - 50 years	2.4				
51 - 59 years	4	51 - 70 years	2.4				
60 - 65 years	4	>70 years	2.4				
>65 years	4						
Pregnancy	4.5	**Pregnancy**	2.6	**Pregnancy**	2.6	**Pregnancy**	4.5
Lactation	5	**Lactation**	2.8	**Lactation**	2.8	**Lactation**	5

12 • Ascorbic Acid (Vitamin C)

12.1 Introduction

Vitamin C, also known as L-ascorbic acid, is an essential water soluble vitamin required for various biological and physiological processes of the body. It has been controversially used to prevent or delay the occurrence of chronic diseases and to treat certain illnesses. L-Ascorbic acid, also known as L-xyloascorbic acid, 3-oxo-L-gulofuranolactone (enol form), L-3-ketothreohexuronic acid lactone and antisorbutic vitamin, has the chemical formula C6H8O6 and a molecular weight of 176.12. The natural form of the vitamin is the L-isomer. Most plants and animals synthesise ascorbic acid for their own requirement. However, human and several other animal species including primates and guinea pig are unable to synthesise it due to a deficiency of L-gulonolactone oxidase, a terminal enzyme in the biosynthetic pathway of ascorbic acid, because of mutation in the gene encoding for the enzyme. Therefore, vitamin C must be solely obtained from the diet, mainly through fruits and vegetables to maintain a normal metabolic functioning of the body (Daud, Ismail, Sarmadi, 2016).

12.2 Functions

Vitamin C is a water-soluble vitamin important for collagen formation, a protein that gives structure to bones, cartilage, muscle and blood vessels. It also helps maintain capillaries, bones, and teeth and aids in the absorption of iron. Ascorbic acid, a reducing agent, is necessary to maintain the enzyme prolyl hydroxylase in an active form, most likely by keeping its iron atom in a reduced state. The precursor molecule to the protein collagen, procollagen, contains an unusual amino acid sequence in that every third amino acid is a glycine and contains a high frequency of two amino acids not found in any other proteins - hydroxyproline and hydroxylysine. These latter two amino acids are converted from proline and lysine, respectively, after the procollagen molecule has been synthesised. The hydroxylation of proline and lysine in procollagen is carried out by the enzyme prolyl hydroxylase using ascorbic acid as a cofactor. Ascorbic acid plays an important role as a component of enzymes involved in the synthesis of collagen and carnitine; however, its most vital role is as a water-soluble vitamin in the human body (Sies & Stahl, 1995; Levine *et al.,* 1995).

Ascorbic acid is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of reactive oxygen and nitrogen oxide species, ascorbic acid has been shown to be effective against the superoxide radical ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen (Weber, Bendich & Schalch, 1996). Ascorbic acid protects folic acid reductase, which converts folic acid to folinic acid, and may help release free folic acid from its conjugates in food. Ascorbic acid facilitates the absorption of iron.

Vitamin C has been controversially used to prevent or delay the occurrence of chronic diseases and to treat certain illnesses. Current evidences on the therapeutic role of vitamin C in several chronic illnesses including cardiovascular disease (CVD), cancer, and respiratory illnesses are not consistent (Fortmann *et al.* 2013).

12.3. Metabolism

Ascorbic acid is present in all parts of the body at varying concentration in the plasma, bone cells, and cellular immune system. Body stores of vitamin C can be affected by dietary intake, excretion rate in renal tubule, faecal losses, and metabolic losses. Upon ingestion, vitamin C is absorbed through passive diffusion mediated or facilitated by facilitative glucose transporters (GLUT) and a saturable-substrate transport mechanism via ascorbate-specific transporter (sodium vitamin C transporter (SVCT)). Oral vitamin C is absorbed for approximately 70-90% at moderate intake (30-180 mg day), but absorption rate falls below 50% at intake above 1000 mg day. Evidence from pharmacokinetics studies indicated that consumption of at least 200 mg day of vitamin C in healthy young adults leads to plasma concentration of more than 50 mM, which is a nearly complete oral bioavailability, indicated by leukocyte saturation and minimum urinary excretion. This suggests that ingestion of vitamin C orally produces plasma concentrations that are tightly controlled. Absorbed vitamin C is transported in the plasma as free anion ascorbate and is distributed to all tissues. At cell levels, particularly in the osteoblast, muscle, and retinal cells, an oxidized form of vitamin C known as dehydroascorbic acid (DHA) is taken up by GLUT and then reduced internally to ascorbic acid. Whilst, active transportation of ascorbate via SVCT is subjected to substrate feedback inhibition indicating a regulatory role in maintaining ascorbate concentration in the cells.

In terms of body store, a total body content of vitamin C in healthy adults ranges from 300 mg to 2 g with higher concentrations maintained in the leukocytes, eyes, adrenal glands, pituitary gland, and brain. Excessive vitamin C intake that is not metabolised is excreted mainly in the urine, and to a lesser extent in the faeces. In healthy non-smoking men, up to 40 to 50 mg per day vitamin C will be excreted. Among smokers, the metabolic losess are higher by 50% than non-smokers. In humans, the main route of removal of ascorbic acid is through urinary excretion, of which ascorbic acid is oxidized to dehydroascorbic acid, then subsequently hydrolysed to diketogulonic acid. Diketogulonic acid is further decomposited to various compounds such as oxalic and threonic acids.

Ascorbic acid is the most potent enhancer of non-heme iron absorption. A study by Hallberg (1987) showed that iron absorption from non-heme food sources can be increased significantly with a daily ascorbic acid intake of at least 25 mg for each meal (estimated for 3 meals/day). Higher ascorbic acid intakes should be considered if meals contain higher contents of nutrient inhibitors such as phytates and tannins.

12.4. Sources

Ascorbic acid is widely distributed in nature, mostly rich in fresh fruits and leafy vegetables followed by animal sources. Fruits rich in vitamin C include guava, mango, papaya, citrus fruits and juices, tomatoes and tomato juice. Vegetables with the most vitamin C include cabbage, cauliflower, mustard leaves, spinach and other leafy greens and 'ulam', broccoli, turnip greens, sweet and white potatoes and winter squash (Tee *et al.,* 1997, Chang, Ismail, Daud, 2016) (Table 12.1). Some cereals and other foods as well as beverages are fortified with vitamin C (Chang et al, 2016). Local herbs and spices including ginger are also rich in vitamin C and has high antioxidant activities (Ghasemzadeh, Jaafar, Rahmat 2010).

Animal sources of this vitamin such as meat, fish, poultry, eggs and dairy products contain smaller amounts and are not significant sources. Most food-based dietary guidelines are similar in that all recommend consumption of 5 servings of fruits and vegetables daily. If this recommendation is followed, daily intake of ascorbic acid will be 210 to 280 mg, depending on food content factors (Levine *et al.,* 1999).Ascorbic acid is the least stable of all vitamins and is easily destroyed during processing and storage. Juices are good foods to be fortified with ascorbic acid because their acidity reduces ascorbic acid destruction.Exposure to oxygen, prolonged heating in the presence of oxygen, contact with minerals (iron and copper) and exposure to light are destructive to the ascorbic acid content of foods.

Table 12.1. Ascorbic Acid (Vitamin C) content of foods

Food	Per 100 g
Fruits	
Guava	152.0
Longan	72.1
Papaya	70.9
Lime	36.9
Tangerine/ mandarin	28.0
Durian belanda (Soursop)	26.9
Mango	20.5
Rambutan	18.8
Honeydew melon	18.0
Banana	17.3
Jambu air (Water apple)	16.7
Pineapple	15.2
Jackfruit	7.9
Pear	7.6
Grape	7.6
Watermelon	5.5
Apple, red	4.9
Starfruit	4.8
Vegetables	
Green leafy	
Tapioca shoots	192.0
Cekur manis	136.0
Kale (kai-lan)	107.0
Mustard leaves (sawi)	89.0
Sweet potato shoots	81.1
Bak Choy	60.0

Food	Per 100 g
Spinach	56.4
Swamp cabbage (kangkong)	48.3
Salad or Ulam	
Cashew leaves (Pucuk gajus)	91.0
Kadok	78.9
Maman	72.0
Ulam raja	65.0
Four-angled bean	11.3
Other vegetables	
Bell peppers	112.0
Broccoli	85.0
Bitter Gourd	53,0
Carrot	53.0
Cabbage	53.0
Cauliflower	42.6
Lettuce	27.6
Tomato	25.8
Okra	19.3
Tubers	
Tapioca	35.9

Source: Tee *et al.,* 1997

12.5 Deficiencies

Plasma vitamin C has a moderate correlation with vitamin C intake. However, the correlation may be affected or influenced by the presence of external factors such as vitamin bioavailability, absorption condition, stress and food processing and storage time, or by error in reporting vitamin C intake (Dehghan *et al.,* 2007). Severe deficiency of ascorbic acid causes scurvy. Symptoms appear when the serum level falls below 0.2 mg/dl. 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 (IOM, 2000).

Several symptoms of ascorbic acid deficiency have been recognized including follicular hyperkeratosis, swollen and inflamed gums, loosening of teeth, dryness of the mouth and eyes, loss of hair and dry itchy skin. These symptoms reflect the role of ascorbic acid in the maintenance of collagen and blood vessel integrity. It is an acute or chronic disease characterised by hemorrhagic manifestations and abnormal osteoid and dentin formation. The psychological manifestations of scurvy include depression and hysteria. This potentially fatal disease can be prevented with as little as 10 mg ascorbic acid per day, an amount easily obtained through consumption of fresh fruits and vegetables.

There are no reports of ascorbic acid deficiency among Malaysian population. Nevertheless, vitamin C intake in various nutrition surveys in Malaysia indicated inadequacy of intake in specific groups. Findings from the Malaysian Adults Nutrition indicated that the mean of vitamin C intake, after excluding over reporters, is 72 ± 1.4 mg/day (103% RNI) and 75 ± 2.9 mg/day (107% RNI), for the year 2003 and 2014, respectively (Zainuddin *et al.,* 2017). Report from MANS 2003 indicated that Indians and those from the East Coast had the lowest intakes but there are no clear differences between gender and locality of rural and urban areas (Mirnalini *et al.,* 2008). Mean of vitamin C intake among Malaysian older adults from a large scale study is 113 mg/d in men and 118 mg/d in women, with a third has inadequate intake (Kamarudin et al. 2016). In children, vitamin C inadequacy is also reported in at least 20% of the subjects (Poh *et al.,* 2013), with more boys aged 7-12 years did not achieve the Malaysian RNI for energy and vitamin C compared with their female counterparts. On the other hand, in rural areas, the percentage of girls aged 4-6 years who did not achieve the Malaysian RNI for vitamins C and A was more than double that of the boys. However, it should be emphasised that there is no data on subclinical and clinical deficiency among Malaysian population.

12.6 Factors affecting ascorbic acid requirements

Various factors have been identified to affect vitamin C requirement. These include bioavailability, nutrient-to-nutrient interactions, and gender. Generally, vitamin C requirement is increased in special population including pregnant and lactating women as well as smokers when compared to their age group counterpart. This is to reflect the different physiological requirements in these groups. In pregnant women, higher requirement is needed to account for expansion of blood volume and active transfer to the foetus, while for lactating women, to account for vitamin C transfer to breast milk. For heavy smokers (i.e., smoking more than 20 cigarettes per day), higher intake is needed to account. As mentioned earlier, data available on vitamin C intake or status and cardiovascular disease related outcomes and vision-related outcomes are not consistent or specific enough as a criterion for deriving the recommendation for vitamin C (EFSA, 2013).

The type of food consumed has not been shown to have a significant effect on the absorption of ascorbic acid. Although absorption of ascorbic acid decreases to about 50% and less with the single doses above 1g, some 70-90% of usual dietary intake of ascorbic acid (30 - 180 mg/day) is absorbed. Bioavailability was completed for 200 mg of ascorbic acid as a single dose. No ascorbic acid was excreted in the urine of six of seven volunteers until the 100 mg dose. At single dose of 500 mg and higher, bioavailability declined and the absorbed amount was excreted (Levine *et al.,* 1996).

Ascorbic acid is very labile, and the loss of ascorbic acid upon boiling milk provides one dramatic example of a cause of infantile scurvy. The ascorbic acid content of food is strongly influenced by season, transportation to market, shelf life, time of storage, cooking practices and chlorination of water.

12.7 Setting requirements and recommended intake of ascorbic acid

Ascorbic acid is already included in the list of vitamins in Malaysian RNI 2005. The Technical Sub-Committee (TSC) on Vitamins noted that in establishing the 2005 recommendations, there were no known local studies on ascorbic acid requirements of communities that the TSC could use as a reference when considering RNI for the vitamin. There were also very few reports of the biochemical status of the vitamin amongst the population groups. The TSC had referred to recommendations available from diverse professional bodies pertaining to vitamin C intake recommendation, mainly the WHO/ FAO (2004) consultation report and the IOM (2000) DRI recommendations. Upon reviewing available information, the TSC on Vitamins decided to adopt the WHO/ FAO (2004) values as the Malaysian RNI 2005.

In the process of preparing for the 2017 review of RNI for ascorbic acid, the TSC on Vitamins sourced for available references. There are still no local studies on ascorbic acid requirements or status. WHO/FAO and IOM too have not updated their ascorbic acid recommendations. It was however found that the European Food Safety Authority had published recommended intakes for this vitamin (EFSA, 2013) which the TSC included as a reference. EFSA recommendation although the latest, was not adopted by the RNI 2017, as it was derived from few small scales metabolic studies among healthy men (EFSA, 2013).

The TSC Vitamins decided to continue to adopt the WHO/FAO (2004) recommendations for ascorbic acid for Malaysia RNI 2017. These recommendations, which are in line with previous RNI (NCCFN, 2005), are given in bold in the following paragraphs, according to age groups and summarised in Appendix 12.1.

Infants

Human milk is recognised as the optimal milk source for infants at least throughout 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. IOM (2000) estimated the AI for infants based on the average volume of milk intake of 780 ml and an average concentration of ascorbic acid of 50 mg/l in human milk. For infants 0-6 months, 40 mg per day was the estimated AI and for the 7-12 months infants, the AI was 50 mg per day, taking into consideration the amount of ascorbic acid from solid foods consumed at this stage.

The FAO/WHO expert consultation (WHO/FAO, 2004) estimated the mean ascorbic acid concentration in human mature milk as 40 mg/l. However, it was felt that the amount of ascorbic acid in human milk appears to reflect maternal dietary intake rather than the infants needs. Moreover, it was noted that 8 mg/day of ascorbic acid is sufficient to prevent scorbutic signs in infants. The Consultation therefore arbitrarily set the recommended intake for infants aged 0-6 months at 25 mg/day. The recommended intake for older infants was gradually increased to 30 mg per day. EFSA (2013) also reported that there are no new data to set an average requirement (AR) for infant, thus a population reference intake (PRI) of infants aged 6 to 11 months at 20 mg/day has been used as DRI. The TSC adopted the recommendation of the WHO/ FAO (2004) as follows:

RNI for infants

0 - 5 months	**25 mg/day**
6 - 11 months	**30 mg/day**

Children and adolescents

No data were available on which to base an estimated average requirement (EAR) for children 1 through 18 years of age. Thus, the IOM (2000) estimated the EARs and RDAs for children on the basis of relative body weight.

The WHO/ FAO (2004) recommended intakes for ascorbic acid for children and adolescents were gradually increased from the recommended intake for infants. EFSA (2013) also recommended similar increment according to age groups. In deciding on recommended intake for older children, eg adolescents, the TSC considered the possible role that ascorbic acid can play in reducing the high prevalence of iron deficiency anemia in the country (Tee *et al.,* 1998). Hallberg (1987) had observed that the additional intake of at least 25 mg ascorbic acid promotes absorption of soluble non-haem iron. In addition, recent studies have pointed towards a possible antioxidant role for ascorbic acid, ie ability to scavenge reactive oxidants in activated leucocytes, lung, gastric mucosa and to protect against lipid peroxidation. The TSC therefore decided to increase the amount recommended by WHO/ FAO (2004) by 25 mg ascorbic acid per day to all age groups from children 10 years and above. This is inline with the fact that iron deficiency is still prevalent among Malaysian population especially children, adolescent and pregnant mothers (Tee *et al.* 1998; Foo *et al.* 2004; Haniff *et al.* 2007; Ngui e*t al.* 2012).

RNI for children

1 - 3 years	**30 mg/day**
4 - 6 years	**30 mg/day**
7 - 9 years	**35 mg/day**

RNI for adolescents

Boys 10 - 18 years	**65 mg/day**
Girls 10 - 18 years	**65 mg/day**

Adults

The classic disease of severe ascorbic acid deficiency, scurvy, is now rare in most countries. Other human experimental data that can be utilized to set a ascorbic acid requirement, based on a biomarker other than scurvy, are limited. The IOM (2000) recommended intakes of ascorbic acid 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. It is however recognised that there is no human data to directly quantify the dose-response relationship between ascorbic acid intake and in vivo antioxidant protection.

Based on ascorbic acid intakes sufficient to maintain near-maximal neutrophil concentrations with minimal urinary loss, IOM (2000) set an EAR of 75 mg/day of ascorbic acid for men. Based on this, and assuming a coefficient of variation of 10%, RDA for ascorbic acid for men was computed to be 120% of estimated requirement or 90 mg/day. Since no similar data were available for women, it is assumed that women will have 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 IOM noted that at a ascorbic acid 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 ascorbic acid prevents LDL oxidation in vivo, if it does this might be relevant in the prevention of heart disease. Also, since neutrophils are at 80 percent 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. EFSA (2013) also used calculation considering metabolic vitamin C losses and allow the maintenance of an adequate body pool characterized by fasting plasma ascorbate concentrations at around 50 umol/L, to arrive at an Average Requirement (AR) of 90 mg/day. Further addition of 10% coefficient of variation lead to a suggestion of a Population Reference Intake (PRI) of 110 mg/day for men. Adjustment for smaller body size of women led to an AR of 80 mg/day and PRI of 95 mg/day.

WHO/ FAO (2004) calculated the dietary intake 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 300-400 mg, and the last signs disappear when the body content reaches about 1000 mg. In these experiments, ascorbate in the whole body was catabolised at an approximate rate of 2.9 percent/day.

There is a sigmoidal relationship between intake and plasma concentrations of ascorbic acid. At low doses, dietary ascorbic acid is almost completely absorbed, but over the range of usual dietary intakes (30-180 mg/day), absorption may decrease to 75 percent because of competing factors in the food. A body content of 900 mg falls halfway between tissue saturation and the point at which clinical signs of scurvy appear. Assuming an absorption efficiency of 85 percent, and a catabolic rate of 2.9, the average intake of ascorbic acid can be calculated as:
900 x 2.9/100 x 100/85 = 30.7 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. WHO/ FAO (2004) therefore made the same recommendation for non-pregnant, non-lactating women as for men. Nevertheless, EFSA (2013) recommended a lower value for women as women reach the plateau of plasma ascorbate concentration at a lower vitamin C intake than men.

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 ascorbic acid would provide at least 1 month's safety interval, even for a zero intake, before the body content falls to 300 mg.

It has been reported that elderly people generally have lower plasma and tissue ascorbate levels than young people, often because of poor dentition or mobility problems. However, WHO/ FAO (2004) 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 recommended intake for the elderly are therefore the same as those for adults (45 mg/day). EFSA (2013) also noted that there is a scarcity of data on the influence of ageing on ascorbic metabolism, thus, the recommendation is similar to adults. Both Austria-Confoederatio Helvetica (D-A-CH, 2013) and the European Food Safety Authority (EFSA, 2013) recommended a higher intake of ascorbic acid (ie. D-A-CH: 100 mg per day for adults regardless of gender; EFSA: 110 mg per day for men and 95 mg per day for women >18 years old) to outweigh the benefit of higher intake on the reduction in the risk of chronic diseases. However, the TSC Vitamins felt that the evidence for this is not conclusive and consistent.

For reasons already mentioned above for the adolescents, the TSC on Vitamins has proposed that 25 mg per day ascorbic acid be added on to the WHO/ FAO (2004) recommended intake of 45 mg per day for all groups above 10 years of age.

RNI for adults

Men	**19 - 65 years**	**70 mg/day**
Women	**19 - 65 years**	**70 mg/day**

RNI for elderly

Men	**> 65 years**	**70 mg/day**
Women	**> 65 years**	**70 mg/day**

Pregnancy and Lactation

During pregnancy, there is a moderate extra drain on ascorbic acid, particularly during the last trimester. It has been reported that 8 mg/day of ascorbic acid is sufficient to prevent scorbutic signs in infants aged 4-17 months. WHO/FAO (2004) therefore provided an extra 10 mg/day throughout pregnancy, to bring the recommended intake to 55 mg/day. This enables reserves to accumulate to meet the extra needs of the growing foetus in the last trimester. EFSA (2013) also suggested a similar quantum of additional intake of 10 mg/ day for pregnancy.

During lactation, it has been estimated that 20 mg/day of ascorbic acid is secreted in milk. For an assumed absorption efficiency of 85 percent, an extra 25 mg will be needed by the mother. WHO/FAO (2004) therefore recommended that the RNI should be set at 70 mg to fulfill the needs of both the mother and infant during lactation. However, EPSA (2013) recommended a higher value, as it was estimated that 40 mg/day of vitamin C is secreted with milk over the first six months post partum. Further, EPSA (2013) assumed an absorption rate of 80%, a mean of 50 mg/ day is required to balance the amount of vitamin C secreted in milk for exclusive breast feeding women during the first six months of lactation. Assuming a CV of 10% and additional intake of 60 mg/ day is recommended for women breastfeeding exclusively.

For the same reasons mentioned for the adolescents, the TSC for Vitamins suggested to add an additional 25 mg per day of ascorbic acid to the WHO/FAO (2004) recommended intake for pregnant and lactating women.

RNI for

Pregnancy	**80 mg/day**
Lactation	**95 mg/day**

Discussions on revised RNI for Malaysia

The 2017 RNI values for ascorbic acid for Malaysia, adapted from WHO/ FAO (2004), but with the addition of 25 mg per day for all age groups above 10 years of age, are also the same as those adopted by the Working Group for the Harmonisation of RDAs in Southeast Asia (Tee & Florentino, 2005). The SEA Group also decided to provide for an additional amount of 25 mg mentioned above. Appendix 12.1 provides a summary of these revised RNI, which is compared with the previous Malaysian RNI (NCCFN, 2005), the WHO/FAO (2004) recommendations, the values recommended by IOM (2000) and recommendations of EFSA (2013). Generally, all of the recommendations adopted calculations based on percentages of ascorbic acid being absorbed minus the amount losses, however, WHO/FAO (2004) quantified based on physiological requirement. Whilst, both IOM (2000) and EFSA (2013) computed based on metabolic studies involving few samples of healthy men. The recommendation from EFSA (2013), set as Population Reference Intake (PRI) is the highest as it added another 10% coefficient variation from the Average Requirement (AR) provided by IOM (2004). WHO/FAO (2004) recommendation is the lowest as it considered as minimum requirement to maintain ascorbic

acid pool, to prevent deficiency. RNI 2017 proposed a similar recommendation as RNI 2005 (NCCFN, 2005), of which 25 mg/ day is added to the WHO/ FAO (2004), in order to increase absorption or iron in the diet among population of which anemia is still prevalent such as the Southeast Asia region as discussed earlier.

12.8 Toxicity and tolerable upper intake levels

The review by IOM (2000) reported no evidence suggesting that ascorbic acid is carcinogenic or teratogenic or that it causes adverse reproductive effects. High intakes of the vitamin have been reported to have low toxicity; adverse effects have been reported primarily after very large doses (greater than 3 g/day). Data obtained showed little increase in plasma steady-state concentrations at intakes above 200 mg/day. Saturable intestinal absorption and renal tubular reabsorption data suggest that overload of ascorbic acid is unlikely in humans. Possible adverse effects associated with very high intakes have been reviewed and include: diarrhea and other gastrointestinal disturbances, increased oxalate excretion and kidney stone formation, increased uric acid excretion, pro-oxidant effects, systemic conditioning ("rebound scurvy"), increased iron absorption leading to iron overload, reduced vitamin B12 and copper status, increased oxygen demand, and erosion of dental enamel. The tolerable upper intake levels (ULs) as proposed by IOM (2000) for various age groups are tabulated in Table 12.2. EFSA (2013) however, did not set any UL.

Table 12.2 Tolerable Upper Intake Levels (UL) of ascorbic acid for various age groups

Age groups	mg/day of preformed ascorbic acid
Infants	Not possible to establish; source of intake should be formula and food only
Children	
1 – 3 years	400
4 – 8 years	650
9 – 13 years	1,200
Adolescents, 14 – 18 years	1,800
Men, ≥ 19 years	2,000
Women, ≥ 19 years	2,000
Pregnant women	
14 – 18 years	1,800
> 19 years	2,000
Lactating women	
14 – 18 years	1,800
> 19 years	2,000

Source: IOM (2000)

The WHO/FAO (2004) report pointed out that the potential toxicity of excessive doses of supplemental ascorbic acid relates to intra-intestinal events and to the effects of metabolites in the urinary system. Intakes of 2-3 g/day of ascorbic acid produce unpleasant diarrhea from the osmotic effects of the unabsorbed vitamin in the intestinal lumen in most people. Gastrointestinal disturbances can occur after ingestion of as little as 1 g because approximately half of the amount would not be absorbed at this dose. Oxalate is an end product of ascorbate catabolism and plays an important role in kidney stone formation. Excessive daily amounts of ascorbic acid produce hyperoxaluria. The risk of oxalate stones formation may become significant at high intakes of ascorbic acid (>1 g), particularly in subjects with high amounts of urinary calcium. The WHO/WHO Consultation felt that 1 g ascorbic acid appears to be the advisable upper limit of dietary intake.

Vitamin C intake at dose beyond 3 g/ day is associated with gastrointestinal disturbance due to the unabsorbed ascorbate. However, participants that were administered intravenous vitamin C up to 100 g per infusion within a few hours in a pharmacokinetics study did not report any adverse effects, demonstrating the safety of this vitamin at megadose. Nevertheless, high doses of vitamin C are contraindicated in special population including chronic kidney disease patients and hyperoxaluria due to inability to excrete excessive oxalate from metabolic conversion of vitamin C. Meanwhile, in patients with glucose-6-phosphate dehydrogenase deficiency, high doses of vitamin C could induce acute hemolysis. Moreover, high doses of vitamin C could hamper copper absorption and thus inhibit copper-containing superoxide dismutase, an important enzyme in antioxidant defence. On the other hand, very high doses of vitamin C could enhance intestinal iron absorption to a dangerous level among individuals with hereditary diseases such as hemochromatosis and thalassemia. It has been shown in some studies that excessive vitamin C intake (>1 g day) may induce severe urine acidification, leading to impaired excretion of weak acids and bases, resulting in deposition of cystinate and urate in the urinary tract and formation of kidney stones, for reduced absorption and increased daily turnover pertaining to higher oxidative stress in this population.

12.9. Research Recommendations

The following priority areas of research are recommended:

- Content of ascorbic acid in breast milk and complementary foods given to infants.
- Ascorbic acid content in a variety of foods especially cooked and processed fruits and vegetables.
- Studies on health benefits of ascorbic acid in the occurrence of chronic diseases and influence on ageing.
- Ascorbic acid status among Malaysian population according to age groups using suitable biomarkers and possibly determine the influence of genotype on the plasma ascorbate concentration.
- Metabolic studies to determine ascorbic acid requirement taking into account factors including gender, age and smoking status.
- Influence of genotype on plasma ascorbate concentration and biomarkers of ascorbate.

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Appendix 12.1 Comparison of recommended intake for Ascorbic Acid (Vitamin C): RNI Malaysia (2017), RNI of WHO/FAO (2004), AI and RDA of IOM (2000), and PRI of EFSA (2013)

Malaysia (2017)*		WHO/ FAO (2004)		IOM (2000)		EFSA (2013)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**PRI (mg/ day)**
Infants		**Infants**		**Infants**		**Infants**	
0 - 5 months	25	0 - 6 months	25	0 - 6 months	40		
6 - 12 months	30	7 - <12 months	30	7 - 12 months	50	7 - 11 months	20
			RDA (mg/day)				
Children		**Children**		**Children**		**Children**	
1 - 3 years	30	1 - 3 years	30	1 - 3 years	15	1 - 3 years	20
						4 - 6 years	30
4 - 6 years	30	4 - 6 years	30	4 - 8 years	25		
7 - 9 years	35	7 - 9 years	35			7 - 10 years	45
Boys		**Boys**		**Boys**		**Boys**	
10 - 18 years	65	10 - 18 years	40	9 - 13 years	45	11 - 14 years	70
				14 - 18 years	75	15 - 17 years	100
Girls		**Girls**		**Girls**		**Girls**	
10 - 18 years	65	10 - 18 years	40	9 - 13 years	45	11 - 14 years	70
				14 - 18 years	65	15 - 17 years	90

Malaysia (2017)*		WHO/ FAO (2004)		IOM (2000)		EFSA (2013)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**PRI (mg/ day)**
Men		**Men**		**Men**		**Men**	
19 - 65 years	70	19 - 65 years	45	19 - 30 years	90	19 - 65 years	1100
> 65 years	70	> 65 years	45	31 - 50 years	90	>65 years	110
				51 - 70 years	90		
				> 70 years	90		
Women		**Women**		**Women**		**Women**	
19 - 65 years	70	19 - 65 years	45	19 - 30 years	75	19 - 65 years	95
> 65 years	70	> 65 years	45	31 - 50 years	75		
				51 - 70 years	75		
				> 70 years	75		
Pregnancy		**Pregnancy**		**Pregnancy**		**Pregnancy**	
80		55		14 - 18 years	80	105	
				19 - 30 years	85		
				31 - 50 years	85		
Lactation		**Lactation**		**Lactation**		**Lactation**	
95		70		14 - 18 years	115	155	
				19 - 30 years	120		
				31 - 50 years	120		

* Recommendations same as RNI 2005 (NCCFN, 2005)

13 • Vitamin A

13.1 Introduction

Vitamin A is a fat-soluble vitamin obtained from the diet either as preformed vitamin A (retinol and retinyl esters) in foods of animal origin or as a provitamin A carotenoid (mainly beta-carotene) in plant-derived foods, primarily in oils, fruits and vegetables. Retinol is composed of a β-ionone ring, a polyunsaturated side chain and a polar end group. This chemical structure makes it poorly soluble in water but easily transferable through membrane lipid bilayers. Preformed vitamin A consists predominantly of retinol and retinyl esters, which are supplied in the diet by animal-derived products and the most abundant forms of vitamin A in the body. Retinol is a transport form and a precursor of the transcriptionally active metabolite all-trans-retinoic acid (EFSA, 2015).

13.2 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. It is also important for maintaining good nutritional status for optimal cognitive function in elderly (WHO/FAO, 2004; IOM, 2001).

A main function of vitamin A is for the normal functioning of the visual system. Retinol is transported to ocular tissue and to the retina of the eye by intracellular binding and transport protein in which it plays an important part in the formation of rhodopsin, an important visual pigment, particularly for dim-light vision. All-trans retinol is converted to retinaldehyde, summarized to the 11-cis form and bound to opsin to form rhodopsin. When there is insufficient amount of retinol available, rhodopsin synthesis is affected and night blindness may result. The condition can, however, also be due to a lack of other nutrients which are critical to the regeneration of rhodopsin such as protein and zinc (WHO/FAO, 2004).

The second main function of vitamin A is in the maintenance of growth and epithelial cellular integrity and immune function in the body. Thus, in vitamin A deficiency, the number of goblet cells are reduced in epithelial tissues, resulting in a reduction in mucous secretions with their antimicrobial components. Cells lining protective tissue surfaces flatten and accumulate keratin because they fail to regenerate and differentiate. All these changes result in diminished resistance to invasion by potentially pathogenic organisms. The immune system is also adversely affected by direct interference with production of some types of protective secretion and cells. As these changes in internal epithelial tissues occur, the external reflections of such changes are seen in the classical eye changes in xerophthalmia and xerosis (WHO/FAO, 2004).

13.3 Metabolism

The absorption of both preformed vitamin A from animal sources (retinol) and provitamin A compounds from plant sources (mainly beta-carotene) occur mainly in the duodenum. The overall absorptive efficiency of preformed vitamin A is high (70%- to 90%) and remains high as intakes increase. On the other hand, the absorption of beta-carotene is only 40% to 60%.

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 (EFSA, 2015).

The chylomicron, 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 (EFSA, 2015).

The main storage form of retinol is retinyl esters. The liver is the main storage organ for vitamin A compounds. While the liver and intestine are the major tissue sites of retinol esterification, other tissues including the eye, lung, adipose tissue, testes, skin and spleen are able to esterify retinol and accumulate retinyl ester stores. The enzyme responsible for most retinyl ester formation is Lecitin retinol acyltransferase (LRAT). In healthy individuals with an adequate vitamin A status, 70%-90% retinol of the body is stored in the liver. The efficiency of storage represents the fraction of ingested retinol which is absorbed and retained in the body (and more particularly in the liver). The efficiency of storage depends on vitamin A status which is the low retinol store are associated with a reduced efficiency of storage (EFSA, 2015).

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.

13.4 Sources

Preformed vitamin A (retinol) is found almost exclusively in animal products. The vitamin A content of several commonly consumed foods as obtained from the Malaysian Nutrient Composition Database (Tee *et al.,* 1997) and USDA (2016) are summarized below and in Table 13.1.

Liver from various animals are extremely rich sources of retinol, containing up to as much as 9,000 µg and 16,000 µg per 100 g of ox liver and chicken liver respectively. Meat of these animals, on the other hand, are generally only medium sources, with levels generally around 30 µg/100 g of meat, chicken, duck or mutton. Whole egg is a very good source of vitamin A, with an estimated content of 350 µg/100 g. Most of this vitamin is in the egg yolk, a 100 g of which contains about 570 µg retinol. Fish in general are not particularly good sources of vitamin A, as only a few species contain the vitamin at a concentration of 20-30 (g/100 g.

Several processed foods have been fortified with vitamin A and are good sources of the vitamin. These include cornflakes, malted milk powder and milk powder.

Carotenoids, especially α- and β-carotene which are potential provitamin A precursors that can be converted into retinol, are present in plant products, especially green leafy vegetables, red palm oil and yellow and orange fruits and some tubers, eg sweet potato. Red palm oil is the richest naturally occurring source of β-carotene and generally contains a total of ~500-800 mg of provitamin A carotenoids/kg oil, which is ~15 times higher than the carotenoid contents of carrots on a weight-by-weight basis (Sundram, Sambanthamurthi &

Tan, 2003). Carotenoids are biologically less available than retinol but they are generally more affordable than animal foods. Thus carotenoids provide most of the vitamin A activity in the diets of lower socio-economic segments of the populations.

Table 13.1 Vitamin A content of foods

Food Category	Vitamin A (µg RE/100g)
Meat/ Poultry/Fish	
Chicken liver	16,000
Ox/ Beef liver	9,000
Hen egg, whole	304
Duck egg, whole	208
Duck, thigh	69
Chicken, thigh	50
Mackarel, Indian *(kembong)*	8
Mackarel, Spanish *(Tenggiri)*	8
Fruits	
Mango	214
Papaya	193
Watermelon	68
Kundang (kemoir)	55
Vegetables	
Cekur Manis (sweet leaf)	1620
Red Capsicum	1510
Kesum (laksa leaf)	1210
Maman (cleome gynandra)	960
Carrot	835
Tomato	700
Processed Foods	
Cornflakes	812
Malted milk powder	767
Full cream milk powder	400
Butter	200
Cheddar Cheese	117
Others	
Sweet potatoes	709

Source: Tee *et al.* (1997), USDA (2016)

13.5 Deficiencies

The main symptoms observed in case of deficiency of vitamin A are intrauterine and post-natal growth retardation and large array of congenital malformations collectively referred to as the foetal 'vitamin A deficiency syndrome', which is well documented in animals. In adults, vitamin A deficiency adversely affects several functions, such as vision, immunity, and reproduction, and has been related to the worsening of low iron status, resulting in vitamin A deficiency anaemia.

Ocular manifestations of vitamin A deficiency (VAD), termed "xerophtalmia" or "dry eye" has been recognized for a long time. The most frequently encountered of these signs is night blindness, which is the earliest manifestation of xerophthalmia. In VAD, the time required to regenerate rhodopsin is prolonged, thereby delaying adaptation time to dark environments. Night-blind young children tend to stumble when going form bright to dimly-lit areas and they tend to remain inactive at dusk and at night.

Vitamin A deficiency can occur as either a primary or a secondary deficiency. A primary vitamin A deficiency occurs among children and adults who do not consume an adequate intake of provitamin A carotenoids from fruits and vegetables or preformed vitamin A from animals and dairy products. Secondary vitamin A deficiency is associated with chronic malabsorption of lipids, impaired bile production and release, and chronic exposure to oxidants (WHO/FAO, 2004).

There are several biochemical methods for estimating sub-clinical vitamin A status, including breast milk retinol and serum retinol levels, as well as percent relative dose response and modified relative dose response methods. These indicators are less specific to VAD than clinical eye signs and less sensitive for measuring sub-clinical vitamin A status. The biochemical indicator of choice for population assessment is the distribution of serum level of retinol.

Vitamin A status can best be expressed in terms of total body pool of retinol or, alternatively, in terms of liver concentration of the vitamin. Hepatic concentration is considered as a marker of vitamin A status because 70-90% of the retinol in the body is stored in the liver in healthy individuals (WHO/FAO, 2004; EFSA, 2015). A minimum concentration of 20 µg retinol/g liver (0.07 µgmol/g) as a criterion to define adequate vitamin A status as well as to derive vitamin A requirements (IOM, 2001; WHO/FAO, 2004; EFSA, 2015).

Prior to the 1950s, vitamin A deficiency in Malaysia was reported to be an important sight-threatening disorder, affecting mainly young children on unbalanced diet, amongst lower socio-economic segments of the population. Vitamin A intake was generally low, with little or no retinol and most of it from the provitamins. Even then, the amount of vegetables and fruits consumed were generally low (Tee, 1993).

No exact estimates of the magnitude of the problem in the country are presently available. However, as seen from reports in the literature, the problem appears to be confined to certain groups, mainly in the rural areas, and does not pose a major health hazard nationwide. The problem appears to have lessened over the years, judging from reports up to the 1990s. There are probably very few cases of children with eye signs past the conjunctival xerosis and with serum vitamin A < 10 (g/dl) (Tee, 1993).

In a nation-wide study of 439 children under 5 years conducted by the Ministry of Health (MOH, 2000), only 3.9% of the children were found to have vitamin A deficiency (serum retinol <0.7(mol/l). A study among 350 rural elderly Malays also showed that there was no sub-clinical vitamin A deficiency (Suzana, 1999). On the other hand, a study of the problem amongst undernourished preschool children included in the nutrition rehabilitation programme in Sabah showed that some 30% of these children had marginal sub-clinical vitamin A deficiency (serum retinol < 30 (g/dl) (Tee *et al.,* 1997). In a clinical study of aborigine children below 15 years, a relatively high prevalence (64.3%) of ocular manifestation of vitamin A deficiency. Night blindness was found in 16.0% of the children, conjunctiva xerosis in 57.3%, Bitot's spot in 2.8%, corneal xerosis in 0.5% and corneal scars in 5.6% (Ngah *et al.,* 2002). It should thus be recognised that although the vitamin A deficiency problem may appear to be small in the country, the problem can be serious amongst malnourished children and underserved communities. The current status of vitamin A among children in the country is unclear as there are no large scale recent investigations of the problem.

13.6 Factors affecting vitamin A requirements

Generally, age and gender may affect the requirements of vitamin A. Children under 3 years of age need vitamin A to support early rapid growth which is the transition period from breastfeeding to dependence on other dietary source of vitamin and increased frequency of respiratory and gastrointestinal infections. Pregnant and lactating women require additional vitamin A to support maternal and fetal tissue growth and lactation losses. Assuming the efficiency of maternal vitamin A absorption to average 70% and vitamin A to be accumulated mostly in the last 90 days of pregnancy, the maternal requirement would be increased during the third trimester (WHO/FAO, 2004; IOM, 2001). Similarly, lactating mothers may require additional vitamin A to maintain vitamin A body stores of the mother. Consideration on whole body retinol stores in the foetus and on retinol secretion in breast milk can be used to derive the additional requirement for, respectively, pregnant or lactating women (EFSA, 2015). Bioavailability of the vitamin A is another factor affecting requirements. Bioavailability is the proportion of an ingested nutrient that is available for utilization in normal physiologic functions and for storage. The bioavailability or preformed vitamin A is very high. More than 90% of retinol added to food as a fortificant is absorbed.

Dietary fat plays an important role in the absorption of ingested vitamin A. Dietary vitamin A is digested in mixed micelles and absorbed with fat. There are indications that increasing the level of fat in a low fat diet can improve retinol and carotene absorption. Particularly for optimal carotenoid absorption, dietary fat must be consumed alongside these provitamin A compounds.

The matrix of the food affects the ability of carotenoids to be released from food and therefore affects intestinal absorption. For example, the rise in serum β-carotene concentration has been observed to be significantly less when individuals consumed carrots than when they received the same amount of the vitamin of β-carotene supplement. The processing of food also greatly affects the absorption of carotenoids. For instance, the absorption of carotene from sliced carrots was much lower than from homogenised carrot. Cooking of carrot and spinach also greatly improved absorption.

Malabsorption of vitamin A can occur with diarrhoea and intestinal infections such as in gastroenteritis and respiratory infections. Malabsorption of the vitamin is also associated with intestinal parasitism.

Units of expression and conversion factors

In blood, tissues and human milk, vitamin A levels are conventionally expressed in (g/dl or (mol/l all-trans retinol. Most of the circulating vitamin A is retinol whereas in most tissues, secretions and other animal food sources it exists mainly as retinyl esters.

To express the vitamin A activity of carotenoids in diets on a common basis, FAO/WHO introduced the concept of retinol equivalent (RE) and established the following relationship among food sources of vitamin A:

1 µg retinol	= 1 RE
1 µg β-carotene	= 0.167 µg RE, (1/6 µg)
1 µg other pro-vitamin A carotenoids	= 0.084 µg RE, (1/12 µg)

The U.S. Institute of Medicine (IOM) has introduced a new term, retinol activity equivalent (RAE) to express the activity of carotenoids in terms of Vitamin A, to take into account new research on the Vitamin A activity (bio-efficacy) of carotenoids (West et. al 2002). IOM established the following conversion factor equivalents:

1 µg retinol	= 1 µg RAE
1 µg β-carotene in oil	= 0.5 µg RAE
1 µg β-carotene in mixed foods	= 0.083 µg RAE
1 µg other pro-vitamin A carotenoids in mixed foods	= 0.042 µg RAE

However, using the new IOM conversion rates, population in developing countries may have difficulty to achieve adequacy (Clive, Ans & Machteld, 2002). Based on the FAO/WHO (1988)) conversion rates, all populations should be able to meet their vitamin A requirements from existing dietary sources. This Technical Sub-Committee on Vitamins and The Scientific Committee for Food (SCF, 1993) for the European populations also decided to maintain the conversion factors that until more definitive data become available. Vitamin A requirement can be met with any mixture of preformed vitamin A carotenoids that provides an amount of vitamin A equivalent to the reference value in terms of µg RE/day.

It has been strongly recommended that weight or molar units replace the use of IU to decrease 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 β-carotene	= 0.6 µg β-carotene
1 IU retinol	= 3 IU µ-carotene

13.7 Setting requirements and recommended intake of vitamin A

Vitamin A is an existing vitamin in the Malaysian RNI 2005. At that time, the Technical Sub-Committee (TSC) on Vitamins found that there was no local data on vitamin A requirements that could be used for arriving at RNI for the vitamin. The TSC therefore referred to the WHO/FAO (2004) consultation report and the IOM (2001) DRI recommendations. The rationale and steps taken in setting requirements and the levels recommended by these organisations as well as available reports of vitamin A status of communities in the country were considered. The TSC on Vitamins decided to adapt the WHO/FAO (2004) values as the revised 2005 RNI for Malaysia.

The FAO/WHO international expert consultation had used the term "safe level of intake" as it was felt that the values arrived at do not strictly correspond to the definition of a recommended nutrient intake. These safe levels of intake are basically the same as those in the 1988 FAO/WHO Expert Consultation (FAO/WHO, 1988).

The safe level of intake for an individual is defined as the average continuing intake of vitamin A required to enable adequate growth and permit other vitamin A-dependent functions to take place as well as 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.

In reviewing the Malaysian RNI 2017 for vitamin A, the TSC noted that there were no large scale recent investigations of the vitamin A status of communities in the country. It was also noted that there have been no updated versions of recommendations of vitamin A intake from FAO/WHO consultation or the IOM DRI committee. The committee however referred to the recently published report of the European Food Safety Authority on Dietary Reference Values for vitamin A (EFSA, 2015).

Upon reviewing all available information, the TSC Vitamins decided to continue to adopt the WHO/FAO (2004) recommendations for vitamin A in the revised Malaysia RNI 2017. The TSC felt that the recommendation intakes set by EFSA (2015) and IOM (2001) are too high for men, women and lactating women and not suitable to be adopted. The recommendations of WHO/FAO (2004) are deemed as still appropriate for Malaysians. Furthermore, with improvement in socio-economic status in the last 30 years, a higher percentage of intakes of vitamin A would be from animal sources as compared to the past, where it was derived mostly from plant sources.

These recommendations for RNI 2017, which are the same as the previous RNI (NCCFN 2005), are given in bold in the following paragraphs, according to age groups and summarised in Appendix 13.1.

Infants

No functional criteria of vitamin A status have been demonstrated that reflect response to dietary intake in infants. Thus recommended intakes of vitamin A are based on an adequate intake that reflects a calculated mean vitamin A intake of infants principally fed human milk.

During the first 6 months of life, exclusive breast-feeding is known to provide sufficient vitamin A to maintain health, permit normal growth and maintain sufficient stores in the liver. The safe level of intake for infants up to 6 months of age is calculated based on observations of breast-fed infants in generally healthy communities. The average breast milk consumption is about 750 ml/day. The average concentration of vitamin A in human milk is about 1.75 µmol/l (range, 0.70-2.45 µmol/l). Thus mean daily intake would then be about 375 µg RE, which is taken as the recommended safe level.

For the second half of infancy, human milk intake averages 650 ml which would provide 325 µg vitamin A daily. In order to reduce risk to death from 6 months onwards, especially in endemic vitamin A-deficient populations, the recommended safe intake is increased to 400 µg (WHO/FAO, 2004). On the other hand, the average intake of vitamin A required for infants aged 7-11 months to maintain a concentration of 20 µg retinol/g liver, but with values for reference body weight and liver/ body weight ration specific to infants, which is 250 µg/day (EFSA, 2015).

RNI for infants

0 - 5 months	**375 µg RE/day**
6 - 11 months	**400 µg RE/day**

Children and adolescents

For older children, the 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 1-9 years was estimated to range from 400-500 µg per day. The safe intake for adolescents was stepped up from that for children. Besides that, for children up to 14 years of aged, it is the same values with infants to maintain a concentration of 20 µg retinol/g liver, that is 250 µg/day. It was considered unnecessary to give sex-specific values for infants and children up to 14 years of aged (EFSA, 2015).

RNI for children

1 - 3 years	**400 µg RE/day**
4 - 6 years	**450 µg RE/day**
7 - 9 years	**500 µg RE/day**

RNI for adolescents

Boys 10 - 18 years	**600 µg RE/day**
Girls 10 - 18 years	**600 µg RE/day**

Adults and elderly

Estimates for the recommended safe intakes for adults are also obtained from those derived from late infancy, i.e. 9.3 µg/kg body weight/ day. The levels recommended are essentially the same as those made during the FAO/WHO expert consultation1988 as the group felt that there were no new published studies to indicate a need to revise the assumptions previously made. The safe intakes recommended are said to be consistent with the per capita vitamin A content in the food supply of countries that show adequate vitamin A status in all sectors of the population.

The FAO/WHO consultation felt that there was no indication that the vitamin A requirement of healthy elderly individuals differed from those of other adults. It should however be borne in mind that the elderly are more commonly affected by several disease conditions that impede vitamin A absorption, storage, and transport which hence affected their requirements for the vitamin.

The EFSA Panel 2015 determines the Adequate Requirement (AR) for vitamin A in healthy adults as the vitamin A intake required to maintain a liver retinol concentration of 20 µg/g (0.07 µmol/g). Assuming a CV of 15% because of the variability in requirement and of the large uncertainties in the dataset, Population Reference Intakes (PRIs) of 750 µg RE/day for men and 650 µg RE/day for women are set.

RNI for adults

Men	**19 - 65 years**	**600 µg RE/day**
Women	**19 - 65 years**	**600 µg RE/day**

RNI for elderly

Men	**> 65 years**	**600 µg RE/day**
Women	**> 65 years**	**600 µg RE/day**

Pregnancy and Lactation

During pregnancy, additional vitamin A is needed for the growth and maintenance of the foetus for providing a limited reserve in the foetal liver and for maternal tissue growth. There are no reliable figures available for the specific vitamin A requirements for these processes. Recognising that a large portion of the world's population of pregnant women live under conditions of deprivation, the FAO/WHO expert consultation recommended that the safe level for women be raised by 300 µg, bringing it to 800 µg RE per day. The Consultation also pointed out that women who are or who might become pregnant should carefully limit their total daily vitamin A intake to a maximum of 3000 µg RE to minimise risk of foetal toxicity.

If the amounts of vitamin A recommended for infants are supplied by human milk, mothers should absorb at least as much in 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 during lactation to 850 µg RE. IOM 2001 recommended intake for pregnant and lactating adolescents are 750 µg RE/day and 1200 µg RE/day, respectively.

The EFSA Panel 2015 assumes that a total amount of 3600 µg retinol is accumulated in the foetus over the course of pregnancy. Consequently, an AR of 540 µg RE/day is estimated for pregnant women by adding the additional requirement of pregnancy to the AR for non-pregnant non-lactating women and rounding. Considering a CV of 15%, a PRI of 700 µg RE/day is derived for pregnant women. For lactating women, based on an average amount of retinol secreted in breast milk of 424 µg/day and considering a CV 15%, a PRI of 1300 µg RE/day is proposed for lactating women.

RNI for

Pregnancy	**800 µg RE**
Lactation	**850 µg RE**

Discussions on revised RNI for Malaysia

The proposed 2017 RNI values for vitamin A for Malaysia, adapted from WHO/FAO (2004), are also the same as those adopted by the Working Group for the Harmonisation of RDAs in SE Asia (Tee & Florentino, 2005). In this revised RNI for Malaysia (2017), the proposed recommended intakes retain same corresponding values to those of RNI (2005). Appendix 11.1 provides a summary of the revised RNI (2017), compared with the RNI (2005), EFSA (2015), the WHO/FAO (2004) recommendations and the values recommended by IOM (2001).

The levels for infants are similar with those recommended by IOM (2001), but the EFSA (2015) recommendations are lower for this age group. On the other hand, the recommendation intakes by IOM (2001) and EFSA (2015) are higher for adolescent, men, women and lactating women. IOM (2001) values recommended for adults are higher than those recommended by WHO/FAO (2004), probably due to the large body size of Americans.

13.8 Tolerable upper intake levels

Being a fat-soluble vitamin, retinol can be stored in the human body, primarily in the liver. Thus long term regular consumption of large amounts of the vitamin A can result in toxic symptoms. These symptoms are varied and non-specific and include central nervous system effects, liver damage, bone abnormalities and joint pain, alopecia, headaches and vomiting and skin desquamation. Rarely does toxicity occur from ingesting food sources of preformed vitamin A. When this occurs, it usually results from very frequent consumption of liver products (WHO/FAO, 2004). Acute toxicity is characterised by nausea, vomiting, headache, increased cerebrospinal fluid pressure, vertigo, blurred vision, muscular incoordination and bulging frontal in infants (IOM, 2001).

Infants, including newborns, administered single doses equivalent to 15,000 and 30,000 µg retinol in oil generally show no adverse symptoms. Daily prophylatic or therapeutic doses should not exceed 900 µg. Most children 1-6 years of age tolerate single oral doses of 60,000 µg vitamin in oil at intervals of 4-6 months without adverse symptoms.

When taken by women at early stages of gestation at daily levels of more than 7500 µg, foetal anomalies and poor reproductive outcomes have been reported. Women who are pregnant or might become pregnant should avoid taking excessive amounts of vitamin A. The WHO expert group recommended that daily intakes in excess of 3,000 µg or weekly intakes in excess of 7,500 µg should not be taken at any period during gestation.

The Tolerable Upper 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 purposes of deriving at a UL, three primary adverse effects of chronic vitamin A intake are: reduced bone mineral density, teratogenecity and liver abnormalities. The ULs recommended by IOM (2001) are slightly different from those of FAO/WHO and are as given in Table 13.2.

Table 13.2 Tolerable Upper Intake (UL) levels of vitamin A for various age groups

Age groups	µg/day of preformed vitamin A
Infants	600
Children	
1-3 years	600
4-8 years	900
9-13 years	1,700
Adolescents, 14-18 years	2,800
Women, ≥ 19 years	3,000
Men, ≥19 years	3,000
Pregnant women	2,800
Lactating women	2,800

Source: IOM (2001)

13.9 Research recommendations

The following priority areas of research are recommended:

- Data on dietary intake of vitamin A among different community groups, particularly children and women, both in terms of amounts of vitamin A and carotene-rich foods as well as the amounts of retinol consumed.
- Comprehensive study of vitamin A status of pre-schoolers using biochemical indicators especially amongst communities of lower socio-economic status.
- Studies on health benefits of carotenoids, eg lycopene, zeaxanthin in local fruits and vegetables/ ulam.
- Bioavailability of provitamin A compounds of food sources.
- Effect of processing on bioavailability of vitamin A and provitamin A compounds.
- Effect of vitamin A status on immune function of children.

13.10 References

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Appendix 13.1 Comparison of recommended intake for Vitamin A: RNI Malaysia (2017), EFSA (2015), "safe intake" of WHO/FAO (2004) AI and RDA of IOM (2001)

Malaysia (2017)*		EFSA (2015)		WHO/FAO (2004)		IOM (2001)	
Age group	**RNI (µgRE/day)**	**Age group**	**PRI (µgRE/day)**	**Age group**	**Safe intake (µgRE/day)**	**Age group**	**AI (µgRE/day)**
Infants		**Infants**		**Infants**		**Infants**	
0 - 5 months	375			0 - 6 months	375	0 - 6 months	400
6 - 12 months	400	7 - 11 months	250	7 - 12 months	400	7 - 12 months	500
							RDA (µgRE/day)
Children		**Children**		**Children**		**Children**	
1 - 3 years	400	1 - 3 years	250	1 - 3 years	400	1 - 3 years	300
4 - 6 years	450	4 - 6 years	300	4 - 6 years	450	4 - 8 years	400
7 - 9 years	500	7 - 10 years	400	7 - 9 years	500		
Boys		**Boys**		**Boys**		**Boys**	
10 - 18 years	600	11 - 14 years	600	10 - 18 years	600	9 - 13 years	600
		15 - 17 years	750			14 - 18 years	900
Girls		**Girls**		**Girls**		**Girls**	
10 - 18 years	600	11 - 10 years	600	10 - 18 years	600	9 - 13 years	600
		15 - 17 years	650			14 - 18 years	700

Malaysia (2017)*		EFSA (2015)		WHO/FAO (2004)		IOM (2001)	
Age group	**RNI (µgRE/day)**	**Age group**	**PRI (µgRE/day)**	**Age group**	**Safe intake (µgRE/day)**	**Age group**	**AI (µgRE/day)**
Men		**Men**		**Men**		**Men**	
19 - 65 years	600	> 18	750	19 - 65 years	600	19 - 30 years	900
> 65 years	600			> 65 years	600	31 - 50 years	900
						51 – 70 years	900
						> 70 years	900
Women		**Women**		**Women**		**Women**	
19 - 65years	600	> 18	650	19 - 65 years	500	19 - 30 years	700
> 65 years	600			> 65 years	600	31 - 50 years	700
						51 - 70 years	700
						> 70 years	700
Pregnancy		**Pregnancy**		**Pregnancy**		**Pregnancy**	
800		700		800		14 - 18 years	750
						19 - 30 years	770
						31 - 50 years	770
Lactation		**Lactation**		**Lactation**		**Lactation**	
850		1300		850		14 - 18 years	1,200
						19 - 30 years	1,300
						31 - 50 years	1,300

*Recommendations similar to RNI 2005
RE- Retinol Equivalents

14 • Vitamin D

14.1 Introduction

Vitamin D or calciferol is a fat-soluble vitamin. It can exist in 2 isoforms which is D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D2 is mostly human-made and added to foods. Vitamin D3 is synthesized in the skin from 7-dehydrocholesterol. The vitamin D3 synthesis can be activated with the exposure of bare skin to sunlight. Vitamin D3 can also be obtained from the diet via the intake of animal-based food. Both D2 and D3 are synthesised commercially and found in fortified foods and dietary supplements. Vitamin D3 is reported to have higher stability in blood compared to vitamin D2 due to the higher ability of binding protein towards vitamin D3.

The D2 and D3 forms differ only in their side chain structure of the chemical compound. The differences do not affect metabolism/activation and both forms function as prohormones. When activated, the D2 and D3 forms have been reported to exhibit identical responses in the body. It was reported that the specific potency related to the ability to cure vitamin D-deficiency rickets is the same between both isoforms (Jones, Strugnell, & DeLUCA, 1998; Jurutka *et al.,* 2001).

14.2 Functions

The dominant function of Vitamin D in its hormonal/active form (calcitriol or 1,25-dihydroxyvitamin D) is with the skeletal system.The main biological function is to maintain normal blood levels of calcium and phosphorus. These minerals are important for the normal mineralization process of the bone (Holick, 1996). Vitamin D also regulates the transcription of a number of vitamin D-dependent genes coding for calcium transporting proteins and bone matrix proteins. Furthermore, the elevation of plasma calcium to normal levels is also required for the functioning of the neuromuscular junction as well as vasodilatation, nerve transmission, and hormonal secretion.

It is noteworthy that the vitamin D receptor (VDR) is present in the nucleus of many tissues that are not involved in the regulation of calcium and phosphate metabolism. For example, the VDR has been clearly described in epidermal keratinocytes, in activated T cells of the immune system, in antigen-presenting cells, in macrophages and monocytes, and in cytotoxic T cells.

Hence, with the discovery of VDR on non-skeletal tissue systems, a large number of research has been focused at the non-skeletal chronic disease outcomes such as diabetes, cancer, cardiovascular disease and metabolic syndrome. However, after extensive and comprehensive review of literature, the Institute of Medicine (IOM, 2011) concluded that the causality was inconsistent and inconclusive and there was insufficient evidence on the non-skeletal chronic disease outcomes to serve as a basis for the development of Dietary Reference Intake (DRI) of vitamin D. Holick *et al.* (2011) performed a meta-analysis and made a scientific statement stating that although many observational studies have identified links of vitamin D with several chronic diseases, but these have neither been evaluated nor replicated in randomised controlled trials and in any cohort studies.

14.3 Metabolism

Vitamin D maintains serum calcium levels by three different mechanisms. The first mechanism, which does not require parathyroid hormone (PTH), is the well-established role of vitamin D in stimulating intestinal calcium absorption throughout the entire length of the intestine, with its greatest activity in the duodenum and jejunum. In the second mechanism, vitamin D plays an essential role in the mobilisation of calcium from bone, a process requiring PTH (Garabedian *et al.,* 1972; Lips, 2006). It induces the formation and activation of the osteoclast to function in the mobilisation of calcium from bone. In the third mechanism, vitamin D together with PTH stimulates the renal distal tubule reabsorption of calcium, ensuring retention of calcium by the kidney when calcium is needed (Sutton & Lazarus, 1976; Yamamoto *et al.,* 1984). Through the VDR, vitamin D suppresses parathyroid gene expression and parathyroid cell proliferation, providing important feedback loops that reinforce the direct action of increased serum calcium levels (Silver *et al.,* 1986; Slatopolsky *et al.,* 1984).

14.4 Sources

Dietary vitamin D can be obtained from three sources, namely natural food, fortified food and supplements. There are a few naturally occurring food sources of vitamin D which includes fatty fish (salmon, tuna, sardine, herring, mackerel), cod liver oil and egg yolk (USDA, 2016). Meat, poultry and vegetables are generally poor sources of the vitamin. Dairy products are fair sources of vitamin D. Other possible sources of vitamin D are from fortified foods which are made available on a voluntary basis by manufacturers. Commercially, vitamin D is fortified in cereals, bread, butter and yoghurt. Beverages such as milk and soy milk are also fortified with vitamin D. In Malaysia, the Malaysian Food Regulation permits the addition of vitamin D according to different categories of foods (MOH, 1985). Currently, voluntary vitamin D fortification of milk powder for children and adults are being carried out by manufacturers. The intake of foods fortified with vitamin D can increase vitamin D in the diet.

In recent years, vitamin D dietary supplements either combined only with calcium or with multivitamin/ multi-mineral formulations have become more common and have been more frequently consumed. Generally, the form of vitamin D used in supplement products can be either from vitamin D2 or vitamin D3 compound. However, the industry is switching from vitamin D2 to D3. Some manufacturers are also found to produce vitamin D with higher concentration. Traditionally, many marketed dietary supplements contain 400 IU (10 ug) per daily dose (IOM, 2011). Direct sunlight exposure to the skin is also a good source to achieve optimum level of vitamin D. Vitamin D content of foods is given in Table 14.1.

Table 14.1. Vitamin D content of foods

Food	Vitamin D µg/100g
Poultry, Meat, Fish	
Fish, salmon, pink	10.9
Fish, mackerel, cooked	7.3
Fish, sardines, cooked	4.8
Egg, whole	2.0
Beef, liver	1.2
Fish, catfish, farmed	0.2
Beef, Meat	0.1
Chicken, Meat	0.1
Lamb, meat	0.1
Dairy	
Milk, cow fortified, low fat	1.3
Yogurt, fortified, low fat	1.2
Cheese, cheddar	1.0
Vegetables	
Mushroom, oyster	0.7
Potatoes, mashed	0.3

Source: USDA, (2016) - 1 ug = 40 IU

14.5 Deficiencies

Vitamin D deficiency is manifested as rickets in children and as osteomalacia in adults. Lack of the vitamin in adults may also contribute to the development of osteoporosis. Higher prevalence of vitamin D deficiency has been found among females compared to males (Moy & Bulgiba, 2011). Infants are the group most at risk of deficiency in vitamin D simply because of their high rate of skeletal growth. Although at birth, vitamin D is acquired in utero, the stores will only be sufficient for the first month of life. In temperate countries, infants born in the autumn months are especially at risk because they spend the first six months of their life indoors. Therefore, they have less opportunity to synthesise vitamin D in their skin during this period.

In the elderly, clinical research studies have suggested age related decline in many key steps of vitamin D action (Holick, 1994). This included rate of skin synthesis, rate of hydroxylation leading to activation to the hormonal form, and response of target tissues (bone) as well as reduced skin exposure. Some studies have indicated that there appears to be vitamin deficiency in a subset of elderly population across the globe (Chapuy, Meunier, & Feldman, 1997). Kok-Yong *et al.* (2016) reported the prevalence of osteoporosis among elderly in Kuala Lumpur as 10.6% in males and 8.0% in females, respectively. A few groups have found that moderate increases in vitamin D intakes (10 to 20 µg/day) reduce the rate of bone loss and

fractures (Chapuy *et al.,* 1997; Dawson-Hughes *et al.,* 1991). These results were used as evidence by IOM (2011) to recommend an increase in vitamin D intakes for the elderly to a value (10-15 (g/day) that is able to maintain normal vitamin D levels.

Since early year 2000, there has been a significant increase in research publications related to vitamin D in Malaysia. Currently, data on several life stages show high prevalence of vitamin D deficiency among adults and children in Malaysia. Appendix 14.1 shows the summary of vitamin D research findings in Malaysia. It is noteworthy that several different cut-off values were used by the respective researchers. Furthermore, varied methodologies were used thus possibly increasing the variability in findings. The cut-off values and laboratory methods in detecting serum vitamin D has been a popular topic of debate among researchers. Perhaps, these have been translated into the weak clinical evidence on non-skeletal system defect even with the presence of vitamin D insufficiency or deficiency in several population studies.

14.6 Factors affecting vitamin D requirements

Technically, individuals living near the equator (sunny regions) could achieve optimum 25OHD in serum from the activation of sun light on the skin. However, this is not the case as majority of individuals such as housewives office and sedentary persons spend most of their day indoor. Other situations that will result in reduced sunlight exposure include the use of sunscreen and cultural clothing cover-up. Darker skin also reduces skin synthesis of pre-vitamin D in response to sunlight exposure. Darker-skinned people may require longer sunlight exposure than light-skinned people. It is noteworthy that all the groups above do not require vitamin D in excess of the RNI because recommendation has been set with the assumption of minimal sunlight exposure.

Across all age groups, obese individuals were also found to have lower serum vitamin D level compared to individuals with normal weight. One of the main reasons could be due to the sequestration of the fat-soluble vitamin D into adipose tissue. However, the IOM (2011) did not recommend higher requirement of vitamin D among this group due to lack of conclusive and beneficial evidence Elderly person's poor diet and low outdoor activities plus poor health condition may also lead to higher requirement for vitamin D among this vulnerable age group. Highest requirement across age group is set by IOM (2011) for elderly person.

The latitude, monsoon season and time of day can affect vitamin D synthesis.The ultraviolet rays that promote vitamin D synthesis are blocked by heavy clouds, smoke or smog. Hence, the change of atmospheric environment should be monitored with caution.

Best foods to obtain vitamin D from the diet are from animal source such as salmon, tuna, sardine, herring, mackerel, liver and egg yolk. These foods are not consumed by vegetarians. Possible plant-based food source for vitamin D is from mushroom. Hence, individuals practicing vegetarianism should try to achieve optimum activation of serum vitamin D from sunlight, from vitamin D fortified foods and possibly supplements.

14.7 Setting requirements and recommended intake of vitamin D

Vitamin A is an existing vitamin in the Malaysian RNI 2005. In making the recommendation for vitamin D intake then, there was no local data on vitamin D requirements that could be used by the Technical Sub Committee (TSC) on vitamin. The RNI Malaysia (2005) referred to the WHO/FAO (2004) consultation report as well as the IOM (1997) DRI recommendations. These organisations had made the same recommended intakes for the vitamin. The rationale and steps taken in setting the requirements and the levels recommended by these organisations were considered. The TSC on Vitamins agreed to adapt the WHO/FAO (2004) values as the RNI for Malaysia 2005.

In preparing for the 2017 review of the RNI for vitamin D, the TSC on vitamins sourced for recent recommendations of the vitamin by international research organisations. It was noted that WHO/FAO has not updated their 2004 recommendations. There were no other new published reports on vitamin D recommendations except by IOM (2011). It was also noted that the European Food and Safety Authority produced a scientific opinion draft on DRI for Vitamin D (EFSA, unpublished) which recommends similar RNI as IOM (2011). Thus, the TSC referred only to the IOM (2011) DRI recommendations as it has the latest published recommendations on Vitamin D. The rationale and steps taken in setting the requirements and the levels recommended by IOM (2011) and available reports on vitamin D status of communities in Malaysia were considered. The TSC on vitamins agreed to adapt the IOM (2011) values as the revised RNI for Malaysia, given in bold in the following paragraphs according to age group and summarised in Appendix 14.2.

The IOM (2011) considered minimal sun exposure in deriving the DRI. This condition is not different with the sun exposure situation in Malaysia even with the availability of sunlight almost throughout the year. This is mainly due to sunlight avoidance practice related to the heat of sunlight among Malaysian. This is evident from a local study which found minimal sunlight exposure and high sun light avoidance practice among Malaysians (Moy & Bulgiba, 2011). Occupation also plays an important roles as a study compared vitamin D level between indoor and outdoor workers (Hamid Jan & Norliyana, 2016) indicating higher serum vitamin D level among outdoor workers compared to indoor workers. Holicks (2002) reported that the best time for sunlight activation of vitamin D is during the noon as the UVB radiation is at peak. In countries near the equator like Malaysia, this is also the time when the heat is at the highest level. Hence, this could be the main reason for sun avoidance practice among majority of Malaysians.

A local study has shown that with minimum surface exposure (face and hand) to sunlight for 30 minutes, 2 times per week at 11am could increase 40% of serum vitamin D (Hamid Jan & Norliyana, 2016). However, caution should be made by individuals who have sensitive skin to heat from sunlight as it may cause skin irritation. The abovementioned study also reported similar effect among participants who took 50,000 IU (1250 ug) (1 vitamin D per week for 3 months. Indicating, alternative source compared to sunlight.

Infants

Data are not sufficient to establish an Estimated Average Requirement (EAR) for infants less than 1 year of age, and therefore an AI has been developed for vitamin D recommendation for this age group. The AI for the 0 to 6 months and 7 to 12 months life stage groups is set at 10 (g of vitamin D per day by IOM (2011). There are no data to suggest that older infants would benefit from higher intakes. There are differences in the volume of milk or formula intake during this 12-month period, with newborns taking in less than older infants. The AI of 10 µg/day, therefore, represents an overall intake for the first year of life, and may vary across the life stages; it also assumes early introduction of a supplement for breast-fed babies. In the case of exclusive formula feeding, there is an assumption of a gradual increase in intake from 800 to 1,000 mL/day during infancy, which for most standard formulas provides about 10 µg /day.

Human milk from vitamin D deficient mother has been shown to contain low amounts of vitamin D (Jan Mohamed *et al.,* 2014). Thus, for the above reasons and assuming that infants are not getting any vitamin D from sunlight, IOM (2011) recommended an intake of at least 10 µg/day for infants 0-6 months. Similarly, for infants 7-12 months, it has been observed that in the absence of any sunlight exposure, an intake of 10 (g/day will result in most of the infants with serum 25(OH)D above 50 nmol/L.

RNI for infants

0 - 6 months	**10 µg/day**
7 - 12 months	**10 µg/day**

Children and adolescents

For children and adolescents, ensuring normal, healthy bone accretion is the main criteria to the DRI values. The requirement distribution developed using serum vitamin D concentrations and the intakes estimated to achieve such concentrations are the basis for the reference values by IOM (2011).

For very young children in this life stage group, currently no data are available to link vitamin D nutriture directly to measures related to bone health outcomes. The Agency for Healthcare Research and Quality based in University of Ottawa (AHRQ-Ottawa) conducted a systematic evidence-based review to examine the relationship between vitamin D and rickets in children 0 to 5 years of age. AHRQ-Ottawa found no studies that evaluated bone mineral content (BMC) and bone mineral density (BMD), or fractures in comparison with measures of vitamin D intake (Cranney *et al.,* 2007).

However, AHRQ-Ottawa found that there was fair evidence that circulating vitamin D levels are associated with a positive change in BMD and BMC in studies in older children and adolescents. Serum 25OHD concentrations of 40 to 50 nmol/L would ideally coincide with bone health benefits such as positive effects on BMC and BMD. A study conducted by Viljakainen et al. (2006) reported that vitamin D intakes of 200 IU/day (5 ug/day) and 400 IU/day (10 ug/day) in adolescent girls were associated with positive BMC measures at serum vitamin D levels of 50 nmol/L and above.

The IOM (2011) refers to several research evidences that indicate an intake of vitamin D of 10 µg/day achieves serum concentrations of 40 nmol/L, and this intake is therefore set as the EAR for persons 1 to 3 years, 4 to 8 years, 9 to 13 years, and 14 to 18 years of age. As this requirement distribution appears to be normally distributed, the assumption of another 30 percent to cover nearly all the population (i.e., 97.5 percent) is appropriate and consistent with a serum vitamin D level of approximately 50 nmol/L as the target for an RDA value. Based on the same analysis relating serum vitamin D levels to intake, and with the assumption of minimal sun exposure, an intake of 15 µg/day is set as the RNI. These reference values assume minimal sun exposure.

RNI for children

1 - 3 years	**15 µg/day**
4 - 6 years	**15 µg/day**
7 - 9 years	**15 µg /day**

RNI for adolescents

Boys 10 - 18 years	**15 µg/day**
Girls 10 - 18 years	**15 µg/day**

Adults

Bone maintenance is the focus for adult stage. The requirement distribution based on serum vitamin D concentrations and the intakes estimated to achieve such concentrations are the basis for the reference values.

Data relating bone health outcomes to vitamin D intake are generally limited for adults 19 to 50 years of ages. Although bone mass measures are, of course, studied in this population, consideration of the dose-response relationship between vitamin D and bone health are not usually included in such studies. In fact, there are no randomized trials in this age group, and whatever data available come from association studies. The results are inconsistent, in part because the confounding inherent in observational studies.

The IOM (2011) recommendation is considered based on the relationship between serum vitamin D levels and calcium absorption, in which serum vitamin D levels of between 30 and 50 nmol/L were consistent with maximal calcium absorption. Based on these considerations as well as the intake versus serum response analysis described above, an RNI of 15 µg/day are established for adults 19 to 65 years of age. These RNI values assume minimal sun exposure.

RNI for adults

Men	**19 - 50 years**	**15 µg/day**
Women	**19 - 50 years**	**15 µg/day**

RNI for adults

Men	**19 - 50 years**	**15 µg/day**
Women	**19 - 50 years**	**15 µg/day**

RNI for adults

Men	**51 - 65 years**	**15 µg/day**
Women	**51 - 65 years**	**15 µg/day**

Elderly

For elderly group, the reduction in fracture risk is the most important indicator of interest, not only because of the actual event, but also because of the high mortality and morbidity associated with fractures. Changes such as impaired renal function, less efficient synthesis of vitamin D in skin, lower endogenous production of active vitamin D, increased PTH as well as age-related changes in body composition affect the daily requirement of vitamin D. Moreover, a sizeable proportion of this population can be categorised as frail compared with other age groups, and the concerns for bone health are increased.

Findings from a large longitudinal study (n = 2,686) carried out by Trivedi, Doll, & Khaw, 2003 was used by IOM (2011) as reference for developing RNI for elderly. Furthermore, upon reviewing available data, IOM (2011) felt that evidence is strong that the elderly are at high risk for vitamin D deficiency, which causes secondary hyperparathyroidism and osteomalacia and exacerbates osteoporosis, resulting in increased risk of skeletal fractures. Based on the available literature and uncertainty due to the elderly physiological changes, a higher value of 20 µg/day was felt prudent for individuals over 70 years of age with limited sun exposure and stores. The TSC on Vitamins agreed to adjust the elderly age starting at 65 years instead of 70 to standardize with other nutrients presented in the updated RNI of Malaysia. It is suggested that the additional 5 ug/day for the lower age group is not expected to have any safety concern.

RNI for elderly

Men	**> 65 years**	**20 µg/day**
Women	**> 65 years**	**20 µg/day**

Pregnancy and Lactation

The EAR for non-pregnant women and adolescents is appropriate for pregnant women and adolescents based on the AHRQ-Ottawa's finding of insufficient evidence on the association of serum vitamin D level with maternal BMD during pregnancy. Furthermore, there is no evidence that the vitamin D requirements of pregnant women differ from those of non-pregnant women. Hence, the RNI values for pregnant women and non-pregnant women are applicable, providing an RDA of 15 µg/day for each group.

The EAR for non-lactating women and adolescents is appropriate for lactating women and adolescents based on evidence from RCTs (Basile *et al.,* 2006; Hollis & Wagner, 2004; Saadi *et al.,* 2007; Wagner *et al.,* 2006). Furthermore, there is no evidence that lactating women require higher serum vitamin D levels than non-lactating women. Hence, the RNI values for lactating women and non-lactating women are applicable, providing an RDA of 15 µg/day for each group.

RNI for

Pregnancy	**15 µg/day**
Lactation	**15 µg/day**

Discussions on revised RNI for Malaysia

There is a one- to two-fold increase in the RNI 2017 compared to RNI 2005. This is due to the reference document that is used in this review which is the IOM 2011. This more recent IOM report reflects comprehensive evidence-based reviews and latest literature searchers which was prepared by experts in vitamin D. Based on the detailed analysis, the IOM 2011 panel suggested higher recommendation as compared to its previous report (IOM 1997) which was referred by the RNI 2005 Technical Sub Committee on vitamin.

Numerous studies conducted by various investigators in different part of Malaysia (Appendix 14.1) have reported high prevalence of Vitamin D deficiency and insufficiency. These findings also lend support for increasing RNI for vitamin D in this country. It is noteworthy that this higher recommendation is based on the assumption of minimal sunlight exposure. Thus, this recommendation is applicable to people in Malaysia as even with abundance of sunlight, high sunlight avoidance practices is found to be very prominent. Furthermore, there are limited choices of food with high level of vitamin D available to Malaysia.

14.8 Tolerable upper intake levels

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 characterised by a considerable increase in plasma 25(OH)D concentration to a level of approximately 160 to 500 ng/ml. Because changes in circulating levels of 1,25(OH)2D 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 (Alshahrani & Aljohani, 2013).

To determine the Tolerable Upper Intake (UL), the IOM (2011) committee considered the emerging evidence of a U-shaped relationship for all-cause mortality, cardiovascular disease, vascular calcification, pancreatic cancer, falls, frailty and fractures, which indicates increased risk at low and high levels and lowest risk at moderate levels of vitamin D. The UL for ages 9 years and older is 100 ug/day with lower value for infants and young children. The UL was derived from the acute possible toxicity from vitamin D of 250 ug/day which is also supported as NOAEL (no observed adverse event concentration). It is adjusted for uncertainty based on chronic disease outcomes and all-cause mortality as well as emerging concerns about risks at serum vitamin D levels more than 50 ng/mL (125 nmol/L). It is noteworthy that acute toxicity is not the appropriate basis for a UL that is intended to reflect long-term chronic intake and to be used for public health (Ross *et al.,* 2011).

The tolerable upper intake for vitamin D for various age groups as proposed by IOM (2011) is given in Table 14.2.

Table 14.2. Tolerable Upper Intake (UL) levels of vitamin D for various age groups

Age groups	ug/day of vitamin D
Infants (0-6 months)	25
Infants (6-12 months)	37.5
Children, 1-13 years	100
Adolescence > 14-18 years	100
Adults > 18	100
Pregnant women	100
Lactating women	100

Source: IOM (2011)

14.9 Research recommendations

- Addition of vitamin D in the Malaysian Food Composition Database as this would encourage more local research on dietary intake of vitamin D.
- Comprehensive nationwide vitamin D status study.
- Rigorous large scale RCT to test the effects of vitamin D on nonskeletal outcomes.
- Investigating the possible variations of biological effect of vitamin D on time and duration of sun exposure, adiposity, ethnicity and genetic factor
- Research on cut-off with skeletal system and chronic disease outcomes.

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Appendix 14.1: Prevalence of Vitamin D status

Author, year	Study Population	Study Location	Participants, n	25 Hydroxyvitamin D (nmol/l)			Cut-off reference used
				% Sufficient [cut off]	% Insufficient [cut off]	% Deficient [cut off]	
Suriah *et al.,* (2004)	Healthy postmenopausal women (50 - 65 years)	Kuala Lumpur	Malay, 101 Chinese, 173	26.7 87.8 [50 – 100 nmol/L]	71.3 12.2 [25 – 50 nmol/L]	2.0 - [<25 nmol/L]	(Washburn, Smith, Jette, & Janney, 1993)
Green *et al.,* (2008)	Nonpregnant women (18 - 40 years)	Kuala Lumpur	Malay, 133 Chinese, 123 Indian, 122	- - -	74 38 68 [< 50.0 nmol/l]	- - 1.0 [< 17.5 nmol/l]	Not mentioned
Khor *et al.,* (2011)	Primary school children (7 - 12 years)	Kuala Lumpur	Total, 402 Boys, 180 Girls, 222	27.6 33.9 22.5 [> 50.0 nmol/l]	37.1 37.8 36.5 [37.5 – 50.0 nmol/l]	35.3 28.3 41.0 [≤ 37.5 nmol/l]	(Rovner & O'Brien, 2008)
Moy & Bulgiba, (2011)	Malay employees (35 years above)	Kuala Lumpur	Total, 380 Male, 158 Female, 222	32.1 - - [> 50.0 nmol/l]	67.9 41.1 86.9 [< 50.0 nmol/l]	- - -	(Zittermann & Gummert, 2010)
Nurbazlin *et al.,* (2013)	Urban and rural adult women	Kuala Lumpur. Negeri Sembilan	Total, 400 Urban, 107 Rural, 293	69.5 18.7 88.1 [≥ 50.0 nmol/l]	18.5 37.4 11.6 [30– 50.0 nmol/l]	12.0 43.9 0.3 [< 30 nmol/l]	IOM (2011)
Jan Mohamed, Rowan, Fong, and Loy (2014)	Malay pregnant women (19-40 years)	USM Hospital & Kubang Kerian Health Clinic, Kelantan.	2nd Trimester 3rd Trimester	5.9 22.5 [≥ 75 nmol/l]	34.3 40.2 [50 - 75 nmol/l]	59.8 37.3 [< 50nmol/l]	(Dawodu & Tsang, 2012)

Author, year	Study Population	Study Location	Participants, n	25 Hydroxyvitamin D (nmol/l)			Cut-off reference used
				% Sufficient [cut off]	% Insufficient [cut off]	% Deficient [cut off]	
Chin, Ima-Nirwana, Ibrahim, Mohamed, and Wan Ngah (2014)	Chinese and Malay adult men (20 years and above)	Klang Valley	Chinese, 233 Malay, 150 Total, 383	84.5 66.0 77.3 [≥ 50.0 nmol/l]	15.5 32.7 85.0 [30– 50.0 nmol/l]	0 1.33 1.92 [< 30 nmol/l]	IOM (2011)
Hamid Jan & Norliyana (2016)	Malay workers	Kelantan	Outdoor workers, 119 Indoor workers, 118	100 45.8 [≥ 50.0 nmol/l]	0 35.6 [30–50.0 nmol/l]	0 18.6 [< 30 nmol/l]	IOM (2011)
Al-Sadat *et al.* (2016)	Adolescents (13 years)	Klang Valley and Perak	Urban, 723 Rural, 638	1.4 14.3 [> 50.0 nmol/l]	9.8 18.2 [37.5 – 50.0 nmol/l]	86.3 67.2 [≤ 37.5 nmol/l]	(Misra, Pacaud, Petryk, Collett-Solberg, & Kappy, 2008)
Bee Koon *et al.* (2016)	Children (0.5-12 years)	Malaysia	Urban Rural	27.5 30.1 [50 – 100 nmol/L]	40.1 38.5 [25 – 50 nmol/L]	4.9 2.7 [<25 nmol/L]	Not mentioned

Appendix 14.2. Comparison of recommended intake for Vitamin D: RNI Malaysia (2017), RNI Malaysia (2005), and IOM (2011)

Malaysia (2017)		Malaysia (2005)		IOM (2011)	
Age group	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**
Infants		**Infants**		**Infants**	
0 - 6 months	10	0 - 5 months	5	0 - 6 months	10
7 - 12 months	10	6 - 12 months	5	7 - 12 months	10
Children		**Children**		**Children**	
1 - 3 years	15	1 - 3 years	5	1 - 3 years	15
4 - 6 years	15	4 - 6 years	5	4 - 8 years	15
7 - 9 years	15	7 - 9 years	5		
Boys		**Boys**		**Boys**	
10 - 18 years	15	10 - 18 years	5	9 - 13 years	15
		-		14 - 18 years	15
		-			
Girls		**Girls**		**Girls**	
10 - 18 years	15	10 - 18 years	5	9 - 13 years	15
	-			14 - 18 years	15
	-				-
Men		**Men**		**Men**	
19 - 50 years	15	19 - 65 years	5	19 - 30 years	15
51 - 65 years	15	51 - 65 years	10	31 - 50 years	15
> 65 years	20	> 65 years	15	51 - 70 years	15
				> 70 years	20

Malaysia (2017)		Malaysia (2005)		IOM (2011)	
Age group	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**
Women		**Women**		**Women**	
19 - 50 years	15	19 - 65 years	5	19 - 30 years	15
51 - 65 years	15	51 - 65 years	10	31 - 50 years	15
> 65 years	20	> 65 years	15	51 - 70 years	15
				> 70 years	20
Pregnancy		**Pregnancy**		**Pregnancy**	
	15		5	14 - 18 years	15
				19 - 50 years	15
Lactation		**Lactation**		**Lactation**	
	15		5	14 - 18 years	15
				19 - 50 years	15

Notes:1 mg = 40 IU

15 • Vitamin E

15.1 Introduction

Vitamin E was first discovered in 1922 but it was not until 40 years later that the vitamin was established as essential to human nutrition (Watson & Preedy, 2008). Vitamin E consists of two classes of molecules known as tocopherols and tocotrienols, which are characterised by a 6-chromanol ring and an isoprenoid side chain. Tocopherols and tocotrienols are differentiated by their phenyl "tails" as these are saturated in the tocopherols but unsaturated in the tocotrienols. The members of each family are designated alpha-(α), beta-(β), gamma-(γ), or delta-(δ) according to the number and position of methyl groups on the chromanol nuclei. Therefore, eight stereoisomers of the large vitamin E family differing in biological activities are possible but only the RRR-form occurs naturally (Combs, 2012).

15.2 Functions

The main function of α-tocopherol is as a lipid-soluble biological antioxidant that prevents propagation of free-radical reactions. The vitamin is a peroxyl radical scavenger and especially protects polyunsaturated fatty acids (PUFAs) within the membrane phospholipids and in plasma lipoproteins as well as reduces the oxidation of low-density lipoproteins (LDLs) (Combs, 2012). It appears that when peroxyl radicals are formed, they react 1000 times faster with α-tocopherol than with PUFAs, thus protecting the latter within membrane phospholipids (Buettner, 1993). By protecting PUFAs, α-tocopherol helps maintain the integrity of cell membranes, plays an important role in the stability of erythrocytes as well as prevents haemolytic anaemia (Muller, 1986).

Like tocopherols, tocotrienols also exhibit antioxidant activities and serve as lipid protectors (Packer *et.al,* 2001). In fact, several studies indicated that these unsaturated forms of vitamin E may offer superior antioxidant activity than α-tocopherol. For examples, tocotrienols were found to be more effective than tocopherols in suppressing lipid peroxidation and protecting against free-radical damage in bone (Maniam *et. al,* 2008; Ahmad *et. al,* 2005). In addition, tocotrienols possess other health benefits that are indeed beyond the antioxidant properties including their beneficial effects in cancer, cardiovascular health, immune modulation and neuroprotection (Meganathan & Fu, 2016). For example, tocotrienols exhibit anti-proliferative, pro-apoptosis and anti-angiogenic activities, all of which are important roles of this vitamin in inhibiting the survival of various tumor cells (Ahsan *et. al,* 2014). In another example, supplementation of tocotrienols-rich fraction among hypercholesterolemic patients ranging from 200 to 300 mg per day was shown to result in a significant reduction in total cholesterol and low density lipoprotein (Meganathan & Fu, 2016).

15.3 Metabolism

While the precise rate of vitamin E absorption is unknown, it is believed that its absorption efficiency is relatively low in humans (IOM, 2000). The ability of an individual to absorb vitamin E is highly dependent on the ability to simultaneously digest and absorb dietary fat. That is, individuals with impaired fat absorption typically exhibit low vitamin E utilization (Ball, 2004). In intestinal mucosal cells, vitamin E is incorporated into chylomicrons, which,

along the lymphatic pathway, are secreted into the systemic circulation. By the action of lipoprotein lipase (LPL), part of the vitamin E transported in chylomicrons is taken up by extra-hepatic tissues, while the remnant chylomicrons transport the remaining vitamin E to the liver (Herrera & Barbas, 2001). In the liver, vitamin E is further packaged into very low-density lipoprotein (VLDL) for its distribution to peripheral tissues.

Vitamin E contents in human tissues vary considerably and tend to be related exponentially to vitamin E intake. It was suggested that the highest vitamin E levels, mostly as (-tocopherol are found in adipose tissue and in the adrenals, and the lowest in the erythrocytes (Combs, 2012). Not all ingested vitamin E is stored in body tissues as the excess is secreted into the bile, which subsequently eliminated via the faeces. However, a small fraction of excess vitamin E may also be excreted through the urine and skin sebaceous glands (IOM, 2000; WHO/FAO, 2004).

15.4 Sources

All eight isoforms of vitamin E occur naturally in foods but in differing amounts, and are synthesised only by plants. The main dietary sources of tocopherols include vegetable oils, fat spreads from vegetable oils, seeds and nuts, and whole grains. However, the proportions of four tocopherols vary accordingly to the food source, the more abundant being α-tocopherol and γ-tocopherol (Dror & Allen, 2011; EFSA, 2015). On the other hand, tocotrienols can be found abundantly in palm oil while cereals such as oat bran also contain small amounts of tocotrienols (Combs, 2012). List of food sources that contain tocopherols and tocotrienols are shown in Table 15.1 & Table 15.2.

In addition, sources of vitamin E also include foods that are added with natural, synthetic and esterified forms of tocopherols including d-α-tocopherol, dl-α-tocopherol, d-α-tocopheryl acetate, dl-α-tocopheryl acetate and d-α-tocopheryl acid succinate (EFSA, 2015).

Table 15.1 Tocopherols content of foods

Food	mg/100g
Oils	
Soybean	94.6
Canola	45.8
Sunflower	41.1
Safflower	34.1
Grapeseed	28.8
Palm Olein*	21.8
Red Palm Olein*	17.1
Olive	15.3
Corn	14.3
Seeds and nuts	
Sunflower	27.5
Pecans	26.7
Almonds	26.6
Hazelnuts	15.4
Peanuts	12.2
Peanut butter	9.1
Cereals and cereal products	
Rice bran, crude	4.9
Wheat bran, crude	1.5
Oat bran, raw	1.2
Wheat flour	0.5

Source: USDA Food Composition Database (2016) & *Dauqan *et al.* 2011

Table 15.2 Tocotrienols content of foods

Food	mg/100g
Oils	
Palm Olein*	79.5
Red Palm Olein*	78.7
Soybean	0.1
Canola	0.04
Cereals and cereal products	
Oat bran, raw	2.3
Wheat flour	0.1

Source: USDA Food Composition Database (2016) & *Dauqan *et. al.* 2011

15.5 Deficiency

Vitamin E deficiency occurs only rarely in humans, even among people living on relatively poor diets (Litwack, 2011). Because there are relatively large human tissue reserves of vitamin E, very low intakes of this vitamin must be maintained for many months before there is any significant fall in circulating vitamin E (Bender, 2003). In contrast, deficiency of vitamin E is more evident in patients with fat malabsorption syndromes, genetic abnormalities in production of the α-tocopherol transfer protein (α-TTP), and protein-energy malnutrition (IOM, 2000; Litwack, 2011). For example, conditions involving the loss of pancreatic exocrine function (i.e. pancreatitis), deficiency of bile (i.e. biliary atresia) and defects in lipoprotein metabolism (i.e. abetalipoproteinemia) can lead to the simultaneous malabsorption of lipids and vitamin E (Combs, 2012). Individuals with disorders causing vitamin E deficiency may exhibit clinical symptoms such as peripheral neuropathy characterised by the degeneration of the large-caliber axons in the sensory neurons. Other symptoms observed include spinocerebellar ataxia, skeletal myopathy, and pigmented retinopathy, increased erythrocyte fragility, and increased ethane and pentane production. However, overt deficiency symptoms in individuals without any disease and who consume diets low in vitamin E have never been described (IOM, 2000).

The most widely used biomarker for estimating vitamin E status is plasma or serum α-tocopherol concentration, partly due to practicality of its use in large surveys and the field setting. To date, there is a lack of data to set a precise cut-off value above which vitamin E status may be considered as adequate. However, plasma or serum α-tocopherol concentrations < 12 µmol/L (0.5 mg/dL) in normal and healthy adults may be indicative of vitamin E deficiency, where the clinical symptoms have been reported (IOM, 2000; EFSA, 2015). Despite this cut-off, studies on vitamin status in diverse populations have used values ranging from 2.8 to 24 µmol/L (0.1 to 1.0 mg/dL) to define deficiency and insufficiency. This has led to huge discrepancies in the reported prevalence of deficiency among different countries (Dror & Allen, 2011). Additionally, vitamin E deficiency can also be assessed by measuring the degree of haemolysis induced by hydrogen peroxide in vitro and 24-hour urinary excretion of alpha-carboxyethyl hydroxychroman (α-CEHC), respectively (Pope *et al,* 2002; Bender, 2008).

Studies on vitamin E deficiency among Malaysian population are rather scarce. In one only example, Shahar *et al.* (1999) showed that approximately 27% of 350 rural elderly Malays residing on the east coast of Malaysia had plasma α-tocopherol ≤ 12 µmol/L, with men at higher risk for deficiency than women.

15.6 Factors affecting vitamin E requirements

Bioavailability of vitamin E is an important factor affecting requirements. As most dietary vitamin E is found in food that also contains fat, its bioavailability is influenced by the amount of fat present in the foods. Vitamin E absorption requires micelle formation and chylomicron secretion by the intestine, although the optimal amount of fat to enhance vitamin E absorption has not been reported.

The dietary intake of PUFAs also directly related to the need for vitamin E, where the latter requirements have been reported to increase when intakes of polyunsaturated fatty acids (PUFAs) are increased. As mentioned earlier, vitamin E functions primarily as an antioxidant in

biological systems by trapping peroxyl free radicals (Combs, 1992; IOM, 2000). In this regard, vitamin E is found in cellular membranes associated with PUFA in phospholipids. In vitamin E deficiency, the oxidation of PUFA is more readily propagated along the membrane, leading to cell damage and eventually symptoms, mainly neurological. It has been suggested that a ratio of at least 0.4 mg (1 µmol) α-tocopherol per gram of PUFA should be consumed by adults. However, the method of determining the vitamin E requirement generated by PUFA intakes is not universally accepted. There are also data to suggest that low-density lipoprotein (LDL) oxidation susceptibility in vitro is dependent upon its PUFA content. Although it is clear that the relationship between dietary PUFA and vitamin E needs is not simple, high PUFA intakes should certainly be accompanied by increased vitamin E intakes.

In addition, the body need for vitamin E is also affected by other dietary factors including selenium and vitamin C. Selenium spares the need for vitamin E and therefore, adequate intake of vitamin E becomes even more important in individuals taking low selenium diets (Combs, 2012). The role of vitamin C is to reduce the oxidised form of vitamin E as a result of the latter free-radical scavenging activity. This interaction has led to the concept of 'vitamin E recycling' where vitamin C helps restore the antioxidant capacity of vitamin E (Combs, 2012).

It is recognised that the requirements for vitamin E differ between sexes with men at higher need than women. The requirements also increase with increasing body weight until adulthood. Besides, increased exercise and physical activity that increase normal oxidative metabolism also increase the need for vitamin E.

Biological activity and units of expression

Biopotencies of tocopherols and tocotrienols are traditionally determined by different assays, namely foetal resorption (rat), haemolysis (rat), myopathy prevention (chick) and myopathy cure (rat). Based on these assays, the different stereoisomers of vitamin E have widely varying biological activities which can be expressed as milligram α-tocopherol equivalents (α-TE) of the most bio-potent natural vitamer, d-α-tocopherol (also called RRR-α-tocopherol). Besides d-α-tocopherol, other forms of vitamin E are also found in a mixed diet and their biopotencies, although weaker by comparison, should also be taken into consideration in the calculation of total vitamin E activity. Thus, total vitamin E activity (IOM 2000; WHO/FAO, 2004) can be calculated as follows:

α-TE = (mg d-α-tocopherol x 1.0) + (mg β-tocopherol x 0.5) + (mg γ-tocopherol x 0.1) + (mg δ-tocopherol x 0.03) + (mg α-tocotrienol x 0.3) + (mg β-tocotrienol x 0.05)

According to IOM (2000) and WHO/FAO (2004), the biological activities of γ- and δ-tocotrienol are not included in the calculation of α-TE as they were considered to be below the limit of detection. In addition, the biological activity of vitamin E is also expressed in international units (IU), in which 1 mg of d-α-tocopherol is equivalent to 1.49 IU.

15.7 Setting requirements and recommended intakes for vitamin E

Vitamin E is an existing vitamin in the Malaysian RNI (2005). In setting recommendations for vitamin E intake for Malaysian RNI 2005, the main references used by the Technical Sub-Committee (TSC) on Vitamins were the WHO/FAO (2004) consultation recommendations and the IOM (2000) publication. There were no local studies of vitamin E requirement or status that could be used as references. The rationale and approaches taken by these consultations were considered. The dietary pattern of the community was also taken into consideration.

In the WHO/FAO (2004) consultation report, it was discussed whether antioxidant property of vitamin E per se should be and can be considered in setting a requirement. It was decided that there was insufficient evidence to enable recommended nutrient intake to be based on the additional health benefits obtainable from nutrient intakes above those usually found in the diet. Even for vitamin E with its important biologic antioxidant properties, there was no consistent evidence for protection against chronic disease from dietary supplements.

In addition, the WHO/FAO (2004) consultation suggested that data available then were not sufficient to formulate recommendations for vitamin E intake for different age groups except for infancy. The Consultation had therefore used the term "accepted intakes" which represents the best estimates of requirements, based on the currently acceptable intakes that support the known function of this vitamin. The TSC was in general agreement with the approaches of the WHO/FAO report and decided to adopt the recommended levels for vitamin E for Malaysian RNI (2005).

In the present review of the vitamin E for RNI 2017, the TSC on Vitamins noted that there was no update of the WHO/FAO (2004) consultation report. The Institute of Medicine has also not published a revised recommendation after the IOM (2000) report. It was however noted that the European Food Safety Authority has recently published Dietary Reference values for vitamin E (EFSA, 2015). As there are no local studies that could be utilised, the three main references used by the Technical Sub-Committee (TSC) on deriving the recommended levels for vitamin E for Malaysian RNI 2017 were the reports from the WHO/FAO (2004), IOM (2000) and EFSA (2015).

In the EFSA (2015) report, the Panel noted that data on the relationship between vitamin E (unspecified form) or α-tocopherol intake and health consequences are inconsistent or limited and cannot be used to derive the requirement for α-tocopherol. The EFSA Panel also considered that there is, at present, insufficient data on biomarkers of vitamin E intake/ status/ function (i.e. plasma/ serum α-tocopherol concentration, hydrogen peroxide-induced haemolysis, urinary α-CEHC excretion and markers of oxidative damage) to derive the requirement for α-tocopherol. Besides, data on α-tocopherol kinetics and body pools are limited and therefore cannot be used to derive α-tocopherol requirement. While the Panel acknowledged that α-tocopherol prevents PUFA oxidation and that PUFA intake should be associated with an adequate α-tocopherol intake, little evidence to support this interaction to be used for deriving the requirement for α-tocopherol. The EFSA Panel, therefore deliberated that available data on biomarkers of vitamin E intake/ status/ function, on the relationship between PUFA intake and α-tocopherol intake as well as on α-tocopherol kinetics and body pools can be used neither on their own nor in combination to derive the requirement for α-tocopherol in adults.

As explained above, the WHO/FAO (2004) consultation had used the term "accepted intakes" to represent the best estimates of requirements for vitamin E. Similarly, the EFSA Panel also deliberated that Average Requirements (ARs) cannot be set for vitamin E (as α-tocopherol) and therefore chose to set Adequate Intakes (AIs) for all population groups, based on observed intakes in healthy populations in nine countries of the European Union (EU) with no apparent vitamin E deficiency. Such intakes were estimated using the EFSA Comprehensive European Food Consumption Database and the EFSA Food Composition Database.

In summary, while the TSC on Vitamins acknowledges the latest recommendation of nutrient intake (as Adequate Intake) from the report by EFSA (2015), the values adopted are only based on observed average intakes of α-tocopherol and α-tocopherol-equivalents in healthy populations in the European countries with no apparent vitamin E deficiency. However, as there are no data available to date especially on vitamin E intake among Malaysian populations, the TSC on Vitamins felt that there is inappropriate to adopt the Adequate Intake set by EFSA (2015). As such, the TSC on Vitamins decided to continue to adopt the WHO/FAO (2004) vitamin E intake recommendations for Malaysian RNI 2017. The TSC opined that these recommendations are still appropriate. The current recommendations, which are the same as the previous RNI 2005, are given in bold in the following paragraphs according to age groups and summarised in Appendix 15.1.

Infants

No functional criteria of vitamin E status have been demonstrated which reflect response to dietary intake in infants. Thus IOM (2000) had recommended intakes for vitamin E based on Adequate Intake, calculated based on vitamin E intake of infants fed principally with human milk.

A similar approach was taken by WHO/FAO (2004). The concentration of vitamin E in early human milk remains fairly constant at 3.2 mg TE/L of milk after 12 days. Therefore, an infant consuming 0.8 L human milk a day would have an intake of 2.7 mg TE of vitamin E. Recommended intake for infants is thus rounded off to 3 mg in the previous RNI for Malaysia 2005. In the EFSA (2015) report, mean α-tocopherol concentration in mature milk of unsupplemented mothers and of full-term infants ranged between 3.5 and 5.7 mg/L (mid-point of 4.6 mg/L). Considering an infant consuming 0.8L/day during the first six months of lactation in exclusively breastfeeding women, and a concentration of α-tocopherol is 4.6mg/L, the secretion of milk during lactation is estimated to be 3.7 mg/day.

As the estimated Vitamin E intakes during lactation are fairly similar based on both WHO/FAO (2004) and EFSA (2015), the TSC proposed that recommended intakes for infants 0 - 5 months and 6-11 months are retained as recommended in RNI (2005).

RNI for infants

0 - 5 months	**3 mg/day**
6 - 11 months	**3 mg/day**

Children and adolescents

As there were previously no data available to guide in the estimation of average requirements for children and adolescents, IOM (2000) decided to set the recommended intakes for vitamin E for these groups only based on extrapolation from adult values based on lean body mass and need for growth.

Recently, the α-tocopherol equivalent (α-TE) intakes of healthy populations in EU countries were made available by EFSA (2015) Panel. In children aged 1 to < 3 years, the average α-TE intakes ranged between 4.7 and 7.3 mg/day in boys and between 4.4 and 6.8 mg/day in girls. In children aged 3 to < 10 years, the average α-TE intakes ranged between 7.1 and 12.4 mg/day in boys and between 6.5 and 11.8 mg/day in girls. Therefore, the EFSA Panel set AIs for α-tocopherol for both sexes at 6 mg/day and 9 mg/day for children aged 1 to < 3 years and 3 to < 10 years, respectively. Meanwhile, in children aged 10 to < 18 years in EU countries, the EFSA Panel noted that the average α-TE intakes ranged between 9.6 and 15.9 mg/day in boys and between 8.8 and 13.8 mg/day in girls. As such, EFSA (2015) set the AIs for α-tocopherol at 13 mg/day for boys and 11 mg/day for girls aged 10 to < 18 years.

Unlike EFSA (2015) report, there are no data available to date especially on vitamin E intake among Malaysian children and adolescents to derive the requirement for vitamin E. As such, the TSC decided that vitamin E requirements for Malaysians follow closely that presently recommended by WHO/FAO (2004) and RNI (2005) for the corresponding age groups.

RNI for children

1 - 3 years	**5 mg/day**
4 - 6 years	**5 mg/day**
7 - 9 years	**7 mg /day**

RNI for adolescents

Boys 10 - 18 years	**10 mg/day**
Girls 10 - 18 years	**7.5 mg/day**

Adults and elderly

The values recommended by IOM (2000) were based largely on studies of induced vitamin E deficiency in humans and the correlation with hydrogen peroxide-induced erythrocyte lysis and plasma α-tocopherol concentrations. The hydrogen peroxide-induced hemolysis was then accepted as the best marker and used by IOM (2000) to estimate intakes for α-tocopherol requirements. IOM (2000) also decided that there was no scientific basis for assuming different requirements for men and women, and although body weights may be greater in men, women have larger fat masses as a percent of body weight, and thus may have similar requirements. The WHO/FAO consultation report however had provided for a lower recommended intake for women.

It is interesting to note that a previous Malaysian report by Ng (2003) had recommended 10 mg TE/day for adults, an estimate using the "Horwitt's equation" (Horwitt, 1974) which was based on dietary PUFA and some allowance for cellular synthesis and retention of PUFA in adipose tissues. In the report, the palm oil-based diets contained 66g total fat (26% energy) and 3.3% energy PUFA (12.7% of total fatty acids or 8.4g PUFA) provided mainly by linoleic acid. It was decided that RNI (2005) to adopt this value as the recommended intake for adult men. This is also the RNI for vitamin E in the WHO/FAO Consultation report (2004).

Meanwhile, the values recommended by EFSA (2015) were based on average α-tocopherol and α-tocopherol equivalent (α-TE) in EU countries. In the report, the average α-tocopherol intakes among adults (≥18 years) ranged between 8.2 and 16 mg/day in men and between 7.8 and 12.5 mg/day in women while average α-tocopherol equivalent (α-TE) intakes ranged between 10.1 and 16.0 mg/day in men and between 8.9 and 13.5 mg/day in women. Using mid-points of the range of mean intakes for α-tocopherol and for α-TEs, the EFSA Panel, therefore decided to set an AI for α-tocopherol at 13 mg/day for men and 11 mg/day for women.

However, as there is no current data available on vitamin E intakes among Malaysian population, the TSC felt that it is necessary to retain the value recommended in RNI (2005) as the recommended intake for adult men and women.

RNI for adults

Men	**19 - 65 years**	**10 mg/day**
Women	**19 - 65 years**	**7.5 mg/day**

IOM (2000) was of the opinion that there is no evidence that the aging process impairs vitamin E absorption or utilization. On the other hand, the limited clinical trial evidence does not justify providing for higher recommendations for higher vitamin E intakes at this time. Similarly, the WHO/FAO (2004) had also provided for the same RNI for elderly subjects. In the EFSA (2015) report, there was no classification for elderly suggesting that the recommended intake is similar to those of adult groups. Therefore, TSC felt that the same intake should be recommended for the elderly adults as recommended in RNI (2005).

RNI for elderly

Men	**> 65 years**	**10 mg/day**
Women	**> 65 years**	**7.5 mg/day**

Pregnancy and lactation

IOM (2000) felt that there is no evidence at the present time that the requirement for women during pregnancy should be increased above the level recommended for women in the non-pregnant state. For lactating women, an additional amount equal to the total quantity of α-tocopherol secreted in human milk (4 mg) was added to the recommended intake for non-pregnant women.

WHO/FAO (2004) consultation report, however, did not provide for increased requirements for vitamin E in pregnancy and lactation as it was felt that there is no evidence of vitamin E requirements different from those of other adults and presumably also as the increased energy intake would compensate for the increased needs for infant growth and milk synthesis. The EFSA (2015) Panel also considered that there is no evidence for an additional dietary α-tocopherol requirement during pregnancy or during lactation. As such, the AI for pregnant or lactating women is set the same as for non-pregnant non-lactating women. The TSC on vitamins decided to follow the same approach and retain the corresponding values as cited by RNI (2005).

RNI for

Pregnancy	**7.5 mg/day**
Lactation	**7.5 mg/day**

Discussions on revised RNI for Malaysia

The RNI for Malaysia (2005) basically follows the same corresponding values recommended by WHO/FAO (2004). In this revised RNI for Malaysia (2017), the proposed recommended intakes also retain similar corresponding values to those of RNI (2005). Appendix 15.1 provides a summary of the revised RNI (2017), compared with the RNI (2005), EFSA (2015), the WHO/FAO (2004) recommendations and the values recommended by IOM (2000).

Recommended intakes by IOM (2000) are on the average, significantly higher than the corresponding values cited by WHO/FAO (2004). This was particularly evident in the adults where WHO/FAO (2004) recommended a RNI of 10 mg TE/day for adult males, compared with 15 mg TE by IOM (2000). As mentioned earlier, the main factor used to assess the adequacy of vitamin E intakes in the United States and United Kingdom advisory bodies was the dietary intake of PUFAs (WHO/FAO, 2004). PUFA intakes in the United States are estimated at slightly above 6% energy which is much higher than the 3.3% energy reported for palm oil-based diets (Ng, 1995). As such, it is not surprising that the IOM (2000) has recommended a RNI of 15 mg TE/day for adults.

On the other hand, recommended adequate intakes by EFSA (2015) are slightly higher than those cited by WHO/FAO (2004), but relatively lower than those recommended intakes by IOM (2000). The recommended intakes by EFSA (2015) were derived based on observed average intakes of α-tocopherol and α-tocopherol-equivalents in healthy populations in the EU countries with no apparent vitamin E deficiency. In addition, recommended intakes cited by other European authorities such as Deutschland-Austria-Confoederatio Helvetica (2015), and The French Food Safety Agency (2001) are either equivalent or slightly higher than those of WHO/FAO (2004) for all age groups.

In the case of Malaysia, there are no data available to date on vitamin E intake among Malaysian populations that can be used to justify the need to follow EFSA (2015) recommended adequate intake. As such, TSC on vitamins decided to retain RNI (2017) similar to those of RNI (2005).

15.8 Tolerable upper intake levels

Unlike most other vitamins, vitamin E can be viewed as one of the least toxic as it exhibits no deposition or saturation thresholds (Combs, 2012). In fact, humans appear to be able to tolerate rather high levels. There is no evidence of adverse effects from the consumption of vitamin E naturally occurring in foods. Therefore, hazard identification by IOM (2000) was focused on evidence concerning intake of α-tocopherol as a supplement, food fortificant, or pharmacological agent. RRR-α-tocopheryl acetate and all rac-α-tocopheryl acetate are the forms of synthetic vitamin E used almost exclusively in supplements, food fortification, and pharmacologic agents. Upon reviewing available data, WHO/FAO (2004) consultation reported that vitamin E appears to have very low toxicity, and amounts of 100-200 mg of the synthetic all-rac-α-tocopherol are consumed widely as supplements. Evidence of pro-oxidant damage has been associated with the feeding of supplements but usually only at very high doses (e.g., >1000 mg/day).

On the other hand, EFSA (2015) considered the impact on blood clotting as the critical adverse effect in the setting of a Tolerable Upper Intake Level (UL), and identified a No Observed Adverse Effect Level (NOAEL) of 540 mg α-TE/day based on a study by Meydani et.al, 1998. In this study, healthy subjects aged ≥ 65 years were reported to develop no adverse effects, including bleeding time after receiving either a placebo, 40, 134 or 537 mg α-TE/day (all-rac-α-tocopherol) for four months. The EFSA set a UL for adults of 270 mg α-TE/day (rounded to 300 α-TE/day) using an uncertainty factor of 2. This UL also applies to pregnant and lactating women as there was no indication from animal studies of a specific risk for these population groups. The ULs for children were derived from the adult UL by allometric scaling on the basis of body weight to the power of 0.75, and ranged from 100 mg α-TE/day (1-3 years) to 260 mg α-TE/day (15-17 years).

Based on considerations of causality, relevance, and the quality and completeness of the database, hemorrhagic effects were selected by IOM (2000) as the critical endpoint on which to base the Tolerable Upper Intake Level (UL) for vitamin E for adults. There is some evidence of an increased incidence of hemorrhagic effects in premature infants receiving supplemental α-tocopherol. The Alpha-Tocopherol, Beta Carotene (ATBC) Cancer Prevention Study in Finnish smokers consuming 50 mg of all rac-α-tocopherol for 6 years, reported a significant 50% increase in mortality from hemorrhagic stroke (ATBC 1994). However, the human data fail to demonstrate consistently a causal association between excess α-tocopherol intake in normal, apparently healthy individuals and any adverse health outcome. In this revised RNI for Malaysia (2017), the TSC decided to follow similar corresponding UL levels for the vitamin for various age groups to those of IOM (2000) as tabulated in Table 15.3.

Table 15.3 Tolerable Upper Intake (UL) levels of vitamin E for various age groups

Age groups	mg/day of any form of supplementary α-tocopherol
Infants	Not possible to establish; source of intake should be formula and food only
Children	
1 - 3 years	200
4 - 8 years	300
9 - 13 years	600
Adolescents	
14 - 18 years	800
Adults	
≥ 19 years	1,000
Pregnancy	
14 - 18 years	800
≥ 19 years	1,000
Lactating women	
14 - 18 years	800
≥ 19 years	1,000

Source: IOM (2000)

15.9 Research Recommendations

The following priority areas of research are recommended:

- Determination of vitamin E content including tocopherols and tocotrienols in local foods, focused primarily on vitamin E rich foods, i.e. fats and oils, and nuts.
- Undertaking studies on the suitability of various biomarkers of status as indicators of the requirement.
- Studies on health benefits of tocotrienols.
- Assessing vitamin E intakes by different age groups of the local population.

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Appendix 15.1 Comparison of recommended intake for vitamin E: RNI Malaysia (2017), Acceptable Intakes of WHO/FAO (2004), AI and RDA of IOM (2000) and AI of EFSA (2015)

RNI Malaysia (2017)*		WHO/FAO (2004)		IOM (2000)		EFSA (2015)	
Age group	**RNI (mg/day)**	**Age group**	**Acceptable intakes (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**
Infants		**Infants**		**Infants**		**Infants**	
0 - 5 months	3	0 - 6 months	2.7	0 - 6 months	4		
6 - 11 months	3	7 - 12 months	2.7	7 - 12 months	5	7 - 11 months	5
					RDA (mg/d)		
Children		**Children**		**Children**		**Children**	
1 - 3 years	5	1 - 3 years	5	1 - 3 years	6	1 - <3 years	6
4 - 6 years	5	4 - 6 years	5	4 - 8 years	7	3 - <10 years	9
7 - 9 years	7	7 - 9 years	7				
Boys		**Boys**		**Boys**		**Boys**	
10 - 18 years	10	10 - 18 years	10	9 - 13 years	11	10 - <18 years	13
				14 - 18 years	15		
Girls		**Girls**		**Girls**		**Girls**	
10 - 18 years	7.5	10 - 18 years	7.5	9 - 13 years	11	10 - <18 years	11
				14 - 18 years	15		
Men		**Men**		**Men**		**Men**	
19 - 65 years	10	19 - 65 years	10	19 - 30 years	15	>18 years	13
> 65 years	10	> 65 years	10	31 - 50 years	15		
				51 - 70 years	15		
				> 70 years	15		

RNI Malaysia (2017)*		WHO/FAO (2004)		IOM (2000)		EFSA (2015)	
Age group	**RNI (mg/day)**	**Age group**	**Acceptable intakes (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**
Women		**Women**		**Women**		**Women**	
19 - 65 years	7.5	19 - 65 years	7.5	19 - 30 years	15	>18 years	11
> 65 years	7.5	> 65 years	7.5	31 - 50 years	15		
				51 - 70 years	15		
				> 70 years	15		
Pregnancy	7.5	**Pregnancy**	7.5	**Pregnancy**		**Pregnancy**	11
				< 18 years	15		
				19 - 30 years	15		
				31 - 50 years	15		
Lactation	7.5	**Lactation**	7.5	**Lactation**		**Lactation**	11
				< 18 years	19		
				19 – 30 years	19		
				31 – 50 years	19		

*Recommendations same as RNI (2005)

16 • Vitamin K

16.1 Introduction

Vitamin K represents a group of fat-soluble vitamins with a common chemical structure of 2-methyl-1, 4-naphtoquinone but differ in the side-chain structures. These natural compounds include vitamin K^1 or phylloquinone, which has a phytyl side-chain. Phylloquinone is synthesised by green plants and is the predominant form in the diet. Vitamin K^2 represents a series of vitamin K forms known as menaquinones. These have side chains based on repeating unsaturated 5-carbon (prenyl) units, and are designated as menaquinone-n (MK-n) according to the number (n) of prenyl units. Most of the menaquinones are synthesised by bacteria in the large intestine, and can also be found in animal products and fermented foods. Vitamin K^2 can be converted from vitamin K^1 by human intestinal microbiota and animals (Shearer, 1992).

A synthetic compound known as menadione (vitamin K^3) is a provitamin with no carbon side-chains but can be alkylated by the liver to produce menaquinone-4 (MK-4) from phylloquinone. This conversion process does not involve bacterial action. The biological potency of menadione is found to be twice of the naturally occurring forms vitamin K^1 and K^2.

16.2 Functions

Vitamin K is essential for a unique post-translational chemical modification in a small group of proteins with calcium-binding properties, collectively known as vitamin K-dependent proteins (Gla-proteins). These proteins are involved in blood coagulation and bone metabolism.

Vitamin K (majorly K^1) acts as a co-factor for the γ-glutamylcarboxylase (GGCX), an enzyme which catalyses the carboxylation of amino acid glutamic acid (Glu) residues in proteins to γ-carboxyglutamyl (Gla) residues, which makes it possible for them to bind calcium and form blood clots. Thus far, the only unequivocal role of vitamin K in health is in the maintenance of normal blood coagulation (for which it was originally designated, koagulation, the Danish word for blood clotting). Factors II (prothrombin), VII, IX, and X (pro-coagulants), and proteins C, S and Z (anti-coagulants) are vitamin-K dependent proteins produced by the liver that is directly involved in coagulation cascade (a series of events that stop bleeding through blood clot formation) (IOM, 2001; WHO/FAO, 2004).

Vitamin K^2 (menaquinone) is required for the γ-carboxylation of bone proteins, most notably osteocalcin. Research has indicated that high serum levels of undercarboxylated osteocalcin are associated with lower bone mineral density (Gundberg, Lian, and Booth, 2012). An adequate intake of vitamin K also assists in reducing bone turnover and protecting against fractures. Epidemiological studies indicate that low vitamin K intake or status is associated with osteoporosis, osteopenia, and increased risk of fracture (EFSA, 2009). Although vitamin K is involved in the metabolism of osteocalcin, the role of vitamin K supplementation in reducing risk for osteoporosis is still being investigated. A systematic review and meta-analysis of randomised clinical controlled trials examined the effect of vitamin K supplementation (phytonadione and menaquinone-4) on bone mineral density and bone fractures (Cockayne *et al,* 2006). It was shown that 12 out of 13 trials included demonstrated that supplementation with either phytonadione (10mg/day) or MK-4 (15mg/day or 45 mg/day) improved bone mineral density. In addition, the incidence of hip, vertebral, and all non-vertebral fractures was significantly reduced with supplementation of MK-4. These findings, however, should be treated

with caution because the studies included were not designed to show a fracture effect. In addition, all the studies with fracture outcomes were undertaken in Japan, and there may be dietary differences which could indicate that these findings are not applicable elsewhere. Indeed, the Japanese people consume a lot of a traditional food called natto. It is a very rich dietary source of vitamin K, almost all of which occurs in the form of MK-7. Supplementation with MK-7 (in the form of natto extract) has been shown to induce more complete carboxylation of osteocalcin compared with phylloquinone (Schurgers *et al.,* 2007).

A more recent meta-analysis involving a total of 21 studies (11 of which exclusively involved Japanese patients) observed a significant decrease in the loss of vertebral and forearm bone mineral density, and reduced incidence of fractures, with supplementation of 45 mg/day of MK-4 (*Huang et al.,* 2015). However, the benefits were only seen in postmenopausal women with osteoporosis, with no significant effect seen in postmenopausal women without osteoporosis. In another development, the European Food Safety Authority (EFSA) has approved a health claim for vitamin K, acknowledging that it contributes to maintenance of normal bone, as reflected by scientific evidence (EFSA, 2009).

Apart from its historical role in blood clotting, and more recent participation in bone building, several other vitamin K-dependent Gla-proteins have also been isolated from kidney, lungs, and other tissues. Matrix Gla-protein (MGP) is a vitamin K-dependent protein that may play a role in the prevention of arterial calcification and stiffening, one of the risk factors for coronary heart disease. An adequate intake of vitamin K^2 has been shown to lower the risk of vascular damage, because it activates MGP, which inhibits the deposit of calcium on the walls (Buchanan *et al.,* 2016). A randomised-controlled trial found that menaquinone supplementation (MK-7, 180 µg/day) significantly decreased arterial stiffness after three years, but the effect was more visible among patients with poorer vascular status at baseline (Knapen *et al.,* 2015). In vitro studies have demonstrated that menaquinone has anti-cancer properties as it is able to induce cell cycle arrest and apoptosis in leukemia, liver, gastric, colorectal, lung, and prostate cancer cells. Intake of menaquinone (MK-4, 45-90 µg/day) has been demonstrated in a few studies, including two meta-analyses, to reduce overall cancer incidence and mortality (Riaz *et al.,* 2012; Zhong *et al.,* 2013).

16.3 Metabolism

Phylloquinones (vitamin K^1) are absorbed by an energy-dependent process in the small intestine. On the other hand, menaquinones (K^2) and menadiones (K^3) are absorbed via passive diffusion in small and large intestines. As a fat-soluble vitamin, the absorption requires a minimal amount of dietary fat and a normal supply of bile salts and pancreatic juices. In healthy adults, the efficiency of phylloquinone absorption in its free form is about 80% (Shearer et al, 2012). The absorbed vitamins are incorporated into chylomicrons in the lymph and taken to the liver. Here they are incorporated into VLDLs and subsequently delivered to the peripheral tissues by LDLs. Absorption of vitamin K is reduced in conditions involving obstructions of the bile duct such as in obstructive jaundice and liver failure. Vitamin K is found in low concentrations in the cellular membranes of many tissues. Because of the metabolism of the vitamin, mixtures of vitamers K can be found in tissues even when a single form is consumed. Most tissues would contain phylloquinones and menaquinones. Human liver stores normally comprise about 90 percent menaquinones and 10 percent phylloquinone (Shearer *et al.,* 2012).

Vitamin K is rapidly and extensively metabolised in the liver. Phylloquinones can be converted into menaquinones before absorption and are excreted via the bile that passes out of the body in the faeces. Menadiones are primarily excreted in the urine. This rapid metabolism accounts for vitamin K's relatively low levels in blood and tissue stores compared to other fat-soluble vitamins.

16.4 Sources

Phylloquinone (vitamin K^1) is present in large amounts (at levels reaching 100 µg/100 g) in fresh, green, leafy vegetables. Excellent sources include spinach, mustard, chives, spring onions, cabbage, lettuce, broccoli, watercress, and sweet potato leaves (Table 16.1). Often the absorption is improved when accompanied by fats such as oils or butter. Phylloquinone obtained from green, leafy vegetables is tightly bound to the membranes of plant chloroplasts, and thus is less bioavailable compared with phylloquinone obtained from plant oils and/or dietary supplements.

Table 16.1: Vitamin K^1 (phylloquinone) content of foods

Food	µg per 100g
Vegetables	
Mustard leaves (cooked)	593
Spinach (cooked)	494
Salad greens (raw)	315
Coriander leaves (raw)	310
Sweet potato leaves (cooked)	302
Watercress (raw)	250
Chives (raw)	213
Spring onions (raw)	207
Broccoli (cooked)	141
Lettuce, green leaf (raw)	126
Cabbage (cooked)	109
Okra (cooked)	40
Celery (cooked)	38
Chinese cabbage, pak-choi (cooked)	34
Fats and Oils*	
Soybean oil	193
Canola oil	127
Olive oil	55
Margarine	42

Food	µg per 100g
Prepared Foods*	
Salad dressings	100
Coleslaw	80
Mayonnaise	41

Source: *Phylloquinone concentration of common foods, IOM (2001)
USDA National Nutrient Database for Standard Reference, Release 28 (2015)

For menaquinones (vitamin K^2) subtypes: MK-4 is produced in animal tissue and can be found in dairy products, egg yolk, chicken, pork, and beef. MK-7 is found in fermented products e.g. cheeses, (Table 16.2) and very high level are found in natto, a Japanese traditional food consisting of fermented soy beans (Kamao *et al.,* 2007). The menaquinone in dairy products and natto is synthesised by fermenting bacteria such as *Bacillus subtilis;* in meats, K^1 and K^3 are converted to K^2 by living tissue. Many species of the bacteria (including *Escherichia coli*) which are present in the large intestine of humans, synthesize a significant portion of this vitamin and thus furnish the needs of the body. Menaquinones (MK-4 and MK-7) are available for supplementation, however, the half-life and bioavailability of MK-7 has been found to be superior to that of MK-4 (Buchanan *et al.,* 2016). Menaquinones, which are primarily derived from animal-based sources, are consumed in food matrices containing more fat that may improve absorption and lead to greater bioavailability than phylloquinone (Beulens *et al.,* 2013). The lack of evidence of a significant vitamin K deficiency in the general population indicates that the required amount of the vitamin can normally be adequately obtained by foods or produced by intestinal microflora. Vitamin K is relatively stable to heat processing, but it can be destroyed by exposure to light.

Table 16.2: Vitamin K[2] (menaquinone) content of foods

Food	µg per 100g
Fermented Products	
Natto*	939
Meat and Poultry	
Beef, pepperoni	41.7
Chicken drumstick, with skin (cooked)	33.2
Chicken, frankfurter	25.0
Chicken breast, with skin (cooked)	15.5
Turkey, breast	9.1
Dairy Products	
Cheese, cream	8.7
Cheese, cheddar	8.6
Cheese, parmesan	7.1
Cheese, mozzarella	4.1

Reference: *Kamao *et al.,* (2007)
USDA National Nutrient Database for Standard Reference, Release 28 (2015)

16.5 Deficiency

Various indicators have been used to assess vitamin K status in humans including prothrombin time, plasma factor VII, plasma and serum phylloquinone concentration, urinary γ-carboxylglutamyl residues, undercarboxylated prothrombin, and undercarboxylated osteocalcin. Of these, the only clinically significant indicator is prothrombin time (the time it takes for blood to clot). Vitamin K deficiency leads to prolonged blood coagulation time and increased incidence of bleeding and haemorrhage. The deficiency is marked by a low level of plasma prothrombin (hypoprothrombinemia) and can be corrected by administration of 2-5 mg/day vitamin K orally.

A primary deficiency is not commonly found in healthy children and adults because of the good supply of vitamin K from plants. In addition, intestinal flora can synthesise a significant portion of the vitamin required daily. The most common causes of vitamin K deficiency are therefore secondary in nature and can occur in two circumstances. First, this can be due to impaired fat absorption for example in inflammatory bowel disease, coeliac disease, ulcerative colitis, and severe liver disease. Second, the synthesis and action of vitamin K can be disrupted by some drugs. For example, prolonged broad-spectrum antibiotics treatment would kill vitamin K-producing bacteria in the intestine, and anticoagulant drugs interfere with vitamin K metabolism and activity.

Unlike healthy adults, newly-born breastfed infants sometimes suffer prolonged prothrombin time due to vitamin K deficiency. The syndrome, known as vitamin K deficiency bleeding (VKDB), represents a significant global health problem (FAO/WHO, 2004). It usually happens in the first week of life due to very little vitamin K is transferred from maternal circulation for storage in foetal tissues. In addition, newborns have a relatively sterile gastrointestinal tract (free from gut bacteria which can synthesise vitamin K). They are also generally fed foods that are relatively free of bacterial contamination. In addition, human milk is not a good source of vitamin K. Preterm babies are more susceptible to vitamin K deficiency than their term counterparts. Based on evidences (Puckett and Offringa, 2007), the WHO recommends that all newborn infants should be given 1mg of vitamin K prophylaxis intramuscularly after the first hour of birth (WHO, 2013).

16.6 Factors affecting vitamin K requirements

Factors to be considered when estimating the requirements for vitamin K include the bioavailability of phylloquinone from various foods, nutrient-nutrient interactions, and drug-nutrient interactions (IOM, 2001).

Very little is known with regards to bioavailability of vitamin K from different foods. The efficiency of absorption of phylloquinone in the form of cooked spinach was reported to be 4%, compared with an estimated 80% when phylloquinone is given in its free form. On the other hand, three times as much phylloquinone was absorbed when the spinach was consumed with butter (Garber *et al.,* 1999). Even though the greatest food source of phylloquinone is from leafy vegetables, its bioavailaibility is poor. This may be due to the poor extraction of phylloquinone which is located in the chloroplast. It is tightly bound to thylakoid membrane where it plays a role in photosynthesis.

High doses of vitamin E and retinoids have been shown to antagonise vitamin K action in animal studies. However, the metabolic basis for this antagonism has not been completely elucidated and adverse responses in human have not been reported (IOM, 2001).

Vitamin K is converted to an inactive form once it has activated the clotting factors (prothrombin). It must then be reactivated for its biological action to persist. Drugs such as warfarin, which strongly inhibits the reactivation process, acts as a powerful anticoagulant and is widely prescribed for the prevention of thrombotic disorders. Patients taking these anticoagulant drugs do not need to eliminate vitamin K from their diets. They should, however, maintain a consistent dietary vitamin K intake and avoid vitamin K supplementation.

16.7 Setting requirements and recommended intakes of vitamin K

Vitamin K is a newly added vitamin in Malaysian RNI (2017). There are no local data on vitamin K requirements that could be used by the Technical Sub Committee (TSC) on Vitamins to arrive at the RNI for vitamin K. Thus, the TSC referred to the WHO/FAO (2004) consultation report as well as the IOM (2001) DRI recommendations. The rationales and steps taken in setting the requirements and the levels recommended by these organisations were considered. IOM (2001) set the Adequate Intake that is based on the median intake for different age groups as reported by the Third National Health and Nutrition Examination Survey (NHANES III). However, adopting a similar approach for setting Malaysian RNI (2017) is not possible as there is no information on vitamin K intake among Malaysian populations. Meanwhile, WHO/FAO (2004) has set the recommended values for vitamin K that is based on a daily intake of 1 µg/kg body weight/day of phylloquinone for its classical function in coagulation. Upon reviewing both reports, the TSC on Vitamins agreed to adopt the WHO/FAO (2004) values as the RNI for Malaysia 2017, given in bold according to age groups in the following paragraphs. The proposed RNI is summarised in Appendix 16.1.

The TSC on Vitamins did not provide a specific recommendation for vitamin K[2] as no such recommendation has been made by WHO/FAO (2004) or IOM (2001).

Infants

Setting the requirement of vitamin K for infants up to age 6 months is complicated by the need to prevent a rare but potentially devastating bleeding disorder (also known as VKDB) due to vitamin K deficiency. In IOM (2001) report, it was proposed that the recommended intakes for vitamin K are based on Adequate Intake (AI), calculated based on mean vitamin K intake of infants principally fed with human milk while assuming that infants also receive prophylactic vitamin K at birth. Considering the average phylloquinone concentration of 2.5 µg/L in human milk and the average intake of milk of 0.78 L/day, the AI for infants 0-6 months was set at 2.0 µg/day after rounding. Meanwhile, the AI for infants 7-12 months was extrapolated at 2.5 µg/day after rounding.

However, the fact that VKDB is epidemiologically associated with breastfeeding, setting of requirements solely based on normal intakes of human milk is not considered prudent. As such, the WHO/FAO expert consultation (WHO/FAO, 2004) has set a higher recommended intake for infants aged 0 to 6 months (the period when VKDB risk is considered highest) at 5 µg/day, and at 10 µg/day for older infants. This is based on extrapolating the adult RDA of 1 µg/kg body weight/day. Nevertheless, exclusively breastfed infants may only meet 40% of this requirement if it is assumed that the vitamin K consumption through human milk is 2 µg/day. To prevent bleeding due to vitamin K deficiency, WHO/FAO recommended that all breast-fed infants should receive vitamin K supplementation as endorsed by WHO, whether in the form of a single intramuscular injection at birth or multiple oral doses given over the first few weeks of life. The provision of vitamin K prophylaxis is a standard practice in government hospitals in Malaysia (Ministry of Health, 2002).

RNI for infants

0 - 5 months	**5 µg/day**
6 - 11 months	**10 µg/day**

Children and adolescents

There were no data available on which to base an Estimated Average Requirement (EAR) for vitamin K for children and adolescents. Therefore, IOM (2001) set the AIs based on the highest median intake for each age group reported by the NHANES III. The AI for children (1-8 years old) was estimated to range from 30-55 µg/day; AI for older children (9-13 years old) was 60 µg/day; and AI for adolescents (14-18 years old) was 75 µg/day. The significant increase in the AI from infancy to early childhood is most likely contributed by increased intakes of fruits and vegetables containing high vitamin K, as the diet becomes more diversified.

On the other hand, WHO/FAO expert consultation (WHO/FAO, 2004) recommended the RNI for these age groups by adopting the RDA (1989) for vitamin K (in the United States), and AI level (in the UK), both of which have been set at a value of 1 µg/kg body weight/day (DoH, 1991).

RNI for children

1 - 3 years	**15 µg/day**
4 - 6 years	**20 µg/day**
7 - 9 years	**25 µg/day**

RNI for adolescents

Boys 10 - 18 years	**35 - 55 µg/day**
Girls 10 - 18 years	**35 - 55 µg/day**

Adults

IOM (2001) set the AI values for adults and elderly at 120 µg/day for men and at 90 µg/day for women. Taking into consideration possible underestimation of actual daily intake of foods, the AIs for each gender were derived from the highest median intake value reported from NHANES III.

WHO/FAO expert consultation (WHO/FAO 2004) recommended the RNI for adults and elderly groups based on the daily intake of approximately 1 µg/kg body weight/day of phylloquinone. The requirements for vitamin K in the past have only considered its classical function in blood coagulation. With more recent evidence demonstrating greater requirements for the optimal carboxylation of vitamin K-dependent proteins in other tissues, the question remains whether the RNI should be raised. Studies have shown that the γ-carboxylation of osteocalcin can be improved by intakes of vitamin K between 100-420 µg/day (Sokoll *et al.,* 1997), which may be even higher than the current intakes of most people in the US or UK.

However, due to lack of clearly-defined metabolic role and biochemical proof of the necessity for fully γ-carboxylated osteocalcin for bone health at the time, the WHO/FAO expert consultation felt it was unwise to increase the recommendation then.

RNI for adults

Men	**19 - 65 years**	**65 µg/day**
Women	**19 - 65 years**	**55 µg/day**

RNI for elderly

Men	**> 65 years**	**65 µg/day**
Women	**> 65 years**	**55 µg/day**

Pregnancy and Lactation

Data pertaining to vitamin K status of pregnant women are limited. However, lack of clinical deficiency signs and comparable circulating vitamin K concentrations suggest that vitamin K status of pregnant women is not different from that of non-pregnant population. In addition, there were no data on the vitamin K content of fetal tissue for estimating additional needs during pregnancy (IOM, 2001). Assessment of phylloquinone dietary intakes among a nationally representative sample of US consumers using 14-day food diaries, data available for a small group (n=17) of pregnant women were found to be similar to those of non-pregnant women (Booth, Webb and Peters, 1999). Therefore, the AI was set based on median NHANES III intake estimates of non-pregnant women.

Furthermore, available studies suggest that the vitamin K status of lactating women is comparable to that of non-lactating women. Reported vitamin K intakes among lactating women (n=23) at 6, 12, and 26 weeks, were not significantly different than non-lactating women. In addition, no significant correlation was found between phylloquinone intake and breast milk concentration (Greer *et al.,* 1991). Therefore, the AI was estimated to be similar to non-pregnant women (IOM, 2001).

WHO/FAO expert consultation (WHO/FAO, 2004) also felt that there is no basis as yet for making different recommendations for pregnant and lactating women. As such, the estimated RNI for these groups shall be similar to those of non-pregnant and non-lactating women, which was derived based on a daily intake of 1 µg/kg body weight/day of phylloquinone.

RNI for

Pregnancy	**55 µg/day**
Lactation	**55 µg/day**

Discussions on revised RNI for Malaysia

There were no recommendations for Vitamin K in the previous version of the Malaysian RNI (2005). The proposed recommendations for the revised recommended intakes for Malaysian RNI 2017 are based on the WHO/FAO (2004) values. Other than for infants, the recommended intakes by WHO/FAO (2004) are generally lower for all age groups as compared to the values adopted by IOM (2001). This is due to the different approaches utilized by these organisations in deriving their recommended intakes. WHO/FAO (2004) set their recommended values for vitamin K based on a daily intake of 1 µg/kg body weight/day of phylloquinone for its classical function in blood coagulation. IOM (2001), on the other hand, set their AI based on the highest median intake values for different age groups as reported by the NHANES III. This contributes to the rather marked differences between the recommended values by WHO/FAO (2004) and IOM (2001).

16.8 Tolerable upper intake levels

No toxicity has been observed with high doses of the two natural forms of vitamin K: vitamins K^1 and K^2. It is recognised that no adverse effects occurred in a small, short-term clinical trial of 10 mg of K^1 per day (Craciun *et al.,* 1998). Hence no tolerable upper intake level for these two forms has been established (IOM, 2001; Expert Group on Vitamins and Minerals, 2003).

However, synthetic preparation of menadione or its salts should be avoided for nutritional purposes, particularly for vitamin prophylaxis in newborns as it has been associated with neonatal haemolysis and liver damage. Infants supplemented with menadione reported toxicity effects such as jaundice, hyperbilirubinemia and anemia (Gropper, Smith & Groff, 2012). Based on EFSA Expert Panel (EFSA, 2008), the lowest observed adverse effect level (LOAEL) recommended for vitamin K^2 intake is 20 mg/kg BW/ day.

16.9 Research Recommendations

The following priority areas of research are recommended:

- Bioavailability of dietary phylloquinone and menaquinones from local foods
- Significance of menaquinones to human requirements for vitamin K
- The physiological roles of vitamin K-dependent proteins in functions other than coagulation
- The significance of under-carboxylated vitamin K-dependent proteins and suboptimal vitamin K status to bone and cardiovascular health

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Appendix 16.1: Comparison of Recommended Intake for Vitamin K: RNI Malaysia (2017), RNI of WHO/FAO (2004) and AI of IOM (2001)

Malaysia (2017)		WHO/FAO (2004)		IOM (2001)	
Age group	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**
Infants		**Infants**		**Infants**	
0 - 5 months	5	0 - 6 months	5	0 - 6 months	2.0
6 - 11 months	10	7 - 12 months	10	6 - 12 months	2.5
Children		**Children**		**Children**	
1 - 3 years	15	1 - 3 years	15	1 - 3 years	30
4 - 6 years	20	4 - 6 years	20	4 - 8 years	55
7 - 9 years	25	7 - 9 years	25		
				Males	
Adolescents		**Adolescents**		9 - 13 years	60
Boys (10 - 18 years)	35 - 55	Males (10 - 18 years)	35 - 55	14 - 18 years	75
Girls (10 - 18 years)	35 - 55	Females (10 - 18 years)	35 - 55	19 - 30 years	120
				31 - 50 years	120
Adults		**Adults**		51 - 70 years	120
Men (19 - 65 years)	65	Males (19 – 65 years)	65	> 70 years	120
Men (> 65 years)	65	Males (65+ years)	65		
Women (19 - 65 years)	55	Females (19 – 65 years)	55	**Females**	
Women (> 65 years)	55	Females (65+ years)	55	9 - 13 years	60
				14 - 18 years	75

Malaysia (2017)		WHO/FAO (2004)		IOM (2001)	
Age group	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**
Pregnancy	55	**Pregnant Women**	55	19 - 30 years	90
				31 - 50 years	90
Lactation	55	**Lactating Women**	55	51 - 70 years	90
				> 70 years	90
				Pregnancy	
				14 - 18 years	75
				19 - 30 years	90
				31 - 50 years	90
				Lactation	
				14 - 18 years	75
				19 - 30 years	90
				31 - 50 years	90

Summary

Minerals and Trace Elements Recommendations

The Technical Sub-Committee (TSC) on Minerals and Trace Elements conducted extensive reviews that included published literature for scientific updates, technical guides and documents on dietary intake recommendations of global authoritative agencies and recommendations for energy and nutrient intakes of various countries.

The TSC agreed to adopt the recommendations of WHO/FAO (2004) as a priority. However, for minerals and trace elements that the WHO/FAO did not have available guidelines, the recommendations of IOM (various years) were used instead. In addition, review of recent dietary intake recommendations of ESFA (2010), as well as those of countries in the region, Australia (2006), Japan (2015) and the Philippines (2015) were also done.

The TSC made the decision to include besides calcium (RNI, 2005), four new minerals namely, phosphorus, sodium, potassium and magnesium for the (RNI, 2017). From the nine trace elements, four (iron, iodine, zinc and selenium (RNI 2005) were retained and five new elements, chromium, copper, manganese, molybdenum and fluoride were included. The scope of the minerals and trace elements recommended by international authorities and various Asia Pacific countries (Table 1) showed wide coverage and the proposed Malaysia RNI (2017) is aligned with these recent developments.

Table 1. Comparison of the scope of recommended intakes of minerals and trace elements

	Ca	Fe	I	Se	Zn	Cr	Cu	F	K	Mg	Mo	Mn	Na	P
Malaysia RNI 2017	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Philippines 2015	√	√	√	√	√	-	-	√	√	√	-	-	√	√
Japan 2015	√	√	√	√	√	√	√	-	√	√	√	√	√	√
Australia 2006	√	√	√	√	√	√	√	√	√	√	√	√	√	√
WHO/FAO 2004	√	√	√	√	√	-	-	-	-	-	√	-	-	-
IOM (various years)	√	√	√	√	√	√	√	√	√	√	√	√	√	√
ESFA DRVs 2010	√	√	√	√	√	√	√	√	-	√	√	√	-	√

17 • Calcium

17.1 Introduction

Calcium is the fifth most abundant element in the human body, with approximately 1000 g present in adults. It plays a key role in skeletal mineralization, as well as a wide range of biologic functions. Nearly all (99%) of this is in the skeleton. The remainder is in the teeth (0.6%), the soft tissues (0.6%), the plasma (0.03%) and the extracellular fluid (0.06%). Calcium is an essential element that is only available to the body through dietary sources.

17.2 Functions

Calcium plays a key role in a wide range of biologic functions, either in the form of its free ion or bound complexes. One of the most important functions as bound calcium is to provide a "structural role" in providing rigidity (structure and strength) to the skeleton. This function is provided by a form of calcium phosphate that is generally known as hydroxyapatite [$Ca^{10}(OH)^2(PO^4)^6$] crystals which are embedded in collagen fibrils. In bone, calcium serves two main purposes: it provides skeletal strength and, concurrently, provides a dynamic store to maintain the intra- and extracellular calcium pools (Munro, 2010).

Calcium ions on the surface of bone can interact with ions in body fluids and act like a large ion exchanger. These properties are important in relation to the role of bone as a reserve of calcium to help maintain a constant concentration of blood calcium. Blood calcium is responsible for a wide range of essential functions, including extra-and intracellular signaling, nerve impulse transmission, and muscle contraction (Campbell 1990; Bootman *et al.,* 2001). Serum calcium ranges from 2.2 to 2.6 mmol/l in healthy subjects. It comprises free ions (51%), protein-bound complexes (40%), and ionic complexes (9%). The concentration of serum ionized calcium is tightly maintained within a physiologic range of 1.10 to 1.35 mmol/L to avoid calcium toxicity. Nonionized calcium is bound to a variety of proteins and anions in both the extra- and intracellular pools. The main calcium binding proteins include albumin and globulin in serum and calmodulin and other calcium-binding proteins in the cell. The major ionic complexes in serum are calcium phosphate, calcium carbonate, and calcium oxalate.

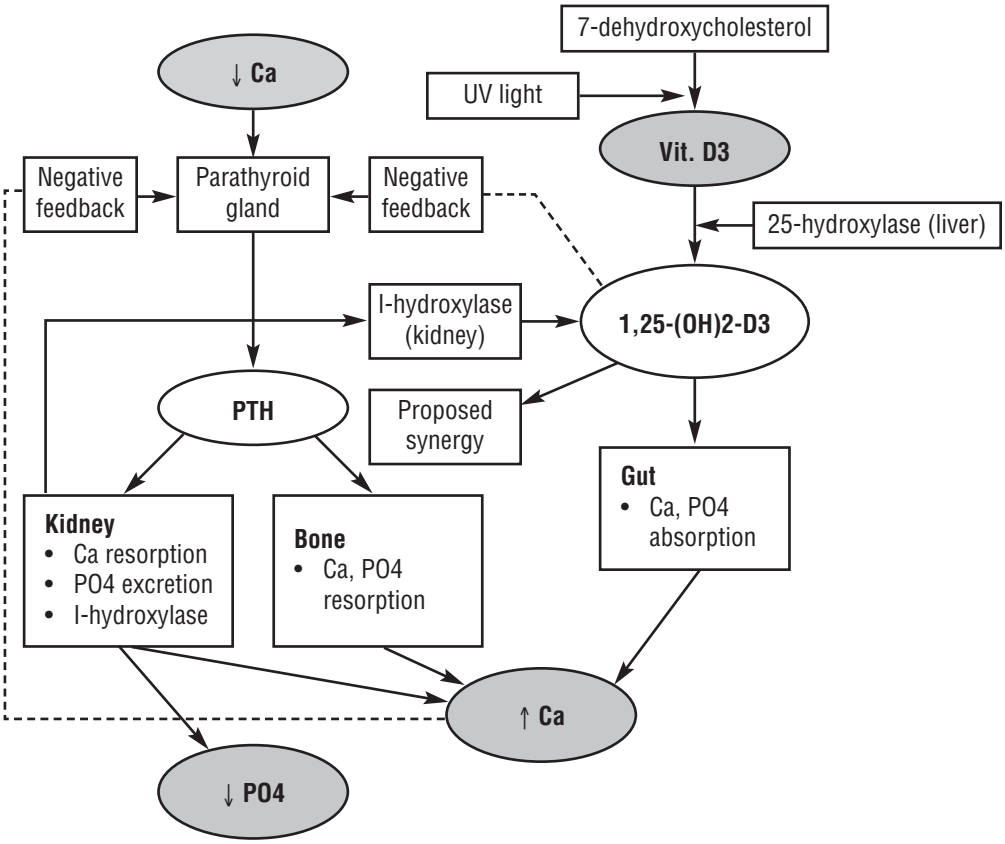
17.3 Metabolism

Calcium requirement is dependent on the state of calcium metabolism, which is regulated by three main mechanisms: intestinal absorption, renal reabsorption, and bone turnover. These in turn are regulated by a set of interacting hormones, including parathyroid hormone (PTH), 1,25-dihydroxyvitamin D ($1,25(OH)^2D$), ionized calcium itself, and their corresponding receptors in the gut, kidney, and bone.

A decrease in serum calcium inactivates the calcium receptors in the parathyroid glands to increase PTH secretion, which acts on the PTH receptor in the kidney to increase tubular calcium reabsorption, and in bone to increase net bone resorption. The increased PTH also stimulates the kidney to increase secretion of $1,25(OH)^2D$, which activates the vitamin D receptor in gut to increase calcium absorption, in the parathyroid glands to decrease PTH secretion, and bone to increase resorption. With a rise in serum calcium, these actions are reversed, and the integrated hormonal response reduces serum calcium. Together, these negative feedback

mechanisms help to maintain total serum calcium levels in healthy individuals within a relatively narrow physiologic range of 10%.

A schematic diagram of calcium homeostasis can be seen below.



Source: Garth FE (2016). http://emedicine.medscape.com/article/874690-overview (accessed 10/3/17)

Dietary intake and absorption provide sufficient calcium to maintain healthy body stores. Absorption occurs mostly in the duodenum and the jejunum. Calcium absorption is a function of active transport that is controlled by 1-25(OH)^{2}D, which is particularly important at low calcium intakes, and passive diffusion, which dominates at high calcium intakes (Bronner, 2009). Typically, at normal calcium intake, 1-25(OH)^{2}D-dependent transport accounts for the majority of absorption, whereas as little as 8 to 23% of overall calcium absorption is caused by passive diffusion (McCormick, 2002). The rate of paracellular calcium uptake is considered non-saturable, while transcellular transport can be upregulated under conditions of low calcium

intake. Recent evidence suggests that paracellular calcium transport is also regulated, at least in part, by 1-25(OH)^2 vitamin D (Christakos, 2012). Since a concentration gradient is not a prerequisite for this process, transcellular transport accounts for most of the absorption of calcium at low and moderate intake levels.

Calcium is excreted in urine, feces, and body tissues and fluids, such as sweat. Calcium excretion in the urine is a function of the balance between the calcium load filtered by the kidneys and the efficiency of reabsorption from the renal tubules. Most of the calcium (-98%) is reabsorbed by either passive or active processes occurring at four sites in the kidney, each contributing to maintaining neutral calcium balance. The majority of the filtered calcium (-70%) is reabsorbed passively in the proximal tubule and the remaining 30% actively in the ascending loop of Henle, the distal tubule, and collecting duct (Munro, 2010).

High intakes of sodium increase urinary calcium excretion. In contrast, adding more potassium to a high-sodium diet might help decrease calcium excretion, particularly in postmenopausal women (Sellmeyer *et al.,* 2002). Studies on human subjects have shown that calcium (Ca) can inhibit iron (Fe) absorption. Both minerals bind to a transporter on the surface of intestinal absorptive cells, but whereas non-haem iron enters the cells this way, calcium hinders further entry of the iron. This effect is mainly relevant when calcium and iron supplements are taken together. However, a thorough review of studies on humans in which Ca intake was substantially increased for long periods shows no changes in hematological measures or indicators of iron status. Thus, the inhibitory effect may be of short duration and there also may be compensatory mechanisms (Lönnerdal 2010; Lynch, 2000).

17.4 Sources

Dietary calcium comes from food sources associated with dairy products, other foods such as vegetables and cereals, foods fortified with inorganic or organic calcium, and from dietary supplements containing calcium.

Other than milk and dairy products, calcium-rich foods in the Malaysian diet can be obtained from fish with edible bones such as canned sardines and anchovies, beans and bean products including yellow dhal, *tofu* and *tempeh* (fermented soybeans), locally processed foods such as shrimp paste, *cincaluk* and *budu*, as well as vegetables like spinach, watercress, mustard leaves, *cekur manis,* tapioca leaves, *kai-lan* and broccoli (Tee *et al.,* 1997). Currently, food manufacturers in Malaysia have also made available in the market calcium fortified products such as high-calcium milk, yogurt, breakfast cereals, biscuits and even rice. Table 17.1 below shows calcium content of local foods in Malaysia.

Table 17.1: Calcium content of foods

Food	mg/100g
Milk and milk products	
High-calcium milk powder	2000
Skimmed milk powder	1169
Low fat cheese	489
Low Fat Milk	132
Low-fat yoghurt	127
Full-cream milk	109
Meat, fish, poultry, legumes, nuts	
Ikan bilis (dried without head & entrails)	500
Sardine (canned)	234
Almonds	222
Cooked dhal	171
Tofu	135
Tempeh	69
Mussels	62
Baked beans	40
Soybean milk	20
Vegetables	
Watercress (sai-yong coy)	200
Kale, Chinese (kai lan coy)	179
Mustard green (sawi),	138
Spinach	69
Calcium fortified products	
High-calcium soybean milk	180
Enriched bread	167
Orange juice (calcium fortified)	146

Ref: Tee *et al.* (1997)

Bioavailability of calcium from plant foods however can be affected by calcium chelators such as oxalate and phytate. Oxalic acids are found in high amounts in plant foods such as spinach, chocolate or cocoa products and in lesser quantities in dried beans, sweet potato, tea infusion, wheat germ, kale, okra and soybean products. However, a clinical study in humans has shown that calcium absorption from low-oxalate high calcium dark green vegetables from the kale species is comparable to milk (Heaney & Weaver, 1990). The authors concluded that the bioavailability from other *Brassica* family vegetables such as broccoli, mustard green, Chinese kale *(kai lan)* and cabbage can be considered as good as milk.

17.5 Deficiencies

Indicators of dietary calcium intakes include calcium balance (i.e., calcium accretion, retention, and loss) which can be measured using stable isotopes techniques. However, bone mineral density can be considered as surrogate marker of fracture risk and calcium status.

Inadequate intake, poor calcium absorption and excessive calcium losses contribute to reduced mineralization of bone. A reduction in absorbed calcium causes serum ionized calcium concentration to decline. This stimulates the parathyroid hormone (PTH) that will act in one of three ways to increase and maintain the level of serum calcium. The parathyroid hormone can increase the production of calcitriol (1,25-dihydroxycholecalciferol), which in turn increases calcium absorption through active transport in the gut and tubular reabsorption in the kidneys. Bone resorption may also increase leading to more calcium being released from the bone. Thus, PTH maintains normal circulating calcium concentration during calcium deprivation. It is noted nonetheless; this is done at the expense of skeletal mass.

Since more than 99% of body Ca is present in the skeleton, an adequate Ca intake during the growth period may be critical in maximizing bone mineral content. Once calcium intake is adequate to prevent rickets (disordered organization of the cartilage matrix) or osteomalacia (defective bone mineralization), provision of additional calcium may increase bone density by affecting bone turnover and the size of the remodeling space. A systematic review concluded that increased calcium/dairy intake significantly increases total body and lumbar spine bone mineral content in children with low baseline intakes (Huncharek *et al.,* 2008).

Chronic calcium deficiency due to inadequate intake or poor intestinal absorption is one of the causes of reduced bone mass and osteoporosis. Osteoporosis is defined as a skeletal disease characterized by reduced bone mass, increased bone fragility and susceptibility to fracture (WHO, 1994). The clinical and public health impact of osteoporosis stems from its association with fractures of the hip, spine and forearm.

There is a dearth of information on the incidence of hip fractures in Malaysia. The Kuala Lumpur Hospital (HKL) had reported the incidence of hip fractures in 1981 as 0.49 per 1,000 and the rate increased to 0.70 per 1,000 in 1989 (Lee, Sidhu & Pan 1993). In that report, the investigators also reported ethnic differences in admissions for hip fractures in HKL, in that Chinese accounted for 58% followed by Indians (27%) and Malays (15%). In 2001, hip fracture incidence from hospital records was reported to be the highest among Chinese (men 94/100,000, women 220/100,000) followed by Indians (men 98/100,000, women 204/100,000) and Malays (men 27/100,000, women 43 /100 000) (Lau *et al.,* 2001). The most recent data

reported that the incidence of hip fractures in Malaysia ranges from 10/100,000 population from age 50 years old to 510/100, 000 populations at age above 75 years old with a sharp increase at age 65 years and above. Similarly, the Chinese had the highest incidence of hip fractures compared to the Malays and Indians. Chinese women accounted for 44.8% of hip fractures. (Lee & Khir, 2007).

Cross sectional surveys in Malaysia often report low calcium intakes among various population. The median intake of calcium among Malaysians was reported to be 353 mg (men 374 mg; women 333 mg) by the Malaysian Adult Nutrition Survey (Institute of Public Health 2014). The SEANUTS study reported that more than one-third did not achieve the Malaysian RNI in 2005 for calcium in children aged 6 months to 12 years (Poh *et al.,* 2013).

Systematic reviews published on the effects of calcium in health and disease showed that in general, low calcium intake is not associated with higher risk of many non-skeletal conditions (Chung *et al.,* 2009; Uusi-Rasi1 et al. 2013). Among hypertensive adults, calcium supplementation (400 to 2000 mg/d) lowered systolic, but not diastolic, blood pressure by a small but statistically significant amount (2 to 4 mm Hg). For body weight, despite a wide range of calcium intakes (from supplements or from dairy and nondairy sources) across the calcium trials, the randomized controlled trials were fairly consistent in finding no significant effect of increased calcium intake on body weight.

There was also no overall effect of calcium intake on cancers, although for breast cancer, subgroup analyses in four cohort studies consistently found that calcium intake in the range of 780 to 1750 mg/d in premenopausal women was associated with a decreased risk for breast cancer. However, no RCTs of calcium supplementation to prevent breast cancer in premenopausal women have been published. For prostate cancer, three of four cohort studies found significant associations between higher calcium intake (>1500 or >2000 mg/day) and increased risk of prostate cancer, compared to men consuming lower amount of calcium (500-1000 mg/day).

On cardiovascular health, six cohort studies of calcium intake suggest that in populations at relatively increased risk of stroke and with relatively low dietary calcium intake (i.e., in East Asia), lower levels of calcium intake under about 700 mg/day are associated with higher risk of stroke. This association, however, was not replicated in Europe or the US, and one Finnish study found a possible association of increased risk of stroke in men with calcium intakes above 1000 mg.

On immunologic disorders; and pregnancy-related outcomes including preeclampsia, there were either few studies or findings were inconsistent.

17.6 Factors affecting calcium requirement

The body's need for calcium relative to skeletal growth and remodeling varies by life stage. The major physiological activities include bone accretion during skeletal growth and maintenance of bone mass after growth is completed. Infancy through late adolescence periods are characterized by positive calcium balance due to enhanced bone formation. After puberty and throughout most of adulthood, bone formation and resorption are balanced. During this

period, bone mass is consolidated, and calcium requirements are relatively stable. Later in life, menopause and age-related bone loss lead to a net loss of calcium due to enhanced bone resorption.

During pregnancy, the foetal need for calcium is met by maternal physiological changes, primarily through increased calcium absorption. Calcium is actively transported across the placenta from mother to foetus, an essential activity to mineralizing the fetal skeleton. Intestinal calcium absorption of the mother doubles in pregnancy (Kovacs, 2005). In teenage pregnancy, whose skeleton is still growing, pregnancy could theoretically reduce peak bone mass and increase the long-term risk of osteoporosis.

Human breast milk is the sole dietary source of calcium to the infant and most of the calcium present in milk was derived from the maternal skeleton. Maternal bone resorption is markedly up-regulated (Specker et al. 1994; Kalkwarf et al. 1997), and it appears that maternal BMD can decline 5 to 10 percent during the 2-to 6-month time period of exclusive breastfeeding. However, it normally returns to baseline during the 6 to 12 months post-weaning (Kalkwarf, 1999). Thus, in the long term, a history of lactation does not appear to increase the risk of low BMD or osteoporosis. The normal loss of BMD during lactation and the post-lactation recovery occurs in adolescents as well (Chantry *et al.,* 2004).

Protein intake stimulates acid release in the stomach, and this, in turn, enhances calcium absorption. However, it has long been known that protein also increases urinary calcium excretion. It is estimated that for every gram of protein metabolized, urinary excretion of calcium increases by 50% or 0.025 mmol calcium taken out. Nevertheless, while protein intake appears to increase urinary calcium excretion, the effect of protein on calcium retention is controversial. While several observational and clinical studies have shown that a higher protein intake (84 to 152 g/day) was positively associated with change in femoral neck and spine BMD (Shapese & Sukumar 2010), other epidemiological studies suggest that high protein diets reduce bone mass; this has been attributed to a higher acid load, leading to a buffering response by the skeleton and greater urinary calcium excretion. A meta-analysis (Darling *et al.,* 2009) concluded that there is a small benefit of protein for bone health, but the benefit may not necessarily translate into reduced fracture risk in the long term. IOM (2011) has not adjusted calcium requirement based on protein intake.

High intakes of sodium increase urinary calcium excretion. In contrast, adding more potassium to a high-sodium diet might help decrease calcium excretion, particularly in postmenopausal women (Sellmeyer *et al.,* 2002). However, available evidence does not warrant different calcium intake requirements for individuals according to their salt consumption.

Caffeine from coffee and tea modestly increases calcium excretion and reduces absorption (Heaney & Recker 1982; Bergman *et al.,* 1990). Two studies have indicated that caffeine intake (two to three or more cups of coffee per day) will result in bone loss, but only in individuals with low milk or low total calcium intake (Barrett-Connor *et al.*1994; Harris & Dawson-Hughes, 1994). The addition of milk into coffee could ameliorate the adverse effect of caffeine.

High dietary intake of phosphorus gives rise to high blood phosphate levels which in turn, reduce the formation of calcitriol in the kidneys, reduce blood calcium and lead to elevated levels of PTH that may be detrimental on bone mineral content. Several observational studies have suggested that the consumption of carbonated soft drinks with high levels of phosphate is associated with reduced bone mass and increased fracture risk, but this is more likely due to the displacement of milk with the carbonated drinks, rather than the phosphorous itself (Heaney & Rafferty, 2001).

Some epidemiological evidence suggests that vegetarians had higher bone loss than omnivores due to the limited quantities of protein, calcium, and phosphorus in their diet (Weaver *et al.,* 1999). In a study of the Seven-Day Adventists (SDAs), however, no significant relationships or trends were found between early or current dietary intake and bone mineral content in that population.

Lacto-ovo vegetarians should be able to obtain adequate calcium intake from milk and milk products. Vegans who eat only plant-based diet may have challenges meeting calcium requirements and should be aware that the bioavailability of calcium may be lower due to plant constituents that can impede calcium absorption.

17.7 Setting requirements and recommended intakes of calcium

Calcium requirements are best derived from balance studies, which is a careful measurement of calcium absorbed and calcium losses across a range of calcium intakes. The intake which provides just enough absorbed calcium to meet losses (zero balance) is then derived and set as the mean calcium requirement of an adult. In children, adolescence and pregnancy, the factorial approach is used to estimate calcium requirement because these groups need to be in positive calcium balance.

The main reference in arriving at this revised recommended intake for calcium for Malaysians is the updated Institute of Medicine, USA (IOM) DRI recommendations in 2011 and the existing FAO/WHO (2002) reference. No other regional RNIs nor updated FAO/WHO data are available at this point in time. There are no known local studies on calcium requirements of communities but several local studies on calcium intake, bone mineral density and calcium supplementation conducted locally has been published (Chee *et al.* 2002; Chee *et al.* 2003; Esra *et al.* 2014; Suriawati *et al.* 2016).

Infants

The optimal source of calcium during the first year of life is human milk. No evidence shows that exceeding the calcium intake of the exclusively breastfed term infant during the first 6 months of life or the intake of the breastfed infant supplemented with solid foods during the second 6 months of life is beneficial to achieving long-term increases in bone mineralization. Calcium requirements for infants are presumed to be met by human milk (IOM, 1997). There are no functional criteria for calcium status that reflect response to calcium intake in infants

(IOM, 1997). According to IOM (2011), data are not sufficient to establish an estimated average requirement (EAR) for infants 0 to 6 and 7 to 12 months of age, and therefore adequate intake (AIs) have been developed based on the available evidence.

Based on infant weighing studies, a reasonable average amount of breast milk consumed is 780 mL/day. The average level of calcium within a liter of breast milk is 259 mg (± 59 mg). It is therefore estimated that the intake of calcium for infants fed exclusively human milk is 202 mg/day (IOM, 2011).

The daily increment of calcium in the skeleton in the first 2 years of life is about 100 mg/day. The urinary calcium loss is about 10 mg/day. Therefore, infants need to absorb about 120 mg of calcium per day for normal growth. Absorption of calcium is assumed to be 60% from human breast milk, while that from infant formula is lower at 40%. It is estimated therefore breastfed infants with an intake of about 202 mg/day would be able to meet the required amount of absorbed calcium of 120 mg, and formula fed babies would require an intake of 250 mg/day of calcium (IOM, 2011).

Based on the above, the proposed AI for infants (0-6 months) who are exclusively breastfed is set at 200 mg calcium/day. Similarly, for formula-fed babies, the AI amount is set higher at 250 mg calcium/day.

As intake of solid foods increases for infants aged 6-12 months, calcium intake from breast milk decreases. There is also lower calcium concentration of breast milk at 6 to 12 months of lactation. Hence, calcium requirement for this age group is expected to be derived increasingly from solid foods. IOM (2011) had proposed the AI for infants 6-12 months to be 260 mg calcium/day.

The figure is derived by assuming that mean human milk intake during the second 6 months of life would be lower at 600 ml/day (Dewey *et al.,* 1984). The calcium concentration in the milk is assumed to be 200 mg/L (Atkinson *et al.,*1995) hence the infant would consume 120 mg/day of calcium. The estimated calcium intake from solid food was assumed to be 140 mg /day and this gives a total of 260 mg/day.

AI for infants

0 - 6 months	**breast fed**	**200 mg/day**
	formula fed	**250 mg/day**
7 - 12 months		**260 mg/day**

Children and adolescents

The amount of calcium that is accumulated in young children is mainly for bone growth. Several studies among Asian children in Hong Kong showed that those with habitually higher calcium intakes during the first 5 years of life had significantly higher bone mineral content (Lee *et al.,* 1993) than in children with lower calcium intakes of less than 400 mg/day. Lee & Leung (1995) also showed that amongst 7-year old Hong Kong Chinese children who habitually

consume a low calcium diet (280 mg/day), gains in radial bone density (3.1 % more than controls) were seen when supplemented with 300 mg/day calcium carbonate for 18 months. No local studies exist for measuring bone calcium accretion in young children.

The IOM (2011) recommendation provided scientific data measuring calcium balance and hence, derive estimated total intake needed for bone accrual across several age groups. For children aged 1- 3 years old, Lynch *et al.* (2007) suggested a target average calcium retention level of 142 mg/day, consistent with the growth needs of this life stage group. Through the factorial method, a calcium intake of 474 mg/day is estimated to meet this need. An estimated EAR was, established as 500 mg of calcium per day, rounded from 474 mg/day. An assumption specified by Lynch et al. (2007) is that an additional 30 percent calcium retention would meet the needs of 97.5 percent of this age group. This results in an estimated RNI for calcium of 700 mg/day calcium for this age-group.

Similarly, for children aged 4 to 8 years old, Abrams *et al.* (1999) and Ames *et al.* (1999) estimated that the total intake of 800 mg would be needed for bone accrual. Again, the assumption that another approximately 30 percent is needed to cover about 97.5 percent of the population- the RNI value for calcium would be 1,000 mg/day for this age group.

Most research in children regarding optimal calcium intakes has been directed toward 9- to 18-year-olds - the efficiency of calcium absorption is increased during puberty, and most bone mineralization occurs. This is the period of growth spurts and the attainment of 'peak bone mass'. Achieving a higher peak bone mass is considered a better approach for prevention of osteoporosis.

Data from studies on calcium balance in this age-group have provided bone calcium accretion levels for children and adolescents ranging from 92 to 210 mg/day (Vatanparast *et al.,* 2010). Average bone calcium accretion was included in the factorial method, and the intake levels can be estimated as ranging between 961-1116 mg/day in females and 1200-1300 mg/day in males. IOM (2011) interpolated an estimated mean need for calcium for boys and girls of 1,100 mg/day with rounding, a value approximately at the midpoint between the two groups. Hence, the RNI is recommended to be set at 1300 mg/day to cover 97.5 percent of the population aged 9 to 18 years old.

RNI for children & adolescents

1 - 3 years	**700 mg/day**
4 - 6 years	**1000 mg/day**
7 - 9 years	**1000 mg/day**

RNI for adolescents

10 - 12 years	**1,300 mg/day**
13 - 16 years	**1300 mg/day**
16 - 19 years	**1300 mg/day**

Adults

After peak bone mass attainment, bone formation and resorption is balanced during adulthood. Bone mass density is relatively stable between ages 20-50, and hence there are relatively few intervention studies on the role of calcium during young and middle adulthood.

FAO/WHO (2002) recommended 750 mg/day for populations with animal protein intake of 20-40 g/day. IOM (2011) cited a study which provides the only evidence for calcium balance studies at this life stage groups. Based on a series of controlled calcium balance studies, they have established an EAR calcium intake level of 741 mg/day to maintain neutral calcium balance, which is rounded to 800 mg/day. Hence, IOM has set the RNI for calcium for this age-group at 1000 mg/day. Based on these considerations, the proposed RNI for calcium for adults aged 19 to 50 years in women and men is revised to 1000 mg/day.

The natural process of bone loss begins to manifest itself in the older age group. It begins earlier for women than for men as a result of the onset of menopause, which usually occurs when women reach 50 to 55 years of age. By the time both men and women have reached 70 or more years of age, they will be experiencing bone loss. Menopause is also associated with a rise in excretion of obligatory calcium or fasting urine of about 20 mg-40-mg daily.

Women 51 through 70 years of age are considered separately from men. Although it is evident that calcium intake does not prevent bone loss during the first few years of menopause, there is the question of whether or to what extent calcium intake can mitigate the loss of bone during and immediately following the onset of menopause. Several studies including a local study of milk supplementation among Malaysian postmenopausal women with 1200 mg calcium per day has been shown to reduce rate of bone loss (Chee *et al.,* 2003).

Available balance data indicate that the EAR for women aged over 51 is 1000 mg/day and 800 mg for men. (IOM, 2011). Therefore, the RNI for calcium is set at 1200 mg/day for women aged 51 years and above and 1000 mg for men.

RNI for adults

20 - 39 years	**1000 mg**
40 - 49 years	**1000 mg**
50-59 years	**1200 mg (women); 1000 mg (men)**
≥60 years	**1200 mg (women); 1000 mg (men)**

Pregnancy & Lactation

The foetal need for calcium is met by maternal physiological changes, primarily through increased calcium absorption. There is still a debate whether the calcium required for foetal bone mineralization can be obtained with no detectable mobilization of maternal bone. Nevertheless, IOM (2011) reported that the EAR for non-pregnant women and adolescents is appropriate for pregnant women and adolescents based on the randomized controlled trials (RCTs) of calcium supplementation during pregnancy that reveal no evidence that additional calcium intake beyond normal non-pregnant requirements has any benefit to mother or foetus (Koo *et al.* 1999; Jarjou *et al.* 2010). Hence the RNI for non-lactating pregnant women and adolescent are at 1000 and 1300 mg/day, respectively.

Post-lactation maternal bone mineral is restored without consistent evidence that higher calcium intake is required, as based on two RCTs (Cross *et al.* 1995; Prentice *et al.* 1995). There is no evidence that calcium intake in lactating women and adolescents should be increased above that of non-lactating women and no additional amount was provided (IOM, 2011). The calcium RNI for lactating women is set at 1000 mg/day and lactating adolescents at 1300 mg/day.

RNI for pregnancy & lactation

13 - 19 years	**1300 mg/day**
20 - 49 years	**1000 mg/day**

Discussions on revised RNI for Malaysia

The previous recommended dietary intakes for calcium (NCCFN, 2005) were lower than the current recommended calcium intake for Malaysian infants, but higher for adolescents, younger adults, pregnant and lactating mothers. This is in light of revised data from IOM (2011) based on studies on calcium balance across different age-groups.

17.8 Tolerable upper intake levels

Calcium levels in the body are very closely controlled so that excessive accumulation in blood or tissues arising from over consumption is unknown. Abnormally high calcium concentrations may occur but usually secondary to diseases such as bone cancer, hyperthyroidism and hyperparathyroidism. The efficiency of calcium absorption decreases with intake, thereby providing the body with a protective mechanism to lessen the chances of calcium intoxication.

The common effects of excessive calcium intakes are kidney stones (nephrolithiasis), milk-alkali syndrome and interaction of calcium with absorption of other essential minerals such as iron, zinc, magnesium and phosphorous. Calcification of vascular tissues has been reported with high calcium intake however, the reports are based on individuals with compromised kidney function. No link has been clearly established for a general population. Similarly, there is no conclusive evidence that the intake of calcium per se in the range of 1000

to 1200 mg/day can be associated with cardiovascular events (IOM, 2011). IOM (2011) had established a tolerable upper level (UL) according to age groups. The calcium tolerable upper intake levels by life stages is shown in Table 17.2.

Table 17.2: Calcium Tolerable Upper Intake Levels by Life Stages

Life Stage Group	UL (mg)
Infants	
0-6 months	1000
6-12 months	1500
Children, adolescents & adults	
1-3 years	2500
4- 8 years	2500
9-13 years	3000
14-18 years	3000
19-30 years	2500
31-50 years	2500
51-70 years	2000
>70 years	2000
Pregnancy & lactation	
14-18 years	3000
19-50 years	2500

Source: IOM (2011).

17.9 Research recommendations

The following priority areas of research are recommended:

1. Nationally representative data on calcium intakes of various population groups such as children, adolescents, adults and elderly.

2. Content of calcium in local foods and absorption efficiency, especially from non-dairy foods.

3. Studies on the effects of increased calcium intakes on skeletal mass and bone loss. In adolescents, it is important to determine to what extent increased calcium intake can influence peak bone mass formation in conjunction with other nutrients and physical activity level.

4. Calcium balance studies on various age and ethnic groups to determine optimal recommendations of calcium intake.

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Appendix 17.1 Comparison of recommended intake for calcium: RNI Malaysia (2017), RNI Malaysia (2005), RNI of IOM (2011) and RNI of FAO/WHO (2004)

Malaysia (2017)		Malaysia (2005)		IOM (2011)		FAO/WHO (2004)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**
Infants		**Infants**		**Infants**	**AI**	**Infants**	
0 - 5 months	200(BF)	0 - 5 months	300 (BF)	0 - 6 months	200(BF)	0 - 6 months	300 (bf)
	250(FF)		400 (FF)		250 (FF)		
6 - 11 months	260	6 - 11 months	400	6 - 12 months	260	7 - 11 months	400 (ff)
Children		**Children**		**Children**		**Children**	
1 - 3 years	700	1 - 3 years	500	1 - 3 years	700	1 - 3 years	500
4 - 6 years	1000	4 - 6 years	600	4 - 8 years	1000	4 - 6 years	600
7 - 9 years	1000	7 - 9 years	700			7 - 9 years	700
Boys		**Boys**		**Boys**		**Boys**	
10 - 12 years	1,300	10 - 12 years	1,000	9 - 18 years	1,300	10 - 18 years	1,300
13 - 15 years	1,300	13 - 15 years	1,000				
16 - 18 years	1,300	16 - 18 years	1,000				
Girls		**Girls**		**Girls**		**Girls**	
10 - 12 years	1,300	10 - 12 years	1,000	9 - 18 years	1,300	10 - 18 years	1,300
13 - 15 years	1,300	13 - 15 years	1,000				
16 - 18 years	1,300	16 - 18 years	1,000				
Men		**Men**		**Men**		**Men**	
19 - 29 years	1,000	19 - 29 years	800	19 - 50 years	1000	19 - 65 years	1,000
30 - 50 years	1,000	30 - 50 years	1,000			> 65 years	1,300
51 - 59 years	1,000	51 - 59 years	1,000	51 - 70 years	1,000		
60 - 65 years	1,000	60 - 65 years	1,000	> 70 years	1,000		
≥ 60 years	1,000	≥ 60 years	1,000				

Malaysia (2017)		Malaysia (2005)		IOM (2011)		FAO/WHO (2004)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**
Women		**Women**		**Women**		**Women**	
20 - 39 years	1,000	19 - 50 years	800	19 - 50 years	1,000	19 - 50 years	1,000
40 - 49 years	1,000	51 - 65 years	1,000			51 - 65 years	1,300
50 - 59 years	1,200	> 65 years	1,000	51 - 70 years	1,200	> 65 years	1,300
≥ 60 years	1,200			> 70 years	1,200		
Pregnancy		**Pregnancy**		**Pregnancy**		**Pregnancy**	
13 - 19 years	1,300						
1st trimester	1,000	1st trimester	1,000	14 - 18 years	1,300	1st trimester	1,000
2nd trimester	1,000	2nd trimester	1,000	19 - 50 years	1000	2nd trimester	1,000
3rd trimester	1,000	3rd trimester	1,000			3rd trimester	1,200
Lactation		**Lactation**		**Lactation**		**Lactation**	
13 - 19 years	1,300						
0 - 3 months	1,000	0 - 3 months	1,000	14 - 18 years	1,300	0 - 3 months	1,000
4 - 6 months	1,000	4 - 6 months	1,000			4 - 6 months	1,000
7 - 12 months	1,000	7 - 12 months	1,000	19 - 50 years	1000	7 - 12 months	1,000

BF=breast fed FF=formula fed

18 • Iron

18.1 Introduction

Iron is a chemical element with symbol Fe and atomic number 26. It is a metal in the first transition series with an atomic mass of 55.8. It is by mass the most common element on Earth, forming much of Earth's outer and inner core. Iron exists in a wide range of oxidation states, –2 to +6, with +2 and +3 forms being central to the biological properties of this mineral. Elemental iron occurs in meteoroids and other low oxygen environments, but is reactive to oxygen and water. Fresh iron surfaces appear lustrous silvery-gray, but oxidize in normal air to give hydrated iron oxides, commonly known as rust.

18.2 Functions

In humans, iron represents approximately 35 and 45 mg/kg of body weight in adult women and men, respectively. According to IOM (2001), functionally important forms of iron in the body exist in four major classes. These includes firstly iron containing heme proteins such as haemoglobin, myoglobin and cytochromes which is important for oxygen transport and storage as well as electron transport. Secondly, iron sulphur enzymes that is involved primarily in energy metabolism (flavoproteins, heme-flavoproteins). Thirdly, iron storage and transport proteins participating in iron uptake, transport and storage in the body such as transferrin, lactoferrin, ferritin and hemosiderin. Lastly, other iron-containing or activated enzymes such as sulphur and nonheme enzymes.

Iron also plays important roles in cellular processes such as the synthesis of DNA, RNA and proteins; electron transport; cellular respiration; cell proliferation and differentiation; and regulation of gene expression. It plays a crucial role in maintaining cellular iron homeostasis by regulating gene expression at the post-transcriptional level. Iron homeostasis is critical for normal brain function, especially in learning and memory.

18.3 Metabolism

The body has no mechanism for the excretion of iron, and it is argued that the acquisition and distribution of iron is tightly regulated, in order to avoid excessive accumulation of the element. This control of body iron depends on an effective co-ordination of intestinal uptake and transfer of iron, with the recycling of iron from the red blood cell mass and other tissues, the storage and release of iron from the liver, and integumental (i.e. loss from the epidermis and epithelia) and, in women, menstrual losses. At the functional level, the cells involved are the enterocytes, hepatocytes and macrophages of the reticulo-endothelial system (RES) (i.e. the monocyte-macrophage system). In macrophages, the uptake and export of iron is mediated by Divalent Metal Transporter 1 (DMT1) and ferroportin, respectively, and as with enterocytes these processes are regulated by hepcidin (Ganz, 2013). A schematic diagram of whole-body iron metabolism is shown in Figure 18.1.

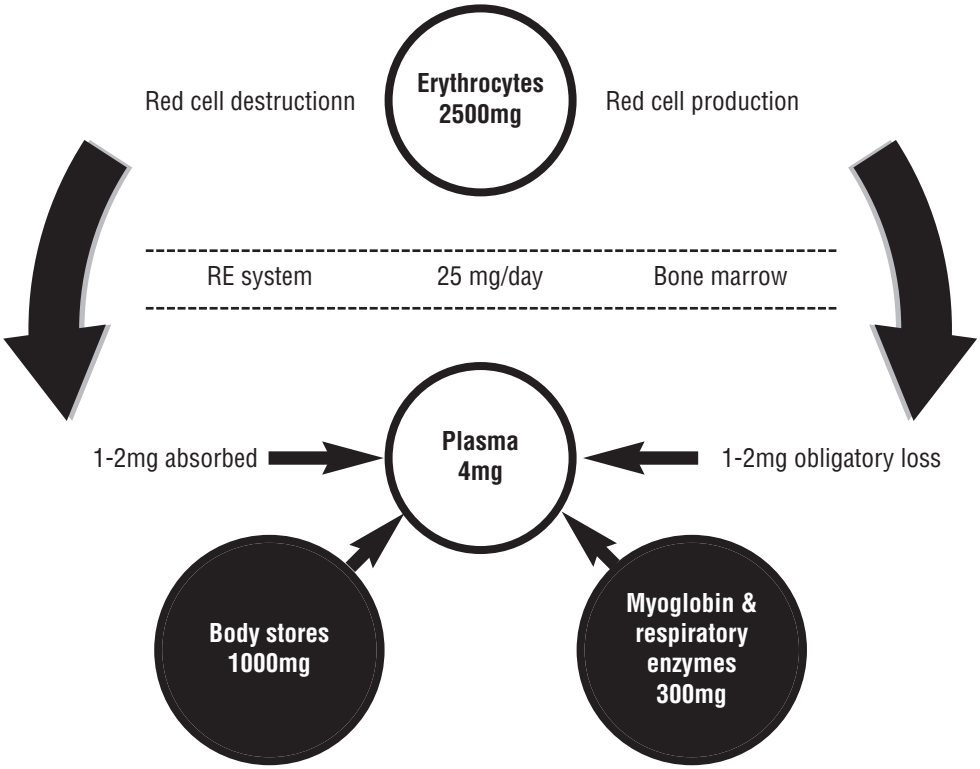

Figure 18.1: Whole-body iron metabolism. RE, reticuloendothelial
Source: EFSA, (2015)

Iron absorption occurs mainly in the duodenum and proximal small intestine. Absorption involves the uptake of iron from the intestinal lumen into enterocytes, its transfer within enterocytes and its subsequent translocation across the basolateral membrane to carriers in the plasma of the portal circulation (EFSA 2015). Iron absorption is regulated by both dietary regulator and store regulator. Dietary regulator regulate short-term increase in dietary iron while stores regulator regulate iron storage in the liver.

About 1-2 mg of iron is lost every day, through skin and enteric desquamation and minor blood losses. This loss is balanced by intestinal absorption. Most of the iron in the body is recycled when old red blood cells are taken out of circulation and destroyed, with their iron scavenged by macrophages in the mononuclear phagocyte system, mainly spleen, and returned to the storage pool for re-use. Iron homeostasis is closely regulated via intestinal absorption. Increased absorption is signalled via decreased hepcidin by decreasing iron stores, hypoxia, inflammation, and erythropoietic activity. The situation is different in menstruating women, pregnant women, during infancy and childhood where increased iron demand occurs due to high blood loss or growth and development demands.

The availability of iron for absorption in the duodenum and small intestine is affected by a number of dietary constituents, which act as either inhibitors (e.g. phytate, polyphenols and calcium) or enhancers (e.g. ascorbic acid and animal tissue). The mechanism of action is the formation of iron complexes in the digestive chyme in the gut lumen, and the strength of binding dictates whether or not the iron can be removed from the complex by DMT1. In addition, ascorbic acid reduces ferric (Fe3+) iron to ferrous (Fe2+), which is the chemical form that is taken up by DMT1 (EFSA, 2015).

Calcium (Ca) is the only micronutrient in the diet that may inhibit both haeme and non-haeme iron absorption, but the mechanisms are unclear and the effect appears to be short-term. There is an adaptation to a high intake of Ca as regulatory mechanism of Fe absorption to maintain Fe homeostasis (Miranda *et al.,* 2014). As for iron and zinc interaction, many combined iron-zinc supplementation trials in humans, have reported varied efficacy (Lind *et al.,* 2003; Wieringa *et al.,* 2007), implying possible negative interactions. A review of published studies on the effects of zinc on iron absorption concluded that the inhibitory effect of zinc occurs at a Zn-Fe (weight/weight) ratio of 1:1 in aqueous solutions but, importantly, there is no inhibitory effect in food matrices (Olivares *et al.,* 2012). Interactions of copper-iron are influenced by age and stage of development (Collins *et al.,* 2010), especially prenatal development (Gambling *et al.,* 2008). In addition to the well-understood effects of copper deficiency on iron metabolism (leading to anaemia), there is some evidence suggesting that copper deficiency results in lower liver iron concentration, and delivery of iron (as well as copper) to the foetus may be compromised (Andersen *et al.,* 2007).

Vitamins such as Vitamin A can affect several stages of iron metabolism, including erythropoiesis and the release of iron from ferritin stores. A number of trials have been undertaken to examine the effect of vitamin A supplementation/ fortification on indices of iron status (Michelazzo *et al.,* 2013), and many report an impact of vitamin A on haemoglobin and other parameters. Studies examining the effect of vitamin A on iron absorption have produced conflicting findings and it is not clear whether vitamin A and/or iron status are key determinants of an effect (Hurrell and Egli, 2010).

Overall, EFSA (2015) considers that interactions between iron and other minerals, vitamins and certain dietary constituents, in the context of a mixed European diet, are not relevant for setting Dietary Reference Value (DRVs) for iron.

18.4 Sources

There are two types of iron in foods - haem iron and non-haem iron. Haem iron is derived primarily from the haemoglobin and myoglobin of flesh foods such as meats, fish, and poultry. About 40 percent of iron from meat, fish, and poultry is in the haem form while the rest is nonhaem iron. Non-haem iron is found in plant foods such as breads, cereals, dark leafy vegetables (such as spinach, fern shoots, kangkung), legumes and eggs. Examples of food sources of iron are given in Table 18.1.

Table 18.1: Iron content of foods.

Food	mg/100 g edible portion
Legumes and legumes products	
Chickpea	6.9
Fried soya bean curd	7.3
Vegetables	
Fern Shoots *(pucuk paku)*	4.8
Bitter gourd	6.1
Spinach *(bayam pasir)*	5.0
Kangkung	5.2
Fruits	
Kedondong	3.4
Jackfruit *(nangka)*	1.1
Rambutan	2.5
Meat	
Lean beef meat	2.2
Liver, ox	9.0
Chicken	2.8
Egg	2.4
Fish	
Dried ikan bilis, whole	5.3
Boiled cockles	7.9
Tenggiri (Spanish mackeral)	1.1
Cereals and cereal products	
Kuih-teow	3.4
Biscuit, wholemeal crackers	4.3
Bread, wholemeal	3.2

Source: Tee *et al.* (1997) and MyFCD (2016).

18.5 Deficiency

Iron deficiency is a serious problem worldwide. It occurs following prolonged negative iron balance, the major causes of which include inadequate intake (owing to insufficient bioavailable iron in the diet or decreased iron absorption), increased iron requirements (for instance, during periods of growth) and chronic blood loss (from heavy hookworm infection or menstrual bleeding). Iron deficiency can be found in various degree from low iron stores; to early iron deficiency to iron-deficiency anaemia (Figure 18.2). These biochemical measures are used as the key indicators in setting the iron requirements.

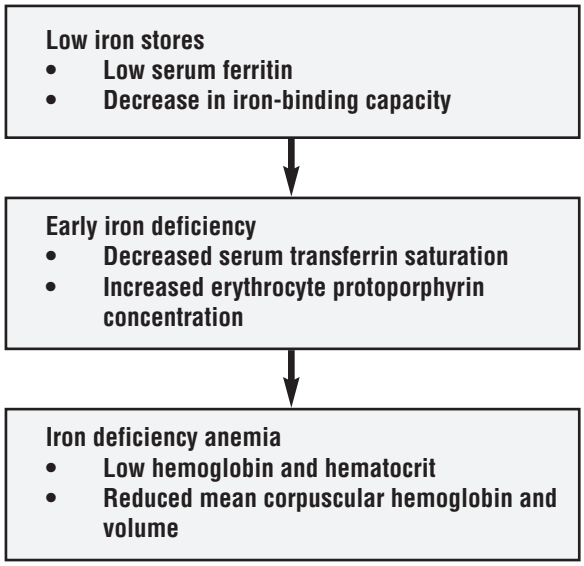

Figure 18.2: Stages of iron deficiency (adapted from WHO, 2007)

Clinical features of iron deficiency include koilonychia (spoon-shaped nails), soft nails, glossitis, cheilitis (dermatitis at the corner of the mouth), mood changes, muscle weakness and impaired immunity. However, these features are not specific to iron deficiency but may also be manifestation of other nutritional deficiencies.

The pathogenesis of iron deficiency may not be dietary. Non-dietary causes of iron deficiency and anaemia include conditions that cause gastrointestinal blood loss or malabsorption, e.g. cancer and inflammatory bowel disease, intestinal infections and parasitism. Blood loss from the genito-urinary and respiratory tracts may also contribute to iron deficiency (Steketee, 2003). Important subclinical and clinical consequences of iron deficiency are impaired physical work performance, developmental delay, cognitive impairment, and adverse pregnancy outcomes (IOM, 2001). The bulk of experimental and epidemiological evidence in humans suggests that functional consequences of iron deficiency (related both to anaemia and tissue iron concentration) occur only when iron deficiency is of a severity sufficient to cause a measurable decrease in haemoglobin concentration.

Iron deficiency is linked to reduced capacity for physical activity, reduced productivity and increased fatigue leading to poorer cognitive work performance (Patterson and Blumfeild, 2009; Greig *et al.,* 2013). The economic costs of iron deficiency anaemia from annual physical productivity losses have been calculated to be around US$ 2.32 per capita, or 0.57% of gross domestic product in low- and middle-income countries (WHO, 2016). Evidence also suggests that iron deficiency may be associated with adverse neurodevelopmental outcomes in young children (Baker and Greer, 2010). Furthermore, in the absence of treatment, iron deficiency may progress to iron deficiency anaemia, which has been found to be associated with irreversible developmental delay in young children (Lozoff *et al.,* 2006). Iron deficiency anaemia during pregnancy has long been associated with an increased risk of low birth weight, preterm delivery, perinatal mortality, and infant and young child mortality as well as maternal mortality (Allen, 2000).

For the year 2011, it is estimated that roughly 43% of children, 38% pregnant women, 29% non-pregnant women and 29% of all women of reproductive age have anaemia globally, corresponding to 273 million children, 496 million non-pregnant women and 32 million pregnant women (WHO, 2015). WHO suggested the classification of public health significance of anaemia in populations on the basis of prevalence estimated from blood levels of haemoglobin, namely 4.9% or lower as "normal", 5.0-19.9% as "mild," 20.0-39.9% as "moderate" and ≥ 40% as "high" (WHO, 2011)

In Malaysia, iron deficiency anaemia was the leading cause of years lived with disability (YLDs) among children and adolescents in 2013 (Global Burden of Disease Pediatrics Collaboration, 2016). The prevalence of children and adolescents with iron deficiency anaemia in Malaysia was 16.2% (Global Burden of Disease Pediatrics Collaboration, 2016). Iron deficiency anaemia among aboriginal schoolchildren in rural Peninsular Malaysia aged 7-12 years was even higher (34%) (Al-Mekhlafi *et al.,* 2008). According to the National Health and Morbidity Survey (NHMS) 2015 study, the overall prevalence of anaemia in Malaysia was 24.6% among population aged 15 years and above (IPH, 2015). Prevalence of mild, moderate, and severe anaemia using haemoglobin level in Table 18.2 were 15.7%, 7.9% and 1.0% respectively. By gender, the prevalence of anaemia was significantly higher among females; 35.5% than males; 14.3% with Indians having the highest prevalence of anaemia at 30.8%, followed by Malays at 25.9% and lastly Chinese at 23.0%.

Table 18.2: Haemoglobin levels to diagnose anaemia at sea level

Population	Non Anaemia*	Anaemia*		
		Mild	Moderate	Severe
Non-pregnant women (15 years of age and above)	120 or higher	110 - 119	80 - 109	lower than 80
Pregnant Women	110 or higher	100 - 109	70 - 99	lower than 70
Men (15 years of age and above)	130 or higher	110 - 109	80 - 109	lower than 80

*Haemoglobin in g/L
Source: WHO, 2001

A study done by Loh and Khor (2010) found that about 10.3% of the women showed iron deficiency anaemia (IDA) (Hb <12g/dL + serum ferritin < 15(g/L + MCV <80fl/) with the Indians showing the highest prevalence (18.0%) followed by Chinese (9.9%) and Malays (4.3%). Among pregnant women in Malaysia, 38% have anaemia according to the WHO survey (McLean *et al.,* 2009). A nationwide, cross-sectional study of more than 1000 pregnant women, 20-30 years of age, found that the prevalence of anaemia increased with increasing gestational age, being 12% in the first, 32% in the second and 43% in the third trimester (Haniff *et al.,* 2007). Among the older population, the prevalence of iron deficiency anaemia among postmenopausal women was 3% (Ambiyya *et al.,* 2014).

18.6 Factors affecting iron requirement

The body iron stores regulator plays an important part in regulating iron absorption in accordance with the body's needs, increasing absorption when stores are low and decreasing when they are high. During the development of a negative iron balance (when absorption is smaller than losses), iron stores are first depleted (serum ferritin is lowered), which is successively associated with a continuous increase in dietary iron absorption. When iron stores are depleted there will be a concomitant reduction in the concentration of Hb. This reduction is also associated with an increase in iron absorption (Hallberg & Hulthen, 2000).

Dietary haem iron is better absorbed because haem is soluble at the pH of the small intestine. For example, the average absorption of haem iron from meat-containing meals is about 25 percent. Haem iron uptake by absorptive enterocytes is not influenced by the dietary constituents that adversely affect the absorption of inorganic iron. However, haem iron can be degraded and converted to non-haem iron if foods are cooked at a high temperature for too long. Non-haem iron compound used for the fortification of foods will only be partially available for absorption. Once iron is dissolved, its absorption from fortificants and food contaminants is influenced by the same factors as the iron native to the food substance.

Several dietary factors have been identified which positively or negatively influenced the absorption of the dietary iron. The absorption of nonhaem iron from a meal depends upon the net effect of factors enhancing iron absorption (ascorbic acid and organic acids; meat, chicken, fish and other seafood; fermented vegetables, fermented soy sauces) and factors inhibiting iron absorption (phytates and inositol phosphates; iron-binding polyphenols; calcium; soy proteins and vegetable proteins) (WHO/FAO, 2004). (Table 18.3)

Table 18.3: Factors influencing dietary iron absorption

Haem iron absorption
Amount of dietary haem iron, especially from meat
Content of calcium in meal (e.g. from milk, cheese)
Food preparation (i.e. time, temperature)
Non-haem iron absorption
Amount of potentially available non-haem iron (includes adjustment for fortification iron and contamination iron)
Balance between the following enhancing and inhibiting factors:
Enhancing factors:
Ascorbic acid (e.g. certain fruit juices, fruits, potatoes, and certain vegetables)
Meat, fish and other seafood
Fermented vegetables (e.g. sauerkraut), fermented soy sauces, etc.
Inhibiting factors:
Phytate and other lower inositol phosphates (e.g. bran products, bread made from high-extraction flour, breakfast cereals, oats, rice - especially unpolished rice
- pasta products, cocoa, nuts, soya beans, and peas) Iron-binding phenolic compounds (e.g. tea, coffee, cocoa, certain spices, certain vegetables, and most red wines)
Calcium (e.g. from milk, cheese)
Soya

Source: WHO/FAO (2004)

The bioavailability of iron in western-type diet is 14 to 16% in borderline iron deficient subjects (Hallberg & Rossander-Hulthen, 1991). Diets that contain smaller portions of meat and fish, high phytates and some vegetarian meals each week was found to have iron bioavailability of 12%. Reducing the meat and fish intake further will reduce the iron bioavailability to about 10%. In the absence of meat and fish and with a high intake of phytate, polyphenols and vitamin C, the bioavailability of iron is about 5%. Table 18.4 shows examples of diets with different iron bioavailability. Common Malaysian diets have iron bioavailability of about 4 to 12% (Ismail *et al.,* 2001).

The amount of dietary iron absorbed is mainly determined by the amount of body stores of iron and by the properties of the diet (iron content and bioavailability). The absorption of haem iron varies from about 40 percent during iron deficiency to about 10 percent during iron repletion (Hallberg, Hulthen & Gramatkovski, 1997). On the other hand, the absorption of non-haem iron differs depending on the presence of other dietary components and physiological conditions. It is important then to adjust absorbed iron requirements according to different types of diets especially in vulnerable groups.

Table 18.4: Examples of diet with different iron bioavailability

Type of diet	Bioavailability mg/kg/day
Very high meat in two main meals daily and high ascorbic acid (theoretical)	75.0
High meat/fish in two main meals daily	66.7
Moderate meat/fish in two main meals daily	53.2
Moderate meat/fish in two main meals daily; low phytate and calcium	42.3
Meat/fish in 60% of two main meals daily; high phytate and calcium	31.4
Low meat intake; high phytate; often one main meal	25.0
Meat/fish negligible; high phytate; high tannin and low ascorbic acid	15.0
Pre-agricultural ancestors	
Plant/animal subsistence: 65/35; very high meat and ascorbic acid intake	150

Source: WHO/FAO (2004)

The WHO and the FAO proposed dietary iron bioavailability values for setting DRVs of 15%, 10% or 5% depending on the composition of the diet, but the evidence base from which these values were obtained was not provided. The highest bioavailability value is for diversified diets with generous amounts of meat and/or foods rich in ascorbic acid. The lowest bioavailability is for diets based on cereals, tubers and legumes with little or no meat or ascorbic acid-containing fruits and vegetables (Allen *et al.,* 2006). Table 18.5 shows data of iron absorption in women of 55 kg body weight with no iron stores.

Table 18.5: Translation of bioavailability expressed as amount of iron absorbed into percent absorbed for two levels of iron intake

Bioavailability, mg/kg/day	Absorption as mg Fe at no iron stores in women of 55 kg body weight	Bioavailability %	
		15 mg	17 mg
150	8.25	55.0	48.8
75	4.13	27.5	24.4
66.7	3.67	24.5	21.8
53.2	2.93	19.5	17
42.3	2.32	15.5	13.5
31.4	1.73	11.5	10
25	1.38	9.2	8.2
15	0.83	5.5	4.7

Source: WHO/FAO (2004)

18.7 Setting requirements and recommended intake of iron

The recommended nutrient intake (RNI) for iron in Malaysia is based on the WHO/FAO (2004) recommendations. The various physiological requirements (basal iron losses, iron for growth and menstrual iron losses) suggested were considered in setting the requirements (Table 18.6). Beside the physiological requirements, the amount of iron in food and its bioavailability in different diets were also taken into consideration. Taking into consideration available local reports on iron intake of communities such as Malaysian Adult Nutrition Survey (MANS) (IPH, 2014) and the magnitude of iron deficiency problem in the country, 10% and 15% iron bioavailability levels of WHO/FAO (2004) were adopted.

Table 18.6: Iron intakes required for growth, median basal iron, menstrual losses in women and total absolute iron requirements

Age/Sex	Growth needs mg/day	Basal loss mg/day	Menstruation mg/day	Absorbed iron required, mg/day (95th percentile)
Infants and children				
0.5 - 1 years	0.55	0.17	-	0.93
1 - 3 years	0.27	0.19	-	0.58
4 - 6 years	0.23	0.27	-	0.63
7 - 10 years	0.32	0.39	-	0.89
Males				
11 - 14 years	0.55	0.62	-	1.46
15 - 17 years	0.60	0.90	-	1.88
18+ years	-	1.05	-	1.37
Females				
11 - 14 years*	0.55	0.65	-	1.40
11 - 14 years	0.55	0.65	0.48	3.27
15 - 17 years	0.35	0.79	0.48	3.10
18+ years	-	0.87	0.48	2.94
Postmenopausal	-	0.87		1.13
Lactating	-	1.15		1.50

* Non-menstruating
Source: WHO/FAO (2004)

The RNIs for iron for Malaysia are given in bold in the following paragraphs according to age groups and summarised in Appendix 18.1.

Infants (0-5 months)

Infants ages 0-5 months have a considerable endowment of iron (80 mg/kg) from the mother and possess a high haemoglobin concentration. Iron requirements during the first 4-6 months of life of full-term infants can be met by iron provided through breast milk as the total body iron of infants at birth and 4 months of age remain essentially the same. Infants born prematurely or with low birth weight should receive additional iron from other dietary sources after two months from birth.

Thus, for 0-5 months old infants, there will be no recommended iron intakes as neonatal stores are adequate to meet iron requirement during this age period, and breast feeding is fully encouraged (WHO/FAO, 2004)

Infants (6-11 months)

Infants aged 6-11 months have an increase in body iron need due to increase in basal iron losses, growth and the decrease in iron store from birth. In addition to breast milk, iron needs must be met from foods. Total iron requirements is calculated by taking into consideration basal iron losses (about 0.17 mg/day) and iron required for growth (0.55 mg/day) which lead to total iron required of 0.93 mg/day (95th percentile) (WHO/FAO, 2004). During this period, the infant should be introduced to complementary foods.

	% Bioavailability	
	10	**15**
RNI for infants, mg/day		
6 - 11 months	**9**	**6**

Children

The mean increase of weight from age 2 till onset of puberty is averaged 2.5-2.75 kg/year (Bothwell *et al.,* 1979) which is equivalent to iron requirement for growth of 0.3 mg/day. Basal iron losses range from 0.2 to 0.4 mg/day. The total iron required (95th percentile) for the age ranges 1-3, 4-6 and 7-9 years old are 0.58 mg/day, 0.63 mg/day and 0.89 mg/day, respectively. It is assumed that children by the second half of their second year of life would have started to eat with the families.

	% Bioavailability	
	10	**15**
RNI for children, mg/day		
1 - 3 years	**6**	**4**
4 - 6 years	**6**	**4**
7 - 9 years	**9**	**6**

Adolescents

The major physiological event occurring in this age group is puberty. The associated physiological processes that have major impacts on iron requirements are growth spurt in both sexes, menarche in girls and major increases in haemoglobin concentrations in boys. Due to differences in requirements, the recommendations are specified separately for boys and girls.

At low bioavailability levels of 5% and 10%, iron needs (especially for female adolescent) are exceedingly high and would be difficult to be met by the usual plant-based diets of Malaysians. Thus, it is important that dietary advice on choice of diets be made or iron supplementation be recommended.

		% Bioavailability	
		10	15
RNI adolescents, mg/day			
Boys	**10 - 14 years**	**15**	**10**
	15 - 18 years	**19**	**12**
Girls	**10 - 14 years***	**14**	**9**
	10 - 14 years	**33**	**22**
	15 - 18 years	**31**	**21**

*non-menstruating

Adults

In men and menopause women, basal iron loss is the only component used to estimate total needs for absorbed iron, which amounts to about 1.37 mg/day and 1.13 mg/day respectively (95th percentile). For menstruating women, the needs derived from basal iron loss (0.87 mg/day) and menstrual loss (1.90 mg/day) giving a total requirement of 2.94 mg/day (95th percentile). Based on the habitual diets of Malaysian adult, it is difficult for a menstruating woman to achieve the RNI for 5% bioavailability.

		% Bioavailability	
		10	15
RNI adults, mg/day			
Men	**19 years and above**	**14**	**9**
Women	**19 years and above**		
	Premenopause	**29**	**20**
	Postmenopause	**11**	**8**

Pregnancy

The components used to estimate requirement for absorbed iron include basal losses, iron deposited in foetus and related tissues, and iron utilized in expansion of haemoglobin mass. Iron requirements were reported to be 300 mg for the foetus, 50 mg for the placenta, 450 mg for the expansion of maternal red cell mass and 240 mg for basal iron losses, totalling to 1040 mg (Table 18.7). Net iron requirement in pregnancy was considered to be 840 mg, assuming sufficient iron stores (i.e. stores of 500 mg available during the last two trimesters). Total daily iron requirements were noted to increase during pregnancy from 0.8 mg to about 10 mg during

the last six weeks, and iron absorption was reported to increase during pregnancy. The FAO/WHO (2004) did not make any recommendation for iron during pregnancy as iron needs increase tremendously and generally beyond that can be adequately supplied by diets. Instead, iron supplementation (about 100 mg) is recommended for pregnant women.

Table 18.7: Iron requirements during pregnancy

	Iron requirements (mg)
Iron requirements during pregnancy	
Foetus	300
Placenta	50
Expansion of maternal erythrocyte mass	450
Basal iron losses	240
Total iron requirement 1040	1040
Net iron balance after delivery	
Contraction of maternal erythrocyte mass +450	+450
Maternal blood loss -250	-250
Net iron balance +200	+200
Net iron requirements for pregnancy[a] 840	840

[a] Assuming sufficient material iron stores are present.
Source: WHO/FAO, 2004

Lactation

For lactating women, iron is secreted in breast milk. Adding this value of 0.3 mg/day to the basal loss of 0.87 mg/day, it is estimated that the iron requirements for lactating women to be about 1.1 mg/d (WHO/FAO, 2004). The Malaysian RNI also assumes that menstruation may resume after 6 months of exclusive breast feeding. Thus, lactating women with menstruation may have a higher iron requirement.

	% Bioavailability	
	10	**15**
RNI for lactation, mg/day		
0 - 3 months	**15**	**10**
4 - 6 months	**15**	**10**
7 - 12 months	**15**	**10**
7 - 12 months*	**32**	**21**

* For lactating women with menstruation

Discussions on revised RNI for Malaysia

The revised RNI for Malaysia for iron retained the 10% and 15% bioavailability levels of RNI (2005) based on the considerations explained in WHO/FAO (2004). It is believed that, the 10% would cover for population taking lower bioavailability and 15% bioavailability approximates the usual level of iron intake among Malaysian population as shown by MANS 2014 study.

18.8 Tolerable upper intake levels

The risk of systemic iron overload from dietary sources is negligible with normal intestinal function. However, iron in excess may lead to health problems due to its ability to generate reactive oxygen species via the Fenton reaction (McCord, 2004). The net effects are DNA damage, impaired synthesis of proteins, membrane lipids and carbohydrates, induction of proteases and altered cell proliferation. Excess free iron can react directly with unsaturated fatty acids and induce lipid hydroperoxidases to form alkoxyl and/or peroxyl radicals which in turn, impair severely cellular integrity leading to cell death. This destructive potential of iron has led to the suggestion that excess iron might play a role in the multi-step processes of carcinogenesis, pathogenesis of atherosclerosis and other lifestyle diseases (Siddique & Kowdley, 2012) as well as neurodegenerative disorders such as Parkinson's or Alzheimer's diseases (Xie *et al.,* 2014).

Acute large intakes of iron (e.g. 20 mg or more elemental iron/kg body weight), particularly without food, cause corrosive haemorrhagic necrosis of the intestinal mucosa, leading to loose stools and blood loss, hypovolaemic shock, damaging failure of systemic organs and death. Early clinical phenomena of this damage (gastritis, nausea, abdominal pain and vomiting) have been used to set exposure levels for health guidance (EFSA 2015). Chronic iron overload may occur in individuals affected by haemolytic anaemias, haemoglobinopathies or one of the haemochromatoses and results in increasing sequestration of iron in ferritin and haemosiderin in all tissues throughout the body. Eventually, the haemosiderin degrades releasing iron, which in turn causes oxidative architectural and functional tissue damage resulting in cardiomyopathy, arthropathies, diabetes mellitus and neurological disease. There is no evidence that heterozygotes for haemochromatoses are at an increased risk of iron overload compared with the rest of the population (EFSA, 2015).

Gastrointestinal side effects were selected as the critical adverse effects on which to base the UL for iron. Although gastrointestinal distress is not a serious side effect when compared with possible risk for vascular disease and cancer, the other side effects considered did not permit the determination of UL (IOM, 2001). According to Nutrient Reference Values for Australia and New Zealand (NHMRC, 2005), for infants and young children, an uncertainty factor (UF) of 3 was used to extrapolate from the lowest observed adverse effect level (LOAEL) to the no observed adverse effect level (NOAEL) based on potential adverse growth effects (Dewey *et al.,* 2002), giving a figure of 20 mg/day. However, according to IOM 2001, the median intake of iron for infants, aged 11 to 14 months, is approximately 10 mg/day. Thus, the above data suggest that an intake of 40 mg/day would be a NOAEL for infants and young children. The NOAEL of 40 mg/day was divided by a UF of 1, resulting ina UL of 40 mg/day of supplemental non-haem iron for infants and young children. Because the safety of excess supplemental non-

haem iron in children aged 4 through 18 years has not been studied, a UL of 40 mg/day is recommended for children 4 through 13 years of age. The upper tolerable level is set at 45 mg/day for adolescents and adults (including pregnant and lactating women) based on a LOAEL for gastrointestinal side effects observed in Swedish adults following supplementation with ferrous fumarate (60 mg/day) in addition to an estimated dietary iron intake of 11 mg/day and using an uncertainty factor of 1.5.

Tolerable upper intake levels (UL)

Infants and children (0 - 13 years)	40 mg/day
Adolescents and adults (14 years and above)	45 mg/day

18.9 Research recommendations

The following priority areas of research are recommended:

- Periodic assessment of the iron status of vulnerable groups, different physiological states on iron requirements such as young children, women of reproductive ages, overweight, obesity and elderly especially in poor communities.
- Dose-response data be generated for iron intake/status and functional outcomes/health endpoints, e.g. growth and development in children, pregnancy outcome, etc.
- Studies on iron bioavailability of mixed diets among various ethnic, socioeconomic and vegetarian groups.
- Intervention studies to determine the efficiency and efficacy of long-term supplementation of iron among women of reproductive age.

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Appendix 18.1 Comparison of recommended intake for Iron:, RNI Malaysia (2017), RNI of WHO/FAO (2004), RDA of IOM (2001), and DRV of EFSA (2015)

Malaysia 2017*			WHO/FAO (2004)					IOM (2001)		EFSA (2015)	
Age group	RDA (mg/day)		Age group	RNI (mg/day)				Age group	RDA (mg/day)	Age group	DRV (mg/day)
	% Bioavailability			% Bioavailability							
	10	15		5	10	12	15				
Infants			**Infants**					**Infants**		**Infants**	
0 - 5 months	a	a	0 - 6 months	a	a	a	a	0 - 6 months	0.27[f]	7 - 12 months	11
6 - 12 months	9	6	7 - 12 months	19	9	8	6	7 - 12 months	11		
Children			**Children**					**Children**		**Children**	
1 - 3 years	6	4	1 - 3 years	13	6	5	4	1 - 3 years	7	1 - 3 years	7
4 - 6 years	6	4	4 - 6 years	13	6	5	4	4 - 8 years	10	4 - 6 years	7
7 - 9 years	9	6	7 - 9 years	18	9	7	6			7 - 11 years	11
Boys			**Boys**					**Boys**		**Boys**	
10 - 14 years	15	10	10 - 14 years	29	15	12	10	9 - 13 years	8	12 - 17 years	11
15 - 18 years	19	12	15 - 18 years	38	19	16	12	14 - 18 years	11		
Girls			**Girls**					**Girls**		**Girls**	
10 - 14 years[b]	14	9	10 - 14 years [b]	28	14	12	9	9 - 13 years	8	12 - 17 years	13
10 - 14 years	33	22	10 - 14 years	65	33	28	22	14 - 18 years	15		
15 - 18 years	31	21	15 - 18 years	62	31	26	21				
Men			**Men**					**Men**		**Men**	
19 - 65 years	14	9	19 - 65 years	27	14	11	9	19 - 30 years	8	≥ 18 years	11
> 65 years	14	9	> 65 years	27	14	11	9	31 - 50 years	8		
								51 - 70 years	8		
								> 70 years	8		

Malaysia 2017)*			WHO/FAO (2004)					IOM (2001)		EFSA (2015)	
Age group	RDA (mg/day)		Age group	RNI (mg/day)				Age group	RDA (mg/day)	Age group	DRV (mg/day)
	% Bioavailability			% Bioavailability							
	10	15		5	10	12	15				
Women			**Women**					**Women**		**Women**	
19 - 50 years [c]	29	20	19 - 50 years [c]	59	29	24	20	19 - 30 years	18	≥ 18 years	
51 - 65 years [d]	11	8	51 - 65 years [d]	23	11	9	8	31 - 50 years	18	Premenopausal	16
> 65 years [d]	11	8	> 65 years	23	11	9	8	51 - 70 years	8	Postmenopausal	11
								> 70 years	8		
Pregnancy			**Pregnancy**					**Pregnancy**		**Pregnancy**	
1st trimester	e	e	1st trimester	e	e	e	e	14 - 18 years	27	As for non-pregnant premenopausal women	
2nd trimester	e	e	2nd trimester	e	e	e	e	19 - 30 years	27		
3rd trimester	e	e	3rd trimester	e	e	e	e	31 - 50 years	27		
Lactation			**Lactation**					**Lactation**		**Lactation**	
0 - 3 months	15	10	0 - 3 months	30	15	13	10	14 - 18 years	10	As for non-lactating premenopausal women	
4 - 6 months	15	10	4 - 6 months	30	15	13	10	19 - 30 years	9		
7 - 12 months	15	10	7 - 12 months	30	15	13	10	31 - 50 years	9		
7 - 12 months [g]	32	21									

*Recommendation similar to RNI (2005)

a - Neonatal iron stores are sufficient to meet the iron requirement for the first six months in full term infants. Premature infants and low birth weight infants require additional iron.

b - Non-menstruating adolescents c - Pre-menopausal d - Menopausal e -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-anaemic pregnant woman, 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. f - Average intake g - Lactating women with menstruation

19 • Iodine

19.1 Introduction

Iodine was discovered by Bernard Courtois in 1811. As the human body is unable to produce iodine for its needs, iodine must come from dietary sources. Oceans are the world's main repositories of iodine and very little of earths iodine is actually found in the soil. The deposition of iodine in the soil occurs due to volatilization from ocean water, a process aided by ultraviolet radiation. The coastal regions of the world are much richer in iodine content than the soils further inland; here the problem gets more compounded by continuous leeching of iodine from the soil including through flooding. Therefore, the crops grown in such soil remain iodine deficient; even ground water in these areas is deficient in iodine.

19.2 Functions

Iodine is an essential trace element required for the synthesis of the thyroid hormones, thyroxine (T^4) and triiodothyronine (T^3). The human body contains 15-20 mg of iodine with 70-80% being located in the thyroid gland. Iodine that is taken up in the thyroid gland is oxidized by hydrogen peroxide and thyroid peroxidase. The oxidized (active) iodine is attached to a glycoprotein called thyroglobulin. The active iodine reacts with the tyrosine components of thyroglobulin to form 3-monoiodotyrosine (MITs) and 3,5-diiodotyrosine (DITs). The MITs and DITs are coupled to form the active thyroid hormones, T^3 and T^4. These thyroid hormones are stored inside the thyroid follicles as the main component of the thyroid colloid. The colloid is a gycoprotein called thyroglobulin. The stored hormones can meet the body requirements for up to 3 months.

Thyroid hormones are essential for life as they regulate key biochemical reactions, especially protein synthesis and enzymatic activities, in target organs such as are the developing brain, muscle, heart, pituitary and kidney.

19.3 Metabolism

Iodine is metabolized in the human body through a series of stages with the thyroid gland playing a central role. Thyroid secretion is regulated by pituitary gland through thyroid-stimulating hormone (TSH), which operates on a feed-back mechanism tuned to T^4 level in blood. A fall in T^4 level stimulates the pituitary to increase its TSH secretion, which in turn stimulates the thyroid gland to release T^4 in circulation to maintain normal level of the hormone in blood.

Proteolytic enzymes subsequently release T^3 and T^4, from thyroglobulin into the blood. When these hormones are utilized in various body cells, they are replaced by T^3 and T^4 from the bound pool. The liberated iodine may be reutilized by the thyroid gland, while the remainder iodine is excreted in the urine. Typically, urine contains more than 90% of all ingested iodine. Most of the remainder is excreted in feces and a small amount may be lost in sweat (IOM, 2001).

19.4 Deficiencies

Iodine insufficiency induces an increase in TSH in response to decreased production of thyroid hormone. Goitre develops as a consequence of the stimulation action of TSH. Serious iodine deficiency also leads to functional and developmental abnormalities such as hypothyroidism. Hypothyroidism causes physical and mental retardation in infants and children. In neonate, iodine deficiency causes perinatal mortality, infant mortality and low birth weight. Severe iodine deficiency in the fetal and neonatal period may lead to cretinism, which is characterized by stunted growth, mental and other neurological retardation, and delay in development of secondary sex characteristics.

Inadequate iodine status in pregnant women can affect cognitive outcomes in chidren. Avon Longitudinal Study of Parents and Children (ALSPAC) found that children of women with an iodine-to-creatinine ratio of less than 150 µg/g were more likely to have scores in the lowest quartile for verbal IQ, reading accuracy and reading comprehension than were those of mothers with ratios of 150 µg/g or more (Sarah *et al.,* 2013).

In adults, iodine deficiency causes reduction in mental function and lethargy (WHO/ FAO, 2004). This diverse array of iodine deficiency problems in different age groups is described as Iodine Deficiency Disorder (IDD). IDD includes mental retardation, hypothyroidism, goitre, cretinism and varying degrees of other growth and developmental abnormalities as a result of iodine insufficiency (IOM, 2001).

Global iodine status continues to improve (IGN, 2015). The number of iodine deficient countries has more than halved in past decade. There have been no countries in the "severely deficient" category (i.e., with a median UIC <20 µg/L). This remarkable progress reflects a growing global awareness of IDD and the tremendous success of iodization programs especially Universal Salt Iodization (USI). However, IDD remains a significant public health problem in 25 countries.

In 1994-96, the Ministry of Health (MOH) Malaysia conducted a National IDD Survey involving almost 12,000 school children aged 8-10 years. Based on the WHO/ICCIDD reference, the survey found a goitre prevalence of 0.7% in Sarawak, 2.2% in Peninsular Malaysia and 18% in Sabah (Kiyu, Zainab & Yahaya, 1998; MOH, 2003). The median urinary iodine concentration was 66.0 µg/l in Sabah, 82.2 µg/l in the Peninsular and 126.0 µg/l in Sarawak. Sarawak was partially gazetted in some districts and sub-district to implement USI in 1990. However, monitoring at the state level found gazetted areas still showed a low iodine intake, and thus, the entire state was gazetted on July 3, 2008 to require the use of iodised salt. The iodine status of the state has improved since the implementation of USI.

As for Sabah, a state-level programme for USI was fully implemented by June 2000. The situation in Sabah has improved as monitoring of school children aged 8-10 years in 2002 found the median urinary iodine level at 240 µg/l.

In 2008, MOH Malaysia conducted another National IDD Survey that included almost 18,078 school children aged 8-10 years (MOH Malaysia, 2009). A total of 18,012 urine samples were collected and analysed for urinary iodine concentration. The overall median urinary iodine concentration for Malaysia was found to be within borderline adequacy at 109.0 µg/l. The median urinary iodine concentration was 101.9 µg/l in Sarawak, 104.1 µg/l in Peninsular Malaysia and 150.2 µg/l in Sabah. In the Peninsula, six states were found to be iodine deficient (median urinary iodine concentration <100 µg/l) namely, Kedah (85.2 µg/l), Penang (85.8 µg/l), Perak (77.6 µg/l) and Pahang (76.5 µg/l), Terengganu (78.7 µg/l) and Kelantan (76.9 µg/l).

Some of the causes for iodine deficiency in Malaysia include low iodine content of soil and water, inadequate iodine content in local foods, consumption of goiterogenic foods and low consumption of marine seafood.

The MOH Malaysia has taken action to prevent and control IDD through the implementation of universal salt iodization for the whole Malaysia, and continuing health and nutrition education.

19.5 Sources

The iodine content of foods depends on the iodine content of the soil in which it is grown. Seawater is a rich source of iodine. Seaweeds and fish, which thrive on seaweeds, are also rich in iodine. Populations living near the sea and consuming seaweeds and reef fish, such as the Japanese, have a high intake of iodine.

A wide variety of food contains iodine including eggs, meat, milk and milk products, cereal grains, dried legumes, dried vegetables and dried fruits, but the good sources are limited to marine fishes and shellfish. The iodine content of foods varies with geographic location, ranging widely from 30 µg/100 g to 800 µg/100 g (WHO/FAO, 2004). Hence, the average iodine content of foods from one country cannot be universally used for estimating the iodine intake for another population. Examples of iodine content in foods are shown in Table 19.1.

Table 19.1: Iodine content of foods

Food	µg/100g
Fish and shellfish	
Siput sedut (Snail)	850.9
Ikan bilis (Anchovy) dried	144.0
Kupang (Mussels)	113.2
Kepah (Clam)	92.7
Kerang (Cockles	92.6
Kembung (Mackerel, Indian) fried	56.1
Kembung (Mackerel, Indian) dried	50.7
Tenggiri (Mackerel, Spanish) raw	48.7
Selar (Trevally) raw - small	44.0
Kerapu (Cod, coral/Grouper) raw	41.7
Pari (Sting ray) raw	41.7
Kembung (Mackerel, Indian) grilled	37.4
Sardin (Sardine) raw	36.7
Selar (Trevally) raw - large	35.1
Ikan Merah (Snapper, red) raw	33.4
Ketam (Crab)	33.3
Kembung (Mackerel, Indian) boiled	33.0
Lala (Clam, Paphia undulata)	30.6
Kerisi (Bream, threadfin, Japanese) raw	25.6
Kembung (Mackerel, Indian) raw	24.8
Mabong (Mackerel, Island) raw	23.5
Udang kecil (Shrimp) raw	19.1
Udang galah (Prawn, Giant freshwater) raw	19.1
Siakap (Perch, sea/Barramundi) raw	14.3
Tilapia Merah (Bream, African, red) raw	12.8
Sotong (Squid)	11.5
Tilapia (Bream, African) raw	9.9
Udang besar (Prawn) raw	7.8
Keli (Catfish) raw	4.1
Eggs	
Telur ayam (Egg, chicken)	26.8
Telur itik (Egg, duck)	14.0
Meat	
Daging lembu (Beef)	7.0
Daging ayam (Chicken)	6.2

Food	Iodine (µg/100g)
Milk and milk products	
Susu Tepung (Milk, powder)	92.1
Keju (Cheese)	62.8
Krimer manis (Sweetened creamer)	39.2
Yogurt (Yogurt)	26.7
Susu segar (Milk, fresh)	20.2
Minuman yogurt (Yogurt drink)	19.5
Mentega (Butter)	10.4
Cereal, legumes and nuts	
Beras (Rice)	13.4
Tepung gandum (Wheat flour)	10.4
Beras pulut (Glutinous rice)	9.65
Barli (Barley)	4.5
Fruits and vegetables	
Rumpai laut (Seaweed) dried	1457.4
Bayam (Spinach)	29.7
Pisang (Banana)	19.6
Biji bunga teratai (Lotus seed)	13.3
Taugeh (Bean sprout)	5.5
Delima (Pomegranate)	4.6
Kedondong (Ambarella)	4.5
Lobak (Carrot)	4.4
Miscellaneous	
Biskut beras dengan rumpai laut (Rice crackers with seaweed)	154.6
Kerepek kentang dengan rumpai laut (Potato chips with seaweed)	46.6
Sushi (Sushi)	24.3
Garam beriodin (Iodized salt)*	2000-4000

Source: Salina Md Taib (2017) – PhD thesis (unpublished)
* Department of Statistics, Malaysia - Regulation 285, Food Act 1983 (Act 281) & Regulations (As at January 2014)

The National IDD Survey 2008 for Malaysia included determining consumption patterns of iodine rich foods. More than 90% of the school children consumed chicken eggs and sea fish as their sources of iodine. Chicken eggs and sea fish constituted the highest mean quantity daily intake of food high in iodine at 26.47 g and 24.05 g respectively. Chicken eggs and sea fish remained as highest mean quantity weekly intake of food high in iodine at 186.6 g and 169.2 g respectively.

Overall, only 7.0% of the households surveyed in Peninsular Malaysia consumed salt with iodine level more than 15 ppm, compared to 83% in Sabah (fully gazetted) and 43% in Sarawak (partially gazetted). Overall, nationally, only 15.5%. of the population consumed salt with iodine level more than 15 ppm.

19.6 Factors Affecting Iodine Requirements

Iodine is ingested in a variety of chemical form 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 traps approximately 60 µg per day iodine 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%. However, several minerals and trace elements (e.g iron, selenium, zinc and vitamin A) are essential for normal thyroid hormone metabolism (Zimmermann & Köhrle, 2002; Sonja, 2010). These micronutrients are necessary for the utilization of iodine for thyroid hormone synthesis. Selenium deficiency for example, inhibits the conversion of T4 to T3 in liver. Iron deficiency impairs thyroid metabolism leading to an inability to control body temperature. Due to vitamin A's role in iron absorption from the intestine and mobilisation of iron from the liver, inadequate vitamin A resulting in low body iron can lead to impaired production of thyroid hormone.

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 that are 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).

There are some medications for an overactive thyroid (anti-thyroid drugs) and high blood pressure that interact with iodine metabolism. These medications should be taken with caution or not to be taken at all when taking iodine supplements. Taking iodine supplements along with anti-thyroid drugs cause too much iodine in the blood. Too much iodine in the blood can cause side effects that affect thyroid functions. Also, as most iodine supplements contain potassium, taking potassium iodide along with some medications for high blood pressure may lead to a rapid loss of potassium from the body.

19.7 Setting Requirements and Recommended Intake

Indicators for estimating requirements for iodine includes using the daily uptake and release (turnover) of iodine in the body, urinary iodine excretion, thyroid size and iodine balance. Urinary iodine excretion, thyroid size using ultrasonography, thyroid stimulating hormone (TSH) and thyroglobulin (Tg) are the indicators recommended by the WHO/UNICEF/ICCIDD (2007) for assessing iodine nutrition worldwide.

The WHO/ FAO (2004) expert consultation and the IOM (2001) reports are the main references used by the Technical Sub-Committee (TSC) for minerals requirement. The rationale and approaches used by these reports were considered. Several local reports on the magnitude of IDD and the intervention measures taken were also considered as useful references.

Since there is lack of scientific data on iodine requirements in Malaysia, the TSC has recommended the approach used by the WHO/FAO (2004). The actual RNIs for Malaysia are based on the body weights of the local population (Introduction Chapter Table 1.1). The recommended intakes for iodine for Malaysians of various age groups are given in bold in the following paragraphs according to age groups.

Infants

No functional criteria of iodine status have been demonstrated that reflect response to dietary intake of iodine in infants. The DRI committee of IOM (2001) therefore based the recommended intake of iodine on an adequate intake that reflects the observed mean iodine intake of infants fed exclusively on human milk. The WHO/FAO (2004) Consultation also used a similar approach to estimating recommended intakes for this age group.

Iodine requirements in infancy are derived from the iodine content in human milk in infants growing at a satisfactory rate. The iodine content in human milk is a function of the iodine intake of the population. It has been reported to range from 20-330 µg/l in Europe and from 30-490 µg/l in the United States. Among population groups with severe iodine deficiency, it could be as low as 12 µg/l. An average human-milk intake of 750 ml/day would give an iodine intake of about 60 µg/day in Europe and 120 µg/day in USA. Positive iodine balance, which is required for the increasing stores of the thyroid, in the infant is achieved only when the iodine intake is at least 15 µg/kg/day in full-term infants and 30 µg/kg/day in pre-term infants (WHO/FAO, 2004). Using the reference weights for Malaysian infants, the TSC for Minerals and Trace Elements had recommended the intake of iodine for children to be as follows.

RNI for infants boys (full term)

0 - 2 months	**67.5 µg/day**
3 - 5 months	**105.0 µg/day**
6 - 8 months	**124.5 µg/day**
9 - 11 months	**138.0 µg/day**

RNI for infants girls (full term)

0 - 2 months	**63.0 µg/day**
3 - 5 months	**96.0 µg/day**
6 - 8 months	**114.0 µg/day**
9 - 11 months	**127.5 µg/day**

Children

The DRI Committee (IOM, 2001) had based the estimated requirement for iodine in children 1-9 years on two balance studies. RDAs were then calculated based on 140% of the requirement.

The daily iodine needs on a body weight basis decreases with age. The iodine recommendation by WHO/FAO (2004) for children is 6 µg/kg/day for ages 1-6 years and 4 µg/kg/day for ages 7-12 years. Using the mean body weight as that is deemed appropriate for Malaysian children, the TSC for Minerals and Trace Elements had recommended the intake of iodine for children to be as follows.

RNI for children boys

1 - 3 years	**73.2 µg/day**
4 - 6 years	**109.8 µg/day**
7 - 9 years	**101.6 µg/day**

RNI for children girls

1 - 3 years	**69.0 µg/day**
4 - 6 years	**109.2 µg/day**
7 - 9 years	**100.0 µg/day**

Adolescents and adults

In adolescents and adults, most dietary iodine eventually appears in the urine, so the urinary iodine concentration is a useful measure for assessing iodine intake. A urinary iodine concentration of above 100 µg/l corresponds to a dietary iodine intake of 150 µg/day. Urinary iodine values below 100 µg/l are associated with increases in thyroid size and serum TSH. WHO/UNICEF/ICCIDD (1996) proposed that 150 µg/day iodine in adolescents and adults (12 years and above) provides the necessary intake to maintain plasma iodide level above the critical limit of 0.10 µg/dL and maintain iodine stores of the thyroid. Similar to WHO/FAO (2004), the Malaysian RNI for iodine for adolescents and adults is calculated based on 2 µg/kg/day, making use of the mean body weights of the local population.

RNI for adolescent boys

10 - 12 years	**133.6 µg/day**
13 - 15 years	**99.2 µg/day**
16 - 18 years	**118.4 µg/day**

RNI for adolescent girls

10 - 12 years	**141.6 µg/day**
13 - 15 years	**93.0 µg/day**
16 - 18 years	**100.6 µg/day**

RNI for adults

Men

19 - 29 years	**122.8 µg/day**
30 - 59 years	**121.2 µg/day**
≥ 60 years	**116.2 µg/day**

Women

19 - 29 years	**105.8 µg/day**
30 - 59 years	**104.4 µg/day**
≥ 60 years	**99.0 µg/day**

Pregnancy & Lactation

The iodine requirement during pregnancy is increased to provide the needs of the foetus and to compensate for the increased loss of iodine in the urine resulting from an increased renal clearance of iodine during pregnancy. These requirements have been derived from studies of thyroid function during pregnancy and in the neonate under conditions of moderate iodine deficiency. These data indicated that the iodine intake required to prevent the onset of subclinical hypothyroidism of mother and foetus during pregnancy, and thus to prevent the possible risk of brain damage of the foetus, is approximately 3.5 µg/kg/day or 200 µg/day (WHO/ FAO, 2004). The TSC for Minerals and Trace Elements on Minerals decided to adopt the same recommendations for the proposed Malaysian RNI.

RNI for

Pregnancy	**200 µg/day**
Lactation	**200 µg/day**

Discussion on comparison of recommended intake of iodine

Compared to the iodine intake recommendations by WHO/ FAO (2004), IOM (2001) had recommended higher intakes for all age groups except for infants (7-11 months) and children (1-13 years). The RDA for pregnancy and lactation, particularly the latter, were also higher in the latter recommendation. The latter had also made the same recommendations for adults of all ages and also for both sexes as it was felt that there is no evidence to suggest that the average iodine requirement is altered with aging, or to have differences based on gender in adults.

The TSC for Minerals and Trace Elements had taken on the approach of the WHO/ FAO (2004) in deriving at RNI for iodine. However, as previously mentioned, the TSC had set the RNI based on the mean body weight of Malaysians. Hence, due to the lower reference body weights of Malaysians, the daily recommended iodine intakes are slightly lower than the values proposed by WHO/ FAO (2004) (Appendix 19.1).

19.8 Tolerable Upper Intake Levels

Most people are very tolerant to excess iodine intake from food with the exception of certain subgroups with autoimmune thyroid disease and iodine deficiency. High intakes of iodine from food, water and supplements have been associated with thyroiditus, goitre (due to increased TSH stimulation), hypothyroidism, hyper-thyroidism, sensitivity reactions, thyroid papillary cancer and acute responses in some individuals. Symptoms of acute iodine poisoning include burning of the mouth, throat, and stomach, abdominal pain, fever, nausea, vomiting, diarrhoea, weak pulse, cardiac irritability, coma and cyanosis (IOM, 2001).

The recommendations of IOM (2001) for the upper limits of intakes for iodine are given in Table 19.2.

Table 19.2: Tolerable upper intake levels (UL) for iodine for various age groups

Group	mg/day iodine
Infants	Not possible to establish
Children	
1 - 3 years	200
4 - 8 years	300
9 - 13 years	600
Adolescents, 14-18 years	900
Adults, ≥ 19 years	1,100
Pregnant women	
14 - 18 years	900
> 19 years	1,100
Lactating women	
14 - 18 years	900
> 19 years	1,100

Source: IOM (2001)

19.9 Research Recommendations

Priority areas of research recommended are as follows

- Determine iodine intake and food sources among communities of various ages.
- Relationship between iodine deficiency and mental/cognitive performance and immune status in children.
- Interactions of common micronutrient deficiencies with iodine and thyroid metabolism in infants and pregnant women.

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Appendix 19.1: Comparison of recommended intake for Iodine: RNI Malaysia (2017), RNI WHO/ FAO (2004), AI and RDA IOM (2001)

Malaysia (2017)		Malaysia (2005)		WHO/ FAO (2004)		IOM (2001)	
Age group	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**
Infants		**Infants**		**Infants**		**Infants**	
Boys							
0 - 2 months	67.5	0 - 5 months	90	0 - 6 months	90	0 - 6 months	110
3 - 5 months	105.0	6 - 11 months	120	7 - 12 months	135	7 - 12 months	130
6 - 8 months	124.5						
9 - 11 months	138.0						
Infants		**Infants**		**Infants**		**Infants**	
Girls		**Girls**					
0 - 2 months	63.0	0 - 5 months	90				
3 - 5 months	96.0	6 - 11 months	120				
6 - 8 months	114.0						
9 - 11 months	127.5						**RDA (µg/day)**
Children		**Children**		**Children**		**Children**	
Boys		**Boys**		**Boys**		**Boys**	
1 - 3 years	73.2	1 - 3 years	72	1 - 3 years	75	1 - 3 years	90
4 - 6 years	109.8	4 - 6 years	108	4 - 6 years	110	4 - 8 years	90
7 - 9 years	101.6	7 - 9 years	104	7 - 9 years	100		
Children		**Children**					
Girls		**Girls**					
1 - 3 years	69.0	1 - 3 years	72				
4 - 6 years	109.2	4 - 6 years	108				
7 - 9 years	100.0	7 - 9 years	104				
Adolescents				**Adolescents**		**Adolescents**	
Boys				**Boys**		**Boys**	
10 - 12 years	133.6	10 - 12 years	144	10 - 11 years	135	9 - 13 years	120
13 - 15 years	99.2	13 - 14 years	106	> 12 years	110	14 - 18 years	150
16 - 18 years	118.4	15 years	106				
		16 - 18 years	118				

Malaysia (2017)		Malaysia (2005)		WHO/ FAO (2004)		IOM (2001)	
Age group	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**	**Age group**	**RNI (µg/day)**
Adolescents		**Adolescents**		**Adolescents**		**Adolescents**	
Girls		**Girls**		**Girls**		**Girls**	
10 - 12 years	141.6	10 - 12 years	148	10 - 11 years	140	9 - 13 years	120
13 - 15 years	93.0	13 - 14 years	98	> 12 years	100	14 - 18 years	150
16 - 18 years	100.6	15 years	98				
		16 - 18 years	104				
Adults				**Adults**		**Adults**	
Men				**Men**		**Men**	
19 - 29 years	122.8	19 - 29 years	124	19 - 65 years	130	19 - 30 years	150
30 - 59 years	121.2	30 - 50 years	124	> 65 years	130	31 - 50 years	150
≥ 60 years	116.2	51 - 59 years	124			51 - 70 years	150
		60 - 65 years	124			> 70 years	150
		≥ 65 years	114				
Adults				**Adults**		**Adults**	
Women				**Women**		**Women**	
19 - 29 years	105.8	19 - 29 years	110	19 - 65 years	110	19 - 30 years	150
30 - 59 years	104.4	30 - 50 years	110	> 65 years	110	31 - 50 years	150
≥ 60 years	99.0	51 - 59 years	110			51 - 70 years	150
		60 - 65 years	110			> 70 years	150
		≥ 65 years	98				
Pregnancy				**Pregnancy**		**Pregnancy**	
1st trimester	200	1st trimester	200	1st trimester	200	14 - 18 years	220
2nd trimester	200	2nd trimester	200	2nd trimester	200	19 - 30 years	220
3rd trimester	200	3rd trimester	200	3rd trimester	200	31 - 50 years	220
Lactation				**Lactation**		**Lactation**	
0 - 3 months	200	1st 6 months	200	0 - 3 months	200	14 - 18 years	290
4 - 6 months	200	2nd months	200	4 - 6 months	200	19 - 30 years	290
7 - 12 months	200			7 - 12 months	200	31 - 50 years	290

20 • Zinc

20.1 Introduction

Zinc is the 24th most abundant element in the Earth's crust and exists as a stable divalent cation. Its essentiality was first established in plants and later in experimental animals and humans. Besides dietary sources, zinc is normally found in small amounts in nature (water, soil and rock). Zinc is also released into the environment (air, water and soil) through human activities such as mining, smelting, metal production and coal burning.

In biological systems, zinc exists as Zn^{2+} and is present in all organs, tissues, fluids and secretions in the body. The total body zinc content has been estimated to be 2 g in adults, ranging from approximately 1.5 g in women to 2.5 g in men. Nearly 90% of total body zinc is in muscle and bone and the rest is distributed in other organs such as prostate, liver, gastrointestinal tract, kidney, skin, lung, brain, heart, and pancreas (WHO/FAO, 2004).

20.2 Functions

Zinc as a nutrient has important catalytic, structural and regulatory functions (King and Cousins, 2014). It is an essential component of the catalytic site or sites of at least one enzyme in each of the six classes of enzymes. As a component of metallo-enzymes, zinc also provides structural integrity to the protein. It serves as a necessary structural component of DNA-binding proteins that contains zinc fingers. Finally, as an intracellular regulatory ion, zinc is involved in gene expression, synaptic signalling and apoptosis (programmed cell death) and protein kinase C activity.

The three general functions of zinc are related to cell division and growth, nutrient metabolism, gene expression, immuno-competence, neurobehavioral development and reproductive function. Zinc stabilises the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. It is a component of various enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. The presence of zinc is necessary in the making of genetic material, including transcription of DNA and translation of RNA. Zinc plays a central role in the immune system, affecting virtually all aspects of cellular and humoral immunity. Zinc also influences behaviour and learning performance as well as quantity and quality of sperms. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms.

20.3 Metabolism

Absorption of dietary zinc mostly occurs in the upper small intestine and is a function of the quantity of bioavailable zinc ingested. Zinc is excreted mainly in the faeces while other minor routes of endogenous zinc losses are urine and integument. There is no major storage site for zinc although liver and bone provide limited store of zinc for release into the circulation as needed. Zinc is transported in both portal and systemic circulation, mainly by albumin. There are metabolic zinc pools in body tissues and organs that exchange with plasma zinc and is thought to represent the most metabolically active portion of total body zinc. The zinc pool in soft tissues, particularly liver, rapidly exchanges with zinc in plasma and accounts for about 10% of total body zinc (Miller, Krebs & Hambidge, 2000).

20.4 Sources

Zinc is widely distributed in foods and is typically associated with the protein fraction and/ or nucleic fraction of food. Zinc is most abundant in foods high in protein such as organ meats, meat, poultry, fish and shellfish and lesser amounts in eggs and dairy products (Table 20.1). Nuts, seeds, legumes and whole grain cereals (especially bran and germ) have relatively high zinc content, while tubers, refined cereals, fruits and vegetables have less. Zinc from foods of animal origin has higher bioavailability than that from foods of plant origin due to the presence of phytate that is inhibitory of zinc uptake by the intestine. Zinc may also be added to processed foods and food supplements as salts of the divalent cation.

Table 20.1 Zinc content of foods.

Food	mg/100 g
Liver, kidney (beef, poultry)	4.2-6.1
Meat (beef, pork)	2.9-4.7
Poultry	1.8-3.0
Seafood	0.5-5.2
Eggs (chicken, duck)	1.1-1.4
Dairy (milk, cheese)	0.4-3.1
Seeds, nuts	2.9-7.8
Beans, lentils	1.0-2.0
Whole-grain cereals	0.5-3.2
Refined cereal grains	0.4-0.8
Bread (white flour, yeast)	0.9
Fermented cassava root	0.7
Tubers	0.3-0.5
Vegetables	0.1-0.8
Fruits	0-0.2

Source: IZiNCG (2004)

20.5 Deficiency/ Excess

Zinc deficiency can occur through at least five mechanisms - inadequate dietary intake, increased requirements, malabsorption, increased losses and impaired utilization. The primary cause of zinc deficiency is inadequate intake that may be due to low dietary intake or dependence on foods with little or poorly bioavailable zinc. As zinc requirement increases with growth and development, infants, children, adolescents, pregnant and lactating women are at high risk of zinc deficiency. Other groups at risk of zinc deficiency include elderly, individuals with malabsorption syndromes (Sprue, Crohn's disease and short bowel syndrome), vegetarians

and infants with acrodermatitis enteropathica. Decreased efficiency of zinc absorption, increased urinary zinc losses, low zinc bioavailability in diet, genetic defects to zinc transporter and reduced food intake attributed to poor appetite and altered taste perception are causes for zinc deficiency commonly observed in these at-risk individuals.

It has been estimated that 17.3% of the world's population is at risk of inadequate zinc intake. The prevalence of inadequate zinc intake could range from 7.5% in high-income regions to 30% or more in regions of South and Southeast Asia, Sub-Saharan Africa and Central America (Wessels and Brown, 2012). These estimations provide suggestive evidence of populations at risk of zinc deficiency. While severe zinc deficiency is rare, mild-to-moderate forms of zinc deficiency may be relatively common worldwide. However, the detection and estimation of prevalence of mild-to-moderate zinc deficiency have been difficult due to the lack of potential biomarkers of zinc status and specific clinical features of zinc deficiency.

Zinc deprivation impairs growth and development of infants, children and adolescents. Poor growth and development due to zinc deficiency has been attributed to its depression on appetite. Although there are multiple causes of linear growth retardation in children, stunting appears to be one of the main adverse effects of zinc deficiency. Zinc deficiency also contributes to child mortality and morbidity due to higher susceptibility to microbial and parasitic infections as the immune system is compromised (Wessels and Brown, 2012). In populations at risk of zinc deficiency, zinc supplementation is reported to prevent growth failure, improve linear growth and weight gain as well as reduce mortality and morbidity due to diarrhoea, lower respiratory tract infection or malaria among infants and children in developing countries (Brown *et al.,* 2009; Mayo-Wilson *et al.,* 2014; McDonald *et al.,* 2015; Suchdev *et al.,* 2016). Zinc supplementation also improves neurobehavioral functions as well as affect and mood of infants and children (IZiNCG, 2004)

Pregnant women are vulnerable to low zinc status due to the additional zinc demands associated with pregnancy and foetal development. It has been suggested that maternal zinc deficiency is associated with adverse maternal and foetal outcomes such as preterm labour and pregnancy induced hypertension, intrauterine growth retardation, low birth weight, poor neurobehavioral development and increased morbidity in low birth weight neonates. A recent review of 21 randomized control trials (Ota *et al.,* 2015) to assess the effects of zinc supplementation in pregnancy on maternal, foetal, neonatal and infant outcomes concluded that zinc supplementation during pregnancy was not significantly associated with improved maternal and pregnancy outcomes, except for preterm birth. However, the observed reduction in preterm births by zinc supplementation was primarily represented by trials involving low-income women with higher risk of poor nutritional status.

Zinc excess is less prevalent than zinc deficiency. Excessive zinc intakes, through either zinc supplement or contact with environmental zinc, can produce acute and chronic effects of toxicity. No evidence of adverse effects of intakes of naturally occurring zinc in food has been reported. Acute effects of zinc toxicity (200 mg zinc or more) can produce metallic taste, gastric distress, dizziness, vomiting, nausea, abdominal cramps and bloody diarrhoea. Chronic effects (100-300 mg zinc daily) in adults can result in reduction of immune functions and HDL cholesterol and impairment of copper status. Severe neurological diseases attributable to copper deficiency are also associated with chronic high zinc intakes (Hedera *et al.,* 2009).

20.6 Factors affecting zinc requirements

Dietary factors can affect the proportion of available zinc for absorption in the intestine and consequently zinc requirement (IOM, 2006; IZiNCG, 2004; Lonnerdal, 2000). There are several main dietary factors influencing zinc absorption and utilization, namely phytate (phytic acid), source and amount of protein in a meal and calcium. While phytate and dietary calcium inhibit zinc absorption, protein enhances absorption. Phytate is present in plant foods with a high content in whole grain cereals, legumes, nuts and seeds and a low content in fruits, leaves and vegetables. As phytate cannot be digested or absorbed, the zinc bound to phytate will also pass through the intestine unabsorbed. The inhibitory effect of phytate on zinc absorption appears to be dose-dependent. Foods that are high in fibre tend to be phytate-rich, however fibre itself is unlikely to negatively affect the zinc absorption.

The amount and source of protein influence zinc absorption. As protein content increases in a meal, a greater percentage of zinc is absorbed. Zinc absorption from a diet high in animal proteins is greater than from a diet high in plant proteins. Other protein sources such as whey protein and casein have enhancing and inhibitory effect on zinc absorption, respectively. In addition, zinc content in a meal will affect zinc absorption in that as the content of zinc in a meal increases, the fractional zinc absorption decreases. Also, low zinc diets will increase zinc absorption and retention.

The inhibitory effect of dietary calcium on zinc absorption occurs only in the presence of phytate-containing meals. Calcium may interact with zinc and phytate to form insoluble complexes in the intestinal tract, thus rendering zinc unavailable. A calcium-rich diet or long term use of calcium supplements appears to have no significant inhibitory effect on zinc absorption or status, provided intake of zinc is adequate. Similarly, the reduced calcium absorption with intake of zinc supplements is only observed when calcium intake is low.

Under most dietary conditions, interactions of zinc and other nutrients may not produce undesirable outcomes. Dietary sources of phosphorous e.g. phytate and phosphorous-rich proteins (milk casein), can bind zinc and decrease zinc absorption. High dose of iron supplements provided simultaneously with zinc supplements can reduce zinc absorption. The effect of iron on zinc will only occur when iron to zinc ratio is very high (e.g. 25:1). However, iron fortification of foods or long-term use of iron supplements does not impair zinc absorption or status. Chronic high zinc intake, particularly through zinc supplementation, could compromise copper status. The effect of zinc-folate interaction on folate absorption has been equivocal. However, folate supplementation does not appear to have a deleterious effect on zinc status. At present, there is no known nutrient-gene interaction that would affect zinc requirement. Although severe genetic polymorphism is responsible for the clinical syndrome of acrodermatitis enteropathica, the primary treatment is zinc supplement.

20.7 Setting requirements and recommended intakes of zinc

General considerations

The present recommended zinc values for Malaysian population are based essentially on the zinc intake recommendations of WHO/FAO (2004). The discussion also considered the zinc intake recommendations by IOM (2001, 2006), IZiNCG (2004) and EFSA (2014).

The factorial approach was used by IOM (2001, 2006), IZiNCG (2004), EFSA (2014) and WHO/FAO (2004) as the basis for computing the EAR of zinc for most age and physiologic groups. The EAR of zinc is the amount of zinc that must be absorbed to match the amount of endogenous zinc losses. The physiologic requirement is the amount of zinc that must be absorbed to counterbalance the sum of endogenous zinc lost through all routes of excretion plus the amount of zinc retained in newly accrued tissue (WHO/FAO, 2004). The estimation of zinc requirements by the factorial approach requires two stages namely; firstly the estimation of physiological requirements and second stage is the determination of the quantity of dietary zinc available for absorption.

Both intestinal and non-intestinal sources (urine, surface losses of skin/hair/nail/sweat) contribute to endogenous zinc losses. The estimated normative physiological requirements for absorbed zinc in adult men and women are estimated to be 1.4 mg/day and 1.0 mg/day, respectively by WHO/FAO (2004). Extrapolations from these values are done to estimate endogenous losses in children (6 months to 18 years). In these growing age groups, the rate of accretion and zinc content of newly formed tissues are also included in the estimation of their physiologic requirements. As for pregnancy and lactation, zinc retention during pregnancy and zinc concentration in human milk at different stages are also added to the requirement estimations.

The estimation of physiologic zinc requirement also requires reference body weights for various age groups. WHO/FAO (2004) and IZiNCG (2004) have utilized the NCHS/CDC 1977 growth reference that is more applicable to the international populations. IOM (2001), on the other hand applied reference body weights which represented those of North American population. For the Malaysian RNI, the reference body weights of Malaysian population are utilised for all age groups.

A summary of the dietary zinc absorption estimates used by the WHO/FAO consultation, the IOM DRI committee and IZiNCG consultation is shown in Table 20.2. The translation of estimates of absorbed zinc to dietary zinc requirement by WHO/FAO (2004) involved two considerations. First, the content of promoters and inhibitors of zinc absorption in the diet determines the fraction of dietary zinc that is potentially absorbable. Second, the efficiency of absorption of potentially available zinc is inversely related to zinc content in the diet. Based on these considerations and other data from zinc absorption studies, three categories of diets (high, moderate and low zinc bio-availability) with their respective fractional absorption rates (50%, 30% and 15%) were identified. For categorizing diets according to their potential bioavailability of zinc, the following criteria were used:

(i). High zinc bioavailability

The diet consists of refined foods low in cereal fibre, phytic acid content and phytate-zinc molar ratio < 5; has non-plant sources of zinc such as meats, fish, certain seafood and poultry.

(ii). Moderate zinc bioavailability

Mixed diet with animal or fish protein; lacto-ovo, ovo-vegetarian 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.

(iii). Low zinc bioavailability

The diet consists of high 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 Technical Sub-Committee (TSC) on Minerals and Trace Elements was of the opinion that the general diet of the Malaysian population is closer to that described for moderate zinc bioavailability. Thus, the TSC recommended adopting the WHO/FAO (2004) zinc values of diet with zinc absorption of 30% (moderate bioavailability).

Table 20.2: Estimated dietary zinc absorption by WHO/FAO (2004), IOM (2001) and IZiNCG (2004)

	WHO/FAO			IOM	IZiNCG	
Diet types represented	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 Years	Men and women 20+years	
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

a Refined diets low in cereal fibre 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)

Recommended intakes by age groups

Upon reviewing available information, the Technical Sub-Committee on Minerals has proposed the RNIs for zinc for Malaysia are given in bold in the following paragraphs according to age groups and summarised in Appendix 20.1.

Infants (0 - 5 months)

No functional criteria of zinc status have been demonstrated that reflect response to dietary intake in infants. In relation to body weight, children appear to have larger losses of zinc than adults (EFSA, 2014). The recommended intakes of zinc were based on observed mean zinc intake of infants exclusively fed human milk. IOM (2006) based its adequate intake (AI) on the average amount of zinc transfer in breast milk from 0-5 months post-partum. Thus, an AI of 2.0 mg/day (2.5 mg/l x 0.78l/day) is proposed for infants in this age group. IZiNCG (2004) concluded that the amount of zinc from breast milk is adequate for the exclusively breastfed, normal birth-weight term infants up to 6 months of age. Consequently, IZiNCG set the AI of zinc

from breast milk as 1.64 mg/day (2.3 mg/l x 0.714 l/day) for 0-2 months and 1.06 mg/day (1.35 mg/l x 0.784 L/day) for 3-5 months. EFSA (2014) estimated physiological zinc requirement for infants aged 7 to 11 months as 0.73 mg/day, whereby the average requirement (AR) for infants as 2.4 mg/day. Due to no reference body weights and no knowledge on the variation in requirement, the population requirement intake (PRI) for infants was estimated at 2.9 mg/day (CV of 10 %)

WHO/FAO (2004) estimated the physiologic requirement of this age group by extrapolating from metabolic rate data for adults and adding the zinc content in newly deposited tissues. In human-milk fed infants, the endogenous zinc losses were assumed to be 20 µg/kg/day while that of infants fed formula or weaning foods was 40 µg/kg/day. For infant growth, estimated zinc increases were set at 120 and 140 µg/kg/day for female and male infants respectively for the first 3 months. The recommended zinc intakes are based on the sources of milk, assuming that human milk has the highest zinc bioavailability followed by a mix of whey adjusted milk formula and partly human-milk or low-phytate food supplements with other liquid milks (moderate bioavailability) and phytate-rich vegetable protein based formula (soy) with or without whole grain cereals (low bioavailability).

For the Malaysian RNI, the TSC for Minerals and Trace Elements recommends zinc intakes to be based on sources of milk with the highest (human milk) and moderate (infant formula) bioavailability levels of 80% and 30% (WHO/FAO, 2004), respectively.

RNI for infants

0 - 5 months	**Breast fed**	**1.1 mg/day**
	Formula fed	**2.8 mg/day**

Infants (6 - 11 months)

To obtain the estimated average requirement (EAR) for infants of 6-11 months of age, IOM (2001) and IZiNCG (2004) have utilized the same fractional zinc absorption from human milk as 50%. The amount of absorbed zinc required from complementary foods is determined as a difference between the required absorbed zinc and the amount of zinc ingested from the milk. The EAR for infants (breastfed and non-breastfed) is then calculated as the amount of zinc from human milk plus the amount of zinc from complementary foods, assuming fractional absorption of zinc is 30% from complementary foods. By 7 and 12 months of age, human milk provides only 0.5 mg zinc/day and 0.39 mg zinc/day, respectively. The NHANES III data indicated that the median zinc intake from complementary foods is 1.48 mg/day for older infants consuming human milk. EFSA (2014) estimated the endogenous faecal zinc loss for this age group as 0.34 mg/day and urine loss as 0.05 mg/day, which were extrapolated from adult values.

WHO/FAO (2004) estimated the physiologic zinc requirement for this age group based on extrapolations from the data used to estimate the endogenous zinc losses in adults. Thus, an average loss of 0.57µg/basal kcal was derived for this age group. The estimated zinc increase for this age group was set at 33 µg/kg/day. Different physiologic requirements (µg/kg body weight/day) were set for different levels of zinc bioavailability.

For the Malaysian RNI, assuming that infants in this age group are already supplemented with diets of moderate zinc bioavailability, the physiologic requirement for zinc is set at 0.311mg/kg body weight/day as recommended by WHO/FAO (2004). Thus, the recommended nutrient intake based on the reference body weight is as per below.

RNI for infants

Boys

6 - 11 months	**4.1 mg/day**

Girls

6 - 11 months	**3.7 mg/day**

Children and adolescents

IOM (2001) and IZiNCG (2004) calculated the endogenous zinc losses and amount of zinc required for growth for children in this age group as 0.034mg/kg body weight/day and 0.020 mg/g of tissue gained, respectively. An additional 0.1 mg/day was included in the estimated physiologic requirements for male and female adolescents 14-18 years to account for zinc losses in the semen and menses as well as zinc required for synthesis of new tissue for growth. These values for endogenous losses and zinc required for growth are then multiplied respectively by the reference body weight and the expected rate of weight gain for the respective age groups. In EFSA (2014), the estimation of physiological zinc requirement for children and adolescent were presented in addition with average requirement (AR).

WHO/FAO (2004) estimated the physiologic zinc requirement of this age group by extrapolating from estimations on endogenous losses in adults. Similar to infants 6-11 months, an average loss of 0.57 µg/basal kcal was utilized. For 1-10 years of age, 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. WHO/FAO consultation did not include zinc loss in semen.

Different physiologic requirements (ug/kg body weight/day) were set for different levels of zinc bioavailability. Assuming Malaysian mixed-diet to have moderate zinc bioavailability, the physiologic requirements for zinc for the various age groups are estimated as follows: 1-3 year (0.230 mg/kg body weight/day); 4-6 year (0.190 mg/kg body weight/day); 7-9 year (0.149 mg/kg body weight/day); 10-12 years (M), 0.140 (mg/day); 10-12 years (F) 0.119 (mg/day); 13-15 years (M), 0.125 (mg/day); 13-15 years (F), 0.111 (mg/day), and 16-18 (M), 0.111 mg/day; 16-18(F), 0.102 (mg/day). Using these age-specific physiologic requirements and the reference body weights of Malaysian children and adolescent, the proposed Malaysian RNIs of zinc are as below:

RNI for children

Boys

1 - 3 years	**4.2 mg/day**
4 - 6 years	**5.2 mg/day**
7 - 9 years	**5.7 mg/day**

Girls

1 - 3 years	**4.0 mg/day**
4 - 6 years	**5.2 mg/day**
7 - 9 years	**5.6 mg/day**

RNI for adolescents

Boys

10-12	**7.0 mg/day**
13-15	**9.3 mg/day**
16-18	**9.9 mg/day**

Girls

10-12	**6.3 mg/day**
13-15	**7.7 mg/day**
16-18	**7.7 mg/day**

Adults

The estimation of physiological requirements for absorbed zinc in adult men and women by WHO/FAO (2004), IOM (2001), IZiNCG (2004) and EFSA (2014) were considered. Total endogenous losses for men and women differed among the three committees with the values set by IZiNCG (M - 2.69 mg/day; F - 1.86 mg/day) intermediate to the low values proposed by WHO/FAO (M - 1.4 mg/day; F - 1.0mg/day) and the high levels of IOM (M - 3.84 mg/day; F - 3.30 mg/day). For most estimation of intestinal and non-intestinal endogenous zinc losses, IZiNCG (2004) and IOM utilized a similar conceptual approach. For example, while WHO/FAO (2004) did not include zinc loss in semen and estimated intestinal loss of endogenous zinc based on the results from one study, IZiNCG (2004) and IOM (2001) included zinc loss in semen and reviewed a larger number of studies to estimate intestinal loss of endogenous zinc. The differences in the calculation of total endogenous losses for men and women by these committees resulted in different estimates of average requirement for zinc and the consequent recommended intake of zinc. In EFSA (2014), the recommended daily allowances of zinc requirement for adults were estimated with different levels of phytate intake namely 300, 600, 900 and 1200 mg/day.

The requirements for the elderly (> 65 years) are estimated in the same way as those for other adults. A higher requirement may be necessary for the elderly due to lower efficiency of zinc absorption. On the other hand, endogenous losses seem to be lower in the elderly. Hence, the WHO/FAO consultation had recommended the same intakes for the elderly and the other adults.

The TSC for Minerals and Trace Elements recommended that the Malaysian RNI of zinc for men and women be based on the approach of WHO/FAO (2004) and the physiologic requirements for diets with moderate zinc bioavailability for men and women were 0.072 mg/kg body weight/day and 0.059 mg/kg body weight/day, respectively.

RNI for adults

Men

19-29 years	**6.6 mg/day**
30-59 years	**6.5 mg/day**
60-64 years	**6.3 mg/day**
> 65 years	**6.2 mg/day**

Women

19-29 years	**4.7 mg/day**
30-59 years	**4.6 mg/day**
60-64 years	**4.4 mg/day**
> 65 years	**4.3 mg/day**

Pregnancy and Lactation

Physiologic zinc requirement increases during pregnancy due to accrual of foetal and maternal tissues. For this purpose, IOM (2001) estimated additional zinc requirements of 0.16 mg/day for the first trimester, 0.39 mg/day for the second trimester and 0.63 mg/day for third trimester. EFSA (2014) estimated an additional value of 1.6 mg/day for all trimesters of pregnancy and 2.9 mg/day for lactation. WHO/FAO (2004) provided estimates of 0.10 mg/day for first trimester, 0.30 mg/day for second trimester and 0.70 mg/day for third trimester. IZiNCG (2004) proposed 0.7 mg/day as additional zinc requirement for all trimesters of pregnancy that may overestimate the average requirements for absorbed zinc in the first and second trimesters. This amount should be added to the age-specific requirement for absorbed zinc of adolescents or adult women.

The amount of zinc transferred from mother to infant in human milk must be added to the physiologic requirement for absorbed zinc in lactating women. IOM (2001) proposed an estimate of 1.35 mg/day as an average additional amount of absorbed zinc required for lactation, after discounting approximately 1 mg/day of endogenous zinc (accumulated during pregnancy) for the first month of postpartum. Using a similar approach as IOM (2001), IZiNCG (2004) proposed an additional amount of absorbed zinc as 1.0 mg/day throughout lactation based on

the age-specific average milk volume transferred to the infants and the zinc concentrations in human milk. This amount should be added to the age-specific requirement for absorbed zinc of adolescents or adult women.

WHO/FAO (2004) estimated zinc concentrations in human milk as 2 - 3 mg/l at 1 month, 0.9 mg/l at 3 months and 0.7 mg/l at 4 months. Based on this, the consultation estimated the average additional zinc amounts during the first year of post-partum as 1.4 mg/day (0-3 months), 0.8 mg/day (3-6 months) and 0.5 mg/day (> 6 months).

The TSC for Minerals and Trace Elements decided to adopt the additional amounts of zinc recommended by WHO/FAO (2004) for pregnancy and lactation. The proposed Malaysian RNI of zinc, based on diets of moderate zinc bioavailability is as follows.

RNI for pregnancy

1st trimester	**5.5 mg/day**
2nd trimester	**7.0 mg/day**
3rd trimester	**10.0 mg/day**

RNI for lactation

0 - 3 months	**9.5 mg/day**
4 - 6 months	**8.8 mg/day**
7 - 12 months	**7.2 mg/day**

Discussion on revised RNI for Malaysia

The inclusion of zinc into the RNI for Malaysia is justified due to its important role in human health and nutrition. The estimate of zinc requirement is required to obtain the obligatory losses and absorption for growth, pregnancy, or lactation. Nevertheless, discrepancies between four expert committees exist due to reference body weights used, studies selected, approaches to estimate endogenous faecal zinc (EFZ) losses, the adjustments in deriving dietary zinc requirements and so forth. The different approaches in estimating physiologic zinc requirement and dietary zinc absorption among WHO/FAO (2004), IOM (2001, 2006), IZiNCG (2004) and EFSA (2014) resulted in similar recommended zinc values by IOM and IZiNCG, which are rather different from those recommended by WHO/FAO. The EFSA recommended values are slightly higher compared to the other expert committees (Appendix 21.1). EFSA is the only expert group that set dietary recommendation based on dietary phytate for adults compared to other expert committees (WHO, IOM and IZiNCG). It is known that phytate impacts zinc requirement, therefore, EFSA adopted tri-variate model of Miller et al (2007) to examine the relationship between total absorbed zinc, total dietary zinc and the dietary phytate content.

From birth until early adolescence, the recommended zinc values set by IOM and IZiNCG tend to lie between the moderate to high zinc bioavailability values of WHO/FAO. During adolescence, adulthood, pregnancy and lactation, the values of IOM and IZiNCG are between the low to moderate zinc bioavailability values of WHO/FAO, however, EFSA values were comparatively higher than others.

The Malaysian RNI is based on the approach by WHO/FAO for diets with moderate zinc bioavailability but adjusted according to the local reference body weights. It is noted that for the same age groups (>10 years, males and females), the RNI values are lower than those in WHO/FAO due to the lower reference body weights of Malaysians.

20.8 Tolerable upper intake levels

The tolerable upper intake levels for zinc as proposed by WHO/FAO (2004), IOM (2006) and IZiNCG (2004) is shown in Table 20.3. The WHO/FAO (2004) set the upper level of zinc intake for an adult man at 45 mg/day.This level was then extrapolated to other groups in relation to basal metabolic rate. For children, this resulted in an upper limit of intake of 23-28 mg/day. IOM (2001, 2006) based the upper intake level for adults on 60 mg zinc/day, which was then extrapolated to 40 mg/day. For children, upper intake level was estimated based on a study of infants fed zinc-fortified infant formula or unfortified infant formula. The value was then extrapolated to older infants and children based on body weight. IZiNCG (2004) adopted the IOM value for upper intake level for adults but did not set upper intake level for children. Instead IZiNCG estimated no adverse effect level (NOAEL) values for children based on an Indonesia study of zinc supplementation for infants. EFSA (2014) has not set UL for any life-stage group but reported UL values based on the recommendations of the SCF (Scientific Committee on Food, 2006. The SCF set an upper level of 25 mg/day for adults, including pregnant and lactating women. For children (1-17 years old), the upper level of zinc intake was extrapolated from that of adults using body weight to the power of 0.75 and reference body weights for European children that yield an upper limit of 7-22 mg/day. The TSC for Minerals and Trace Elements recommended that the tolerable upper intake levels for zinc for Malaysian population be based on the approach of WHO/FAO (2004).

Table 20.3: Tolerable Upper Intake Level (UL) for zinc according to age groups by WHO/FAO (2004), IOM (2006) and IZiNCG (2004)

FAO/WHO (2004)		IOM (2006)		IZiNCG (2004)	
Age, Sex	Upper Limit (mg/day)	Age, Sex	Upper Limit (mg/day)	Age, Sex	Upper Limit (mg/day)
0-6 months	-	0-6 months	4	0-6 months	-
7-12 month	13	7-12 months	5	6-11 months	6
1-3 years	23	1-3 years	7	1-3 years	8
4-6 years	23	4-8 years	12	4-8 years	14
7-9 years	28				
10-12 years, M	34	9-13 years	23	9-13 years	26
10-12 years, F	32				
12-15 years, M	40				
12-15 years, F	36				
15-18 years, M	48	14-18 years, M	34	14-18 years, M	44
15-18 years, F	38	14-18 years, F	34	14-18 years, F	39
18-60 + years, M	45	> 19 years, M	40	> 90 years, M	40
18-60 + years, F	35	> 19 years, M	40	> 90 years, M	40

Source: Gibson, King & Lowe (2016)

20.9 Research recommendations

The following priority areas of research are recommended:

- Estimation of zinc requirement using radio or stable isotopes in different age and physiological groups.
- Identification of potential biomarkers of zinc status.
- Assessment of zinc status and dietary zinc intakes of population groups at risk of zinc deficiency.
- Assessment of possible outcomes of zinc deficiency (e.g. growth retardation, impaired immune status, pregnancy outcomes).
- Determination of zinc content and bioavailability of typical mixed diets of various ethnic and socioeconomic groups
- Investigation of the effects of zinc supplementations in specific population groups e.g. infants, young children, pregnant women and elderly.

20.10 References

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Appendix 20.1 Comparison of recommended intake for Zinc: RNI Malaysia (2017), RNI of WHO/FAO (2004), RDA of IOM (2000), IZiNCG (2004) and EFSA (2014)

Malaysia (2017)		Malaysia (2005)		WHO/FAO (2004)				IOM (2001)		IZiNCG (2004)			EFSA (2014)		
Age group	RNI (mg/day) Moderate bio-availability	Age group	RNI (mg/day)	Age group	RNI (mg/day) High bio-availability	RNI (mg/day) Moderate bio-availability	RNI (mg/day) Low bio-availability	Age group	AI (mg/day)	Age group	AI (mg/day)		Age group	AI (mg/day)	PRI (mg/day)
Infants		**Infants**		**Infants**				**Infants**		**Infants**			**Infants**		
0-5 mths	1.1 (BF) 2.8 (FF)	0-5 mths	1.1 (BF)	0-5 mths	1.1 (BF)	2.8 (FF)	6.6 (FF)	0-6 mths	2	0-2 months		1.64	7-11 mths		2.9
Boys (mth)		**Boys (mth)**		6-12 mths	0.8 (FF) 2.5 *	4.1	8.3			3-5 months		1.06			
6-11	4.1	6-11	3.7												
Girls (mth)		**Girls (mth)**							**RDA (mg/day)**		**RDA (mg/day)** Mixed or refined vegetarian diets	Unrefined, cereal-based diets			
6-11	3.7	6-11	3.7												
								7-12 mths	3	6-11 mths	4	5			
Children		**Children**		**Children**				**Children**		**Children**			**Children**		
Boys		**Boys**		1-3 years	2.4	4.1	8.4	1-3 years	3	1-3 years	3	3	1-3 years		4.3
1-3 years	4.2	1-3 years	4.1	4-6 years	3.1	5.1	10.3	4-8 years	5	4-8 years	4	5	4-6 years		5.5
4-6 years	5.2	4-6 years	5.1	7-9 years	3.3	5.6	11.3			9-13 years	6	9	7-10 years		7.4
7-9 years	5.7	7-9 years	5.8												
Girls		**Girls**													
1-3 years	4	1-3 years	4.1												
4-6 years	5.2	4-6 years	5.1												
7-9 years	5.6	7-9 years	5.8												
Boys		**Boys**		**Boys**				**Boys**		**Boys**			**Boys**		
10-12 years	7	10-12 years	9.0	10-18 years	5.7	9.7	19.2	9-13 years	8	14-18 years	10	14	11-14 years		9.4
13-15 years	9.3	13-15 years	9.0					14-18 years	11				15-17 years		12.5
16-18 years	9.9	16-18 years	9.0												

Malaysia (2017)		Malaysia (2005)		WHO/FAO (2004)				IOM (2001)		IZiNCG (2004)		EFSA (2014)		
Age group	RNI (mg/day) Moderate bio-availability	Age group	RNI (mg/day)	Age group	RNI (mg/day) High bio-availability	Moderate bio-availability	Low bio-availability	Age group	AI (mg/day)	Age group	AI (mg/day)	Age group	AI (mg/day)	PRI (mg/day)
Girls		**Girls**		**Girls**				**Girls**		**Girls**		**Girls**		
10-12 years	6.3	10-12 years	7.5	10-18 years	4.6	7.8	15.5	9-13 years	8	14-18 years	9 11	11-14 years		9.4
13-15 years	7.7	13-15 years	7.5					14-18 years	9			15-17 years		10.4
16-18 years	7.7	16-18 years	7.5											
Men		**Men**		**Men**				**Men**		**Men**		**Men**		
19-29 years	6.6	19-29 years	6.7	19-65 years	4.2	7	14	19-30 years	11	> 19 years	13 19	> 18 years	300	9.4
30-59	6.5	30-59	6.7	>65 years	4.2	7	14	31-50 years	11				600	11.7
60-64	6.3	60-64	6.7					51-70 years					900	14
>65	6.2	>65	6.2					> 70 years					1200	16.3
Women		**Women**		**Women**				**Women**		**Women**		**Women**		
19-29 years	4.7	19-29 years	4.9	19-65 years	3	4.9	9.8	19-30 years	8	> 19 years	8 9	> 18 years	300	7.5
30-59	4.6	30-59	4.9	>65 years	3	4.9	9.8	31-50 years	8				600	9.3
60-64	4.4	60-64	4.9					51-70 years	8				900	11
>65	4.3	>65	4.3					> 70 years	8				1200	12.7
Pregnancy		**Pregnancy**		**Pregnancy**				**Pregnancy****		**Pregnancy**		**Pregnancy**		
1sttrimester	5.5	1sttrimester	5.5	1sttrimester	3.4	5.5	11	14-18 years	12	14-18 years	11 15			+1.6
2ndtrimester	7	2ndtrimester	7.0	2ndtrimester	4.2	7	14	19-30 years	11	> 19 years	10 13			13
3rdtrimester	10	3rdtrimester	10.0	3rdtrimester	6	10	20	31-50 years	11					
Lactation		**Lactation**		**Lactation**				**Lactation*****		**Lactation**		**Lactation**		
0-3 months	9.5	0-3 months	9.5	0-3 months	5.8	9.5	19	14-18 years	13	14-18 years	10 11			+2.9
4-6 months	8.8	4-6 months	8.8	4-6 months	5.3	8.8	17.5	19-30 years	12	> 19 years	9 10			
7-12 months	7.2	7-12 months	7.2	7-12 mths	4.3	7.2	14.4	31-50 years	12					

BF=breast fed FF=formula fed

* – not applicable to infants consuming human milk only

** – throughout the 1st, 2nd and 3rd trimesters

*** – throughout the first year of post-partum

21 • Selenium

21.1 Introduction

Selenium, a trace mineral found mainly in the soil, has received much attention in human nutrition in the last few decades due to better understanding of its role in human health and disease. Its essentiality was first observed in farm animals and later in humans when selenium-responsive diseases were attributed to the migration of the nutrient from the soil to the food chain. In the diet, the organic compounds of selenium (seleno-methionine and seleno-cysteine) predominate with lesser amounts in inorganic compounds (selenate and selenite). While there is a variation in selenium content of normal adult humans depending on geographic location, generally selenium body content is distributed in skeletal muscle (30-50%), bones (15%), plasma (10%), liver (8%), kidneys (3%) and brain (3%) (Zachara *et al.*, 2001).

21.2 Functions

Selenium from plant and animal food sources is absorbed in the intestine with selenium in the form of organic compounds is more efficiently absorbed than inorganic compounds. In the forms of selenomethionine and selenocysteine, more than 90% of selenium is estimated to be efficiently absorbed. Selenate and selenite, two inorganic forms of selenium, have roughly equivalent bioavailability which generally exceeds 50%. Most dietary selenium is highly bioavailable (50-80%) with absorption efficiency of selenium from usual diets is estimated at 70%. However, the absorption efficiency of selenium is not affected by selenium status or influences the homeostatic regulation of selenium (Sunde, 2012).

The physiological function of selenium is mainly due to selenocysteine which is integral for the synthesis of selenoproteins. Selenomethionine, on the other hand, has a physiological function similar to methionine in that it acts as a substitute for methionine residue in proteins. Currently there are 25 identified selenoproteins in humans that are involved in antioxidant activity against oxidative damage and inflammation, T-cell immunity for protection against infections, thyroid hormone metabolism, selenium homeostasis and transport, skeletal and cardiac muscle growth and function (Mehdi *et.al.,* 2013).

As selenium is part of the antioxidant system of the human body, it is likely to interact with other nutrients that protect against or promote oxidative damage to the cells (Sunde, 2012).

21.3 Metabolism

Upon absorption, selenocysteine, selenate and selenite are available for the synthesis of selenoproteins, the functional form of selenium. Selenomethionine is incorporated into body proteins in organs such as the skeletal muscles, red blood cells, pancreas, liver, kidney, stomach and the gastrointestinal mucosa. Selenium from selenomethionine may be mobilized for metabolic processes through conversion of selenomethionine to selenocysteine in the liver or kidney. Selenoprotein P (SEPP1) appears to play a central role in the delivery of selenium from the liver, via the plasma, to other tissues in the body. Selenium is excreted predominantly in the urine with other routes include breath, faeces and breastmilk (EFSA, 2014).

The interaction between selenium and vitamin E is commonly observed in many deficiency diseases in animals. In humans such data are still limited (Hurst *et al.,* 2013) although there is much interest in the interaction between vitamin E and selenium in relation to prevention of prostate cancer, with both being antioxidants (Kristal *et al.,* 2014). Other nutrients that interact with selenium include iodine for thyroid hormone metabolism, ascorbic acid for absorption and metabolism of selenium and methionine for body protein synthesis in methionine-deficient state WHO/FAO (2004).

21.4 Sources

Selenium content of many foods, particularly plant food sources, generally depends on environmental conditions and agricultural practices. For plant food sources, the transfer of selenium from the soil to the plants is influenced by selenium content of the soil as well as soil pH, redox potential and water content. For animal food sources, selenium content varies according to the diet of the animals. Table 21.1 shows selenium content of selected animal and plant food sources. Fish, meat (especially organ meats) eggs, milk and shellfish are good sources of selenium. In general, the higher the protein content of a food, the more selenium it contains. Other sources of selenium include water, selenium-enriched foods and food supplements. Food processing (e.g. milling) and cooking (e.g. boiling) can contribute to loss of selenium from foods.

Table 21.1: Selenium content of foods

Food	µg/100g
Meat and Meat Products	
Chicken, liver, boiled	100.20
Beef, liver, boiled	55.80
Mutton, raw, lean and fat	32.20
Chicken, drumstick, baked, lean	31.10
Sandwiches and burgers, roast beef sandwich with cheese	19.50
Beef, blade steak, raw, lean and fat	10
Satay, beef, frozen	5
Seafoods	
Ikan bilis	598.90
Oyster, raw	69.4
Prawn, king, cooked	52.9
Salmon, steamed	46.2
Kembong raw	10.2

Food	Selenium (µg/100g edible portion)
Cereal And Cereal Products	
Bread roll, wholemeal	11.60
Cream cracker, wholemeal	4.70
White rice cooked	4
Egg And Egg Products	
Egg, duck, whole, salted	24.04
Egg, hen, whole, hard boiled	11
Nuts And Seeds, Pulses And Products	
Nuts, brazil nuts, dried, unblanched	1917
Nuts, cashew nuts, oil roasted, with salt added	20
Lentils, dhal, yellow	8.30

Sources: Health Promotion Board (2011) Singapore

Dietary intake of selenium varies according to populations as factors such as selenium content of foods (i.e. amount and types of selenium compound), food consumption patterns (i.e. vegetarians) and the geographic origin of the food supply could (i.e. whether it comes from regions with selenium-rich or selenium-poor soils) have profound effect on this variability (WHO/FAO, 2004). The mean per capita/day intake of selenium of adults in most countries (except China) ranged from a low of 28 µg/day to a high of 220 µg/day (NRC, 1989; IOM, 2000). In the United States, the overall mean dietary selenium intake of adults between 1974 and 1982 was 108 µg/day with mean daily intake for each year ranged from 83 µg to 129 µg (NRC, 1989). Another study in the US reported selenium intake of 81 µg/day while a Canadian survey reported higher selenium intakes of 113 µg to 220 µg/day (Harshman and Aldoori, 2007). In Malaysia, Chinese (94.3+ 39.7µg) had the highest selenium intake followed by the Malays (83.4+28.9µg) and Indians (76.5+33.9µg) (Suzana *et al.,* 2009).

21.5 Deficiency/ Excess

The expression and function of seleno-proteins are compromised with selenium deficiency. Common observed symptoms of selenium deficiency in humans include skeletal myopathy, muscle weakness and cardiomyopathy. Keshan and Keshan-Beck diseases, characterized by degeneration of organs and tissues, are manifestations of selenium deficiency (Fairweather-Tait *et al.,* 2011). These conditions are prevalent among children, adolescence and young women in selenium-deficient areas or with low selenium intake within China, Mongolia, Siberia and North Korea. Other conditions whereby selenium deficiency is observed include children with phenylketonuria (PKU) on a low-protein diet and patients receiving selenium-free

total parenteral nutrition (TPN) (Cabrera. and Barba 2005). Various selenium supplementation studies, suggestive of correction of selenium-deficient state, have been conducted for prostate cancer, cardiovascular disease, diabetes mellitus, rheumatoid arthritis and Alzheimer disease (Loef,. Schrauzer & Walach, 2011; Stranges. *et al.,* 2007; Kristal *et al.,* 2014).

Excess intake of dietary selenium (> 1000 ug/day) over a long period of time can lead to selenosis, a condition of chronic excess of body selenium. Intakes of selenium as high as 6690 ug/day have been reported in China where areas are with naturally seleniferous soils (Kuila, Mukhopadhyay and Banerje, 2012). Characteristics of selenosis include skin rash, headache, garlicky breath, tooth decay and discoloration, hair loss, nausea, diarrhoea, fatigue, numbness, paralysis and hemiplegia and changes in nails (Cabrera and Barba, 2005). However, the levels of dietary exposure considered excessive or toxic and can lead to selenosis are difficult to establish due to selenium compounds in foods and interactions with other dietary components as well as genotype (Fairweather-Tait *et al.,* 2011).

As estimation of selenium intake from dietary assessment is imprecise due to variation in selenium content of foods, biomarkers of selenium intake or status become essential in human health and disease. These important markers of selenium intake, status or function include plasma/serum and whole blood selenium concentration, glutathione peroxidase (GPxs) activity in various blood components, plasma SEPP1 concentration, urinary selenium excretion, selenium concentration in hair and toe nails (Fairweather-Tait *et al.,* 2011). Other potential biomarkers are ratio of plasma triodothyroxine to thyroxine (T3:T4), plasma thyroxine and plasma total homocysteine concentration (Ashton *et al.,* 2009).

21.6 Factors affecting selenium requirement

As dietary selenium, both organic and inorganic forms, is highly bioavailable, selenium requirement is less likely to be affected by selenium bioavailability. Although selenium may interact with vitamin E, iodine, ascorbic acid, other antioxidants and methionine, the scarcity of available data may not allow suggestion that such interactions affect selenium requirement. In addition, the present knowledge on the effect of selenium-gene interaction on selenium status and requirement is limited (EFSA, 2014).

21.7 Setting Requirements and recommended intake of selenium

In Malaysia, hardly any nutritional study on the status of selenium has been carried out. Similarly, data on selenium content of Malaysian foods are equally lacking. Hence, in setting recommended intakes for Malaysians, the TSC on Minerals had referred to several major publications, namely the WHO/FAO Expert Consultation report of 2004, the DRI Committee of IOM (2000) and the WHO/FAO/IAEA (1996). The rationale and steps taken in setting requirements and the levels recommended by these organizations were considered. The TSC decided to adapt the approach and the recommendations of WHO/FAO (2004) as the revised RNI for Malaysia, given in bold in the following paragraphs according to age groups and summarized in Table 21.2.

Infants

No functional criteria of selenium status have been demonstrated that reflect response to dietary intake of infants. Thus the IOM (2000) had based the recommended intakes of selenium on adequate intake that reflects the observed mean selenium intake of infants fed principally with breast milk. Assuming an average selenium concentration of milk of well-nourished but unsupplemented mothers to be 18 g/l and the average volume of human milk to be 0.78 l, the adequate intake of selenium for this age group would be 14 µg/day, rounded to 15 µg by the DRI Committee. For the older infants, the IOM report had computed the adequate intake based on selenium in human milk plus that in infant foods. The computed intake was 20 g per day.

A similar approach was taken by WHO/FAO (2004) although the actual intakes recommended were lower than those of the IOM. The Consultation felt that the estimates of RNI for infants are compatible with estimates of the international reference range of selenium content in breast milk (18.5 µg/l) with data from an extensive international survey of breast milk selenium of WHO/FAO/IAEA (1996) and with WHO data on the milk consumption of exclusively human-milk-fed infants in developed and developing countries. Data from the WHO/FAO/IAEA (1996) survey from six countries suggest that the human milk from all countries met the RNI for infants aged 0-6 months. In two of six countries, Hungary and Sweden, the human milk selenium was marginal with respect to the RNI for infants aged 7-12 months.

The TSC on Minerals had recommended that intakes for infants 0-5 months and 6-11 months be calculated based on the WHO/FAO (2004) estimated selenium requirements of 0.85 µg/kg/day and 0.91 µg/kg/day, respectively, whilst making use of the reference weight for Malaysian infants.

RNI for Infants

Boys	
0 - 5 months	**6 µg/day**
6 - 11 months	**10 µg/day**
Girls	
0 - 5 months	**6 µg/day**
6 - 11 months	**9 µg/day**

Children

The IOM (2000) found no data that could be used to derive an estimated average requirement for selenium for children or adolescents. In the absence of additional information, the requirements and recommended intakes for children and adolescents were estimated based on extrapolation from adult values. The requirement was thus based on the same criteria of adequacy for adults, in that it would be expected to maximize plasma glutathione peroxidase activity.

In the case of the WHO/FAO (2004) consultation, 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 1-3 years, 4-6 years and 7-9 years, the estimated selenium requirements are 1.13 µg/kg/day, 0.92 µg/kg/day and 0.68 µg/kg/day, respectively. The TSC then used the reference weight for Malaysian children to compute the RNI for selenium for these age groups.

RNI for Children

Boys

1 - 3 years	**17 µg/day**
4 - 6 years	**21 µg/day**
7 - 9 years	**22 µg/day**

Girls

1 - 3 years	**16 µg/day**
4 - 6 years	**21 µg/day**
7 - 9 years	**21 µg/day**

Adolescents

The requirement for selenium is calculated as in children 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. Table 21.2 shows the estimated selenium requirements are based on 0.50 µg/kg/day for male adolescents 10-12 years, 13-15 years and 16-18 years (WHO/FAO, 2004) and the body weights of adolescents from local data. For female adolescents 10-12 years, 13-15 years and 16-18 years the estimated selenium requirements are based on 0.42 µg/kg/day (WHO/FAO, 2004) and the body weights of adolescents from local data.

RNI for Adolescents

Boys

10-12 years	**21 µg/day**
13-15 years	**31 µg/day**
16-18 years	**37 µg/day**

Girls

10-12 years	**19 µg/day**
13-15 years	**24 µg/day**
16-18 years	**26 µg/day**

Adults and elderly

IOM (2000) had determined the estimated requirements for selenium for adults based on the results of two intervention studies that were done in different countries but with similar designs. The Chinese study (Yang *et al.,* 1987) suggests that a plateau of plasma glutathione peroxidase activity was reached with a selenium intake of 41 µg/day. The New Zealand study (Duffield *et al.,* 1999) seem to suggest an estimated requirement of 38 µg/day. The DRI Committee took the average of these two studies and made a weight adjustment for North American males and arrived at a requirement of 45 µg/day. The RDA, computed as 120% of the requirement, is 55 µg for both men and women. For the elderly group, IOM did not recommend for additional intakes, noting that the aging process does not appear to impair selenium absorption or utilization.

Studies have been conducted with adult male subjects initially of low selenium status given a carefully monitored diet providing selenium at 11 µg/day together with supplements of seleno-methionine given orally which provided 0, 10, 30, 60, or 90 µg/day. Starting at frankly deficient levels, total daily selenium intakes of above 41 µg/day were found sufficient to increase plasma GSHPx substantially and to saturate plasma activity in 60-kg male subjects within 5-8 months. It was estimated that satisfactory levels of plasma selenium and of GSHPx indicative of adequate selenium reserves would be attained after intakes of approximately 27 µg/day by 65-kg male subjects (WHO/FAO/IAEA, 1996). Such criteria satisfying the definition of average normative requirements for selenium was used as the basis for calculating recommended nutrient intake (RNI) values by the FAO/WHO (2004) consultation after interpolating estimates of average requirements by allowing for differences in weight and basal metabolic rate of age groups to up to 65 years. A 25 percent increase (2 x assumed SD) was next added to allow for individual variability in the estimates of RNI.

Table 21.2 shows WHO/FAO (2004) estimated selenium requirement for adult men, 19-65 years of age is 0.42 µg/kg/day, while those > 65 years old is 0.41 µg/kg/day. For adult women (19-65 years and > 65 years), the requirement is set at 0.37 µg/kg/day. Thus, using reference body weights for adult Malaysian men and women respectively, the TSC on Minerals and Trace Elements had recommended that the Malaysian RNI for selenium for men and women are as follows.

RNI for adults

Men	
19-29 years	**32 µg/day**
30-59 years	**32 µg/day**
Women	
19-29 years	**25 µg/day**
30-59 years	**24 µg/day**

RNI for elderly

Men	
60-64 years	**31 µg/day**
≥65 years	**30 µg/day**
Women	
60-64 years	**23 µg/day**
≥65 years	**23 µg/day**

Pregnancy

Upon reviewing the literature, IOM (2000) found few studies that could provide information on the selenium requirements of pregnant women. However, the pregnancy requirement should allow accumulation of enough selenium by the foetus to saturate its seleno-proteins. Based on an estimated foetal deposition of 4 µg/day throughout pregnancy, the estimated requirement is increased by this amount during pregnancy. Since most selenium is highly bioavailable, no adjustment for absorption is felt necessary.

WHO/FAO/IAEA (1996) attempted to predict the increase of dietary selenium needed for pregnancy by factorial estimation of the likely quantity of selenium incorporated into the tissues of the foetus. It was assumed that the total products of conception amount to 4.6-6 kg lean tissue with a protein content of approximately 18.5-20 percent. If the selenium content of this protein resembles that of a skeletal muscle, growth of these tissues could account for between 1.0 and 4.5 µg/day of selenium. With an assumed absorption and utilization rate of 80 percent dietary selenium and allowing for a variability of estimates (CV 12.5 percent) an increase of 2 µg/day was felt appropriate for the second trimester and 4 µg/day for the third trimester of pregnancy (WHO/FAO, 2004).

RNI for pregnancy

1st trimester	**25 µg/day**
2nd trimester	**27 µg/day**
3rd trimester	**29 µg/day**

Lactation

Based on an estimated human milk selenium concentration of about 18 µg/l and a milk volume of 0.78 l per day, the average amount of selenium secreted in milk was estimated by the DRI committee to be 14 µg. Since most selenium in human milk is present as seleno-methionine, which has a bioavailability of greater than 90%, no adjustment was made for absorption. The IOM (2000) hence added 14 µg/day of selenium to the estimated requirement of non-pregnant and non-lactating women.

WHO/FAO (2004) estimated selenium requirement for lactating women from the estimated RNI for infants aged 0-6 months and 7-12 months. Assuming that the selenium of maternal milk is used with an efficiency of 80%, an individual variability of 12.5% and an estimated RNI for 0-6 months as 6 µg/day, the increase in maternal dietary selenium for the first 6 months of lactation is 9 µg/day. Using similar calculations, the increase in dietary selenium intake for months 7-12 is recommended to be 16 µg/day.

RNI for Lactation

0 - 3 months	**34 µg/day**
4 - 6 months	**34 µg/day**
7 - 12 months	**41 µg/day**

Table 21.2: Recommended Nutrient Intakes for Selenium, by age group.

Group	Reference body weight[a] (kg)	Average normative requirement[b]		RNI (µg/day)[c]
		Se $R^{normative}$ (kg/day)	Se $R^{normative}$ (total/day)	
Infants				
Boys				
0-5 months	5.8	0.85	4.9	6
6-11 months	8.8	0.91	8	10
Girls				
0-5 months	5.3	0.85	4.5	6
6-11 months	8.1	0.91	7.4	9
Children				
Boys				
1-3 years	12.2	1.13	13.8	17
4-6 years	18.3	0.92	16.8	21
7-9 years	25.4	0.68	17.3	22
Girls				
1-3 years	11.5	1.13	13	16
4-6 years	18.2	0.92	16.7	21
7-9 years	25.0	0.68	17	21
Adolescents				
Boys				
10-12 years	33.4	0.50	16.7	21
13-15 years	49.6	0.50	24.8	31
16-18 years	59.2	0.50	29.6	37
Girls				
10-12 years	35.4	0.42	14.9	19
13-15 years	46.5	0.42	19.5	24
16-18 years	50.3	0.42	21.1	26

Group	Reference body weight[a] (kg)	Average normative requirement[b]		RNI (µg/day)[c]
		Se $R^{normative}$ (kg/day)	Se $R^{normative}$ (total/day)	
Adults				
Men				
19-29 years	61.4	0.42	25.8	32
30-59 years	60.6	0.42	25.5	32
≥60 years	58.1	0.42	24.4	31
60-64 years	58.5	0.42	24.6	31
≥65 years	57.7	0.41	23.7	30
Women				
19-29 years	52.9	0.37	19.6	25
30-59 years	52.2	0.37	19.3	24
≥60 years	49.5	0.37	18.3	23
60-64 years	50.2	0.37	18.6	23
≥65 years	48.8	0.37	18.1	23
Pregnancy				
1st trimester				25
2nd trimester				27
3rd trimester				29
Lactation				
0-3 months				34
4-6 months				34
7-12 months				41

[a] reference body weights for the Malaysian population
[b] derived from WHO/ FAO/ IAEA values by interpolation.
[c] recommended nutrient intake (RNI) derived from the average Se $R^{normative}$ + 2 X assumed standard deviation (of 12.5%).

21. 8 Discussion on Comparison of Recommended Intake of Selenium

In general, the recommended selenium intakes for most age groups by IOM (2000) are 1.5 - 2.0 times more than the values recommended by FAO/WHO (2004). The differences could be due to the different approaches in estimating physiologic selenium requirement by both committees. The approach used by the Malaysian RNI committee to derive the recommended selenium intakes is similar to that of FAO/WHO (2004). However, for most age groups, the Malaysian RNI values are slightly lower than those in the FAO/WHO report due to the lower reference body weights of Malaysians (Table 21.2).

21.9 Tolerable Upper Intake Levels

The tolerable upper intake level (UL) for various age groups as suggested by IOM (2000) are given in Table 21.3. WHO/FAO (2004) has also proposed a UL of 400 µg/day for adults but the UL for children and for pregnant or lactating women has yet to be determined. The European Commission (2000) adopted a UL of 300 ug/day for adults including pregnant and lactating women and extrapolated the UL of adults to children based on reference body weights.

Table 21.3 Tolerable Upper Intake Level for selenium according to age group

Age groups	µg/day of selenium
Infants	
0-6 months	45
7-11 months	60
Children	
1-3 years	90
4-8 years	150
9-13 years	280
Adolescents 14 – 18 years	400
Adult women ≥19 years	400
Adult men ≥ 19 years	400
Pregnant women	
14-18 years	400
19-50 years	400
Lactation women	
14-18 years	400
19-50 years	400

(Source: IOM, 2000)

21.9 Research recommendations

The following priority areas of research are recommended:

- Assessment of selenium intakes among various ethnic, socioeconomic, age and gender groups
- Bioavailability studies of selenium from the local food sources.
- Cross-sectional and longitudinal studies on the prevalence of selenium deficiency in the
- Relationship between low Se intake and diet related chronic diseases.
- Influences of soil composition and agricultural practices on selenium content of plant and animal food sources. The information can then be used to document selenium content in local foods.

21.10 References

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Appendix 21.1 Comparison of recommended intake for Selenium: RNI Malaysia (2017) and (2005), RNI of FAO/WHO (2004), AI and RDA of IOM (2000) and EPSA (2014)

Malaysia (2017)		Malaysia (2005)		FAO/WHO (2004)		IOM (2000)		EFSA (2014)	
Age groups	**RNI (µg/day)**	**Age groups**	**RNI (µg/day)**	**Age groups**	**RNI (µg/day)**	**Age groups**	**AI (µg/day)**	**Age groups**	**AI (µg/day)**
Infants (Boys)		**Infants**		**Infants**		**Infants**			
0 - 5 months	6	0 - 5 months	6	0 - 6 months	6	0 - 6 months	15	7 - 11 months	15
6 - 11 months	10	6 - 12 months	9	7 - 11 months	10	7 - 12 months	20	1 - 3 years	15
								4 - 6 years	20
Infants (Girls)									
0 - 5 months	6						**RDA (mg/day)**	7 - 10 years	35
6 - 11 months	9							11 - 14 years	55
Children		**Children**		**Children**		**Children**			
(Boys)									
1 - 3 years	17	1 - 3 years	17	1 - 3 years	17	1 - 3 years	20	15 - 17 years	70
4 - 6 years	21	4 - 6 years	21	4 - 6 years	22	4 - 8 years	30	≥18 years	70
7 - 9 years	22	7 - 9 years	22	7 - 9 years	21			pregnancy	70
								lactation	85
(Girls)									
1 - 3 years	16								
4 - 6 years	21								
7 - 9 years	21								
Boys		**Boys**		**Boys**		**Boys**			
10 - 18 years	21	10 - 18 years	28	10 - 18 years	32	9 - 13 years	40		
13 - 15 years	31			14 - 18 years	55				
16 - 18 years	37								
Girls		**Girls**		**Girls**		**Girls**			
10 - 18 years	19	10 - 18 years	23	10 - 18 years	26	9 - 13 years	40		
13 - 15 years	24			14 - 18 years	55				
16 - 18 years	26								

Malaysia (2017)		Malaysia (2005)		FAO/WHO (2004)		IOM (2000)		EFSA (2014)	
Age groups	**RNI (µg/day)**	**Age groups**	**RNI (µg/day)**	**Age groups**	**RNI (µg/day)**	**Age groups**	**AI (µg/day)**	**Age groups**	**AI (µg/day)**
Adults		**Adults**		**Adults**		**Adults**			
Men		**Men**		**Men**		**Men**			
19 - 29 years	32	19 - 65 years	33	19 - 65 years	34	19 - 30 years	55		
30 - 59 years	32	> 65 years	29	> 65 years	33	31 - 50 years	55		
> 60 years	31					51 - 70 years	55		
60 - 64 years	31					> 70 years	55		
≥ 65 years	30								
Women		**Women**		**Women**		**Women**			
19 - 29 years	25	19 - 65 years	25	19 - 65 years	26	19 - 30 years	55		
30 - 59 years	24	> 65 years	23	> 65 years	25	31 - 50 years	55		
> 60 years	23					51 - 70 years	55		
60 - 64 years	23					> 70 years	55		
≥ 65 years	23								
Pregnancy		**Pregnancy**		**Pregnancy**		**Pregnancy**			
1st trimester	25	1st trimester	25	1st trimester	26	14 - 18 years	60		
2nd trimester	27	2nd trimester	27	2nd trimester	28	19 - 30 years	60		
3rd trimester	29	3rd trimester	29	3rd trimester	30	31 - 50 years	60		
Lactation		**Lactation**		**Lactation**		**Lactation**			
0 - 3 months	34	0 - 3 months	34	0 - 3 months	35	14 - 18 years	70		
4 - 6 months	34	4 - 6 months	34	4 - 6 months	35	19 - 30 years	70		
7 - 12 months	41	7 - 12 months	41	7 - 12 months	42	31 - 50 years	70		

22 • Phosphorus

22.1 Introduction

At 0.099%, phosphorus is the most abundant pnictogen in the Earth's crust. It is a chemical element with symbol P and an atomic number of 15. Phosphorus exists in two major forms, namely the white and the red phosphorus. As phosphorus is highly reactive, it is not found as a free element on Earth but is widely distributed in many minerals, mainly as phosphates. The largest use of phosphorus compounds is for fertilizers. It is also important in the production of steels, special glasses and fine chinaware. Phosphorus comprises approximately 1% of total body weight (Farrow and White, 2010; Penido and Alon, 2012) and is the second most abundant macro mineral that existed in human body after calcium.

22.2 Functions

Phosphorus plays a vital role in the maintenance of human health and involves in various physiological processes. Phosphate acts as component of high-energy molecules such as adenosine triphosphate (ATP) and creatine phosphate. When cleavage occur between the bond of phosphate molecules, energy is release for the body to function normally. Besides that, phosphorus provides structure to the cell membranes by acting as component of phospholipids. In bone and teeth, it acts as component of hydroxyapatite and calcium phosphate, which provides strength for bone and teeth. In addition, it acts as an important element in the structural component of DNA and RNA, underpins its function in cell regulation and signaling. Lastly, phosphate also acts as buffer in the body by regulating the pH of the extracellular fluid (Kalantar-Zadeh *et al.,* 2010).

22.3 Metabolism

Intestinal phosphorus absorption occurs through both cellular and paracellular pathways (Sabbagh *et al.,* 2011; Penido and Alon, 2012), and at least two mechanisms, i.e. passive diffusion and sodium-dependent active transport (Eto *et al.,* 2006) are involved. Dietary phosphate, 1α, 25-dihydroxyvitamin D ($1,25(OH)^2D^3$), and parathyroid hormones (PTH) are thought to be the most important physiological regulators of intestinal phosphate absorption (Penido and Alon, 2012). Dietary phosphorus is absorbed by the epithelium of the small intestine (duodenum and jejunum) via both a passive diffusional, load-dependent process (depends on the amount of phosphorus in the gut) and an active sodium dependent process (increased by $1,25(OH)^2D^3$) (Amanzadeh & Reilly., 2006).

Intestinal phosphatases hydrolyze the organic forms contained in ingested protoplasm, and thus most phosphorus absorption occurs as inorganic phosphate. Phosphorus is absorbed with high efficiency. Net absorption of total phosphorus ranges from 55 to 80% in adults (Heaney, 2012; Lemann, 1996; Nordin, 1989; O'Brien *et al.,* 2014) and from 65 to 90% in infants and children (Wilkinson, 1976; Heaney, 2012; O'Brien *et al.,* 2014). Phosphorus absorption is affected by the total amount of phosphorus in the diet and also by the type of phosphorus (organic versus inorganic), the food origin (animal-versus plant-derived) and the ratio of phosphorus to other dietary components (EFSA, 2015). Absorption of phosphorus is reduced by ingestion of aluminum-containing antacids and by pharmacologic doses of calcium carbonate. There is, however, no significant interference with phosphorus absorption by calcium

at intakes within the typical adult range. There is no evidence that absorption efficiency of phosphorus varies with dietary intake and there is no apparent adaptive mechanism that improves phosphorus absorption at low intakes (Lemann, 1996), unlike calcium where absorption efficiency increases as dietary intake decreases and adaptive mechanisms exist that improve calcium absorption still further at habitual low intakes.

Excretion of endogenous phosphorus is mainly through the kidneys. In the healthy adult, urine phosphorus is essentially equal to absorbed diet phosphorus, less small amounts of phosphorus lost in shed cells of skin and intestinal mucosa. The proximal tubule reabsorbs most of the filtered phosphorus (75%) followed by distal tubule (10%) and the rest (15%) is excreted through urine (Noori *et al.,* 2010). Several factors such as phosphate depletion, calcitriol, volume depletion, metabolic alkalosis, chronic hypocalcemia and the hormones insulin, estrogen, thyroid hormone, and growth hormone will increase the reabsorption of renal tubular phosphorus. On the other hand, parathyroid hormone, phosphatonins, acidosis, hyperphosphatemia, chronic hypercalcemia, and volume expansion will reduce the reabsorption of renal tubular phosphorus (Uribarri, 2007).

Phosphorus homeostasis is tightly regulated by the bone-kidney-parathyroid gland axis, involving PTH, the active metabolite of vitamin D (1,25 $(OH)^2$ D) and the phosphatonin fibroblast growth factor-23 (FGF-23), mainly produced and secreted by osteocytes in bone (Berndt and Kumar, 2009; Bergwitz and Jüppner, 2010). In adults, under normal physiological conditions, the amount of phosphorus entering the phosphorus pool from bone resorption equals that exiting the pool for bone formation (Hruska *et al.,* 2008). Intestine and the kidneys are involved in phosphate homeostasis by regulating absorption of phosphorus from the diet and phosphorus excretion (Berndt and Kumar, 2007). Figure 22.1 below depicts the metabolism of phosphorus (Uribarri, 2007).

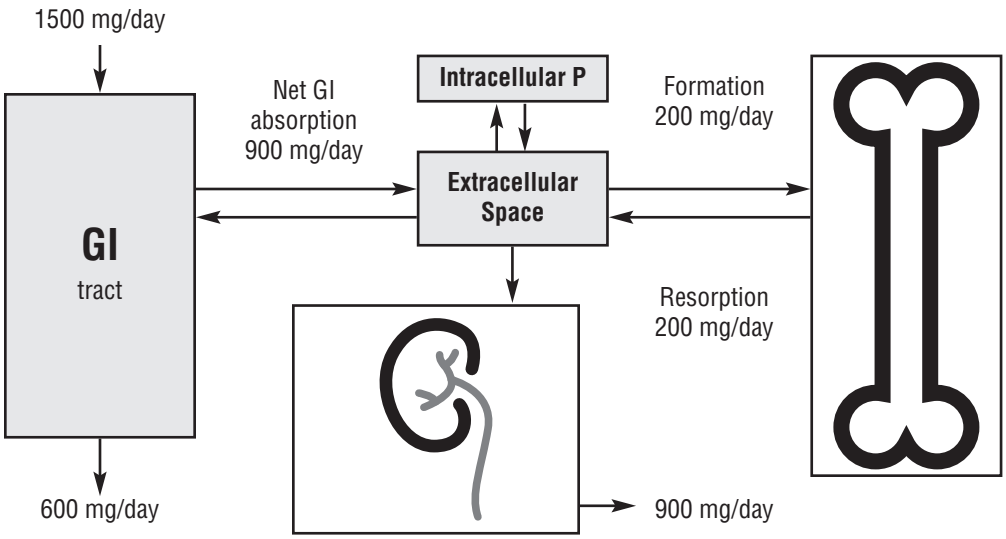

Figure 22.1: Phosphorus Homeostasis

About 85% of the phosphorus in human body is present in teeth and bones, with the remaining 14% in soft tissues, including muscle, liver, heart and kidney, and only 1% in the extracellular fluid (O'Brien *et al.,* 2014). Hence, serum measurements may reflect only a minor fraction of total body phosphorus, and do not consistently reflect total body stores (Moe, 2008). At birth, a neonate contains approximately 20 g phosphorus (0.5 g/100 g fat free tissue), most of which is accumulated during the last 8 weeks of pregnancy (Widdowson & Spray, 1951). Assuming continuous growth and maturity at 18 years, phosphorus accretion rates have been estimated at 66 mg/day at age 4-12 months and at 107 mg/day in boys and 80 mg/day in girls, (Prentice and Bates, 1994). In adults, using total body neutron activation analysis, total body phosphorus was reported to range from 374 ± 60 g to 439 ± 70 g in Caucasian women aged between 20 and 74 years and from 461 ± 82 g to 561 ± 69 g in Caucasian men aged between 20 and 90 years in the USA (Ellis, 1990). Hence, total body phosphorus has been reported to be in the order of 400-800 g (Moe, 2008).

In the past, a great deal of emphasis was placed on the calcium-phosphorus ratio (Ca:P) of diets (Chinn, 1981), particularly those of infants (Fomon & Nelson, 1993). This is a useful concept during periods of rapid growth but has little relevance in adults when assessing requirements. Also, the ratio does not take into account differing bio-availabilities and adaptive responses of the two nutrients. In balance studies in human adults, Ca:P molar ratios ranging from 0.08 to 2.4 (a 30 fold range) had no effect on either calcium balance or absorption (Heaney & Recker, 1982). For this reason, there is little or no evidence for relating the two nutrients during most of human life. Other indicators are now used to assess phosphorus requirements, including measurement of inorganic phosphorus in serum (serum Pi) or phosphorus balance.

Animal studies have shown that niacinamide, which is a circulating form of niacin, prevents an increase in serum phosphate in animals with renal failure by reducing sodium-phosphate 2b transporter expression in the jejunum and inhibiting intestinal phosphorus absorption (Katai *et al.,* 1999; Eto *et al.,* 2005). Limited human clinical trials have also shown that niacinamide and niacin accomplish clinically significant reductions in serum phosphate in patients undergoing dialysis (Takahashi *et al.,* 2004; Muller *et al.,* 2007; Cheng *et al.,* 2008; Sampathkumar *et al.,* 2006; Restrepo Valencia *et al.,* 2008) or dyslipidemia patients with advanced renal failure (Maccubbin *et al.,* 2010). To the best of knowledge, there is no study available on the effect of niacin or niacinamide on phosphorus absorption among healthy individual.

22.4 Sources

Food phosphorus is a mixture of inorganic and organic forms. Phosphates are found in foods as naturally occurring components of biological molecules and as food additives in the form of various phosphate salts. Phosphorus is widely distributed in foods. All foods from animal tissues contain different amounts of phosphorus (Noori *et al.,* 2010). Rich food sources of phosphorus include cheese, meat, fish, poultry and nuts. Meanwhile, there is only small amount of organic phosphorus in plants. In addition, phosphorus in plant (about 75%) exists

in the form of phytic acid or phytate (Uribarri & Calvo, 2003; Noori *et al.,* 2010), which has poor bioavailability. In Europe, milk and dairy products (30-53%), grains and grain-based products (27-38%) are the main food groups contributing to phosphorus intake. The contribution of meat and meat products was between 10 and 25 % in the age groups from 10 years and above (EFSA, 2015).

Besides organic dietary phosphorus, sources of phosphorus include inorganic phosphorus, mainly contributed by phosphate additives which are widely use during industrial food processing to extend conservation, enhance color or flavor, and retain moisture. The use of phosphorus-containing food additives in processed foods and cola beverages contribute substantially to total phosphorus intake. In recent studies, it is estimated that the extra burden of phosphorus coming from processed food may reach 700-800 mg per day (León, Sullivan & Sehgal, 2013; Carrigan *et al.,* 2014). However, phosphate additives does not usually appear in the common databases and food compositions tables (Benini *et al.,* 2011; Cupisti *et al.,* 2012). Table 22.1 shows the phosphorus content of common foods in Malaysia.

Table 22.1: Phosphorus content of foods.

	mg/100g Edible Portion
Milk and milk products	
Skimmed milk powder	930
Cheddar cheese	460
UHT chocolate milk	95
Malted Milk Drink	91
Fresh Cow Milk	82
Meat, fish, poultry	
Dried Prawn	727
Hen egg yolk	586
Duck egg yolk	360
Ikan Kembong (Indian Mackerel)	263
Beef frankfurter*	245
Black Pomfret	237
Century egg	218
Chicken breast meat	205
Chicken frankfurter*	205
Beef burger patty*	201
Ikan Kurau (Threadfin)	181
Canned sardine	159
Chicken burger patty*	156
Lean Mutton	146
Fish ball*	100

	mg/100g Edible Portion
Lean Beef	82
Legumes and nuts	
Fucok (Soya bean curd sheet)	577
Cashew nuts	359
Walnut	333
Fried Tofu (soya bean curd)	287
Tau-hoo-pok (Soya bean curd)	264
Peanut / groundnut	246
Cooked Dishes	
Fried Chicken	467
Beef Satay	462
Papadam (Black gram cracker)	382
Beef curry	349
Chicken Satay	335
Vadai (Yellow dhall)	302
Murtabak (meat egg pancake)	258
Chappatti (unleavened flatbread)	244
Yau-Char-Kue (fried breadstick)	169
Green gram porridge with coconut milk	163
Roti telur (Indian flatbread with eggg	154
Ham Chin Peng (Cantonese fried doughnut)	151
Roti Canai (Indian flatbread)	143
Pizza (with Chicken & Pineapple)	114
Mee Bandung (spicy noodle dish)	81
Cola drinks*	69
Fried rice	52
Nasi lemak (Coconut milk rice)	51

Source: Malaysian Food Composition Database (MyFCD) 2016

* May contain higher phosphorus as indicated due to presence of phosphate additives

22.5 Deficiency

In the presence of normal kidney function, fasting serum phosphorus is maintained within a tight range despite wide fluctuations in dietary phosphorus intake through variations in the urinary phosphorus excretion (Nadkarni & Uribbari, 2014), hence phosphorus deficiency (hypophosphatemia) or excess (hyperphosphatemia) is extremely rare among general population. However, public health concern for excess phosphorus intake beyond nutrient requirements stems from the growing epidemiologic evidence that elevated serum phosphorus and its associated health outcomes have been extended to the general population. This becomes even more important in view of the increasing dietary phosphorus intake attributed to the increased consumption of foods processed with phosphate additives.

Dietary phosphorus intake is increasing as a result of the growing consumption of foods processed with phosphate additives. Using the National Health and Nutritional Examination Survey (NHANES 2005-2006), 50% of the US population consumes 1000 mg/d, far exceeding the requirements (Moshfegh *et al.,* 2009). NHANES surveys over the past three decades had consistently reported phosphorus intakes are generally in excess of the requirements for all ages, except for rapidly growing young adults (Calvo & Uribarri, 2013). In nine European Union countries, mean phosphorus intakes range from 265 to 531 mg/day in infants, from 641 to 973 mg/day in children aged 1 to < 3 years, from 750 to 1202 mg/day in children aged 3 to < 10 years, from 990 to 1 601 mg/day in children aged 10 to < 18 years and from 1000 to 1 767 mg/day in adults ((18 years) (EFSA, 2015), which were in general higher than the recommended intakes. Studies are scarce on the phosphorus intake among Malaysian. The available nationwide study reported that the phosphorus intake was 1195mg/day and 1220mg/day for children aged 6-9 year old and 10-12 year old, respectively (Jan Mohamed *et al.,* 2015). An estimated intake of 842 mg/day was reported among early adolescents (Abdul Majid *et al.,* 2016). A precise assessment of dietary phosphorus intake is difficult because of the questionable accuracy of dietary instruments and databases used to estimate phosphorus in foods in all its forms, particularly inorganic sources from phosphorus-based food additives and dietary supplements (Calvo and Uribarri, 2013). Hence dietary phosphorus intake may be underestimated by 20% as nutrient content databases are unable to capture contribution by phosphate additives (Oenning, Vogel & Calvo, 1988; Sullivan, Leon, Sehgal, 2007). Hyperphosphatemia may further progress to secondary hyperparathyroidism (Martin & González, 2011), skeletal deformations, bone loss and/or ectopic calcification in animal studies. However, such effects were generally not observed in human studies, except in patients with end-stage renal disease. Excessive phosphorus intake from supplements with dosages greater than 750 mg/day has been associated with gastrointestinal symptoms, such as osmotic diarrhoea, nausea and vomiting in healthy subjects (EFSA, 2005).

As phosphorus is widely distributed in the food supply that phosphorus deficiency is extremely rare, the exception being individual on long-term and severe food restriction. Also, as aluminum-containing antacids will bind diet phosphorus in the gut, when consumed in high doses, it may produce hypophosphatemia and aggravate phosphate deficiency related to other problems. Symptoms of hypophosphatemia may include anorexia, increase susceptibility to infection (Lotz *et al.,* 1968), skeletal demineralization and muscular weakness (Takeda *et al.,* 2012).

With regards to suitability as marker of phosphorus intake or phosphorus status, in view of the lacking of accurate dietary instruments and nutrition databases to estimate total dietary phosphorus in foods, particularly inorganic sources from phosphorus-based food additives and dietary supplements, several biomarkers including serum/plasma inorganic phosphorus (IOM, 1997) and urinary phosphorus excretion had been proposed as surrogate markers of phosphorus intake beyond dietary estimates. Concentration of serum or plasma inorganic phosphorus shows only minimal modifications as a result of tight regulation of homeostatic mechanisms, even in the presence of wide variations in intake (EFSA, 2015). As there is only approximately 1% of total body phosphorus found in extracellular fluid, hence serum inorganic phosphorus is generally inadequately reflects body stores. Serum inorganic phosphorus is also influenced by age, sex, lactation, diurnal and seasonal variations, vitamin D status and pathological conditions such as malabsorption syndromes and insulin-dependent diabetes mellitus (Gibson, 2005), hence making it unsuitable as biomarker for phosphorus intake and status. New markers of phosphorus balance such as fibroblast growth factor 23 (FGF23) had been recently proposed as a better biomarker of disturbances of phosphorus homeostasis in general (Gutierrez, 2013), attributed by its greater magnitude and strength of the association with adverse outcomes than that of serum phosphorus itself and its less random variation than other markers of phosphorus metabolism such as serum phosphorus concentrations (Isakova, Gutierrez & Wolf, 2009). However, studies to determine the effect of dietary phosphorus intake on FGF23 concentrations in community-dwelling individuals and whether the reduction of FGF 23 has a salutary effect on cardiovascular health are scarce. To date, the EFSA (2015) concluded there is currently no reliable biomarker of phosphorus intake and status that may be used for deriving the requirement for phosphorus.

22.6 Factors Affecting Phosphorus Requirement

There is no direct studies available showing that phosphorus requirement varies according to physiological changes. Both pregnancy and lactation are associated with physiological adaptive changes in mineral metabolism that are independent of maternal mineral supply within the range of normal dietary intakes. These adaptive processes provide the minerals including phosphorus, necessary for fetal growth and breast milk production without requiring an increase in maternal dietary intake or compromising maternal bone health in the long term (Prentice, 2003). Phosphorus requirement is also similar between men and women. Across the age groups, phosphorus requirement is higher in adolescents compared to adult men and women, while there is no different in phosphorus requirement among young or older adults.

The bioavailability of phosphorus from an individual food may differ substantially depending on the form of phosphorus found in the food and the presence of other nutrients in the meal (Uribarri 2007; Kalantar-Zadeh *et al.* 2010). Dairy foods contain abundant calcium, which build complexes with phosphate in the intestine, thereby reducing phosphorus absorption (Heaney & Nordin 2002). Phosphorus from casein was found to affect phosphate metabolism more profoundly than the same amount of phosphorus from grains in a rat study, implying relatively good phosphorus bioavailability from casein (Moe *et al.,* 2009). With regards to type of milk, the efficiency of absorption is highest from human milk (85 to 90%) (Williams *et al.,* 1970), intermediate from cow milk (72%) (Ziegler and Fomon, 1983), and lowest from soy formulas (~59%) (Ziegler and Fomon, 1983). However infant formulas contain substantially

greater amounts of phosphorus than human milk, the absorbed phosphorus from cow milk and soy formulas is twice that attained by human milk-fed infants (Moya *et al.,* 1992). Relatively low intakes of phosphorus, as occur in human milk, may actually confer an advantage to the infant by virtue of the low residual phosphorus in the lower bowel, reduce the proliferation of potentially pathogenic microorganisms and provide an immune protective effect for infant fed on human milk. Data on phosphorus bioavailability from meat are scarce (Schuette & Linkswiler, 1982), even though generally phosphorus from meat is considered to be well absorbed (Uribarri, 2007).

In general, most food sources exhibit good phosphorus bioavailability except plant seeds (beans, peas, cereals, nuts) which contain phytic acid. Phytic acid cannot be hydrolyze by the digestive systems of most mammals and hence limits its availability. The phosphorus bioavailability of plant seeds can be improved with the presence of phytase, bacterial enzymes and yeast. Hence, while phosphorus from animal sources is easily absorbed, the bioavailability of phosphorus from plant foods is relatively low, usually less than 50% (Uribarri & Calvo, 2003; Noori *et al.,* 2010). Meanwhile, it is believed that over 90% of inorganic phosphate added in the processed foods is absorbed by our intestinal tract as compared to organic phosphate. This is because the phosphate additives are salts that are not protein bounded hence, it is more easily disassociate and absorbed by the intestinal tract (Noori *et al.,* 2010).

22.7 Setting Requirements and Recommended Intake of phosphorus

The dietary recommendations for phosphorus set by the Institute of Medicine (2006) used studies on serum inorganic phosphorus (serum Pi) concentrations. The level required to maintain serum Pi within an optimal range has been considered an indicator of phosphorus nutrition. The National Health and Medical Research Council (NHMRC) for Australia and Ministry of Health New Zealand (NHMRC, 2006) set their phosphorus requirements based on graphical transformation technique assessing intake level required to reach lowest point for normal plasma phosphorus level. On the other hand, the recommendation by European Food Safety Authority (European Food Safety Authority 2015) was based solely on the range of the molar ratio of calcium to phosphorus in the whole body. As noted earlier, this approach is unsatisfactory. For this reason, the Technical Sub-Committee (TSC) on Minerals and Trace Elements decided to adopt values from IOM (2006) where the primary criterion used to set an Estimated Average Requirement (EAR) is serum Pi concentration (Appendix 22.1).

Infants

The AI for 0-6 months was calculated by multiplying the average intake of breast milk (0.78 L/day) by the average concentration of phosphorus in breast milk (124 mg/L) from 10 studies reviewed by Atkinson *et al.* (1995), and rounding (IOM, 1997). The AI for 7-12 months was set by adding an estimate for phosphorus from breast milk at this age to an estimate of intake from supplementary foods. A breast milk volume of 0.60 L/day (Dewey *et al.* 1984, Heinig *et al.* 1993) and the average concentration of phosphorus in breast milk at this age of 124 mg/L (Atkinson *et al.,* 1995) give a contribution of 75 mg phosphorus/day from breast milk that is added to 200 mg/day from complementary foods (Specker *et al.,* 1997).

AI for infants:

0 - 6 months	**100 mg /day**
7 to 12 months	**275 mg /day**

Children and Adolescents

In the absence of data on serum Pi or phosphorus balance in children from 1-8 years, estimation of body accretion for these age groups was used on known tissue composition and growth rates (Fomon *et al.,* 1982; IOM 1997) using a conservative estimate of phosphorus absorption of 70%. The equation used to set EAR was equal to the sum of accretion and urinary loss, divided by fractional absorption. This gave an EAR of 380 mg for children aged 1-3 years which, with an assumed coefficient of variation (CV) of 10% for the EAR and rounding, gives an RDI of 460 mg/day. For children aged 4-8 years, the EAR and the RDI were estimated to be 405 mg/day and 500 mg/day, respectively. For 9-13 year olds, longitudinal data and a large cross-sectional database (Slemenda *et al.,* 1994) allowed estimation of phosphorus requirement from tissue accretion data using a factorial approach (IOM, 2006) that was then also adopted for the 14-18 year-olds. The Phosphorus requirement for the healthy adolescents (9-18 years) is higher than the adult value in view of the intense growth, with growth rate, absorption efficiency, and normal values of inorganic phosphorus in the extracellular fluid changing at this period of time (IOM, 2006). The EAR for both age groups (9 to 13 years and 14 to 18 years) was set at 1,055 mg/day. Assuming a CV of 10% for the EAR and rounding gave an RDI of 1,250 mg.

RNI for children and adolescents

1 to 3 years	**460 mg/day**
4 to 8 years	**500 mg/day**
9 to 13 years	**1250 mg/day**
14 to 18 years	**1250 mg /day**

Adults

The EAR for adults was based on average dietary intake of phosphorus required from a typical mixed diet to reach the lowest point of the normal range for serum Pi (Nordin, 1989; IOM; 2006). The estimates assume an absorption efficiency of 62.5% (Heaney & Recker, 1982; Wilkinson, 1976). By definition, at this level of intake, only half the population will achieve a Pi above the bottom of the normal range. A CV of 35% for the EAR was derived from consideration of the increase in ingested intake required to raise serum Pi from the bottom end of the normal range to a level of 3.1 mg/dL (1 mmol/L), the fasting level attained by most well-nourished adults (Nordin, 1989; IOM 1997) giving an RDI of 1,000 mg.

RNI for adults

Adults	**700 mg/day**

Pregnancy and Lactation

As there are no direct studies showing increased needs in pregnancy, the EAR and RDI were set at those of the non-pregnant state. Similarly, increased bone resorption and decreased urinary excretion occurring independently of dietary intake provide the additional needs for milk production (Kent *et al.,* 1990; 1991) and thus there is no evidence of increased needs in lactation. Hence, the EAR and RDI were set at those of the non-lactation state.

RNI for pregnancy and lactation

14-18 years	**1250 mg/day**
Other age group	**700 mg/day**

22.8 Tolerable upper intake levels

Excess

The UL is set at the intake associated with the upper boundary of normal values of serum Pi. The upper boundaries are higher in infants than in adults and there is no evidence that intakes at the adult upper boundary cause harm. The higher boundaries in infants are obviously tissue-safe and assuming they approximate the upper normal human value, the corresponding ingested intake in an adult would be more than 10,000 mg/day. A No-Observed-Adverse-Effect Level (NOAEL) of 10,000 mg/day was therefore set (IOM, 1997). Information concerning adverse effects in the area between normal Pi and levels associated with ectopic mineralization is lacking. In keeping with pharmacokinetic practice when relationships between intake and blood level are known (Petley *et al.,* 1995), an uncertainty factor (UF) of 2.5 was chosen, taking the UL for adults to 4,000 mg/day. For adults over 70 years, because of increased prevalence of kidney damage, a larger UF of 3.3 was applied, giving a UL of 3,000 mg/day. In pregnancy, absorption efficiency rises by about 15% so the UL was set 15% lower at 3,500 mg/day. In lactation, phosphorus metabolism is the same as in the non-pregnant state, so the UL stays at 4,000 mg/day.

For children, an upper level of intake of 3,000 mg/day was set by dividing the NOAEL for adults by an uncertainty factor of about 3.3 for potentially increased susceptibility related to smaller body size. For children, 9-18 years, the adult UL was applied as there was no evidence to suggest increased susceptibility.

No harm is known to occur for some groups in the community, especially those with high energy intakes if dietary phosphorus intakes go above the UL limits. Nevertheless, dysfunction of phosphorus homeostasis will lead to serious clinical consequences in healthy individuals and those with conditions, such as advanced kidney diseases, in which hyperphosphatemia is associated with increased risks of cardiovascular morbidity and mortality (Ritter & Slatopolsky, 2016). Excessive dietary phosphorus intake can increase the risk of bone fractures by 9% with every 100 mg of phosphorus intake in a normal healthy person. This is attributed to the hormonal changes equivalent to mild hyperparathyroidism and reduces calcitriol concentrations associated with excessive dietary phosphorus intake (Takeda *et al.,* 2012). Besides that, serum phosphate concentration above normal range is associated with the development of atherosclerosis in human with normal kidney function. In healthy human with normal kidney function, although long term excessive phosphorus intake does not cause hyperphosphatemia, it can increase the risk of getting cardiovascular disease and carotid intima media thickness (Takeda *et al.,* 2012). Table 22.2 depicts the UL of phosphorus according to age groups.

Table 22.2. Tolerable Upper Intake Levels (UL) of Phosphorus for various age groups

	UL
Infants	Not possible to establish. Sources of intake should be from breast milk and supplementary foods
Children	
1 – 3 years	3000 mg/day
4 – 8 years	3000 mg/day
Adolescents	
9 - 13 years	4000 mg/day
14 – 18 years	4000 mg/day
Adults	
19 – 30 years	4000 mg/day
31 - 50 years	4000 mg/day
50 – 70 years	4000 mg/day
> 70 years	4000 mg/day
Pregnancy	
≤ 18 years	3500 mg/day
19 - 50 years	3500 mg/day
Lactation	
≤ 18 years	4000 mg/day
19 - 50 years	4000 mg/day

Source: IOM (2006)

22.9 Research recommendations

The scarcity of dietary intake data on phosphorus intake signifies future national dietary surveillance to include the assessment of dietary phosphorus at the population level. Future updates on the Malaysian Food Composition Database should consider to analyze phosphorus content of processed foods and carbonated beverages as these are not extensively covered on the current database. On the other hand, as the current food composition database does not adequately take into account the current use of phosphate additives, the nutrient contents of foods should be revised to reflect the actual amount of phosphate additives.

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Appendix 22.1: Comparison of recommended intake for Phosphorus: RNI Malaysia (2017), RNI of EFSA (2015) and AI of IOM (2006)

Malaysia (2017)			EFSA (2015)			IOM (2006)		
	Age	AI (mg/day)		Age	RNI (mg/day)		Age	AI (mg/day)
Infants (boys)	0 - 6 months	100	**Infants (boys)**	7 - 12 months	160	**Infants (boys)**	0 - 6 months	100
	7 - 12 months	275					7 - 12 months	275
Infants (girls)	0 - 6 months	100	**Infants (girls)**	7 - 12 months	160	**Infants (girls)**	0 - 6 months	100
	7 - 12 months	275					7 - 12 months	275
Children (boys)	1 - 3 years	460	**Children (boys)**	1 - 3 years	250	**Children (boys)**	1 - 3 years	460
	4 - 8 years	500		4 - 10 years	440		4 - 8 years	500
Children (girls)	1 - 3 years	460	**Children (girls)**	1 - 3 years	250	**Children (girls)**	1 - 3 years	460
	4 - 8 years	500		4 - 10 years	440		4 - 8 years	500
Adolescent (boys)	9 - 13 years	1250	**Adolescent (boys)**	11 - 17 years	640	**Adolescent (boys)**	9 - 13 years	1250
	14 - 18 years	1250					14 - 18 years	1250
Adolescent (girls)	9 - 13 years	1250	**Adolescent (girls)**	11 - 17 years	640	**Adolescent (girls)**	9 - 13 years	1250
	14 - 18 years	1250					14 - 18 years	1250
Men	19 - 29 years	700	**Men**	18 - 29 years	550	**Men**	19 - 29 years	700
	30 - 50 years	700		30 - 50 years	550		30 - 50 years	700
	51 - 59 years	700		51 - 59 years	550		51 - 59 years	700
	60 - 69 years	700		60 - 69 years	550		60 - 69 years	700
	> 70 years	700		> 70 years	550		> 70 years	700

Malaysia (2017)			EFSA (2015)			IOM (2006)		
	Age	AI (mg/day)		Age	RNI (mg/day)		Age	AI (mg/day)
Women	19 - 29 years	700	**Women**	18 - 29 years	550	**Women**	19 - 29 years	700
	30 - 50 years	700		30 - 50 years	550		30 - 50 years	700
	51 - 59 years	700		51 - 59 years	550		51 - 59 years	700
	60 - 69 years	700		60 - 69 years	550		60 - 69 years	700
	> 70 years	700		> 70 years	550		> 70 years	700
Pregnancy	14 - 18 years	1250	**Pregnancy**	14 - 18 years	640	**Pregnancy**	14 - 18 years	1250
	19 - 30 years	700		19 - 30 years	550		19 - 30 years	700
	31 - 50 years	700		31 - 50 years	550		31 - 50 years	700
Lactation	14 - 18 years	1250	**Lactation**	14 - 18 years	640	**Lactation**	14 - 18 years	1250
	19 - 30 years	700		19 - 30 years	550		19 - 30 years	700
	31 - 50 years	700		31 - 50 years	550		31 - 50 years	700

23 • Sodium

23.1 Introduction

Sodium is a chemical element with symbol Na and atomic number 11. It is a soft silver white, highly reactive metal. It has a single electron in its outer shell that it readily donates, creating a positively charged atom-a cation. Its only stable isotope is 23 Na. The metal does not occur in nature, but must be prepared from compounds. Sodium is the sixth most abundant element in the earth's crust and exists in numerous minerals such as feldspars, sodalite and rock salt (Nacl).

Sodium is found in most foods as sodium chloride (Nacl), generally known as 'salt' and 1.0 g sodium is equivalent to 2.55 mg NaCl while 1 mmol sodium is equivalent to 23 mg sodium (NaCl consists of Na at 40 %), Thus, 1 teaspoon or 5 g salt provides 2000 mg or 88 mmol sodium. In addition to NaCl, sodium may also be present in other forms, such as monosodium glutamate, sodium nitrate and sodium benzoate. (Food Act, 1983). Other form of sodium are food additives such as sodium phosphate and sodium carbonate. Sodium bicarbonate is used as an ingredients in food and can be used as a treatment of metabolic acidosis .Still the major form of dietary sodium is sodium chloride. (IOM, 2004)

23.2 Functions

Sodium is the principal cation in extracellular fluid in the body and is an essential nutrient necessary for maintenance of plasma volume, acid-base balance, transmission of nerve impulses and normal cell function (WHO, 2012). Sodium is the most important cation in regulation of fluid and electrolyte balance in the body due to its abundance and osmotic pressure. Since all body fluids are in chemical equilibrium, any change in sodium levels causes a compensatory shift in water, affecting plasma volume, blood pressure and intracellular and interstitial volumes.

The transmission of a nerve impulse along a neuron from one end to the other occurs as a result of electrical changes across the membrane of the neuron. The membrane of an unstimulated neuron is polarized-that is, there is a difference in electrical charge between the outside and inside of the membrane. The inside is negative with respect to the outside. Polarization is established by maintaining an excess of sodium ions (Na +) on the outside and an excess of potassium ions (K +) on the inside. A certain amount of Na + and K + is always leaking across the membrane through leakage channels, but Na +/K + pumps in the membrane actively restore the ions to the appropriate side. The main contribution to the resting membrane potential (a polarized nerve) is the difference in permeability of the resting membrane to potassium ions versus sodium ions. The resting membrane is much more permeable to potassium ions than to sodium ions resulting in slightly more net potassium ion diffusion (from the inside of the neuron to the outside) than sodium ion diffusion (from the outside of the neuron to the inside) causing the slight difference in polarity right along the membrane of the axon. Other ions, such as large, negatively charged proteins and nucleic acids, reside within the cell. It is these large, negatively charged ions that contribute to the overall negative charge on the inside of the cell membrane as compared to the outside.In addition to crossing the membrane through leakage channels, ions may cross through gated channels. Gated channels open in response to neurotransmitters, changes in membrane potential, or other stimuli (cliffnotes.com) Figure 23.1 below show the events that characterize the transmission of a nerve impulse.

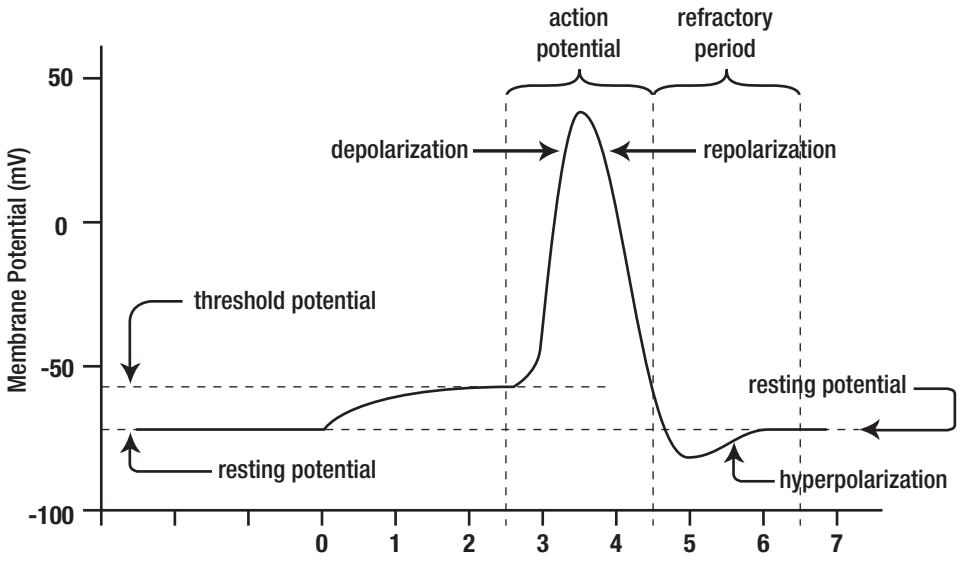

Figure 23.1. Events that characterize the transmission of a nerve impulse.
Source: cliffsnotes.com

23.3 Metabolism

Dietary sodium is virtually completely absorbed along the length of the intestine and the active transport of sodium is closely linked to the wider ability of the small intestine to absorb other nutrients (EFSA, 2006). In healthy Individual, nearly 100% of ingested sodium is absorbed during digestion, and urinary excretion is the primary mechanism for maintaining sodium balance. Even in hot humid climates, there are only minimal loses through faeces and sweat. Acclimation to heat occurs rapidly; thus, within a few days of exposure to hot and humid conditions, individuals lose only small amounts of sodium through sweat. Under conditions of extreme heat and intense physical activity that result in high sweat production, sodium losses in sweat are increased and appreciable; nonetheless, most individuals can replace the necessary sodium through food consumption, without dietary alterations, supplements of specially formulated products (WHO, 2012).

There are various system and hormones that influence sodium and chloride balance. These include the renin-angiostensin-aldosterone axis, the sympathetic nervous system, atrial natriuretic peptide (ANP), the kallikrein-kinin system, various intrarenal mechanisms and other factor's that regulate renal and medullary blood flow. Angiostensin II, a potent vasoconstrictor, regulates the proximal tubule of nephron to promote sodium and chloride retention and also stimulate the release of aldosterone from the adrenal cortex. Aldosterone promotes the renal reabsorption of sodium in the distal tubule of nephron by mineralocorticoid receptor-mediated exchange for hydrogen and potassium ions. With reduced salt intake, reduced blood volume, or reduced blood pressure, the renin angiostensin-aldosterone axis is stimulated. When the

renin-angiostensin-aldosterone system is less responsive, as with advancing age, there is a greater blood pressure reduction from a reduced intake of sodium chloride.

ANP is released in response to elevated blood volume and serves as a counter-regulatory system to the renin-angiostensin-aldosterone system. ANP decreases the release of renin and therefore the release of angiostensin II and aldosterone and increases the glomerular filtration rate. These action contribute to reductions in blood volume and blood pressure. The sympathetic nervous system is another major regulatory system for sodium and chloride excretion through at least three mechanisms alteration in renal medullary blood low, release of renin, direct effects on the renal tubules. Similar to the renin-angiostensin-aldosterone system, the sympathetic nervous system is activated during sodium depletion and suppressed during sodium excess. With Increased extracellular fluid volume, there is a decreased sodium concentration of the fluid delivered to the ascending limb of Henle's loop in the renal tubule. This decrease leads to reduced sodium reabsorption of the kidney's nephron so that more sodium is delivered to the distal tubules for excretion. Intrarenal mechanisms include locally released prostaglandins, kinins, angiostensin, endothelial relaxing factor and other less well defined factors (IOM, 2004).

Administration of potassium salts has been shown to increase urinary sodium excretion. In Normal human volunteers studied under controlled metabolic conditions, both potassium bicarbonate and potassium chloride have demonstrated substantial and comparable effects on increasing urinary sodium excretion, at least until equilibrium is reached. At a new steady state, sodium intake and excretion become equivalent. Animal experiment suggest that potassium may inhibit sodium reabsorption in the distal tubule of the kidney. By reducing extracellular volume and plasma volume, this effect is generally considered to be important of the antihypertensive effect of potassium, particularly in patients with hypertension A substantial body of evidence has documented that higher intakes of sodium result in increased urinary excretion of calcium. Data on effect of calcium intake on sodium excretion, however are limited (IOM, 2004)

23.4 Sources

Sodium is found naturally in a variety of foods, such as milk, meat and shellfish. It is often found in high amounts in processed foods such as breads, crackers, processed meats and snacks foods. High amounts of sodium are also found in many condiments (e.g soy and fish sauces), thus, a diet high in processed foods and low in fresh fruits and vegetables is often high in sodium (WHO, 2012).

Both the study on sodium intake among health staff in 2015(My Salt, 2015) and 2012 found that light soy sauce was the most popular seasoning consumed daily which contributed to the highest daily sodium intake. Fried rice, omelette, nasi lemak, thick soy sauce, roti canai, meat soups and fried mee were also major sources of sodium, followed by oyster sauce, and tomato/chilli sauce. Most sources of sodium in the diet were mainly contributed by cooked food (grain products), sauces/seasonings, meat & meat products, fish/seafood products, fast foods, eggs ,cooked foods (others), snacks, local kuih-muih ,canned foods and spreads, (IPH, 2016).

Malaysian Adult Nutrition survey (MANS, 2014) showed that foods sources of high sodium content mostly came from local kuih (79%), breads(76.9%), mee hoon, kueh teow, laksa, laksam, lohsi fun(76%), ketchup (75.6%) and followed by mee (75.2%).The list of sources and content of sodium in selected foods in Malaysia are listed in Table 23.1.

Table 23.1: Sodium content of foods

Food Sources	mg/100g
Cereal And Cereal Product	
Noodle snack,flavoured	613 mg
Rice porridge,instant	467 mg
Bread ,white	340 mg
Bread,wholemeal	311 mg
Biscuits, cream crackers [7 pcs]	110 mg
Noodle, Instant	103 mg
Rice,plain, cooked	6 mg
Starchy Roots, Tubers & Product	
Potato chip [12 pieces]	712 mg
Potato [2 whole]	33 mg
Legumes & Legumes Products	
Soya bean paste,fermented [1 tablespoon]	7299 mg
Soya sauce'thick'	2807mg
Soya bean cake,fermented 7 mg	
Nut, Seeds & Products	
Watermelon seeds, dried, black [3 cups]	358 mg
Vegetables & Vegetables Products	
Seaweed,dried [Half cup]	2025 mg
Cabbage,chinese,salted [1 tablespoon]	1863 mg
Peas,salted,fried. [1 cup]	414 mg
Fruits & Fruits Products	
Fruits, mixed, spicy pickled [1 tablespoon]	1488 mg
Durian fermented [2 tablespoon]	577 mg
Meat & Poultry Products	
Beef rendang,canned [Half cup]	507 mg
Mutton curry,canned [Half cup]	424 mg
Beef burger,regular [1 whole]	416 mg
Chicken frankfurter [2 pieces]	283 mg
Beef frankfurter[12x2cm] [2 pieces]	281 mg
Mutton,lean,raw [1 cup]	91 mg

Food Sources	mg/100g
Chicken,breast meat [1 cup]	42 mg
Chicken,thigh [2 medium]	42 mg
Fish, Shellfish & Products	
Fish sauce [Quarter tablespoon]	6329 mg
Shrimp,fermented [Half tablespoon]	4485 mg
Anchovy,dried [Quarter cup]	3969 mg
Prawn,salted,dried [1 tablespoon]	2554 mg
Prawn paste [1 tablespoon]	1742 mg
Fish ball	665 mg
Shrimp paste [Half piece]	1430 mg
Cuttlefish,fried[1 whole,small]	1038 mg
Prawn crackers [1 small packet]	750mg
Fish crackers, fried [5 pieces]	736 mg
Cuttlefish crackers [1 large packet]	527 mg
Sardine,canned [1 small can]	307 mg
Bream,threadfin [1 whole]	246 mg
Carp,common	67 mg
Mackerel,Spanish [1 slice]	73 mg
Milk & Milk Products	
Cheese,processed,cheddar [1 slice]	1452 mg
Oils & Fats	
Chili sauce [1 tablespoon]	1144 mg
Butter [2 tablespoons]	609 mg
Margerine [3 tablespoons]	384 mg

Source: My FCD, (1997 FCD)

Sodium content in different types of salt

Regardless of the cost, whether it comes in crystals or grains, from the sea or from the Himalayas, a new Consensus Action On salt and Health (CASH) in UK survey has found they all contain an equally high sodium chloride content as table and cooking salt.Garlic salt and celery salt are also popular alternatives to standard table salt. These products are made predominantly of table, rock or sea salt combined with small amounts of dried garlic or celery. The salt component is still sodium chloride so these too should be limited as with rock and sea salt (WASH, 2017). A market survey of sodium content and nutrient composition in a few types of salt in Malaysia is shown in Table 23.2.

Table 23.2: Nutrients composition in different type of salts in Malaysia

Nutrient composition /100 gm	Type Of Salt		
	Regular Single Refined Rock Salt	Natural, Himalayan Pink Rock Salt	Pure Rock Salt
Sodium chloride – NaCl (dry weight basis)	97.0%	98.68%	99.9%
Sulfate (Ca SO4)	%0.5Max		0.7%
Potassium(K)		0.14%	0.19%
Calcium (Ca)		0.07%	0.04%
Calcium Chloride & Magnesium Chloride (CaCl2 & MgC12)	0.25%		
Magnesium(Mg)		0.07%	0.08%
Iron (fe)		2.5ppm	2.88 mg/kg
Copper(cu)		0.85 ppm	0.93 mg/kg
Manganese		1.5 ppm	
Zinc(Zn)			3.98 mg/kg
Selenium (se)			10.15 mg/kg
Insoluble matter (in cold water)	%0.1max		

Source: Viola Michael (Personal Communication) 2017

Salt substitute are referred to as light salts, in which all or some of the sodium is replaced with other mineral such as potassium or magnesium(Food Act, 1983) Salt substitute like potassium salt have up to 70% less sodium than standard table salt so do not carry the same high risks as sodium based salts (WASH,2016). Iodised Salt is a form of salt that has been fortified with iodine. It contains 20 to 30 ppm or 20 to 30 mg iodine per kg of salt (FAO, 1995). Low-salt, very low salt and salt free food are defined as food with sodium concentration not more than 0.12g/100g, 0.04 g/100g, 0.005 g/100 g, respectively, and 1 mmol sodium = 23 mg sodium, 1 gram of sodium chloride (salt) contains 390 mg (17 mmol) of sodium. (Food Act, 1983)

23.5 Excess

Data obtained from 24-hour urine analysis is considered as the "gold standard" or method used by World Health Organization (WHO) to estimate sodium intake in the population. Other method used are 24 hours dietary recall and spot urinary sodium analysis.

According to Powles *et al.* (2013), in 2010 the estimated mean intake of sodium in 181 out of 187 countries which consisted 99.2 % of the world adult population exceeded the WHO recommendation of 2.00 g/day sodium or 5 gram of salt per day. Asian regions had the highest

mean sodium intake where Central Asia recorded intake of 5.51 g per day or (14.01 gram of salt), followed by Asia Pacific High income countries mainly Japan and South Korea with mean sodium intake of 5 gram per day or 12.71 gram salt and East Asia with mean sodium intake of 4.8 gram per day or 12.21 gram of salt.

A recent local study, My Salt, 2015 using the 24 hours urinary sodium among 1027 health staff in Malaysia showed that, the mean sodium intake was 2860 mg or 7.15g salt per day (IPH, 2016). There was a reduction of sodium intake compared to the pilot study which was done among 445 health staff in 2012 which showed an average intake of 3.4 gm/day sodium or 8.7 gram of salt per day. Data obtained through dietary surveys generally tend to underestimate sodium intakes, as compared to data obtained from 24-hour urine analysis. The mean sodium intake among health staff in 2012, 2015 and Malaysian Adult Nutrition Survey (MANS, 2003, MANS, 2014) is shown in Table 23.3.

Table 23.3: Mean sodium intake in Malaysia using different methods of sodium analysis.

Mean sodium intake per day by different methods.						
Name of study	**Sample size (n)**	**24 hours urinary sodium analysis (mg/day)**	**Sample size (n)**	**24 hours dietary survey sodium (mg/day)**	**Sample size (n)**	**Spot urinary sodium analysis (mg/day)**
My Salt 2015 (IPH, 2016)	1027	2860	1096	2761	1013	2383 mg
Pilot study among health staff in 2012						
(Rashidah et.al, 2014)	445	3429	445	2372		
MANS 2014 (NHMS, 2014)		-	3880	1935		-
MANS 2003 Mirnalini et al. (2008)		-	6886	2575		-

Data on sodium intake among children and adolescents is very limited. A Cohort study consisting of 794 adolescents, aged 13 years (attending 15 public schools from the Central (Kuala - Lumpur and Selangor) and Northern (Perak) Regions of Peninsular Malaysia using a 7 days historical assessment of habitual food intakes showed that sodium intake was (2289.5 mg/day) which exceeded the recommendation by WHO (Abdul Majid *et al.,* 2016).

Reducing sodium intake significantly reduced systolic and diastolic blood pressure in adults and children. The reduction in blood pressure was detected across a wide range of intake levels, and was independent of baseline sodium intake. Reducing sodium intake to <2 g/day was more beneficial for blood pressure than reducing sodium intake but still consuming > 2 g/day. Reducing sodium intake had no significant adverse effect on blood lipids, catecholamine levels in renal function.

Higher sodium intake was associated with higher risk of incident of stroke, fatal stroke and fatal coronary heart disease. There was no association between sodium intake and all cause mortality, incident cardiovascular disease and non-fatal coronary heart disease. However, the

strong positive relationship between blood pressure and these outcomes provides indirect evidence that reducing sodium intake can improve these outcomes through a beneficial effect on blood pressure. (WHO, 2012)

Higher sodium intake is also linked with stomach cancer. A meta-analysis of 7 prospective studies, involving 270,000 individuals who were followed up over 6-15 years, showed that those with high salt/sodium intake had a 68% higher risk of developing gastric cancer than those with low intake (D 'Elia *et al.,* 2012). Similiarly, a 2007 meta-analysis of cohort studies found that for every gram of salt intake per day, the risk of developing stomach cancer increased by 8% (WCRF, 2007).

The prevalence of hypertension in Malaysia is currently at 30.3% in adult age 18 years and above. Based on the latest age-standardised adjusted estimates published by WHO, Malaysia's prevalence of high BP is higher compared to Singapore and Thailand. Based on Malaysia's latest Burden of Disease Study published in 2014, high blood pressure is estimated to cause 42.2% of deaths and 21.6 % of Disability-adjusted life years (DALY), the largest contributor for both men and women (MOH, 2015) Figure 23.2 shows the prevalence of hypertension in Malaysia.

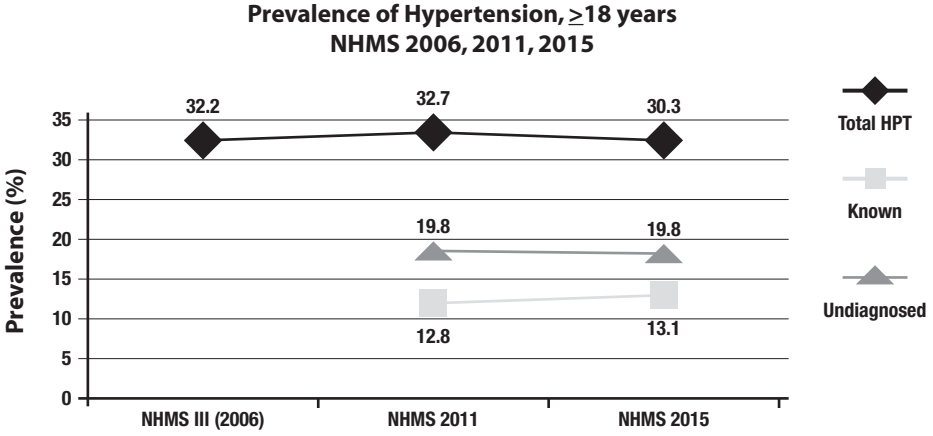

Figure 23.2: Prevalence of Hypertension in Malaysia (NHMS 2006-2015)
(Source: NHMS 2015)

The prevalence of Hypertension among children and adolescent were found to be increasing in Malaysia. A study from Sabah, Malaysia has reported that the prevalence of hypertension among children aged 8-9 years old was 14% (Chong, Soo & Rasat, 2012). Similarly, a study by Rampal et al (2010) among 1778 Malay adolescents in Putrajaya Secondary Schools showed that the prevalence of pre-hypertension and hypertension among male and female was 16.2 % and 12.9 %, and 5.8% and 10.2 %, respectively. The overall prevalence of prehypertension and hypertension was 11.1 % and 11.6 %, respectively.

The minimum intake level of sodium necessary for proper bodily function is not well defined. It is estimated to be as little as 200-500 mg/day. Data around the world suggest that the population average sodium consumption is well above the minimal physiological needs and in many countries is above the value recommended by the Joint World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO, 2002) Expert consultation of 2g sodium/day or equivalent to 5 g salt/day.

Decreased sodium intake results in reduced blood volume and thus activates the renin-angiotensin- aldosterone and sympathetic nervous systems which help to control blood volume (WHO, 2012)

23.6 Factors Affecting Sodium Requirements.

Indicators that have been used for assessing the need for sodium include Sodium balance, urine and faeces, skin and sweat, chloride balance, serum or plasma concentration, plasma renin activity, and blood lipid concentration. When substantial sweating does not occur, total obligatory sodium losses are very small, up to 0.18g/day or 8mmol/day (Table 23.4). For this reason, in a temperate climate or even a tropical climate, acclimatized persons can survive on extremely low sodium intake.

Table 23.4: Obligatory losses of sodium

	g/d	mmol/day
Urine	0.005-0.035	0.2-1.5
Skin (non sweating)	0.025	1.1
Faeces	0.010-0.125	0.4-5.4
Total	0.040-0.185	1.7-8.0

Source: DHAL (1958)

In non sweating individuals living in a temperate climate who are in a steady - state of sodium and fluid balance, urinary sodium excretion is approximately equal to sodium intake (90 to 95 percent of total intake is excreted in urine). Obligatory urinary losses of sodium in adults are approximately 23 mg (1mmol)/day. Excretion of sodium in the stool is minimal. When sodium intakes ranged from 0.05 to 4.1g/day of sodium, only about 0.01 to 0.125 g (0.4 to 5.4 mmol/day) appeared in the stool. Still faecal excretion of sodium was less than 5 percent of intake even at the highest level of sodium intake (IOM, 2004). Daily dermal losses of sodium have been reported to average less than 0.025 g (1.1mmol/day). Sweat sodium loss depends on a number of factors, including the sweat rate, sodium intake and heat acclimation. For these reasons, the sodium concentration in sweat varies widely. Healthy persons sweating 5 to 9 L/day could maintain sodium chloride balance on intakes ranging from as low as 1.9 g (83 mmol/day) to 3.2g (139mmol/day) of sodium chloride, the maximum intake provided. In aggregate available data indicated that healthy, free living individuals can achieve sodium balance following acclimation under a variety of conditions, including low sodium intake and extreme heat (IOM, 2004)

Chloride losses usually accompany sodium losses. Hence conditions and diseases in which sodium is lost are likewise associated with chloride loss. Excess chloride depletion marked by hypochloremia, results in hypochloremis metabolic alkalosis in which loss of hydrochloric is the primary form of chloride loss (IOM, 2004).

Changes in sodium intake can influence serum or plasma levels of sodium, but the changes are relatively small and do not lead to pathological conditions, such as hyponatremia. When observed, hyponatremia is often caused by excessive sodium loss from the body, which occurs with impaired renal function, increased vasopressin release, or excessive consumption of water. Diuretic use is an infrequent cause of hyponatremia. Renin is released from the juxtaglomerular cells of the kidney response to a perceived reduction in blood volume, blood pressure, or tubular sodium concentration. As a result, renin induces the production of angiotensin 11, which stimulates renal sodium reabsorption via a direct tubular effect, as well as by increasing the production of aldosterone. Elevation in blood pressure (IOM, 2004). Several trials on the effects of reduced sodium intake on blood lipid concentration showed that it will increase the total and low density lipoprotein (LDL) cholesterol concentration (IOM, 2004).

23.7 Setting Requirements and Recommended Intakes of Sodium

Based on the entire body of evidence, WHO (2012) recommends a reduction in sodium intake to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults. For adults, WHO recommends a reduction of < 2 g/day sodium (5 g/day). For children, WHO recommends a reduction in sodium intake to control blood pressure. The recommended maximum level of intake of 2 g/day sodium in adults should be adjusted downward based on the energy requirement of children relative to those of adults (WHO, 2012).

This recommendation applies to all individuals with or without hypertension including pregnant or lactating women, except for individuals with illnesses or taking drug therapy that may lead to hyponatraemia or acute build-up of body water, or require physician- supervised diets (e.g. patients with heart failure and those with type 1 diabetes). In these subpopulations, they may be a particular relationship between sodium intake and the health outcomes of interest. For this recommendation adults includes individuals >16 years of age and children includes individuals 2-15 years of age inclusive. The recommendation for children does not address the recommended period of excusive breastfeeding (0-6) month or the period of complementary feeding with continued breastfeeding (6-24 months) (WHO, 2012).

Because of insufficient data from dose response trials, an Estimated Average Requirement (EAR) could not be established, thus Recommended Dietary Allowance could not be derived, hence an Adequate Intake (AI) is provided.

Technical Sub-committee (TSC) on mineral & traces of elements decided to adapt the IOM (2006) recommendation for sodium.

For the prevention and control of Non Communicable Diseases, the TSC on mineral & traces of elements is agreeable to the current recommendation by WHO (2012) which was based on the maximum level of 2 g/ day sodium for adult and for children it should be adjusted downward based on the energy requirement). Anyway, since this was not clearly stated based

on age range and was not stated in any proper reference, with justification the TSC decided to adapt to the IOM (2006) for the recommendation of sodium which is stated in AI (Table 23.5) and still below the recommendation of 2 gram sodium per day for all the age range. It is worth noting that even the earliest recommendation by SCAN in 2003 stated that the RNI for sodium in adult should be 1600 mg/day.

Table 23.5: Sodium requirement (AI mg/ day) for all age group

	Sodium Requirement (AI mg/day)
Infants	
0 - 6 months	120 mg/day (5 mmol/day)
7 - 12 months	370 mg/day (16 mmol/day)
Children & adolescents	
1 - 3 yr	1000 mg/day (42 mmol/day)
4 - 8 yr	1200 mg/day (53 mmol/day)
9 - 13 yr	1500 mg/day (65 mmol/day)
14 - 18 yr	1500 mg/day (65 mmol/day)
Adults	
Men	1500 mg/day (65 mmol/day)
Women	1500 mg/day (65 mmol/day)
>70 yr	1200 mg/day (53 mmol/day)
Pregnancy	
14 - 18 yr	1500 mg/day (65 mmol/day)
19 - 30 yr	1500 mg/day (65 mmol/day)
31 - 50 yr	1500 mg/day (65 mmol/day)
Lactation	
14 - 18 yr	1500 mg/day (65 mmol/day)
19 - 30 yr	1500 mg/day (65 mmol/day)
31 - 50 yr	1500 mg/day (65 mmol/day)

Source: IOM 2006

23.8 Tolerable Upper Intake Levels

The scientific rationale for setting the Tolerable Upper intake levels should be based on the adverse effects of higher levels of sodium intake on blood pressure in adults and children. The lowest observed adverse effect level (LOAEL) for the dietary sodium is set at 2.3 g/day (100 mmol/day) based on a preliminary three trials in which the lowest level of dietary sodium intake was close to the AI (Macgregor *et al.,* 1989; Sacks *et al.,* 2001). When dietary sodium was provided at the average level of 1.2 g (50 mmol)/day, blood pressure was significantly less than

when the target average sodium intake was 2.3 g (100 mmol/day. It is recognized that the term LOAEL as applied to dietary sodium is a point on a continuous relationship with blood pressure, a point that correspondents to the next level above the AI that was tested in dose-response trials. As with other nutrients, a no observed adverse effect level (NOAEL) would have been preferable. However in the setting of a progressive, dose-response relationship without a threshold, a NOAEL cannot be set. Note that the UL is not a recommended intake. As with other ULs, there is no apparent benefit to consuming levels above the AI. The UL recommendation based on age range is shown below.

UL for sodium

Age	mg/day (mmol)
Infants	
0 - 12 months	Not possible to establish. Source of intake should be through breast milk, formula and food only.
1 - 3 year	1000 mg/day(43 mmol)
4 - 8 yr	1,400 mg/day(60 mmol)
9 - 13 yr	2000 mg/day(86 mmol)
14 - 18 yr	2300 mg/day(100 mmol)
Adults 19 + yr	
Men	2300 mg/day(100 mmol)
Women	2300 mg/day(100 mmol)

23.9 Research Recommendations

The Technical subcommittee identified a number of data gaps or lack of evidence in relation to mean sodium intake for population, database on sodium content in foods, adverse effect of lowering sodium intake and association between sodium intake and other diseases or condition as stated below.

i) Data on salt (sodium) intake using the 24 hours urinary sodium which is the Gold Standard as recommended by WHO is very limited in Malaysia. The available data is only confined from data of a few specific group such as the salt intake among MOH staff and a few other studies among urban poor and University students which is still unpublished. All these data are not representing the population salt intake and can only be used as a proxy to develop any programs for salt (sodium) recommendation.

   A population based study using the 24hours urinary sodium is very much needed to help to provide novel and valuable data for the sodium /salt recommendation in Malaysia.

ii) There is a need for sodium to be mandatory labelled in all processed foods in Malaysia for the purpose of educating the public and monitoring the content of sodium in all processed foods. Research or market survey on the current status of sodium labelling need to be done to accelerate the process of labelling.

iii) In addition to RCT Research, mechanistic studies are needed to examine the potential physiological changes associated with lowering sodium intake and adverse health outcomes.

iv) Finally, additional observational research is needed to examine associations between sodium intake and cancer, especially gastric cancer, as well as association between sodium intake and caloric intake in both short- term and longitudinal studies.

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Appendix 23.1: Comparison of recommended intake for Sodium: RNI of Malaysia (2017), SACN (2003) and AI of IOM (2006)

Malaysia 2017			SACN 2003			IOM 2006		
	Age	AI (mg/day)		Age	RNI (mg/day)		Age	AI (mg/day)
Infants (boys)	0 - 6 months	120	**Infants (boys)**	0 - 3 months	210	**Infants (boys)**	0 - 6 months	120
	7 - 12 months	370		4 - 6 months	280		7 - 12 months	370
				7 - 9 months	320			
				10 - 12 months	350			
Infants (girls)	0 - 6 months	120	**Infants (girls)**	0 - 3 months	210	**Infants (girls)**	0 - 6 months	120
	7 - 12 months	370		4 - 6 months	280		7 - 12 months	370
				7 - 9 months	320			
				10 - 12 months	350			
Children (boys)	1 - 3 years	1000	**Children (boys)**	1 - 3 years	500	**Children (boys)**	1 - 3 years	1000
	4 - 8 years	1200		4 - 6 years	700		4 - 8 years	1200
				7 - 10 years	1200			
Children (girls)	1 - 3 years	1000	**Children (girls)**	1 - 3 years	500	**Children (girls)**	1 - 3 years	1000
	4 - 8 years	1200		4 - 6 years	700		4 - 8 years	1200
				7 - 10 years	1200			
Adolescent (boys)	9 - 13 years	1500	**Adolescent (boys)**	11 - 14 years	1600	**Adolescent (boys)**	9 - 13 years	1500
	14 - 18 years	1500		15 - 18 years	1600		14 - 18 years	1500
Adolescent (girls)	9 - 13 years	1500	**Adolescent (girls)**	11 - 14 years	1600	**Adolescent (girls)**	9 - 13 years	1500
	14 - 18 years	1500		15 - 18 years	1600		14 - 18 years	1500

Malaysia 2017			SACN 2003			IOM 2006		
	Age	AI (mg/day)		Age	RNI (mg/day)		Age	AI (mg/day)
Men	19 - 29 years	1500	**Men**	19 - 50 years	1600	**Men**	19 - 29 years	1500
	30 - 50 years	1500		> 50 years	1600		30 - 50 years	1500
	51 - 59 years	1500					51 - 59 years	1500
	60 - 69 years	1500					60 - 69 years	1500
	> 70 years	1200					> 70 years	1200
Women	19 - 29 years	1500	**Women**	19 - 50 years	1600	**Women**	19 - 29 years	1500
	30 - 50 years	1500		>50 years	1600		30 - 50 years	1500
	51 - 59 years	1500					51 - 59 years	1500
	60 - 69 years	1500					60 - 69 years	1500
	> 70 years	1200					> 70 years	1200
Pregnancy	14 - 18 years	1500				**Pregnancy**	14 - 18 years	1500
	19 - 30 years	1500					19 - 30 years	1500
	31 - 50 years	1500					31 - 50 years	1500
Lactation	14 - 18 years	1500				**Lactation**	14 - 18 years	1500
	19 - 30 years	1500					19 - 30 years	1500
	31 - 50 years	1500					31 - 50 years	1500

24 • Potassium

24.1 Introduction

Potassium is a chemical element with symbol K (Kalium) and atomic number of 19. The name is derived from the collection of wood ash in metal pots when the beneficial fertilizer properties of this material were first recognized. Potassium is a soft, silvery-white metal and a member of the alkali group of the periodic chart. Potassium oxidizes rapidly in air and is generally stored under oil or grease. High solubility of potassium in water makes it applicable in industry goods, such as fertilizers, liquid soaps and detergents. Potassium in the form of potassium chloride is used in pharmaceuticals, medical drips and saline injections. The human body contains about 3.5-5 mmol potassium. Only 2% of potassium is in the extracellular fluid and the rest is held in the cells by complex mechanisms (Ringer & Bartlett, 2007).

24.2 Functions

Potassium is classified as a macro-mineral and is the major intracellular cation required for normal cellular functions in the body. The ratio of intracellular to extracellular potassium is the major determinant of muscular and neuronal excitability. Relatively small changes in the concentration of extracellular potassium can greatly affect the extracellular to intracellular potassium ratio. This will affect neural transmission, muscle contraction and vascular tone. Potassium helps muscles contract, regulates fluids and acid base balance in the blood and tissues along with sodium. In the nerve cells, the sodium-potassium flux generates the electrical potential that aids the conduct of nerve impulses (Institute of Medicine, IOM, 2006).

Potassium changes the membrane potential and allows the nerve impulse to progress. This electrical potential gradient created by the Na^+/K^+-ATPase pump helps to generate muscle contractions and regulates the heartbeat. The high intracellular concentration of potassium is maintained via the activity of the Na^+/K^+-ATPase pump. Approximately 77 to 90% of dietary potassium is excreted in urine, while the remainder is excreted mainly in faeces, with much smaller amounts being lost in sweat. There is no effective method for potassium conservation, unlike sodium. The kidneys continue to excrete potassium even when a shortage occurs. It is essential to maintain potassium balance in the body for regular heart contractions and healthy nervous system (Palmer & Clegg, 2016).

Increased potassium intake is known to reduce blood pressure, decrease risk of cardiovascular diseases and lessen the negative impacts of high sodium intake. This is due to the function of potassium being tightly bound to sodium. Excess of sodium intake will deplete potassium, thus, the balance of potassium and sodium is important (Aburto *et al.,* 2013; Oberleithner *et al.,* 2009). The ratio of sodium and potassium is reported as an important factor in cardiovascular disease and mortality and is a better predictor of cardiovascular outcome than either potassium or sodium individually (Cook *et al.,* 2009; Yang *et al.,* 2011). Recent meta-analysis demonstrated that sodium-to-potassium ratio is more strongly associated to blood pressure outcomes than either individual nutrients (Perez & Chang, 2014).

24.3 Metabolism

In healthy persons, approximately 85% of dietary potassium is absorbed. There is 50 mEq/kg of potassium in the body which mean total body potassium in a 70 kg person is 3,500 mEq. Potassium is found mainly within cells (98%) and the rest of the potassium is in the extracellular fluid. The normal concentration of potassium in the extracellular fluid is around 3.5-5.3 mEq/l. About 90% of the daily potassium intake is excreted in the urine, whereas a smaller percentage (10%) is excreted through the gastrointestinal tract. The colon contains about 30 mmol/L of potassium but it is reabsorbed in the final part of the bowel. Thus, the faeces contain a small amount of potassium. Excretion in faeces and sweat is usually negligible. Potassium is also cofactor for many enzymes and it is also required for creatine phosphorylation, carbohydrate metabolism and protein synthesis (Perez & Chang, 2014).

Potassium in urine results mainly from secretion of potassium into the cortical collecting duct. This secretion is regulated by a number of factors, including the hormone aldosterone. The latter is released by adrenal cortex when it is stimulated due to elevated plasma concentration of potassium. Insulin, catecholamine, and aldosterone are critical factors responsible for maintaining the normal internal distribution of potassium. The kidney is the major organ responsible for buffering the movement of potassium into or out of skeletal muscle cells. Internal potassium balance is a term used to refer to the regulation of potassium between the intracellular and extracellular space. The kidney facilitates potassium homeostasis by adjusting renal potassium excretion over several hours in response to a potassium load. Initial changes in extracellular potassium concentration are buffered by movement of potassium into or out of skeletal muscle cells (Palmer & Clegg, 2016).

Almost all cells possess the Na^+-K^+-ATPase, which pumps sodium out of the cell and potassium into the cell that leads to a potassium gradient across the cell membrane that is partially responsible for maintaining the potential difference across the membrane. This potential difference is critical to the function of cells, particularly in excitable tissues, such as nerve and muscle. The body has developed numerous mechanisms for defense of serum potassium. These mechanisms serve to maintain a proper distribution of potassium within the body as well as regulate the total body potassium content (Palmer & Clegg, 2016).

24.4 Sources

Potassium normally can be obtained from one's diet and thus, the body usually does not need to draw from intracellular potassium (Ringer & Bartlett, 2007). It is important to note that the beneficial effects of potassium in some studies appear to be mainly in the forms of potassium that are associated with bicarbonate precursor; the forms found naturally in foods such as fruits and vegetables. Eating diets high in potassium has been linked to reducing blood pressure, decreasing the risk of stroke, improving bone health, and reducing the risk of nephrolithiasis more than taking potassium supplements. This suggests that not only is potassium critical, but also consumption of the foods enriched in potassium provides benefits. Several food groups have been recommended as excellent food sources of potassium (Table 24.1).

Table 24.1: Potassium content of foods.

Food Sources	mg/100g
Legumes & legume products	
Soya bean	397
Chick pea	345
Kidney bean	315
Tempeh	178
Fermented soya bean paste	233
Fish	
Tuna	344
Nuts	
Cashew nut	410
Dried walnut	260
Vegetables	
Spinach	385
Cabbage	103
Carrot	136
Potato	203
Sweet potato	210
Onion (small)	279
Pumpkin	113
Tomato	129
Cucumber	76
Milk product	
Yoghurt	153
Fruits and fruits products	
Exotica papaya	137
Banana	379
Dates	62
Papaya	39
Tomato juice	84

Sources: USDA, 2010; WHO, 2012; Tee *et al.* 1997.

24.5 Deficiency

Severe potassium deficiency is characterized by hypokalemia, a situation when serum potassium concentration is less than 3.5 mmol/L. It can be induced by diuretics, chloride-depletion associated forms of metabolic alkalosis and increased aldosterone production (Kjelden, 2010). Hypokalemia can affect the functions of pancreas through reduction of the insulin secretions that causes reversible glucose intolerance.

A low potassium diet, as low as 0.58 g (15 mmol/day) can cause a decrease in plasma insulin concentration and caused a resistance to insulin action. This can be reversed when dietary potassium is supplemented at 4.8 g (64 mmol/day). The adverse consequences of hypokalemia include cardiac arrhythmias, muscle weakness and glucose intolerance. Moderate potassium deficiency is characterized by increased blood pressure, increased salt sensitivity, increased risk of kidney stones and increased bone turnover (Ringer & Bartlett, 2007).

An inadequate intake of dietary potassium may also increase the risk of cardiovascular disease, particularly stroke (D'Elia *et al.,* 2011). The adverse effects of inadequate potassium intake can result from a deficiency of potassium. It is a deficiency of its conjugate anion, or both. Potassium deficiency due to low dietary intake only is very uncommon. This is due to the widespread occurrence of potassium in food. Potassium deficiency can develop as a consequence of increasing losses from the gastrointestinal tract and kidneys, e.g. during prolonged diarrhoea or vomiting, and in connection with use of laxatives or diuretics.

24.6 Factors affecting potassium requirements

Increased losses of potassium, primarily through heavy sweating can occur with heat exposure and intense activity level. Previous studies showed there was a decline in sweat loss over time which demonstrated that acclimation occurs. This suggests that potassium balance might be achieved over a period of time. However, non-acclimated individuals engaged in intense physical activities especially at high temperatures for extended periods of time experience profuse sweating. Thus, these individuals should have higher potassium intake to replace the loss of potassium.

Another factor will be diuretics which is one of the most frequently prescribed drugs for hypertension and congestive heart failure. This kind of drugs increases urinary excretion of potassium. Diuretic-induced hypokalemia may need potassium supplement to offset the adverse effects related to hypokalemia. The interaction of potassium and sodium is also important. The requirement for potassium depends on the level of dietary sodium, and the deleterious effects of sodium are attenuated by higher intakes of potassium IOM (2006).

24.7 Setting requirements and recommended intake

Data generated from the NHANES study conducted in 2007-2008 estimated that both men and women in the USA were obtaining substantially lower levels of potassium from their diet than recommended; the mean intake for women and men were estimated to be 2290 mg/day, and 3026 mg/day respectively (USDA, 2007). In fact, the most recent Dietary Guidelines

for Americans and the Food and Drug Administration have now designated potassium as a nutrient of public health concern since people are not meeting their estimated recommended dietary intake (DeSalvo et al. 2016).

European Food Safety Authority (EFSA, 2013) did not have any recommendation for potassium. World Health Organization (WHO, 2012) recommends an increase in potassium intake from food to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart disease in adults. Hence, WHO suggested that potassium intake should be at least 90 mmol/day (3510 mg/day) for adults and this intake should be adjusted downward for children, based on the energy requirements of children relative to those of adults. Malaysian Health and Adolescents Longitudinal Research Team Study reported that the potassium intakes among the male and female adolescents (aged 13 years) were 1096.6 mg/day and 998. 1 mg/day, respectively (Abdul Majid *et al.* 2015). The latter's potassium intake was considered low when compared to a recent study in USA (Buendia *et al.,* 2015) that classified low potassium intake among female adolescents to be less than 1800 mg potassium.

IOM (2006) has reviewed the amount of dietary potassium required for normal homeostasis. An adequate intake levels for dietary potassium was established at 4.7 g (120 mmol)/day for all adults when based on available data (IOM, 2006). This level is based on an intake of naturally occurring potassium from dietary sources, primarily fruits and vegetables. Available evidence indicates that this level of potassium intake should lower blood pressure, blunt the adverse effects of sodium chloride on blood pressure, reduce the risk of kidney stones, and possibly reduce bone loss. Thus, the Technical Sub-Committee (TSC) on Minerals and Trace Elements suggest to adapt the recommendations from IOM (2006) for the RNI values for potassium since it has proposed a set of dietary reference intake values for different age groups as well as pregnant and lactating women. Comparison of recommended intakes for potassium from RNI Malaysia (2017), AI of IOM (2006) and RNI of WHO (2012) are as shown in Appendix 24.1.

Infant

The recommended intakes of infants are based on the average amount of potassium in human milk that is consumed. For infants from 0 - 6 months, the intake is estimated based on the average volume of milk intake (0.78 L/day) and an average concentration of potassium in human milk (0.5 g/L) during the first 6 months of lactation. For the older infant, it can be determined by estimating the intake from human milk (0.3 g/day) and complementary food (0.44 g /day). Thus, the total potassium intake is estimated to 0.74 g and rounded to 0.7 g. The TSC recommendations are as follows:

RNI for infants

0-6 months	**0.4 g (10 mmol)**
7-12 months	**0.7g (18 mmol)**

Children and adolescent

RNI for children and adolescents ages 1 to 18 years was derived by extrapolating from the adults RNI on the basis of the average of median energy intake levels. This is due to the concern that adjustment based on weight might lead to relatively low and potentially inadequate intake of potassium. Given the high energy intake of children relative to their weight and the potential for a high sodium intake as results of their high energy intake, a greater intake of dietary potassium would be appropriate as a means to reduce the adverse effects of sodium. The TSC recommendations are as follows:

RNI for children and adolescents

1 - 3 years	**3.0 g (77 mmol)**
4 - 8 years	**3.8 g (97 mmol)**
9 - 13 years (Girls/boys)	**4.5 g (115 mmol)**
14 - 18 years (Girls/boys)	**4.7 g (120 mmol)**

Adults

As for adults from age 19 to 50 years, the available data are nonetheless insufficient for setting an EAR which requires data at multiple intake levels. Present available data are also insufficient to set gender specific requirements. Thus, the RNI was set at 4.7 g that could give beneficial effects such as decreasing the risk of kidney stones, lowering the blood pressure and decreasing bone loss as findings from epidemiological studies. The TSC recommendations are as follows:

RNI for adults

Men

19 - 30 years	**4.7 g (120 mmol)**
31 - 50 years	**4.7 g (120 mmol)**

Women

19 - 30 years	**4.7 g (120 mmol)**
31 - 50 years	**4.7 g (120 mmol)**

Older adults

As for adults from age 50 years and above, they consume less energy than younger adults. However, due to increased risk of elevated blood pressure with aging, the potassium needs to be greater and not adjusted down for older adults. There is a lack of evidence to suggest that the requirement for potassium differs in normal healthy older adults and elderly compared with younger individuals, the RNI is set at the same level of intake as for young adults. However, this RNI does not apply to individuals with medical conditions or taking drugs that impair the potassium excretion. The TSC recommendations are as follows:

RNI for older adults

Men

51 - 60 years	**4.7 g (120 mmol)**
> 60 years	**4.7 g (120 mmol)**

Women

51 - 60 years	**4.7 g (120 mmol)**
> 60 years	**4.7 g (120 mmol)**

Pregnancy

Potassium accretion during pregnancy is very small and there is an absence of data to suggest that the requirement for potassium is different during pregnancy. Thus, it is set at 4.7 g, the same as non-pregnant women. The TSC recommendations are as follows:

RNI for pregnancy

14 - 18 years	**4.7 g (120 mmol)**
19 - 30 years	**4.7 g (120 mmol)**
31 - 50 years	**4.7 g (120 mmol)**

Lactation

The potassium content of human milk averages around 0.5 g/L (13 mmol/L) during the first 6 months of lactation. Average milk production during the first 6 months of lactation is about 7.8L/d. Thus approximately, 0.4 g (10 mmol)/ day of potassium is needed for lactation during this period (0.5 g/L X 0.78 L/day = 0.4 g/day). Due to the lack of information, it is assumed that the efficiency of conversion dietary potassium to milk produced is almost 100%. The TSC recommendations are as follows:

RNI for lactation

14 - 18 years	**5.1 g (130 mmol)**
19 - 30 years	**5.1 g (130 mmol)**
31 - 50 years	**5.1 g (130 mmol)**

24.8 Tolerable upper intake level

In a generally healthy population with normal kidney function, a potassium intake from foods above the RNI poses no potential for increased risk because excess potassium is readily excreted in the urine. Therefore, a Tolerable Upper Intake Level (UL) was not set. However, in individuals in whom urinary excretion of potassium is impaired, a potassium intake below 4.7 g (120 mmol)/day is appropriate because of adverse cardiac effects (arrhythmias) from the resulting hyperkalemia (a markedly elevated serum potassium concentration). Such individuals are typically under medical supervision. However, potassium supplement should be avoided as it can cause acute toxicity. As for infants, potassium intake should be only from formula and complementary foods as their renal secreting ability is not fully developed.

24.9 Research recommendations

In recent years, potassium has received substantial attention due to its ability to attenuate deleterious effects of sodium. Future research should work to determine the optimal ratio of sodium to potassium intake. Local based studies should be encourage to determine the recommended levels for Malaysians as well as safe dosage of potassium in order to ensure optimum physiological functioning without adverse effects.

24.10 References

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Appendix 24.1. Comparison of recommended intakes for Potassium: RNI of Malaysia (2017), AI of IOM (2006) and RNI of WHO (2012)

Malaysia (2017)		IOM (2006)		WHO (2012)	
Age	**RNI (g/day)**	**Age**	**AI (g/day)**	**Age**	**RNI (g/day)**
Infants		**Infants**		**Infants**	
0 - 6 months	0.4	0 - 6 months	0.4	0 - 6 months	-
7 - 12 months	0.7	7 - 12 months	0.7	7 - 11 months	-
Children		**Children**		**Children**	
1 - 3 years	3.0	1 - 3 years	3.0	1 - 3 years	-
4 - 8 years	3.8	4 - 8 years	3.8	4 - 6 years	-
				7 - 10 years	-
Boys		**Boys**		**Boys**	
9 - 13 years	4.5	9 - 13 years	4.5	11-14 years	-
14 - 18 years	4.7	14 - 18 years	4.7	15 -17 years	-
Girls		**Girls**		**Girls**	
9 - 13 years	4.5	9-13 years	4.5	11-14 years	-
14 - 18 years	4.7	14 -18 years	4.7	15 -17 years	-
Men		**Men**		**Men**	
>19 years	4.7	>19 years	4.7	≥18 years	3.5
Women		**Women**		**Women**	
>19 years	4.7	>19 years	4.7	≥18 years	3.5

Malaysia (2017)		IOM (2006)		WHO (2012)	
Age	**RNI (g/day)**	**Age**	**AI (g/day)**	**Age**	**RNI (g/day)**
Pregnancy		**Pregnancy**		**Pregnancy**	-
< 18 years old	4.7	< 18 years old	4.7		
>19 years old	4.7	>19 years old	4.7		
Lactation		**Lactation**		**Lactation**	-
< 18 years old	5.1	< 18 years old	5.1		
>19 years old	5.1	>19 years old	5.1		

25 • Magnesium

25.1 Introduction

Magnesium is the eleventh most abundant element in the human body. An adult body contains approximately 25 g magnesium, with 50% to 60% present in the bones and most of the rest in soft tissues (Volpe, 2012). Less than 1% of total magnesium is in blood serum, and this level is kept under tight control. Normal serum magnesium concentrations ranges between 0.75 and 0.95 mmol/L. Approximately 0.3 % of body magnesium is in the serum, as free cations (about 54 %), which is the bioactive form, as a protein-bound form (about 33%) mainly to albumin (75 %) and as anion complexes (about 13 %), Elin (2010). Magnesium concentrations in blood cells are higher than in the serum: eight times in reticulocytes, three times in red blood cells.

25.2 Functions

Magnesium is a cofactor of more than 300 enzymatic reactions, acting either on the substrates (especially for reactions involving adenosine triphosphate (ATP) by facilitating the transfer of phosphate) or on the enzyme itself as a structural or catalytic component. As ATP utilization is involved in many metabolic pathways, magnesium is essential in the intermediary metabolism for the synthesis of carbohydrates, lipids, nucleic acids and proteins, as well as for specific actions in various organs such as the neuromuscular or cardiovascular system. Magnesium can interfere with calcium at the membrane level or bind to membrane phospholipids, thus modulating membrane permeability and electrical characteristics. Magnesium has an impact on bone health through its role in the structure of hydroxyapatite crystals in bone.

25.3 Metabolism

Magnesium absorption takes place in the distal intestine, mainly as the ionized form. Percentage absorption are generally considered to be between 40% to 50 %. The majority of the body magnesium content is stored in bone (about 60 %) and muscle (about 25 %). A small amount is present in the serum, mainly as the free cation. Most cells are able to actively and rapidly buffer magnesium loss or accumulation through the involvement of specific magnesium transporters.

The kidney plays a major role in magnesium homeostasis and maintenance of serum concentration. Urinary magnesium excretion is increased by high natriuresis, osmotic load and metabolic acidosis, and reduced by metabolic alkalosis, parathyroid hormone and, possibly, calcitonin. A large proportion of the magnesium content of faeces stems from unabsorbed magnesium. Endogenous magnesium is lost through bile, pancreatic and intestinal juices, and intestinal cells, and part of this can be re-absorbed. Magnesium losses through sweat are modest and very variable, depending on the techniques used for sweat collection, and losses through menstruation are negligible.

25.4 Sources

The magnesium content in Malaysian foods is not available in local food composition table and data on dietary intakes are scarce.

Magnesium is widely distributed in plant and animal foods and in beverages. Green leafy vegetables, such as spinach, legumes, nuts, seeds, and whole grains, are good sources. In general, foods containing dietary fibre provide magnesium. Magnesium is also added to some breakfast cereals and other fortified foods. Some types of food processing, such as refining grains in ways that remove the nutrient-rich germ and bran, lower magnesium content substantially.

Tap, mineral, and bottled waters can also be sources of magnesium, but the amount of magnesium in water varies by source and brand (Azouly, 2001). Azrina *et al.* (2012) reported that the magnesium content in Malaysian drinking water ranges from 0.07 mg/L to 4.11 mg/L). Food sources and magnesium content (mg/100g) is shown in Table 25.1.

Table 25.1. Magnesium content of foods

Food	mg/ 100g
Cereals & grains	
Wheat bran	156
Bran flakes cereal	109
Whole wheat bread	100
Brown rice	37
Milk & products	
Low fat milk	14
Nuts, legumes & lentils	
Cashew nuts	292
Dry roasted almonds	225
Peanut butter	153
Cooked lentils	32
Fruits & vegetables	
Spinach	79
Baked potato with skin	42
Banana	27
Green beans	25
Broccoli	21
Fish & shellfish	
Fish (mackerel, cod, halibut)	76
Tuna fish	40

Sources: USDA National Nutrient Database (2012)

25.5 Deficiencies

Assessing magnesium status is difficult because most magnesium is inside cells or in bone. The most commonly used and readily available method for assessing magnesium status is measurement of serum magnesium concentration, even though serum levels have little correlation with total body magnesium levels or concentrations in specific tissues (Gibson, 2005). Other methods for assessing magnesium status include measuring magnesium concentrations in erythrocytes, saliva, and urine; measuring ionized magnesium concentrations in blood, plasma, or serum; and conducting a magnesium-loading (or "tolerance") test (in which urinary magnesium is measured after parenteral infusion of a dose of magnesium). No single method is considered satisfactory (Witkowski, Hubert & Mansu, 2011) and both laboratory tests and a clinical assessment might be required to comprehensively evaluate magnesium status.

Symptomatic magnesium deficiency due to low dietary intake in otherwise-healthy people is uncommon because the kidneys limit urinary excretion of this mineral (Rude, 2012). However, habitually low intakes or excessive losses of magnesium due to certain health conditions, chronic alcoholism, and/or the use of certain medications can lead to magnesium deficiency.

Early signs of magnesium deficiency include loss of appetite, nausea, vomiting, fatigue, and weakness. As magnesium deficiency worsens, numbness, tingling, muscle contractions and cramps, seizures, personality changes, abnormal heart rhythms, and coronary spasms can occur (IOM, 1997; Rude, 2010). Severe magnesium deficiency can result in hypocalcemia or hypokalemia (low serum calcium or potassium levels, respectively) because mineral homeostasis is disrupted (Rude, 2010).

Habitually low intakes of magnesium induce changes in biochemical pathways that can increase the risk of illness over time.

A diet containing more magnesium because of added fruits and vegetables, more low-fat or non-fat dairy products, and less fat overall was shown to lower systolic and diastolic blood pressure by an average of 5.5 and 3.0 mmHg, respectively (Champagne, 2006). However, this Dietary Approaches to Stop Hypertension (DASH) diet al.so increases intakes of other nutrients, such as potassium and calcium that are associated with reductions in blood pressure, so any independent contribution of magnesium cannot be determined. A systematic review and meta-analysis of prospective studies found that higher serum levels of magnesium were significantly associated with a lower risk of cardiovascular disease, and higher dietary magnesium intakes (up to approximately 250 mg/day) were associated with a significantly lower risk of ischemic heart disease caused by a reduced blood supply to the heart muscle. Nevertheless, large, well-designed clinical trials are needed to better understand the contributions of magnesium from food and dietary supplements to heart health and the primary prevention of cardiovascular disease.

Diets with higher amounts of magnesium are associated with a significantly lower risk of diabetes, possibly because of the important role of magnesium in glucose metabolism (Larsson & Wolke 2007; Rodriguez-Moran *et al.* 2011). Most investigations of magnesium intake and risk of type 2 diabetes have been prospective cohort studies. A 2011 meta-analysis

of prospective cohort studies on the association between magnesium intake and risk of type 2 diabetes included 13 studies with a total of 536,318 participants and 24,516 cases of diabetes (Evert *et al.,* 2013). Only a few small, short-term clinical trials have examined the potential effects of supplemental magnesium on control of type 2 diabetes and the results are conflicting. The American Diabetes Association states that there is insufficient evidence to support the routine use of magnesium to improve glycemic control in people with diabetes (Evert *et al.,* 2013). It further notes that there is no clear scientific evidence that vitamin and mineral supplementation benefits people with diabetes who do not have underlying nutritional deficiencies.

Magnesium is involved in bone formation and influences the activities of osteoblasts and osteoclasts (Rude, Singer & Gruber 2009). Magnesium also affects the concentrations of both parathyroid hormone and the active form of vitamin D, which are major regulators of bone homeostasis. Several population-based studies have found positive associations between magnesium intake and bone mineral density in both men and women (Tucker, 2009). Other research has found that women with osteoporosis have lower serum magnesium levels than women with osteopenia and those who do not have osteoporosis or osteopenia (Mutlu *et al.,* 2007). These and other findings indicate that magnesium deficiency might be a risk factor for osteoporosis (Rude *et al.,* 2009). Diets that provide recommended levels of magnesium enhance bone health, but further research is needed to elucidate the role of magnesium in the prevention and management of osteoporosis.

Magnesium deficiency is related to factors that promote headaches, including neurotransmitter release and vasoconstriction (Sun-edelstein & Mauskop 2009). People who experience migraine headaches have lower levels of serum and tissue magnesium than those who do not. In their evidence-based guideline update, the American Academy of Neurology and the American Headache Society concluded that magnesium therapy is "probably effective" for migraine prevention (Holland *et al.,* 2012). Because the typical dose of magnesium used for migraine prevention exceeds the UL, this treatment should only be used under the direction and supervision of a healthcare provider.

25.6 Factors affecting magnesium requirement

Approximately 30% to 40% of the dietary magnesium consumed are typically absorbed by the body. High levels of dietary fibre from fruits, vegetables, and grains decrease magnesium absorption and/or retention (Wisker *et al.,* 1991)

Balance studies performed either in children or in adults did not detect an interaction between magnesium and calcium balances (Spencer *et al.* 1994; Andon *et al.* 1996; Abrams *et al.*1997; Nielsen & Milne 2000; Klevay & Milne 2002). However, in two studies, calcium balance was significantly higher under conditions of negative magnesium balance (at magnesium intakes of 107 and 118 mg/day) than with a positive magnesium balance (at magnesium intakes of 318 and 327 mg/day) (Nielsen 2004; Nielsen *et al.* 2007).

Manganese shares physical properties with magnesium that enable it to be interchangeable with magnesium in enzymatic phosphate transfer reactions and it has been used as a probe to study the role of magnesium in these processes, particularly in energy metabolism. However, the relevance of this interrelationship to human dietary requirements is uncertain.

There are some studies indicating a relationship with protein intake, possibly through increased apparent magnesium absorption. Wisker et al. (1991) showed that percentage faecal magnesium excretion and balances differed significantly between low-fibre and high-fibre diets containing adequate amounts of protein; however, Kelsay & Prather (1983) showed there was no clear effect of diets low and high in fibre and oxalic acid on magnesium balances. Overall, data on interactions between magnesium and other minerals, protein or fibre are limited.

Gastrointestinal diseases such as chronic diarrhea and fat malabsorption resulting from Crohn's disease, gluten-sensitive enteropathy (celiac disease), and regional enteritis can lead to magnesium depletion over time (Rude, 2010). Resection or bypass of the small intestine, especially the ileum, typically leads to malabsorption and magnesium loss.

Magnesium deficits and increased urinary magnesium excretion can occur in people with insulin resistance and/or type 2 diabetes (Chaudrey *et al.,* 2010; Tosiello 1996.). The magnesium loss appears to be secondary to higher concentrations of glucose in the kidney that increase urine output.

People with chronic alcoholism often have poor dietary intake and nutritional status; gastrointestinal problems, including vomiting, diarrhea, and steatorrhea (fatty stools) resulting from pancreatitis; renal dysfunction with excess excretion of magnesium into the urine; phosphate depletion; vitamin D deficiency; acute alcoholic ketoacidosis; and hyperaldosteronism secondary to liver disease which can all contribute to decreased magnesium status (Chaudhary *et al.,* 2010).

Older adults have lower dietary intakes of magnesium than younger adults (Tosiello, 1996). In addition, magnesium absorption from the gut decreases and renal magnesium excretion increases with age (Musso, 2009). Older adults are also more likely to have chronic diseases or take medications that alter magnesium status, which can increase their risk of magnesium depletion (Barbagallo, 2009).

25.7 Setting requirements and recommended intakes of magnesium

The requirements for magnesium set by IOM in 1997 were retained in IOM (2006), while EFSA has published values for 2015 based on data from European countries.

The IOM (1997), WHO /FAO (2004), and EFSA (2015) committees have acknowledged that results of balance studies are difficult to interpret owing to methodological limitations in some studies, takes a long time to achieve equilibrium and the potential for physiological adaptations to low magnesium intakes. Nevertheless, most countries set their recommendations for magnesium based on balance studies and urinary magnesium excretion before and after magnesium loading for adults.

Infants

Several countries have assumed that magnesium retention is about 3 mg/kg body weight per day during growth, which could be achieved from an intake of 6 mg/kg body weight per day (IOM, 1997). Dietary Reference Intakes (DRIs) for magnesium was derived on the basis of this value and taking into account reference body weights of the age groups. Values were set as Adequate Intake (AI).

In breast-fed infants aged 0-6 months, magnesium requirement is set based on amount in breast milk reported to be between 25-34 mg/L. IOM has set the AI requirement as follows:

During the second 6 months of life, solid foods become a more important part of the infant diet and add a significant but poorly defined amount of magnesium. The requirement for infants above the age of 6 months is estimated based on intake from solid foods and breast milk. EFSA recommends an intake of 80 mg/day based on the mean observed intakes in four EU countries where available data showed a range 72-120 mg/day.

The TSC on Minerals recommended that the Malaysian RNI of magnesium be based on the AI and RDA recommendations of IOM (1997, 2006).

AI for infants
0 - 6 months **30 mg/day**

AI for infants
7 - 12 months **75 mg/day**

Children and adolescents

Unlike calcium, for which maximal retention can be associated with benefit to bone mass accretion, and in the absence of adequate balance or usual accretion data in children aged 1 through 8 years, most countries including IOM extrapolated magnesium EAR for children in various age groups based on changes in body weight and linear growth. Then a CV of approximately 10 percent is assumed for each EAR.

Requirements for boys ages 9 through 13 years appear to be similar with girls per kg per day (Abrams *et al.* 1997). Several countries also set the requirements for magnesium based on dietary intake reported in Western countries. The values are similar to what IOM had set based on EAR and included a CV of 10%.

For girls aged 10 to 18 years, the midpoint of average intakes for non-pregnant girls was considered and AI was set at 250 mg/day. However, IOM recommends EAR values of 340 mg for boys and 300 mg for girls aged 14-18 years and added a CV of 10% to obtain the RNI values.

EFSA recommendations set AIs based on observed intakes in EU countries. For boys and girls aged 1 to < 3 years, the midpoint of average intakes was considered and AI of 170 mg/day was recommended for boys and girls. For boys and girls aged 3 to < 10 years, on the same basis as for children aged 1 to < 3 years, the midpoint of average intakes was used to set

an AI of 230 mg/day for boys and girls. EFSA approached the recommendation for older children where for boys aged 10 to 18 years, they considered the distribution of the observed average intakes among European countries, and selects the midpoint of average intakes and sets an AI of 300 mg/day.

RNI for children and adolescents

1 - 3 years	**80 mg/day**
4 - 8 years	**130 mg/day**
9 - 13 years	**240 mg/day**
Boys	
14 - 18 years	**410 mg/day**
Girls	
14 - 18 years	**360 mg/day**

Adults

A pooled analysis of well-controlled balance studies in adults suggests that zero magnesium balance may occur at a magnesium intake of 165 mg/day (Hunt & Johnson, 2006). According to IOM, available balance data indicate that the EAR for men and women aged over 51 years is similar and a CV of 10% is assumed to determine the RNI. Declining renal function with age was also taken into consideration for older adults.

However, although EFSA considers magnesium balance studies to be the most suitable basis for setting reference values, the overall results of these studies were considered inconsistent and requirement by EFSA were based on average intake. Thus, for men, EFSA proposed an AI of 350 mg/day and for women, on the same basis, proposed an AI of 300 mg/day. The Panel considered that these AIs apply to all adults, including older adults.

RNI for adults

Men	
19 - 30 years	**400 mg/day**
31 - 50 years	**420 mg /day**
51 - 70 years	**420 mg/day**
>70 years	**420 mg/day**
Women	
19 - 30 years	**310 mg/day**
31 - 50 years	**320 mg/day**
51 - 70 years	**420 mg/day**
>70 years	**320 mg/day**

Pregnancy & lactation

EFSA considered that pregnancy induces only a small increase in magnesium requirement probably covered by adaptive physiological mechanisms and increases in energy intake in pregnancy. Hence, the Panel considered that the AI for non-pregnant women also applies to pregnant women.

IOM stated that inconsistent findings on the effect of magnesium supplementation on pregnancy outcome make it difficult to determine whether magnesium intakes greater than those recommended for non-pregnant women are beneficial. In addition, there are no data indicating that magnesium is conserved during pregnancy or intestinal absorption is increased. IOM however assumes that the gain in weight associated with pregnancy alone may result in a greater requirement for magnesium. Hence, IOM sets the EAR with additional 35mg/day for pregnancy over the requirements of non-pregnant women. The RNI is calculated with a CV of 10% over the EAR as follows:

About 25 mg/day is secreted with exclusive breastfeeding during the first six months after birth. The EFSA Panel considered the possibility of adaptive processes in magnesium metabolism, at the level of both absorption and elimination and concluded that the AI for non-pregnant non-lactating women also applies to lactating women.

IOM also reported that there was no consistent evidence to support an increased requirement for dietary magnesium during lactation. Therefore, the EAR and RNI are estimated to be the same as that obtained for non-lactating women of similar age and body weight.

RNI for pregnancy

14 - 18 years	**400 mg/day**
19 - 30 years	**350 mg/day**
31 - 50 years	**360 mg/day**

RNI for lactation

14 - 18 years	**360 mg/day**
19 - 30 years	**310 mg /day**
31 - 50 years	**320 mg/day**

25.8 Tolerable upper intake levels (UL)

Magnesium, when ingested as a naturally occurring substance in foods, have not been shown to exert any adverse effects. However, adverse effects of excess magnesium intake have been observed with intakes from non-food sources such as various magnesium salts used for pharmacologic purposes.

Diarrhoea is the primary manifestation as a result of excessive magnesium intake from non-food sources. Individuals with impaired renal function are at greater risk of magnesium toxicity. Hypermagnesemia can occur in individuals with impaired renal function and is most commonly associated with the combination of impaired renal function and excessive intake of non food magnesium such as from antacids.

The values below show the UL from magnesium supplements as recommended by IOM (1997):

Infants	
0 - 12 months	Not possible to establish
Children & adolescents	
1 - 3 years	65 mg/day
4 - 8 years	110 mg/day
9 - 13 years	350 mg/day
14 - 18 years	350 mg/day
Adults	
Men	350 mg/day
Women	350 mg/day
Pregnancy	350 mg/day
Lactation	350 mg/day

25.9 Research recommendations

The following priority areas of research are recommended.:

- Database on magnesium content of local foods and absorption efficacy.
- Reliable data on population intakes of magnesium are required based on dietary surveys that include estimates of intakes from food, water, and supplements in healthy
- Biomarkers of magnesium status must be investigated in order to assess their ability to predict functional outcomes that indicate adequate magnesium status over prolonged periods.
- Studies on the effects of magnesium on health such as the association of low magnesium status with carbohydrate and lipid metabolism, and sequelae of the metabolic syndrome including diabetes mellitus.
- Magnesium balance studies on various age and ethnic groups to determine optimal recommendations of intake.

25.10 References

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Appendix 25.1 Comparison of recommended intake for Magnesium: RNI of Malaysia (2017), FAO/WHO (2002), AI of EFSA (2015), DRI of IOM (1997) and (2006)

Malaysia (2017)		FAO/WHO (2002)		EFSA (2015)		IOM (1997; 2006)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**DRI (mg/day)**
Infants		**Infants**		**Infants**		**Infants**	
0 - 6 months	30			0 - 6 months	25	0 - 6 months	30
7 - 12 months	75	< 1 year		7 - 12 months	80	7 - 12 months	75
Children		**Children**		**Children**		**Children**	
1 - 3 years	80	1 - 3 years		1 - 3 years	170	1 - 3 years	80
4 - 8 years	130	4 - 6 years		3 - 10 years	230	4 - 8 years	130
		7 - 9 years					
Boys		**Boys**		**Boys**		**Boys**	
9 - 13 years	240	10 - 12 years		10 - 18 years	300	9 - 13 years	240
14 - 18 years	410	13 - 15 years				14-18 years	410
		16 - 19 years					
Girls		**Girls**		**Girls**		**Girls**	
9 - 13 years	240	10 - 12 years		10 - 18 years	250	9 - 13 years	240
14 - 18 years	360	13 - 15 years		14 - 18 years	360		
		16 - 19 years					

Malaysia (2017)		FAO/WHO (2002)		EFSA (2015)		IOM (1997; 2006)	
Age group	**RNI (mg/day)**	**Age group**	**RNI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**DRI (mg/day)**
Men		**Men**		**Men**		**Men**	
19 - 30 years	400	20 - 39 years		19 - 65 years	350	19 - 30 years	400
31 - 50 years	420	40 - 49 years		> 65 years		31 - 50 years	420
51 - 69 years	420	50 - 59 years				>51 years	420
> 70 years	420	≥ 60 years					
Women		**Women**		**Women**		**Women**	
19 - 30 years	310	20 - 39 years		19 - 50 years		19 - 30 years	310
31 - 50 years	320	40 - 49 years		51 - 65 years		31 - 50 years	320
51 - 69 years	420	50 - 59 years		> 65 years	300	>51 years	320
>70 years	320	≥ 60 years					
Pregnancy		**Pregnancy**		**Pregnancy**		**Pregnancy**	
14 - 18	400	1st trimester			300	14 - 18 years	400
		2nd trimester				19 - 30 years	350
19 - 30	350	3rd trimester				31 - 50 years	360
31 - 50	360						
Lactation		**Lactation**		**Lactation**		**Lactation**	
14 - 18	360	1st 6 months			300	14 - 18 years	360
19 - 30	310	2nd 6 months				19 - 30 years	310
31 - 50	320					31 - 50 years	320

26 • Chromium

26.1 Introduction

Chromium (Cr) is a chemical element with a symbol Cr and atomic mass of 51.9961 Da. It exits in each of the oxidation states from -2 to +6, with only trivalent Cr (+3) and hexavalent Cr (+6) being the most often studied in relation to human health (Nielsen, 2012). Other forms such as chromium III and chromium VI are associated with industrial exposure. A trivalent Cr is the most common form found in nature due to its oxidation stability state and biologically active form, whereby all forms of chromium can be toxic at high levels, but hexavalent compounds, a by-product of manufacturing stainless steel, pigments, chromate chemicals and other industrial products, is the most oxidizing states and fairly unstable. It is more toxic in the body than its trivalent form.

26.2 Functions

Chromium is an essential trace element in regulating many metabolic processes as metalloenzymes in the body. Chromium also helps in regulating metabolism of fat and carbohydrates. In addition, it stimulates fatty acid and cholesterol synthesis, which are crucial for brain function and other body processes (Mertz, 1993). A growing body of evidence indicates that Cr is essential for normal glucose tolerance that has been found to aid in optimal insulin action regulation, by enhancing the activity of insulin receptor and thus increasing insulin signal transduction and sensitivity (Chen *et al.,* 2009; Yin & Phung, 2015). Moreover, it was also reported that chromium supplementation may improve glucose tolerance in children, adults and elderly with impaired glucose tolerance and/or dyslipidaemia (Amato, Morales & Yen, 2000; Kim *et al.,* 2011). A current understanding of the positive effect of dietary Cr on insulin action is based on several in-vitro and in vivo experimental studies. Vincent and his colleagues (2004) proposed a first model of the role of Cr as enhancer on insulin activity, in which the movement of Cr into the cell is stimulated by an activation of the insulin receptor. The intracellular Cr binds to a peptide to become the low-molecular-weight chromium-binding substance (LMWCr). In proportion to its Cr concentration, the LMWCr activates the insulin receptor and enhance its activity. When the blood glucose level becomes normal and the insulin level decreases, the LMWCr is released from the cell and this terminates its effects (Vincent, 2004). Moreover, Cr may enhance insulin action by improving insulin signaling in skeletal muscle. This was reported by Chen *et al.* (2009), in a study of genetically obese and insulin resistant of male mice that had been given a chromium-containing milk powder. In 7 weeks result showed that chromium supplementation improved insulin signaling in skeletal muscle in these diabetic mice.

26.3 Metabolism

Chromium is present in the diet both in the inorganic and organically complexed forms. Cr forms coordination compounds and chelates that have made it more available for absorption and transport in small intestines. Based on the metabolic balance studies both in animal and humans, the rate of chromium absorption from food was estimated to range between 0.4% and 2.5% and varies depending on the chromium complex ingested (DiSilvestro & Dy, 2007). Once absorbed, Cr is distributed to various tissues such as liver, spleen, kidney, soft tissue and bone. It is estimated that normal levels of blood Cr in humans is between 20 to 30mg/L. In general, the Cr bioavailability is relatively low, with most orally ingested Cr in human appears

to be unabsorbed and is excreted in the faeces (Mertz, 1993), whereas most absorbed Cr is excreted rapidly in the urine, primarily through the kidney and also small amounts is excreted in perspiration and bile.

The absorption of Cr is inversely proportional to the Cr intake in humans. The efficacy of absorption of chromium depends on other factors including the chemical properties of the ingested source and on the presence of other dietary constituents. Both intestinal (endogenous) and dietary (exogenous) factors have been demonstrated to alter the bioavailability of chromium in the small intestine. Most chromium compounds are soluble at the pH of the stomach and less soluble hydroxides may form when the pH is increased. The environment of the gastrointestinal tracts and ligands provided by foods and supplements are important for mineral absorption. Bioavailability of the dietary Cr can be influenced by several dietary substances such as vitamin C, carbohydrates, oxalate and iron intake (Mertz, 1993; Vincent, 2004).

A higher bioavailability of dietary Cr can be achieved by increasing the content of vitamin C. absorption of dietary Cr is enhanced in animals fed concomitantly with chromium III chloride and ascorbic acid (Dowling, Offenbacher & Pi-Sunyer, 1989). Similar effect was found in human, in which the absorption of Cr as measured by blood plasma Cr levels was increased significantly in women who was given 1mg chromium and 100mg ascorbic acid simultaneously compared to women without ascorbic acid (Offenbacher, 1994).

Carbohydrate intake has been shown to alter the Cr bioavailability. The effects of types of carbohydrates (simple vs. complex carbohydrates) on urinary Cr excretion were also investigated in animal and human studies. Animals fed ^{51}Cr-labelled chromium III chloride concomitantly with starch found a significant higher level of Cr in blood and tissue than that of the mice fed with chromium III chloride mixed with sucrose, fructose or glucose (Seaborn & Stoecker, 1989). Similar effect was also reported in human Cr supplementation trial in healthy adults, the urinary Cr excretion was significantly higher during the simple sugar diets period than after consuming the complex diet (Kozlovsky *et al.,* 1986). In general, diets high in simple sugars such as sucrose, fructose and/or glucose significantly increased urinary excretion of Cr by 10 to 200%, without any changes in absorption rates (Kozlovsky *et al.,* 1986). Phytate, a salt of phytic acid, is widely distributed in plant-based foods. High intake of phytate impaired Cr absorption, whereas oxalate that is also widely found in vegetables and plants-based products significantly increases Cr transport by increases in Cr in the blood and tissues levels.

An interaction between Cr and iron is thought to be linked to the shared binding sites on transferrin, in which Cr competes for one of the binding sites on transferrin. The negative effects of iron on Cr absorption was demonstrated for the first time by Sargent and his colleagues (1979) that iron overload in hereditary hemochromatosis may interfere with Cr transport by competing for transferrin binding sites, consequently leading to the pathogenesis of diabetes mellitus among patients with hemochromatosis. A significant low level of plasma Cr was found in patient with hemochromatosis than in iron-depleted patients. This observation was further supported in numerous studies, whereby high levels of urinary unbound Cr excretion and a smaller blood pool of Cr due to the saturation of transferrin by iron among patients with hemochromatosis (Lim, Sargent & Kusubov, 1983). Hence, it has been hypothesised that the diabetes associated with hemochromatosis is caused by the decreased binding and transport of Cr and subsequently increases losses of Cr from the body (Lukaski, Siders & Penland, 2007).

26.4 Deficiency

Although there is no clinically defined cut-off point of Cr deficiency, several clinical cases associated with low Cr levels have been previously reported among patients with prolonged intravenous parenteral nutrition without supplementation of dietary chromium. Symptoms of Cr deficiency include peripheral neuropathy, ataxia, weight loss and hyperglycaemia; however, re-administration of chromium supplementation to the parenteral nutrition solution would help to correct these abnormalities (Jeejeebhoy *et al.,* 1977; Mertz, 1993). In animal models, it showed that Cr deficiency causes a syndrome of glucose intolerance similar to clinical diabetes (Chen *et al.,* 2009). Certain groups of people such as the elderly and the young are at high risk of dietary Cr deficiency, especially when their diets are low in chromium. Another underlying cause attributed to the fact that tissue levels of chromium tend to decrease with age that could predispose the older people to have an increased risk of type-II diabetes.

Although it is generally thought that Cr is essential in enhancing the insulin action and therefore Cr deficiency may result in impaired glucose tolerance. Cr insufficiency has been hypothesized to be a contributing factor to the development of diabetes mellitus (Sharma *et al.,* 2011). In a Cr supplementation trial conducted in China, where 180 diabetic adults were assigned into three groups namely as 200µg, 1,000µg chromium picolinate daily and the controls for 4 months to assess the effect of daily chromium on glucose homeostasis status indicators, as assessed by fasting glucose and insulin, glycosylated hemoglobin (HbA1c) and 2-hour glucose tolerance test (Anderson *et al.,* 1997). A significant reduction was found in fasting and 2-hour insulin levels in both supplemented groups compared to those in the control group. In addition, a higher daily dosage of 1,000mg group showed significantly greater decreases in the levels of HbA1c and 2-hour glucose levels than those in other groups. The significant reductions in both fasting glucose and insulin levels and HbA1c levels were also observed at the end of 4-month trial.

Another study among patients with newly onset type-II diabetes showed a beneficial effect of chromium supplementation on glycaemia control and blood lipid profile among those who had been supplemented 42µg Cr daily for 3 months period (Sharma *et al.,* 2011). On the contrary, in another recent meta-analysis of nine randomized controlled trials, comprising a total of 440 diabetic patients who were supplemented with daily chromium between 200(g and 1,000µg failed to show any beneficial effects on fasting glucose levels, as only single indicator used for glucose homeostasis status (Bailey, 2014). A large scale randomized controlled trials of Cr supplementation are warranted to further investigation on the dosages and duration needed to demonstrate the effectiveness of chromium on the treatment of type-II diabetes.

26.5 Sources

Although chromium is a naturally occurring element that are mainly found in soil, rocks and plants, but the amount in food is very variable, depending on the chromium content of the soils. Chromium in foods can be degraded during physical refining and processing. Several common foods that have significant amount of chromium based on micrograms per 100gram of food are given in Table 26.1. In general, meat and meat products, high bran cereals, pulses, legumes, nuts, egg yolk and some fruits and vegetables are good sources of dietary chromium. Whole grains have more Cr content than that of a refined grain. Diets high in simple sugars such

as sucrose and fructose are usually low in chromium content and it may actually promote chromium excretion. Moreover, several chromium compounds have been added in food such as chromium chloride and its hexahydrate, chromium sulphate and its hexahydrate (EFSA, 2014).

Table 26.1. Chromium content of foods

Types	µg/100g
Cereal and cereal products	
Wholemeal flour	21
Barley (wholegrain)	13
Maize (wholegrain)	9
Starchy root and tuber products	
Potatoes (mashed)	1
Nut and seed products	
Brazil nut	100
Hazelnut	12
Vegetable and vegetable products	
Tomato	20
Mushroom	17
Broccoli	16
Fruit and fruit products	
Date (dried)	29
Pear	27
Meat and poultry products	
Pork chop	10
Egg yolk	6
Beef	3
Fish, seafood and shellfish products	
Mussel	128
Oyster	57
Brown shrimp	26
Herring	2

Source: Food Composition and Nutrition Table of Germany (2008)

26.6 Setting Requirements and Recommended Intake

Limited information is available to determine an Estimated Average Requirement (EAR) and in order to calculate an RDA values for chromium, an adequate intake (AI) was instead developed by the Food and Nutrition Board of the US Institute of Medicine (IOM, 2001). On the contrary, ESFA guidelines should not be followed since there is no specific requirement guideline defined for dietary chromium at this moment due to uncertainty in terms of the functional essentiality of trivalent chromium on health outcomes in both animals and humans (ESFA, 2014).

One of the major challenging issues is related to assessment of Cr nutritional status in biological tissues and fluids due to its very low levels in these tissues. Currently there is no reliable, sensitive and specific indicator or biomarker to assess nutritional Cr status, making it difficult to estimate and compare the prevalence and severity of Cr deficiency in the population. However, the current status of nutritional Cr assessment is primarily based on Cr concentrations in blood, urine and hair as proxy measures of Cr nutritional status (Gibson, 2005). It is generally established that serum Cr levels is relatively high in newborns and the Cr levels, in particularly trivalent Cr levels has been reported to decrease with advancing age and pregnancy. In addition, a significant age-related decrease in the Cr levels in hair, sweat, and urine has also been reported.

The primary criterion used to establish an Estimated Safe and Adequate Daily Dietary level of dietary Cr that sufficient to meet the chromium requirements for populations throughout the lifespans (IOM, 2001). There was no documentation on the functional criteria to assess chromium status in response to dietary chromium intakes. The determination of RDA for dietary chromium is derived from AI values, which was obtained from an Estimated Safe and Adequate Daily Dietary Intake ranges reported in various studies among different populations across the lifespans (IOM, 2001).

Infants

The recommended intakes of dietary chromium in infants from aged 0 through 12-months of age was based on an average dietary Cr intake of infants principally fed human milk (IOM, 2001). It is estimated that daily energy intake for infants between 0 to 12 months is 845kcal, in which human milk provides 750 kcal/ L. During the first 6 months of exclusive breastfeeding, the average volume of human milk consumed by the infant is approximately 0.6L/ day. An estimated chromium intake from human milk based on an average concentration of 0.25 µg/ L would be 0.15 µg per day (0.6 x 0.25). Additional dietary chromium from complementary foods was taken into account for infants after 6-months of age and it is estimated to be 5.36 µg per day. Hence the adequate amount of chromium consumed from both human milk and complementary foods would be 5.5 µg per day (0.15 + 5.36).

AI for infants

0 - 6 months	**0.2 µg/ day**
7 - 12 months	**5.5 µg/ day**

Children and Adolescents

AI for these children and adolescents from different age groups has been extrapolated from adults aged between 19 years and 30 years according to gender.

AI for children

1 to 3 years	**11 µg/ day**
4 to 8 years	**15 µg/ day**

AI for Boys

9 to 13 years	**25 µg/ day**
14 to 18 years	**35 µg/ day**

AI for Girls

9 to 13 years	**21 µg/ day**
14 to 18 years	**24 µg/ day**

Adults

Similar approach was used for adults ages from 19 years through 50 years based on the mean daily intakes of dietary chromium. It has been documented that the mean chromium intake of 13.4 µg/ 1,000 kcal and an estimated mean daily energy intake of 2,800 kcal and 1,850 kcal for men and women aged between 19 and 30 years, respectively, and 2,550 kcal and 1,750 kcal for men and women aged 31 to 50 years from the Third National Health and Nutrition Examination survey (NHANES III, 1988-1994) (Briefel *et al.,* 1995). These highest intake levels of these adults reported were then used to establish AI estimates for dietary chromium according to gender. Thus, the AI for men is 35 µg/ day (2,800 x13.4) and 25 µg/ day (1,850 x 13.4), after rounding.

AI for Men

19 to 50 years	**35 µg/day**

AI for Women

19 to 50 years	**25 µg/day**

Adults Ages 51 years and older

An estimated chromium intake of well-balanced diet in a day for adults was 13.4 µg/ 1,000 kcal and median energy intakes for adult men and women aged 51 and older were 2,100 and 1,500 kcal per day, respectively. The energy needs for older men and women aged more than 70 years are 1,700 and 1,300 kcal per day, respectively (Briefel *et al.,* 1995). Calculation

of gender-specific AI for these older adults is based on the highest intake level for adults aged 51 years and older. Thus, the AI is calculated for men and women are 30 µg/ day (2,100 x 13.4) and 20 µg/ day (1,500 x 13.4) after rounding.

AI for Men

51 - 70 years	**30 µg/ day**
>70 years	**30 µg/ day**

AI for Women

51 - 70 years	**20 µg/day**
>70 years	**20 µg/day**

Pregnancy and Lactation

It is established that chromium depletion is common among pregnant women and also among women with multiple pregnancies. Since there was no information pertaining to additional chromium requirement for women during pregnancy, the AI is set up based on extrapolated values from adolescent girls and adult women by taking into consideration additional weight gain observed during pregnancy. Numerous evidences have been well documented that a median weight gain of 16 kg is significantly associated with good pregnancy outcomes. Hence, additional 16 kg is included to the reference weight for adolescent girls and adult women for extrapolation.

On the other hand, establishment of AI for women during lactation is based on the chromium intake needed to compensate the chromium secreted in human milk and the AI for non-pregnant women. Based on the absorption rate of chromium of 1%, additional 20 µg of chromium must be consumed in a day in order to compensate for the milk losses. Hence, approximately 20 µg is required to be added to the AI for adolescent girls and adult women.

AI for Pregnancy

14 to 18 years	**29 µg/day**
19 to 30 years	**30 µg/day**
31 to 50 years	**30 µg/day**

AI for Lactation

14 to 18 years	**44 µg/day**
19 to 30 years	**45 µg/day**
31 to 50 years	**45 µg/day**

26.8 Tolerable upper intake levels

Little evidence has been documented that trivalent Cr is toxic to humans. Scant information of serious adverse effects associated with excess intake of Cr from foods has been found. In general, the toxicity from the nutritional Cr levels is considered to be low and rare because ingested Cr is poorly absorbed, and most absorbed Cr is rapidly excreted from the body through urine (Nielsen, 2012). Moreover, very limited evidence on adverse effects with excess intake of chromium from food or supplements up to a dose of 1 mg per day has been reported on health outcomes (ESFA, 2014), therefore, a tolerable upper intake level (UL) for chromium was not established.

26.9 Research recommendations

Although it is generally recognised nutritional Cr deficiency appeared to be widespread in populations throughout the lifecycle, particularly in older adults and pregnant women, but several major issues and challenges in Cr research still remain unresolved. The challenges, such as the optimal Cr status of the populations, the chemistry of Cr as a dietary component and its bioavailability and the optimal levels of Cr in the body in achieving and maintaining good health and well-being of the populations. Therefore, a several priority areas of research are highly recommended namely,

- A development of reliable, sensitive and specific biomarker to assess the Cr nutritional status in humans in order to substantially improve the scientific basis on the optimal requirement of chromium on health in any populations of interest
- To estimate the Cr requirement using reliable method of assessment among populations of different age groups and of different physiological groups
- To assess the possible negative health effects associated with a marginal Cr deficiency in populations throughout the lifespans

26.10 References

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Appendix 26.1 Comparison of recommended intake for Chromium: RNI Malaysia (2017) and RDA of IOM (2001)

Malaysia (2017)*		IOM (2001)	
Age group	**RNI (µg/day)**	**Age group**	**RDA (µg/day)**
Infants			
0 - 6 months	0.2	0 - 6 months	0.2
7 - 12 months	5.5	7 - 12 months	5.5
Children			
1 - 3 years	11.0	1 - 3 years	11.0
4 - 8 years	15.0	4 - 8 years	15.0
Boys			
9 - 13 years	25.0	9 - 13 years	25.0
14 - 18 years	35.0	14 - 18 years	35.0
Girls			
9 - 13 years	21.0	9 - 13 years	21.0
14 - 18 years	24.0	14 - 18 years	24.0
Men			
19 - 30 years	35.0	19 - 30 years	35.0
31 - 50 years	35.0	31 - 50 years	35.0
50 - 70 years	30.0	50 - 70 years	30.0
>70 years	30.0	>70 years	30.0
Women			
19 - 30 years	25.0	19 - 30 years	25.0
31 - 50 years	25.0	31 - 50 years	25.0
50 - 70 years	20.0	50 - 70 years	20.0
>70 years	20.2	>70 years	20.2
Pregnancy			
≤1 years	29.0	≤18 years	29.0
19 - 30 years	30.0	19 - 30 years	30.0
31 - 50 years	30.0	31 - 50 years	30.0
Lactation			
≤18 years	44.0	≤18 years	44.0
19 - 30 years	45.0	19 - 30 years	45.0
31 - 50 years	45.0	31 - 50years	45.0

* Refer to Adequate Intake (AI).

27 Copper

27.1 Introduction

Copper (Cu) is a soft, ductile and conductive metal with symbol Cu and atomic number of 29. Found in the earth crust, it is the first metal mined by humans and has since been used widely as electrical wires and industrial alloys. Copper is also an essential trace element that is required in the cellular processes of electron transfer in humans. A typical adult contains approximately 100mg Cu, which is distributed in the skeletal muscle, skin, bone marrow, liver and plasma. While copper mainly exists as Cu (II) in the human body, its ability to shift between oxidation forms of cuprous (Cu^{1+}) and cupric (Cu^{2+}) underpins its important roles in oxidation-reduction reactions (Collins and Klevay 2011, EFSA, 2015).

27.2 Functions

As a component of a number of metallo-enzymes, copper primarily functions as oxidases in the reduction of molecular oxygen. There are approximately 12 copper-containing enzymes in human body, including the cytochrome C oxidase, amine oxidases, ceruloplasmin, copper/zinc superoxide dismutase (Cu/Zn SOD), dopamine beta-hydroxylase, and others. Therefore, copper is involved in diverse body functions including cellular respiration, iron metabolism, antioxidant defense, connective tissue formation, and neurotransmitter biosynthesis.

Ceruloplasmin is the principal copper-carrying protein in human body. It accounts for 80-95% of the copper in plasma (EFSA, 2015) and 3% of total copper in the body. Besides functioning as the transporter of copper to cells, ceruloplasmin is a ferroxidase enzyme that oxidizes ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) which is essential in the mobilization of iron for red blood cell synthesis.

27.3 Metabolism

The uptake, distribution, storage and excretion of copper is tightly regulated under homeostatic control by the human body. Dietary copper is absorbed primarily as cuprous (Cu^{1+}) in the small intestines, mainly at the duodenum. Copper is taken into the enterocytes via the copper transporter I (Ctr1), and subsequently transported by albumin, transcuprein, and low molecular weight copper-histidine complexes in the portal circulation to the liver. In the liver, copper is either stored in metallothionein, or bound to one of 3 series of copper chaperones (CCS, Cox17, Atox1) for circulation to target tissues. CCS and Cox 17 are the copper chaperones for superoxide dismutase 1 and cytochrome c oxygenase, respectively. Atox1 is the chaperone for the two ATPases, of which ATP7A delivers copper to tyrosinase while ATP7B delivers copper to lysyl oxidase and ceruloplasmin (de Romana *et al.,* 2011). Copper is excreted mainly through the bile (98%) (Wijmenga and Klomp 2004), and hence presence of copper exporter ATP7B is especially important for biliary excretion of copper. Less copper is excreted in the urine, skin and sweat.

In general, the absorption of copper depends on the amount ingested and the presence of other dietary components (Ellingsen, Horn and Aaseth, 2007). It is estimated that 50% of dietary copper is absorbed in humans across all ages and life stages (EFSA, 2015). The increase

in copper absorption will also increase the turnover of copper. In cases of deficiency or low dietary intake such as intakes below 1 mg/day, absorption of copper is more than 50%. However, the efficiency of absorption reduced to less than 20% when copper intakes are above 5mg/day (Turnlund, 1998). Therefore, copper bioavailability is inversely related to dietary copper intake.

The enhancers of copper absorption are amino acids (especially histidine and cysteine), and organic acids such as citric, gluconic, lactic, acetic and malic acids. On the contrary, presence of divalent cations such as iron and zinc and alkaline environment may inhibit copper absorption.

High levels of dietary iron intake may reduce copper absorption. Copper absorption has been found to be lower in infants fed a formula with higher iron concentration compared to those fed the same formula with lower iron concentration (IOM, 2006). It has been postulated that some copper may enter enterocytes through the iron transport channel (divalent metal transporter 1, DMTI) besides copper transporter 1 (Ctr1) at the apical membrane of enterocyctes. Therefore, high levels of iron may reduce copper absorption (Sharp, 2004).

High intake of zinc (≥50mg/day or 760µmol) for extended periods can affect intestinal absorption of copper (WHO/FAO, 2004; Linus Pauling Institute, 2016). Excessive zinc intake have been associated with excess intake from supplements (Maret and Sandstead, 2006) and inappropriate use of denture cream (Hedera *et al.,* 2009). High zinc intake has been shown in animal studies to increase the synthesis of metallothionein (EFSA, 2015). Because copper has higher affinity than zinc to metallothionein, copper will be trapped in enterocyctes and subsequently shed and excreted in faeces.

27.4 Sources

Copper is found in a wide variety of foods. Most copper in foods is found in the cupric state (Cu^{2+}) bound to organic compounds especially amino acids. The two rich dietary sources of copper are organ meats and shellfish, especially liver and oyster. Nuts and seeds are good plant sources of copper (Table 27.1). On the other hand, milk and dairy products are poor sources. Copper is supplemented in fortified food or supplements mainly in the form of copper sulfate (contains 25% copper). The other bioavailable forms of copper include cupric chloride (47% copper), cupric acetate (35% copper) and copper carbonate (57% copper) (Baker, 1999).

Table 27.1. Copper content of Foods

	µg/100g edible portion
Organ meats	
Beef, liver, cooked, pan-fried	14587
Beef, liver, raw	9755
Duck, liver, raw	5961
Beef, sweetbread, cooked, boiled	5100
Beef, kidneys, cooked, simmered	563
Shellfish	
Oyster, cooked, moist heat	5707
Lobster, cooked, moist heat	1550
Crab, blue, cooked, moist heat	814
Legumes, nuts, and seeds	
Cocoa, dry powder, unsweetened	3788
Cashew nuts, dry roasted	2220
Chocolate malt powder	1818
Soy bean curd, sheet (tim-cok)	1600
Mixed nuts, dry roasted, with peanuts	1520
Pistachio nuts, dry roasted	1293
Soy bean, white (kacang soya putih)	980
Kidney beans, mature seeds, raw	958
Shitake mushrooms	896
Kacang kuda (chickpea)	880
Kacang hijau (Gram, green / mung bean)	820
Lentils, raw	754
Dal kuning (dhal, yellow)	710
Dal Mysore (dhal, Mysore / orange)	700
Kacang merah (gram, red)	670
Tempeh (fermented soy bean cake)	500
Barley, hulled	498

Sources: USDA National Nutrient Database for Standard Reference, Nutrient Composition of Malaysian Foods (Tee *et al.,* 1997)

Drinking water, including water-based beverages is another important contributor to dietary copper. Due to the widespread use of copper pipes, drinking water may contribute up to 50% of total copper intake if the copper content in drinking water is high (>1-2 mg/L) (EFSA, 2015). According to the Malaysian Food Regulations (1985), the maximum permitted concentration of copper in packaged drinking water is 1000µg. This standard limit is lower than the international standard limits of 2000µg (WHO Guidelines for Drinking Water Quality (2006)). A study examining drinking water in Malaysia found that copper levels in drinking water are below the national and international standard limits. The copper concentration were 2.99 µg/L (in drinking water), 12.77 µg/L in mineral water, and 8.54 µg/L in tap water (Azlan *et al.,* 2012). Given the low copper content in drinking water (<0.1mg/L), contribution of water to daily copper intake of Malaysians is unlikely to be high (or higher than 10%).

However, the dietary intake of copper in Malaysia remains unknown. This is mainly due to the non-availability of information on copper content for most foods in the local food composition database (Tee *et al.,* 1997). On the other hand, according to dietary surveys in seven European countries, the average intake of copper ranged between 1.47 to 1.67mg/day (median intake 1.57mg/day) for men, and 1.20mg to 1.44mg/day (median intake 1.32mg/day) for women aged 18 to less than 65 years. The main food group contributing to dietary copper intake is grains and grain-based products in these countries due to the high consumption of this food group (EFSA, 2015).

27.5 Deficiency

Because copper is found ubiquitously in foods, dietary copper deficiency is rare in humans. In early studies, cases of dietary copper deficiency have been documented in malnourished infants, premature and low-birth-weight infants fed cows' milk or on a poor diet lacking in copper, and patients receiving total parental nutrition un-supplemented with trace minerals (Williams, 1983). Nonetheless, copper deficiency is likely with excessive consumption of zinc, prolonged use of certain medications, renal or gastrointestinal disorders that alter the excretion or absorption of copper.

Given the various functions of copper in human body, deficiency in copper can result in a broad spectrum of clinical presentations. The more commonly recognized signs and symptoms of deficiency include anemia (that is unresponsive to iron therapy), neutropenia, impaired immune function, skeletal demineralization, osteoporosis and other abnormalities of bone development (de Romana *et al.,* 2011). Other less common symptoms include hypo- or depigmentation of skin and hair, aneurysms, and neurological symptoms.

Severe deficiency of copper can be a result of genetic defects in copper metabolism. Menkes disease, also known as the kinky hair syndrome, is an inherited disorder of copper deficiency caused by mutations of the copper export pump ATP7A expressed in the placenta, gut and brain (Tumer and Moller, 2010). This X-linked lethal disorder of copper metabolism typically presents in males at 2-3 months of life with failure to thrive, delay in development, and seizures. Although copper are absorbed into the intestinal cells, they cannot be released into the circulation resulting in systemic copper deficiency. Consequently, copper accumulates in the blood-brain barrier and the cuproenzyme activity in the neurons reduced. Menkes disease can be recognized by the characteristic short, sparse, coarse, twisted, often-described-as 'kinky'

or 'steel' hair (Kaler, 2013). While Menkes disease is an extremely rare disease (1 in 300,000 incidence rate), it causes progressive neurological impairment, connective tissues disturbances, and death before the third year of life.

Copper deficiency has been diagnosed based on serum and plasma copper concentrations, ceruloplasmin concentration, and erythrocyte superoxide dismutase activity. These biomarkers' levels respond to copper supplementation and are low with copper depletion, however they may not be sensitive to marginal copper deficiency. No single biomarker is sufficiently robust to determine requirements for copper (IOM,2001; EFSA, 2015).

27.6 Factors affecting copper requirements

The requirement for copper increases with age and is influenced by growth. During pregnancy, copper requirement increased due to the synthesis of pregnancy products and increased fetal needs. Although metabolic adaptation could lead to increased copper absorption, limited data is available on the efficiency of this upregulation in meeting additional requirement during pregnancy. Similarly during lactation, copper requirement increases due to the secretion of copper (approximately 0.2mg/day) in breast milk (EFSA, 2015).

In addition, copper requirement may be affected by prolonged use of certain drugs such as proton pump inhibitors (PPIs) and penicillamine. Low pH environment induced by excessive antacid ingestion and prolonged use PPIs, may interfere the absorption of copper and decrease zinc body stores (Farrell *et al.,* 2011, Plantone *et al.,* 2015). Penicillamine is prescribed to enhance copper elimination in Wilson's disease. It binds copper with sulfhydryl groups to form complex that can be excreted via urine (Hordyjewska, Popiolek and Kocot 2014). For individuals taking this medication without copper overload, they may have increased copper requirement due to the increased urinary excretion of Cu.

27.7 Setting requirements and recommended intakes of copper

Setting requirements for copper is a challenging one, due to the absence of robust, sensitive and specific biomarkers or other indices to assess copper status in human (EFSA, 2015). The IOM (2001) set the requirement for copper based on a combination of indicators, including serum concentration of copper and ceruloplasmin, erythrocyte superoxide dismutase activity and platelet copper concentration in human depletion/repletion studies. On the other hand, the National Health and Medical Research Council (NHMRC) for Australia and Ministry of Health New Zealand (NHMRC, 2006), and the European Food Safety Authority (EFSA, 2015) set their requirements based mainly on observed copper intake of their populations. For this reason, the Technical Sub-Committee (TSC) on Minerals and Trace Elements decided to adopt values from FNB-IOM (2001).

Infants

The recommended intake of infants is based on observed mean intake of copper among breast-fed infants. For younger infants aged 0 to 6 months, the AI reflects the usual copper intake from human milk, which is approximately 200 µg per day (250 µg/L x 0.78 L/day) after rounding. Taking into account the reference weight of 7kg, the AI is rounded up to 30 µg/kg/day or 200 µg/day. For the older infants aged 6 to 12 months, the AI is based on the average copper intake from complementary foods (estimated to be 100 µg/day) in addition to human milk. With the declining concentration of copper in human milk after six months (200 µg/L) and human milk intake of 0.6 L/day, the copper intake from human milk is 120 µg per day. Therefore the AI is 24 µg/kg/day or 220 µg/day for a reference weight of 9kg.

AI

0-6 months	**200 µg/day**
7-12 months	**220 µg/day**

Children and Adolescents

In the absence of data for children and adolescents, EAR for copper are extrapolated from adult EAR based on metabolic weight (kg $^{0.75}$) for ages 1-18 years (FNB-IOM, 2001). The EAR of the ages 1-3 years, 4-8 years, 9-13 years, and 14-18 years are 260 µg/day, 340 µg/day, 540 µg/day, and 685 µg/day, respectively. The RNI is set as equal to EAR plus twice the coefficient variation (CV) of 15 percent (130 percent of the EAR).

RNI

1 - 3 years	**340 µg/day**
4 - 8 years	**440 µg/day**
9-13 years	**700 µg/day**
14-18 years	**890 µg/day**

Adults

The EAR for adults is 700 µg/day and is set based on a combination of biochemical indicators. There are no differences in requirements between males and females (IOM, 2001).

RNI

19 - 30 years	**900 µg/day**
31 - 50 years	**900 µg/day**
50 - 70 years	**900 µg/day**
> 70 years	**900 µg/day**

Pregnancy and Lactation

The EAR for pregnancy was based on EAR for adults plus additional requirement for fetal needs and pregnancy products. The amount of copper deposited in a full-term infant is estimated to be 13.7mg, while the copper content in products of pregnancy including placenta amniotic fluids and maternal tissues are estimated to be 4.6g. These together add to a total 18mg copper over the course of pregnancy or 67 µg/day (18mg/266 days). Taking into account 65-70 percent bioavailability, the additional requirement for copper during pregnancy is estimated to be 100 µg/day.

The EAR for lactation is determined based on the requirement to cover losses of copper secreted in human milk in addition to adult EAR. Assuming that 200 µg per day of copper is secreted in human milk and bioavailability of 65-70 percent, an additional 300 µg of copper is required per day during lactation. Additional requirements for pregnancy (100 µg/day) and lactation (300 µg/day) were added to the EARs of adolescent girls (14-18 years) and women (19 years and above), and rounded to the nearest 100 µg.

RNI

Pregnancy

≤ 18 years	**1000 µg/day**
19 - 50 years	**1000 µg/day**

Lactation

≤ 18 years	**1300 µg/day**
19 - 50 years	**1300 µg/day**

27.8 Tolerable upper intake level

Tolerable upper intake level (UL) refers to the highest level of copper intake that is likely to have no risk of adverse effect to almost all individuals in a general population FNB-IOM (2001) set UL for copper based on potential liver damage as the critical adverse event. Doses up to 10mg daily have not reported to result in liver damage, and is hence set as the UL for adults including pregnant and lactating women. It was not possible to establish UL in infants due to the lack of sufficient data on adverse effects in this age group and concern for infants' inability to cope with excess copper. For children and adolescents, UL is extrapolated from UL of adults based on reference body weight are as follows:

Age	UL
Infants	Not possible to establish. Sources of intake should be from food and formula only.
Children	
1 - 3 years	1000 µg/day
4 - 8 years	3000 µg/day
Adolescents	
9 - 13 years	5000 µg/day
14 - 18 years	8000 µg/day
Adults	
19 - 30 years	10000 µg/day
31 - 50 years	10000 µg/day
50 - 70 years	10000 µg/day
> 70 years	10000 µg/day
Pregnancy	
≤ 18 years	8000 µg/day
19 - 50 years	10000 µg/day
Lactation	
≤ 18 years	8000 µg/day
19 - 50 years	10000 µg/day

Excessive intake of dietary copper is not a concern as copper level is under tight homeostatic control under normal physiological condition. Copper toxicity is rare in general population without hereditary defect in copper homeostasis. However, acute copper poisoning may occur through industrial exposure to fumes, intake of contaminated beverages and contaminated water supplies (Bremner, 1998). Toxicity symptoms range from abdominal pain, nausea, vomiting and diarrhea to more severe symptoms such as liver damage, kidney failure, coma and death.

Individuals with immature liver function (Lonnerdal, 1996) and genetic disorders (Wilson's disease, Indian childhood cirrhosis, idiopathic copper toxicosis) affecting copper metabolism may be at risk of copper toxicity at significant lower intake levels. In the case of Wilson's disease, copper overloading may happen due to the deficiency of copper export pump ATP7B caused by gene mutation. As results of impaired incorporation of copper into ceruloplasmin and decreased biliary excretion of copper, copper accumulate in many organs including the liver and brain. The clinical presentations are very diverse, consisting hepatic symptoms such as acute and chronic liver diseases, liver cirrhosis, neurological symptoms such as dystonia, tremor and cognitive and mood disorders (Chen *et al.,* 2015).

27.9 Research recommendations

- Currently, the Malaysian Food Composition Database contains only copper content of 103 legumes and vegetables extracted from both published and unpublished reports (Tee *et al.,* 1997). To assess dietary intake of copper, there is a need to determine the copper content of local foods from varying food groups.

- Locally, the lack of dietary intake data on copper intake precluded identifying the most appropriate requirement for the Malaysian population. Food consumption data or nationwide dietary survey should access dietary intake of copper at the population level.

- At global level, there is a need to collect dose-response data to evaluate the health effects of copper supplements.

27.10 Reference

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Appendix 27.1 Comparison of recommended intake for Copper: RNI Malaysia (2017), RDA of IOM (2001) and AI of ESFA (2015)

Malaysia (2017)		IOM (2001)		ESFA (2015)	
Age group	**RNI (µg/day)**	**Age group**	**RDA (µg/day)**	**Age group**	**AI (mg/day)**
Infants					
0 - 6 months	200*	0 - 6 months	200*		
7 - 12 months	220*	7 - 12 months	220*	7 - 11 months	0.4
Children					
1 - 3years	340	1 - 3years	340	1 - < 3years	0.7
4 - 8years	440	4 - 8years	440	3 - < 10years	1.0
Boys					
9 - 13 years	700	9 - 13 years	700	10 - <18years	1.3
14 - 18 years	890	14 - 18 years	890		
Girls					
9 - 13 years	700	9 - 13 years	700	10 - <18years	1.1
14 - 18 years	890	14 - 18 years	890		
Men					
19 - 30 years	900	19 - 30 years	900	≥18years	1.6
31 - 50 years	900	31 - 50 years	900		
50 - 70 years	900	50 - 70 years	900		
>70 years	900	>70 years	900		

Malaysia (2017)		IOM (2001)		ESFA (2015)	
Age group	RNI (µg/day)	Age group	RDA (µg/day)	Age group	AI (mg/day)
Women		**Women**		**Women**	
19 - 30 years	900	19 - 30 years	900	≥18years	1.3
31 - 50 years	900	31 - 50 years	900		
50 - 70 years	900	50 - 70 years	900		
>70 years	900	>70 years	900		
Pregnancy		**Pregnancy**		**Pregnancy**	
≤18 years	1,000	≤18 years	1,000		1.5
19 - 30 years	1,000	19 - 30 years	1,000		
31 - 50 years	1,000	31 - 50 years	1,000		
Lactation		**Lactation**		**Lactation**	
≤18 years	1,300	≤18 years	1,300		1.5
19 - 30 years	1,300	19 - 30 years	1,300		
31 - 50 years	1,300	31 - 50 years	1,300		

* Refer to Adequate Intake (AI).

28 • Manganese

28.1 Introduction

Manganese is a chemical element with the atomic symbol Mn. The atomic number and standard weight of manganese are 25 and 54.9 respectively. The appearance of manganese is in the form of a solid metal and silvery metallic in colour. The manganese metal was first isolated from manganese dioxide by Johan Gottlieb Gahn in 1774 (Royal Society of Chemistry, 2017). In humans, manganese is a trace element, usually present in low quantities (12-20mg), predominantly in either +2 or +3 oxidation states, and can be found mainly in the bones, liver, kidneys, pancreas, and small intestine.

28.2 Functions

The functions of manganese include as component of several metalloenzymes (e.g. superoxide dismutase), and a co-factor of other enzymes involved particularly in macro-nutrient (carbohydrate, protein, and lipid) metabolism (e.g. pyruvate carboxylase in glycolysis); and in cartilage tissue and bone formation. The following are specific examples of the major role of manganese in human metabolism:

Manganese superoxide dismutase

Manganese superoxide dismutase (MnSOD/SOD2) is a member of the superoxide dismutase family together with iron. The major role of this enzyme is its protection against oxidative stress as this enzyme is able to convert toxic superoxide to form hydrogen peroxide and diatomic oxygen (Figure 28.1). Hence, MnSOD is often associated with chronic diseases/ condition such as ischemic heart disease, cancer, and ageing.

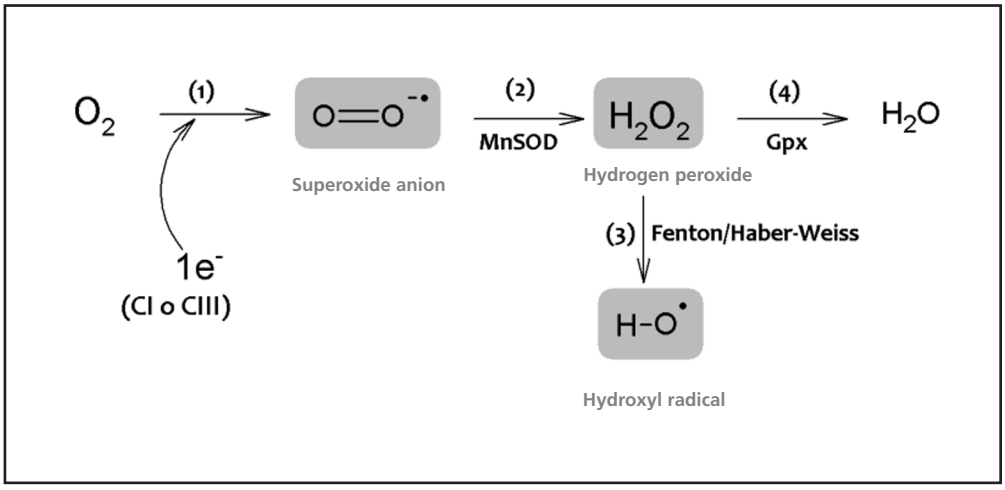

Figure 28.1. The catalyzed reaction of MnSOD in prevention of oxidative stress
Source: Cantala A (2012)

Arginase contains four atoms of manganese, is an important enzyme in the urea cycle, which functions to convert ammonia from the breakdown of proteins into urea for excretion. There are a total of five steps in the urea cycle and arginase is involved in the last step in which it hydrolyzes arginine to form ornithine and urea.

Glutamine synthetase is an important enzyme in nitrogen metabolism for the ATP-dependent condensation of glutamate and ammonia to produce glutamine. In this catalyzed reaction, ATP will phosphorylate glutamate to produce ADP and γ-glutamyl phosphate, which then reacts with ammonia for the formation of glutamine and inorganic phosphate.

Phosphoenolpyruvate carboxykinase (PEPCK) is involved in carbohydrate metabolism as one of the key enzymes in the gluconeogenesis pathway (formation of glucose from non-carbohydrate sources). PEPCK is the rate-limiting enzyme, therefore the main control point of the gluconeogenesis pathway. In this step, the substrate oxaloacetate is decarboxylated and phosphorylated by PEPCK to form the product, phosphoenolpyruvate (PEP). Plasma glucose levels were found to be lower in manganese-deficient rats, which may suggest the important role of manganese for the regulation of carbohydrate metabolism (Baly, Keen & Hurley, 1985).

The other important roles of manganese include cartilage and bone development, and in would healing. Manganese is a co-factor for the enzymes glycosyltransferases, which are required for the synthesis of proteoglycans in the formation of cartilage and bone. Besides glycosyltransferases, manganese also activates the enzyme prolidase which synthesizes proline for collagen formation in wound healing.

28.3 Metabolism

The total amount of manganese in the human body range between 12mg to 20mg, and it is distributed widely and uniformly in the tissues. In cells, manganese is concentrated in the mitochondria and nucleus. Manganese is transported in the blood by the liver and is distributed to the extrahepatic tissues via bound transferrin. In terms of storage, manganese is mostly found in bones as part of hydroxyapatite, and is also concentrated in the liver, kidneys, and pancreas. The absorption of manganese from dietary sources in the body is small and unabsorbed manganese is excreted in the faeces.

In terms of interactions of manganese with other nutrients or compounds, it is reported that high intakes of calcium, phosphorus, and phytates impair the absorption of manganese (SCF, 1993; IOM, 2001; ATSDR, 2012). However, this may not be a serious effect because manganese deficiency is rare among humans.

28.4 Sources

Manganese is mainly found in plant-based foods with the greatest contribution from whole grains. Other food sources include nuts, fruits, leafy vegetables, legumes, and tea. Animal based foods are low in manganese so vegetarian diet may be richer in manganese when compared to omnivorous diet. Table 28.1 shows the manganese content of some manganese-rich food and beverage sources.

Table 28.1. Manganese content of food and beverage

Food/Beverage	mg/ 100g edible portion
Nuts	
Pecans	4.51
Almonds	2.29
Peanuts	1.94
Cereals & cereal products	
Instant oatmeal (prepared with water)	2.41
Whole wheat bread	2.07
Raisin bran cereal	1.32 - 5.12
Brown rice, cooked	1.07
Legumes	
Navy beans, cooked	0.53
Lima beans, cooked	0.52
Pinto beans, cooked	0.46
Vegetables	
Spinach, cooked	0.93
Sweet potato, cooked	0.27
Fruits & fruits products	
Pineapple, raw	0.93
Pineapple juice	0.50
Beverages	
Tea (green)	0.33 - 1.29
Tea (black)	0.15 - 0.63

Sources: The Linus Pauling Institute's Micronutrient Information Center (MIC) (2010) and USDA Food Composition Database (2015)

Tap water do contain some amounts of manganese but is not a good source of this trace element. In a study by Azrina *et al.* (2011), the concentrations of majority of the inorganic elements in the tap water samples from all the 12 states of Peninsular Malaysia were found to be low, and below the maximum permitted levels recommended by the international standard limits for drinking water except for iron and manganese. The result showed that the maximum level of manganese in the tap water samples was 0.09mg/L, which was slightly higher than the international standard limit of 0.05mg/L.

28.5 Deficiency

Deficiency of manganese rarely occurs in humans. The documented symptoms of manganese deficiency include nausea, vomiting, dermatitis, decreased growth of hair and nails, and changes in hair color.

Manganese deficiency in animal studies have shown possible effect of impaired growth, impaired reproductive function, impaired glucose tolerance, and interference of normal skeletal development (IOM, 2001).

However, low dietary intakes of manganese were found to be associated with certain metabolic pathway and may have a possible role in the cholesterol synthesis. This is shown in a study by Friedman et al. (1987) which examined the effects of manganese depletion. In this study, seven male subjects were first fed with a manganese-adequate diet (2.59mg Mn/day) for 3 weeks to establish baseline data, followed by a depletion phase of 39 days with diet of 0.11mg Mn/day, and finally a repletion of two 5 days periods (total 10 days) with a diet of 1.53 and 2.55mg Mn/day. The results showed that plasma cholesterol concentrations decreased during the depletion period, and there was no response to the repletion phase.

Another study by Penland and Johnson (1993) examined the effects of dietary calcium and manganese on menstrual cycle symptoms in young women. In this study, ten women were assigned to each of the four combinations for 39 days: 587 or 1336 mg calcium per day with 1.0 or 5.6 mg manganese per day. The results showed that increased calcium intake helped to reduce the menstrual cycle symptoms but subjects in the low manganese intake experienced symptoms of altered mood and increased pain during premenstrual phase despite higher intake of calcium.

Up to date, there are no reliable and validated biomarkers to determine manganese intake or status (EFSA, 2013). Based on the metabolism of manganese, the suggested biomarkers for inadequate supply of manganese include a combination of serum manganese concentration and MnSOD activity, and possible blood arginase activity (Greger, 1999). However, there are several biomarkers to determine manganese intoxication primarily due to environmental factor such as inhalation.

28.6 Factors Affecting Manganese Requirements

Majority of studies have shown that serum or plasma manganese concentrations respond to dietary intakes of individuals. However, there are some studies which showed that the serum or plasma levels could be sensitive to large variations in manganese intake. This relationship requires further investigation.

There are significant differences in absorption of manganese, with lower absorption in men compared to women. This could be due to the iron status and high ferritin concentrations, which have shown to be associated with low absorption of manganese (Finley Johnson & Johnson, 1994).

In terms of excretion, it is still unclear whether urinary manganese corresponds to low intakes of manganese in the diet. This is because there were studies which showed significant decrease in urinary manganese excretion when study subjects were in a depletion diet of manganese (Friedman *et al.,* 1987; Freeland-Graves *et al.,* 1988) while other studies showed no change in either high or low intakes of manganese (Greger *et al.,* 1990; Davis and Greger 1992).

28.7 Setting requirements and recommended intakes of manganese

It is difficult to determine the manganese intake and status of the population since there are no reliable and validated biomarkers. There is also insufficient data to set Estimated Average Requirement (EAR) for manganese. Hence, Adequate Intake (AI) was proposed in both Institute of Medicine (IOM) (2006) and European Food Safety Authority (EFSA) (2013). The major difference in AI values between IOM (2006) and EFSA (2013) is the latter did not discriminate the AI values by sex and stages of life-cycle for pregnancy and lactation. In the EFSA (2013), the AI values for pregnancy and lactation stage are the same as the AI values for adults. Based on the US Food and Drug Administration Total Diet Study (1991-1997), there were differences in median intakes between males and females with the former having higher intakes. In addition, there is also sex difference in absorption for manganese with females absorbing significantly more than males. Hence, the Technical Sub-Committee (TSC) on Minerals and Trace Elements had decided to adopt the IOM Recommendations (2006) for the AI values for manganese. The median intakes reported from the US Food and Drug Administration Total Diet Study (US TDS) was used as the basis to set the AI values for manganese.

Infants

The AI value for infants aged 0-6 months in the IOM Recommendations (2006) was determined by multiplying the average milk volume consumption (0.78 L/day) with the average manganese concentration in human milk (3.5 µg/L). This totalled up to 3 µg/day after rounding up. As for the older infants aged 7-12 months, the AI value is much higher compared to the younger infants. This is due to the higher amounts of manganese available in complementary foods when compared to human milk. The AI value for the older infants was determined by using the following two methods: 1) multiplying the average consumption of manganese for 6- and 12-month-old infants with the respective reference body weight for these two ages; and 2) the average intake of manganese using a reference body weight method extrapolated from the adults. The values obtained from the first method ranged from 500 - 720 µg/day and the value from method 2 came up to 567 µg/day (IOM 2006). Hence 600 µg/day was set as the AI value for the older infants based on these two methods.

AI for Infants

0-6 months	**0.003 mg/day**
7-12 months	**0.6 mg/day**

Children and Adolescents

The AI values for manganese are the same for both sexes in the age group 1-3 years old (1.2 mg/day) and 4-8 years old (1.5 mg/day). However, the recommended intakes for manganese are more for the boys compared to the girls aged 9-18 years old. The AI values in the IOM Recommendations (2006) were based from the data obtained from US TDS which reported median intakes of 1.22 mg/day for children aged 1-3 years, 1.48 mg/day for children aged 4-8 years, 1.91 mg/day for boys aged 9-13 years, 1.57 mg/day for girls aged 9-13 years, 2.17 mg/day for adolescent boys aged 14-18 years, and 1.55 mg/day for adolescent girls aged 14-18 years. The final AI values were set by rounding up the median intakes of the respective groups.

AI for children and adolescents

1 - 3 years	**1.2 mg/day**
4 - 8 years	**1.5 mg/day**
Boys	
9 - 13 years	**1.9 mg/day**
14 - 18 years	**2.2 mg/day**
Girls	
9 - 13 years	**1.6 mg/day**
14 - 18 years	**1.6 mg/day**

Adults

The recommended intakes for manganese are also higher for adult men than for women. This is due to sex differences in the absorption and consumption of manganese. The AI values in the IOM Recommendations (2006) were based on the median intakes of 2.1 to 2.3 mg/day for men and 1.6 to 1.8 mg/day for women obtained from the US TDS. The final AI values were set by taking the upper limit from the range of median intakes for both adult men and women.

AI for adults

Men

19 - 30 years	**2.3 mg/day**
31 - 50 years	**2.3 mg/day**
50 - 70 years	**2.3 mg/day**
> 70 years	**2.3 mg/day**

Women

19 - 30 years	**1.8 mg/day**
31 - 50 years	**1.8 mg/day**
50 - 70 years	**1.8 mg/day**
> 70 years	**1.8 mg/day**

Pregnancy and Lactation

According to the IOM Recommendations (2006), the recommended intakes for manganese are slightly higher in the stages of pregnancy and lactation when compared to adult women and adolescent girls. The AI values for pregnancy stage were determined via extrapolating up from adolescent girls and adult women. The values totalled up to around 2 mg/day. This value is also consistent with the median intakes of manganese obtained from the US TDS for this group. The AI value in the IOM Recommendations (2006) for lactation stage was determined via rounding up the median intake of manganese in lactating women (2.56 mg/day) obtained from the data in the US TDS.

AI for pregnancy and lactation

Pregnancy

14 - 18 years	**2.0 mg/day**
19 - 50 years	**2.0 mg/day**

Lactation

14 - 18 years	**2.6 mg/day**
19 - 50 years	**2.6 mg/day**

28.8 Tolerable upper intake levels

The occurrence of toxicity from manganese usually occurs due to environmental factor such as contaminated air rather than from dietary intake. Manganese toxicity is rare, but individuals who inhale manganese-rich dust are known to experience Parkinson-like symptoms. Manganism is a permanent neurological disorder resulting from manganese toxicity and the symptoms include tremors, difficulty walking, and facial muscle spasms.

Children tend to be more sensitive to manganese toxicity when compared to adults. A review by Zoni and Lucchini (2013) concluded some evidence of possible developmental effects (cognitive, motor, and behavioural) when children are exposed to high levels of manganese from either the environment or from drinking water.

The following are examples of biomarkers used to determine manganese intoxication (Zheng *et al.,* 2011):

The common biomarker is by measuring blood manganese. This method can only be used for group comparisons e.g. to distinguish between groups exposed to manganese vs unexposed groups, therefore only useful for epidemiological studies.

Another more sensitive biomarker to determine manganese exposure at an individual level will be the use of Mn/Fe ratio (MIR) in plasma or red blood cells. This method was established on the notion that manganese exposure can alter iron homeostasis.

A non-invasive method will be the use of magnetic resonance imaging (MRI), in combination with (-aminobutyric acid (GABA) by magnetic resonance spectroscopy (MRS). This method is able to quantify manganese accumulation in the brain based on the images, and detect manganese exposure, even without any clinical symptoms of manganese intoxication.

Finally, an emerging method is the use of X-ray fluorescence spectroscopy or neutron-based spectroscopy. This method is able to detect low levels of a variety of metals (Nie *et al.,* 2006).

However, since manganese is excreted via bile, potential toxicity is likely to occur in neonate and individuals with liver disease (Hauser *et al.,* 1994). In addition, due to the bioavailability of manganese which may be more in drinking water and supplements than food, extra caution should be considered.

The UL levels for manganese was based on the IOM Recommendations (2006). The UL level for adults above 19 years old for manganese is 11mg/day and Table 28.2 shows the UL levels by age group.

Table 28.2. Tolerable upper intake (UL) levels by age group for manganese

Age group		Tolerable upper intake (UL) mg/day
Infants	0 - 12 months	Unable to establish
Children	1 - 3 years	2
	4 - 8 years	3
	9 - 13 years	6
Adolescents	14 - 18 years	9
Pregnancy	14 - 18 years	9
	19 - 50 years	11
Lactation	14 - 18 years	9
	19 - 50 years	11

Source: IOM (2006)

28.9 Research Recommendations

The following are three areas for research recommendations:

- There is no data on the manganese status in the Malaysian population, whether we are consuming enough manganese from our diet, or reports of manganese deficiency.
- The Malaysian Food Composition do not have data on the manganese content of Malaysian foods and its inclusion is recommended.
- There are some reports which showed potential roles of manganese in the prevention of the following chronic diseases/conditions: brain disorders; diabetes mellitus; lipid disturbances; and some cancers so further investigations in this area are needed.

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Appendix 28.1 Comparison of recommended intake for Manganese: RNI Malaysia (2017), AI & RNI of IOM (2006) and AI& RNI of EFSA (2013)

Malaysia (2017)		IOM (2006)		EFSA (2013)	
Age group	**AI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**
Infants		**Infants**		**Infants**	
0 - 6 months	0.003	0 - 6 months	0.003	0 - 6 months	-
7 -12 months	0.6	7 - 12 months	0.6	7 -11 months	0.02 - 0.5
	RNI (mg/day)		**RNI (mg/day)**		**RNI (mg/day)**
Children		**Children**		**Children**	
1 - 3 years	1.2	1 - 3 years	1.2	1 - 3 years	0.5
4 - 8 years	1.5	4 - 8 years	1.5	4 - 6 years	1.0
				7- 10 years	1.5
Boys		**Boys**		**Boys**	
9 - 13 years	1.9	9 - 13 years	1.9	11 - 14 years	2.0
14 -18 years	2.2	14 -18 years	2.2	15 - 17 years	3.0
Girls		**Girls**		**Girls**	
9 - 13 years	1.6	9 - 13 years	1.6	11 - 14 years	2.0
14 - 18 years	1.6	14 -18 years	1.6	15 - 17 years	3.0

Malaysia (2017)		IOM (2006)		EFSA (2013)	
Age group	**AI (mg/day)**	**Age group**	**AI (mg/day)**	**Age group**	**AI (mg/day)**
Men		**Men**		**Men**	
19 - 30 years	2.3	19 - 30 years	2.3	≥18 years	3.0
31 - 50 years	2.3	31 - 50 years	2.3		
50 - 70 years	2.3	50 - 70 years	2.3		
>70 years	2.3	>70 years	2.3		
Women		**Women**		**Women**	
19 - 30 years	1.8	19 - 30 years	1.8	≥18 years	3.0
31 - 50 years	1.8	31 - 50 years	1.8		
50 - 70 years	1.8	50 - 70 years	1.8		
>70 years	1.8	>70 years	1.8		
Pregnancy		**Pregnancy**		**Pregnancy**	
14 - 18 years old	2.0	14 - 18 years old	2.0		3.0
19 - 50 years old	2.0	19 - 50 years old	2.0		
Lactation		**Lactation**		**Lactation**	
14 - 18 years old	2.6	14 - 18 years old	2.6		3.0
19 - 50 years old	2.6	19 - 50 years old	2.6		

29 • Molybdenum

29.1 Introduction

Molybdenum is a metallic element with the atomic symbol Mo. Molybdenum is a hard, high-melting high-density dark gray metal or black powder. Practically insoluble in alkali hydroxides and insoluble in water, molybdenum is used to make structural alloys and as a catalyst.

In humans, molybdenum is an essential trace element, being a cofactor of a limited number of key enzymes involved in the catabolism of sulphur amino acids and heterocyclic compounds, including purines and pyridines (Brychkova *et al.,* 2015).

Most of the body's molybdenum is stored in the liver, bones, glands and kidneys. It is also found in the skin, muscles, spleen and lungs. While the body is usually able to absorb more molybdenum than it needs, the majority of what is absorbed is released as waste in urine.

29.2 Functions

As a dietary trace element, molybdenum is known to function as a cofactor for the following important enzymes:

1. Sulphite oxidase (SO) is crucial for degradation of sulfur-containing compounds in animals and plants. In the metabolism of sulphur amino acids (methionine and cysteine), sulphite oxidase catalyses the transformation of sulphite to sulphate. Sulphite is present in many chemically preserved foods and specific food proteins, and is a toxic nucleophile, the level of which must be carefully controlled. Thus, the action of sulphite oxidase helps to reduce toxic build-up of sulphites.

2. Xanthine oxidase (XO) catalyses the breakdown of nucleotides (precursors to DNA and RNA) to form uric acid. Uric acid is a final enzymatic product in the degradation of purine nucleosides and free bases in humans, and is known to be a major antioxidant in the human plasma. On the other hand, a strong association has been demonstrated between high levels of serum uric acid and the development of hypertension, diabetes type II, kidney disease, cardiovascular disease, metabolic syndrome and its components (Sautin & Johnson, 2008; Nejatinamini *et al.,* 2015).

3. Aldehyde oxidase (AO) is a xanthine oxidase (XO)-related enzyme with emerging importance due to its role in the metabolism of drugs. It has been increasingly recognized in this past decade that AO, through its unique structure, distribution, and substrate recognition, has an important role to play in the metabolism of drugs (Pryde *et al.,* 2010). In addition to the oxidation of aldehydes to carboxylic acids, AOX is also responsible for the metabolism of new chemical entities emerging from modern drug discovery programmes, primarily through nitrogen-containing heterocycle oxidation including pyridines and purines and a wide variety of other fused heteroaromatic systems.

4. Mitoochondrial amidoxime reducing component (mARC) is the newly discovered fourth molybdenum enzyme in mammals. While its precise function is under investigation, initial studies showed that mARC forms a three-component enzyme system with

cytochrome b5 and NADH cytochrome b5 reductase. This mARC's N-reductive enzyme system plays a a major role in drug metabolism, especially in the activation of so-called "amidoxime-prodrugs" and in the detoxification of N-hydroxylated xenobiotics, though its physiological relevance is largely unknown (Plitzko et al, 2013).

29.3 Metabolism

Besides being a cofactor of key enzymes, molybdenum also plays a role in the excretion of excess acetaldehydes in the body. Yeast (candida) feeds on sugars in the intestine and continuously releases by-products, most important of which is acetaldehyde. Detrimental effects attributed to acetaldehyde include damages to the membranes of red blood cells, and it can negatively affect the respiratory, immune and endocrine systems. A buildup can lead to muscle aches, an overall sensation of weakness, and joint pain. Those who drink alcohol excessively often experience increased levels of acetaldehyde in their bodies. Molybdenum is able to convert acetaldehyde into acetic acid, which is easily excreted, or alternatively, acetic acid is converted into acetyl coenzyme A, which is an essential part of the body's metabolism.

The biosynthesis of molybdenum cofactor involves the complex interaction of six proteins and is a process of four steps, which also require iron, ATP, and copper. Mutations in the molybdenum cofactor biosynthetic pathway lead to the combined deficiency of all molybdenum-dependent enzymes.

Nutrient-nutrient interactions

Molybdenum-copper

Excess molybdenum intake causes fatal copper deficiency diseases in ruminant animals. The interaction of molybdenum with high sulfide generation in the rumen results in the formation of thiomolybdates. Thiomolybdate with four sulfur atoms called tetrathiomolybdate is a molecule that can form high-affinity complexes with copper, preventing the absorption of copper and blocking the activity of copper-dependent enzymes (Suttle, 2012).

However, there is a lack of evidence in humans ingesting enough dietary molybdenum to cause depletion of copper. Hence, the National Academy of Sciences did not consider this copper interaction to be relevant for humans in their determination of a Tolerable Upper Intake Limit (UL) for molybdenum.

Interestingly, thiomolybdate's ability to lower free copper levels is taken advantage in the treatment of Wilson's disease, a genetic disorder characterised by copper accumulation in tissues responsible for hepatic and neurologic disorders. Tetrathiomolybdate therapy (TM) seems able to prevent neurologic deterioration in patients (Brewer *et al.,* 2009).

Molybdenum-iron

Adequate iron status is essential to ensure proper molybdenum utilization. All enzymes that use molybdenum as a cofactor require iron in the formation of these enzymes. Additionally, the sulfite oxidase enzymes use iron in the form of heme (the same iron-containing protein found in red blood cells) along with molybdenum in their detoxification activity.

29.4 Sources

Legumes, such as beans, lentils, and peas, are the richest sources of molybdenum. Grain products and nuts are considered good sources, while animal products, fruit, and many vegetables are generally low in molybdenum. The molybdenum content of plants depends on the soil molybdenum content and other environmental conditions in which the plant is grown and may differ considerably. Traces of molybdenum are also found in natural water sources (Table 29.1).

Molybdenum is stored in the body, particularly in the liver, kidneys, glands, and bones. It is also found in the lungs, spleen, skin, and muscles. Molybdenum is absorbed very efficiently over a wide range of intakes by passive transport and urinary excretion reflects intake (Turnlund, Keyes, Peiffer, 1995). About 90% of the molybdenum eaten in foods is eliminated by the body through the urine

Table 29.1: Molybdenum content of foods

Food sources	µg/100 g fresh weight
Oats (rolled)	115.4
Soybeans (cooked)	75
Black beans (cooked)	75
Lentils	74.3
Kidney beans	73.8
Barley	41.5
Eggs	17
Carrots	4.8
Tomatoes	4.5
Cucumber	2.3

Source: www.whfoods.org (2017)

29.5 Deficiency

Since only a very small amount of molybdenum is required, deficiency is very rare unless due to genetic disorders. Xanthinuria is a rare genetic defect with excess urinary excretion of xanthine. There are two inherited forms of xanthinuria. Type I xanthinuria caused by a deficiency of xanthine dehydrogenase, while Type II xanthinuria is characterised by a deficiency of xanthine dehydrogenase, aldehyde oxidase in addition to xanthine oxidase. Plasma accumulation and excess urinary excretion of the highly insoluble xanthine may lead to arthropathy (a collective term for any disease of the joints), myopathy, crystal nephropathy, urolithiasis (formation of stony concretions in the urinary system), or renal failure.

Molybdenum cofactor deficiency is a rare inborn error of metabolism that is estimated to occur in 1 in 100,000 to 200,000 new-borns worldwide. It is thought that the condition is underdiagnosed, so the number of affected individuals may be higher (Bayram *et al.,* 2013). The major clinical symptoms are intractable neonatal seizures, progressive encephalopathy, facial dysmorphic features and feeding difficulties. Molybdenum cofactor deficiency Type A is due to mutations in the molybdenum cofactor synthesis 1 (MOCS1) gene, while Type B deficiency is caused by mutations in MOCS2. Both Type A and Type B deficiencies result in the loss of sulfite oxidase activity, also observed in isolated sulfite oxidase deficiency and characterized by severe neurologic abnormalities in affected patients (Mendel, 2013).

In the genetic disorder that prevents sulphite oxidase synthesis, sulphite is not oxidised to sulphate, resulting in severe neurological damage and early death. Patients with Crohn's Disease (inflammatory bowel disorder) are known to excrete excess molybdenum leading to molybdenum deficiency. Crohn's Disease is believed to be implicated by both genetic and environmental factors.

29.6 Factors affecting Molybdenum requirements

The gastrointestinal tract readily absorbs soluble, but not insoluble, molybdenum compounds. Absorption rate of molybdenum from the diet of both patients and healthy volunteers averaged about 50% in one study and 88-93% in another study in which the patients received 22-1490µgm Mo/day for 24 days (Wester, 1971).

Studies on molybdenum bio-kinetics in humans using the radionuclide 99Mo provided the following conclusions (Giussani *et al.,* 1998):

- Intestinal absorption of molybdenum supplied as an aqueous solution is almost complete (>90%, Mo < 5 mg).
- Intestinal absorption of molybdenum supplied with a solid meal is less than 50% of the administered amount.
- Urinary excretion regulates rapidly the body content of molybdenum and its pattern is independent of the administered form (injection or ingestion, extrinsic or intrinsic tracer).

- The excreted amount rises strongly with increasing molybdenum dietary level.
- Gastric emptying is much slower with solid meals than with aqueous solutions of molybdenum: residence half-times for solids 30 minutes, for liquids 10 minutes.

29.7 Setting requirements and recommended intake of molybdenum

The primary criterion used to set an Estimated Average Requirement (EAR) is molybdenum balance in controlled studies with specific amounts of molybdenum consumed. Adjustments are made for the bioavailability of molybdenum and criteria for determining Molybdenum requirement is shown in Table 29.2.

Table 29.2: Criteria for determining molybdenum requirements, by life stage group.

Life stage group	Criterion
0 through 6 months	Average molybdenum intake from human milk
7 through 12 months	Extrapolation from 0 through 6 months AI
1 through 18 years	Extrapolation from adult EAR
19 through 30 years	Balance data
31 through > 70 years	Extrapolation of balance data from 19 through 30 years
Pregnancy	
≤ 18 years	Extrapolation of adolescent female EAR based on body weight
19 through 50 years	Extrapolation of adult female EAR based on body weight
Lactation	
≤ 18 years	Adolescent female EAR plus average amount of molybdenum secreted in human milk
19 through 50 years	Adult female EAR plus average amount of molybdenum secreted in human milk

Source: IOM (2006)

The European Food Safety Authority (EFSA) in 2013 proposed adequate intake (AI) level for molybdenum as part of its effort to provide dietary reference values (DRVs) for micronutrients. The EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) after a public consultation, proposed AI for molybdenum as 65 micrograms per day for all adults and 10-65 micrograms per day for infants, children and adolescents.

The TSC on Minerals and Trace Elements decided on adoption of the IOM (2006) values for the 2017 RNIs.

Recommended intakes by age groups

AI of molybdenum for infants:

0 - 6 months	**2 µg/day**
7 - 12 months	**3 µg/day.**

Information on dietary intake of molybdenum is limited because of lack of a simple and reliable analytical method for determining molybdenum in foods (IOM, 2006). For children and adolescents, a RNI has been set for both male and female:

1 - 3 years	**17 µg/day**
4 - 8 years	**22 µg/day**
9 - 13 years	**34 µg/day**
14 - 18 years	**43 µg /day**

It is estimated that a typical US adult's diet supplies 120 µg/day to 210 µg/day. For men and women above 19 years and pregnancy and lactation (14-50 years), the RNI are:

Adults Ages ≥19 years

Men and women ≥19 years	**45 µg/day**

Pregnancy and Lactation

14 - 50 years	**50 µg/day**

29.8 Tolerable upper intake levels

There is little cause for concern about excessive dietary intake of toxic levels of molybdenum in foods, except in situations involving environmental contamination. Molybdenosis is a rare disorder caused by excessive consumption of molybdenum supplement with symptoms of impairment of growth in children, diarrhoea and anaemia. At extreme amounts in animal studies, molybdenum can cause stunted growth, kidney damage, bone loss, anemia, and infertility. None of these potential problems has been observed in humans, as it is

practically impossible to consume such high levels of molybdenum from food, unless there is industrial contamination of the environment, and humans living in the area consume the contaminated plants and animals.

The National Academy of Sciences (NAS) has established a Tolerable Upper Intake Level (UL) of 2 mg for adult men and women 19 years and older. For younger persons, the ULs are as follows.

- 1-3 years: 0.6 mg (600 µg)
- 9-13 years: 1.1 mg (1,100 µg)
- 14-19 years: 1.7 mg (1,700 µg)
- 19+ years: 2.0 mg (2,000 µg)
- Pregnant or lactating women, under 19 years: 1.7 mg (1,700 µg)
- Pregnant or lactating women, over 19 years: 2.0 mg (2,000 µg)

29.9 Research recommendations

The most recently discovered molybdenum-containing enzyme in mammals. "mitochondrial amidoxime reducing component" (mARC) has been found to be capable of reducing a great variety of N-hydroxylated substrates, in the activation of prodrugs containing an amidoxime structure, and in detoxification pathways, e.g., of N-hydroxylated purine and pyrimidine bases. However, its physiological relevance is largely unknown (Ott, Havemeyer, Clement, 2015).

There continues to be research into examining the roles of molybdenum in various forms of cancer and cancer prevention.

Controversy prevails about uric acid, gout and other inflammatory diseases, which feature high uric acid. These may include: heart disease, arthritis, kidney disease, diabetes, autoimmune conditions, and GI inflammation. When uric acid precipitates into tissues, this can lead to the aggravation of gouty arthritis. Very high levels of molybdenum in the diet such as 10mg to 15 mg/day, and industrial exposure to molybdenum might cause gout. More studies are suggested to clarify the roles dietary molybdenum, sulfur, iron and copper in uric acid biosynthesis.

As molybdenum is found in tooth enamel, many believe that healthy molybdenum levels help prevent the formation of cavities and even some other gum disorders. Studies are proposed to elucidate the roles of molybdenum in gum health.

29.10 References

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Appendix 29.1: Comparison of recommended intake for Molybdenum RNI Malaysia (2017) and AI of IOM (2006)

RNI Malaysia (2017)		IOM (2006)	
Age group	**AI (µg/day)**	**Age group**	**AI (µg/day)**
Infants		**Infants**	
0 - 6 months	2	0 - 6 months	2
7 -12 months	3	7 - 12 months	3
Children		**Children**	
1 - 3 years	17	1 - 3 years	17
4 - 8 years	22	4 - 8 years	22
Boys		**Boys**	
9 - 13 years	34	9 - 13 years	34
14 -18 years	43	14 -18 years	43
Girls		**Girls**	
9 - 13 years	34	9 - 13 years	34
14 - 18 years	43	14 - 18 years	43
Men		**Men**	
19 - 30 years	45	19 - 30 years	45
31 - 50 years	45	31 - 50 years	45
50 - 70 years	45	50 - 70 years	45
>70 years	45	>70 years	45
Women		**Women**	
19 - 30 years	45	19 - 30 years	45
31 - 50 years	45	31 - 50 years	45
50 - 70 years	45	50 - 70 years	45
>70 years	45	>70 years	45
Pregnancy		**Pregnancy**	
14 - 18 years old	50	14 - 18 years old	50
19 - 50 years old	50	19 - 50 years old	50
Lactation		**Lactation**	
14 - 18 years old	50	14 - 18 years old	50
19 - 50 years old	50	19 - 50 years old	50

30 • Flouride

30.1 Introduction

Fluorine was discovered as an element in the early 1500s by the German physician Georgius Agricola. The element was isolated in its pure form by Henri Moissan in late 1800s. Fluorine occurs naturally as the negatively charged ion, (F-). Fluoride is found throughout the earth's crust and occurs naturally in all water sources and some foods.

In the 1930s, researchers found that people who grew up drinking naturally fluoridated water had up to two-thirds fewer cavities than people living in areas without fluoridated water. Studies since then have repeatedly shown that in the right amount, fluoride provides significant protection against tooth decay and dental cavities. Subsequently, the American Dental Association, the World Health Organization and the American Medical Association, among many other organizations, have endorsed the addition of fluoride in drinking water supplies.

30.2 Functions

Fluorine is present in almost all tissue, especially the teeth and bones. Ingested fluorides are completely ionized and absorbed in the stomach and intestines. Fluoride is rapidly excreted in the urine.

Fluoride rapidly enters the mineralized tissue, mainly bones and developing teeth. About 95% of the total body fluoride is stored in bones and teeth, where fluoride reacts with hydroxyapatite in the bones and teeth to form fluoroapatite. Fluoride's high chemical reactivity and small radius allow it to either displace the larger hydroxyl (-OH) ion in the hydroxyapatite crystal, forming fluoroapatite, or to increase crystal density by entering spaces within the hydroxyapatite crystal. (Cerklewski, 1998).

Fluoride helps prevent cavities in two different ways:

- Fluoride concentrates in the growing bones and developing teeth of children, helping to harden the enamel on child and adult teeth before they emerge

- Fluoride helps to maintain the hardened enamel on adult teeth.

Fluoride works during the demineralization and remineralisation processes that naturally occur in the mouth. Acids in the saliva cause demineralization, dissolving of the calcium and phosphorous under the tooth's surface. At other times, one's saliva is less acidic, it does just the opposite, replenishing the calcium and phosphorous that keep teeth hard. This process is caused remineralisation. Fluoride present during remineralisation, calcium and phosphorus deposited are harder than they would otherwise be, helping to strengthen teeth and prevent dissolution during the next demineralisation phase.

Fluoride is considered a trace element because only small amounts are present in the body (about 2.6 grams in adults). The daily requirement for maintaining dental health is only a few milligrams a day. Although its role in the prevention of dental caries is well established, fluoride is not generally considered an essential mineral element because humans do not require it for growth or to sustain life.

30.3 Metabolism

Fluorine is present in almost all tissues especially the teeth and bones. Ingested fluorides are completely ionized and absorbed in the stomach and intestines. Fluoride is rapidly excreted in the urine.

Both calcium and magnesium form insoluble complexes with fluoride and are capable of significantly decreasing fluoride absorption when present in the same meal. However, the absorption of fluoride in the form of monofluorophosphate is unaffected by calcium. Also, a diet low in chloride (salt) has been found to increase fluoride retention by reducing urinary excretion of fluoride (Cerklewski, 1997).

Aluminum-containing antacids can decrease the absorption of fluoride. It is best to take these products two hours before or after fluoride supplements. The use of small amounts of aluminium-containing antacids increases faecal fluoride significantly, thereby decreasing the intestinal absorption of fluoride. The dual effects of aluminium, namely in causing calcium loss and inhibition of the intestinal absorption of fluoride, can result in adverse effects on bone which may contribute to bone loss.

30.4 Deficiency

In humans, the only clear effect of inadequate fluoride intake is an increased risk of dental caries for individuals of all ages. Epidemiological investigations of patterns of water consumption and the prevalence of dental caries across various regions in United States with different water fluoride concentrations led to the development of a recommended optimum range of fluoride concentration of 0.7-1.2 milligrams/liter (mg/L) or parts per million (ppm); the lower concentration was recommended for warmer climates where water consumption is higher, and the higher concentration was recommended for colder climates.

This recommendation was revised recently "all community water systems to adjust the fluoride concentration to 0.7 mg/L as recent data do not show a convincing relationship between fluid intake and ambient air temperature, and to reduce the risk of dental fluorosis and in light of the widespread availability of fluoride from other sources, including fluoride-containing oral-care products" (Department of Health and Human Services, 2015).

In Malaysia, the level of 0.7 ppm (parts per million) was recommended in 1971 by a special committee of inquiry appointed to report upon the fluoridation of public water supply. In 1972, the government gave approval for nationwide implementation of the fluoridation programme Presently an estimated 76% of the Malaysian population benefit from fluoridated water (MOH, 2011).

An analysis of drinking water in nine areas in Malaysia reported that water fluoride levels varied from 0.71 +/-0.12 mg/L in Seri Serdang, Selangor to 0.08 +/- 0.06 mg/L in Sabah, where water fluoridation was stopped in 1989 (Shaharuddin *et al.,* 2010).

30.5 Sources

The major source of dietary fluoride globally is drinking water through controlled addition of fluoride to water used by communities as a public health measure to adjust fluoride concentration. An adult male residing in a community with fluoridated water has an intake range from 1-3 mg/day. Intake is less than 1 mg/day in non-fluoridated areas.

The fluoride content of most foods is low (less than 0.05 mg/100 grams or 0.5 ppm). Rich sources of fluoride include tea, which concentrates fluoride in its leaves, and marine fish that are consumed with their bones (e.g., sardines). Foods made with mechanically separated (boned) chicken, such as canned meats, hot dogs, and infant foods, also add fluoride to the diet. In addition, certain fruit juices, particularly grape juices, often have relatively high fluoride concentrations. Foods generally contribute only 0.3-0.6 mg of the daily intake of fluoride.

In Malaysia, Zubaidah *et al.* (2014) reported the mean fluoride contents in packet and hawkers' drinks, at 7.64±1.88 mg/L and 7.51±1.60 mg/L respectively, and in bottled drinking water (1.05±0.35 mg/L). Tea packet drinks were found to contain the highest mean amount of fluoride (13.02±0.23 mg/L).

Drinking water in the US has an optimal level of 0.7 to 1.2 milligrams (mg) per liter, which corresponds to 0.7-1.2 ppm. This concentration range has been found to decrease the incidence of dental caries while minimizing the risk of dental fluorosis and other adverse effects. The US Department of Health and Human Services in 2015 has recommended that the optimal concentration in drinking water be set at 0.7 ppm.

Water fluoridation

The common types of fluoride additives used in water fluoridation are:

Fluorosilicic acid: a water-based solution used by most water systems in the United States. Fluorosilicic acid is also referred to as hydrofluorosilicate, FSA, or HFS.

Sodium fluorosilicate: a dry additive, dissolved into a solution before being added to water.

Sodium fluoride: a dry additive, typically used in small water systems, dissolved into a solution before being added to water

(http://www.cdc.gov/fluoridation/factsheets/engineering/wfadditives.htm).

Most fluoride additives used in the United States are produced from phosphorite rock. Phosphorite contains calcium phosphate mixed with limestone (calcium carbonates) minerals and apatite-a mineral with high phosphate and fluoride content. It is refluxed (heated) with sulfuric acid, releasing hydrogen fluoride (HF) and silicon tetrafluoride (SiF4) gases, which are condensed to a water-based solution of approximately 23% FSA. Approximately 95% of FSA used for water fluoridation comes from this process.

All additives used by water treatment plants, including fluoride additives, must meet strict quality standards that assure the public's safety. In United States, these additives are subject to a stringent system of standards, testing, and certificates by the National Sanitation Foundation/American National Standards Institute (NSF/ANSI). The NSF International is a not-for-profit standards development and conformity assessment organization. Products used for drinking water treatment are evaluated to the criteria specified in NSF/ANSI Standard 60. This standard was developed by an NSF International-led consortium, including the American Water Works Association (AWWA) (http://www.cdc.gov/fluoridation).

NSF/ANSI Standard 60 requires, when available, that the U.S. Environmental Protection Agency (EPA) Maximum Contaminant Level (MCL) be used to determine the acceptable level for a chemical of interest. The EPA MCL for fluoride ion in water is 4 mg/L. The allowable maximum use levels (MUL) for NSF 60 Certified fluoridation products are:

- Fluorosilicic Acid: 6 mg/L
- Sodium Fluorosilicate: 2 mg/L
- Sodium Fluoride: 2.3 mg/L

Studies suggest that human exposure to fluorosilicates due to the use of hexafluorosilicic acid or hexafluorosilicate for drinking water fluoridation, if any, is very low as fluorosilicates in water are rapidly hydrolyzed to fluoride (Finney et al. 2006).

While water softeners are not thought to change water fluoride levels, reverse osmosis systems, distillation units, and some water filters have been found to remove significant amounts of fluoride from water.

Bottled water sales have grown exponentially and studies have found that most bottled waters contain sub-optimal levels of fluoride, although there is considerable variation (Cutrufelli *et al.,* 2005).

Table 30.1 shows examples of countries with a varying extent of flouridation of drinking water supplies.There are also geographical variations within some countries in terms of availability of fluoridated water at home. In Malaysia, it is estimated about 76% of the population or almost 21 million received fluoridated water in 2011, which is an increase from about 5 million in 2003 (MOH, 2011).

Table 30.1. Water fluoridation (artificial and natural) in selected countries.

	Number of people supplied with fluoridated water (millions)	Percentage of population supplied with optimally fluoridated water
Singapore	5.1	100
Vietnam	7	100
Australia	17.7	80
Malaysia	20.7	76
United States	204.3	74
New Zealand	2.3	61
Canada	14.6	44
Brazil	73.2	41
Spain	3.4	11
United Kingdom	6.1	10
Republic of Korea (South Korea)	2.8	6

Source: British Fluoridation Society (2013) bfs@bfsweb.org

30.6 Factors Affecting fluoride Requirements

The Adequate Intake (AI)

The Food and Nutrition Board (FNB) of the US Institute of Medicine updated its recommendations for fluoride intake in 1997. As data were insufficient to establish a Recommended Dietary Allowance (RDA), AI levels were set based on estimated intakes that have been shown to reduce the occurrence of dental caries most effectively without causing the unwanted side effect of tooth enamel mottling known as dental fluorosis (0.05 mg/kg of body weight) (Table 30.2) (IOM, 1997).

Fluoridation is most protective when the tooth is being formed during the mineralisation process. This coincides with ages 1 to 1.5 years for incisors, at about 6 years for premolars, and ages 16 - 17 years for molars. Excess fluoride on the other hand may result in fluorosis. The World Health Organization (WHO) concluded that at a fluoride level of 0.9 mg/L to 1.2 mg/L, very mild fluorosis occurs (WHO, 1997). Therefore, ensuring optimum levels of water fluoride is important especially during the mineralisation periods.

Table 30.2. Adequate intake (AI) for fluoride

Life Stage	Age	Males (mg/day)	Females (mg/day)
Infants	0-6 months	0.01	0.01
Infants	7-12 months	0.5	0.5
Children	1-3 years	0.7	0.7
Children	4-8 years	1.0	1.0
Children	9-13 years	2.0	2.0
Adolescents	14-18 years	3.0	3.0
Adults	19 years and older	4.0	3.0
Pregnancy	all ages	-	3.0
Breast-feeding	all ages	-	3.0

Source: http://lpi.oregonstate.edu/mic/minerals/fluoride

Fluoride supplements

Fluoride supplements in the US are intended for infants six months and older and children up to 16 years of age living in areas with suboptimal water fluoridation for the purpose of bringing their intake to approximately 1 mg/day (IOM, 1997). The American Dental Association Council on Scientific Affairs recommends the prescription of fluoride supplements only to children at high risk of developing dental caries (Rozier *et al.,* 2010). The supplemental fluoride dosage schedule in Table 30.3 was recommended by the American Dental Association, the American Academy of Pediatric Dentistry, and the American Academy of Pediatrics. It requires knowledge of the fluoride concentration of local drinking water, as well as other possible sources of fluoride intake.

Table 30.3. American Dental Association Fluoride Supplement Schedule

Age	Fluoride Ion Level in Drinking Water (ppm)*		
	<0.3 ppm	0.3-0.6 ppm	>0.6 ppm
Birth - 6 months	None	None	None
6 months - 3 years	0.25 mg/day**	None	None
3 years - 6 years	0.50 mg/day	0.25 mg/day	None
6 years - 16 years	1.0 mg/day	0.50 mg/day	None

*1.0 part per million (ppm) = 1 milligram/liter (mg/L)
(Micronutrient Information Centre, Linus Pauling Institute, Oregon State University)

Nutrient interactions

Both calcium and magnesium form insoluble complexes with fluoride and are capable of significantly decreasing fluoride absorption when present in the same meal. However, the absorption of fluoride in the form of monofluorophosphate is unaffected by calcium. Also, a diet low in chloride (salt) has been found to increase fluoride retention by reducing urinary excretion of fluoride (Cerklewski, 1997).

Drug interactions

Aluminum-containing antacids, can decrease the absorption of fluoride. It is best to take these products two hours before or after fluoride supplements. The use of small amounts of aluminium-containing antacids increased faecal fluoride significantly, thereby decreasing the intestinal absorption of fluoride. The dual effect of aluminium, namely in causing calcium loss and inhibition of the intestinal absorption of fluoride, can result in adverse effects on bone which may contribute to bone loss.

30.7 Setting requirements and recommended intake of flouride

The European Food Safety Authority (EFSA)'s Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) (2014) proposed AI for fluoride at 0.05 mg/kg body weight per day for children aged 7 months to 17 years as well as adults, including pregnant and lactating women.

According to IOM (2006), the AI for fluoride for 0-6 moths is based on human milk fluoride content. The AIs for 6 months and older are based on the intake values that maximally reduce the occurrence of dental caries in a group of individuals without causing unwanted effects including moderate tooth enamel mottling known as dental fluorosis. The IOM (2006) proposed the following dietary Reference Intakes (refer Table 30.4) for various age groups:

Table 30.4: Dietary Reference Intakes by Age Groups

	DRI values (mg/day)	
	AI	
	males	females
Life Stage Group		
0 through 6 month	0.01	0.01
7 through 12 month	0.5	0.5
1 through 3 years	0.7	0.7
4 through 8 years	1	1
9 through 13 years	2	2
14 through 18 years	3	3
19 through 30 years	4	3
31 through 50 years	4	3
51 through 70 years	4	3
> 70 years	4	3
Pregnancy		
≤ 18 years		3
19 through 50 years		3
Lactation		
≤ 18 years		3
19 through 50 years		3

Reference: IOM (2006)

Upon reviewing both reports, the TSC on Minerals and Trace Elements agreed to adapt the IOM (2006) values as the RNI for Malaysia 2017.

30.8 Tolerable Upper Intake Levels (UL)

Adverse effects

Fluoridation of public drinking water in the US was initiated nearly 70 years ago. A number of adverse effects have been attributed to water fluoridation, but extensive scientific research has uncovered no evidence of increased risks of cancer, heart disease, kidney disease, liver disease, thyroid disease, Alzheimer's disease, birth defects, or Down's syndrome (Whitford, 2011; US Department of Health and Human Services, 2015; Committee on Fluoride in Drinking Water NRC. 2006).

Toxicity may arise due to ingestion of excess fluoride from multiple sources: drinking water supplies, food processed in areas containing high levels of fluoride, use of toothpastes with high fluoride contents, intake of plant foods grown in soil with fluorides added to fertilizers, and high consumption of sea foods.

Dental flourosis

Children exposed to too much fluoride while the enamel of their teeth is being formed may develop dental fluorosis. In its mild form, this leads to discolouration. When it is more severe, the tooth enamel is pitted. It can affect both the first set of teeth (baby or milk teeth) and the second set, which remain through adult life. The mildest form of dental fluorosis is detectable only to the trained observer and is characterized by small opaque white flecks or spots on the enamel of the teeth. Moderate dental fluorosis is characterized by mottling and mild staining of the teeth, and severe dental fluorosis results in marked staining and pitting of the teeth. In its moderate to severe forms, dental fluorosis becomes a cosmetic concern when it affects the incisors and canines (front teeth). It is also a dose dependent condition, with higher fluoride intakes being associated with more pronounced effects on the teeth. The incidence of mild and moderate dental fluorosis has increased over the past decades, mainly due to increasing fluoride intake from reconstituted infant formula.

Fluoridated toothpastes are effective in preventing dental caries but also add considerably to fluoride intake of children, especially young children who are more likely to swallow toothpaste. Researchers estimate that children under six years of age may ingest an average of 0.3 mg of fluoride from toothpaste with each brushing and are at increased risk of a white speckling or mottling of the permanent teeth, known as dental fluorosis. The American Dental Association (2014) recommended that parents supervise children under six years of age while brushing with fluoridated toothpaste.

Skeletal fluorosis

Intake of fluoride at excessive levels for long periods of time may lead to changes in bone structure known as skeletal fluorosis. Fluoride weakens the bone structure by incorporation in the mineral matrix of bone. The early stages of skeletal fluorosis are characterized by increased bone mass, detectable by x-ray. If very high fluoride intake persists over many years, joint pain and stiffness may result from the skeletal changes. The most severe form of skeletal fluorosis is known as "crippling skeletal fluorosis," which may result in calcification of ligaments, immobility, muscle wasting, and neurological problems related to spinal cord compression.

Skeletal fluorosis has been reported in India, China and Africa in regions with high concentrations of fluoride in drinking water, or where coal containing fluoride is burnt indoors. In China, a meta-analysis of 27 studies, mainly conducted in China, found lower intelligence quotients (IQs) in children exposed to fluoride concentrations ranging from 1.8 mg/L to 11.5 mg/L of drinking water (Choi *et al.,* 2012). In Europe it has only been seen in some workers in the chemical and mineral processing industries.

Serious limitations, including substantial heterogeneity among studies and co-occurrence of other neurotoxicants in drinking water, hinder the strength of the finding and its application to US settings. The Academy of Nutrition and Dietetics has recently estimated that only limited evidence supports an association between fluoride content in water and the IQs of children (Palmer and Gilbert, 2012). A recent prospective study in a New Zealand population-based cohort followed for nearly four decades found no association between fluoride exposure in the context of community water fluoridation programs and IQs measured during childhood and at 38 years of age (Broadbent *et al.,* 2015).

Water fluoridation has not been linked with bone fractures, and modest fluoridation may even lower bone fracture risk. Animal and human studies have examined other possible risks from fluoride intake. They have not found clear links between fluoride and bone cancer, or with other suggested risks such as neurological or reproductive effects.

Acute toxicity

Fluoride is toxic when consumed in excessive amounts, so concentrated fluoride products should be used and stored with caution to prevent the possibility of acute fluoride poisoning, especially in children and other vulnerable individuals. The lowest dose that could trigger adverse symptoms is considered to be 5 mg/kg of body weight, with the lowest potentially fatal dose considered 15 mg/kg of body weight. Nausea, abdominal pain, and vomiting almost always accompany acute fluoride toxicity. Other symptoms like diarrhea, excessive salivation and tearing, sweating, and generalized weakness may also occur.

In order to prevent acute fluoride poisoning, the American Dental Association (2014) has recommended that no more than 120 mg of fluoride (224 mg of sodium fluoride) be dispensed at one time and toothpaste, although inappropriate for use as fluoride supplements may also contribute. In 1997, the US Food and Nutrition Board (FNB) of the Institute of Medicine set the tolerable upper intake level (UL) for fluoride based on the prevention of moderate enamel fluorosis (Table 30.4).

The UL for fluoride for individuals aged 9 years and older was derived using data on the risk of developing early signs of skeletal fluorosis, which is associated with a fluoride intake greater than 10 mg/day for a period of 10 years or longer. The UL for infants and children younger than 8 years old was based on a critical adverse effect of developing fluorosis of the anterior teeth, not skeletal fluorosis.

Table 30.4. Tolerable upper intake level (UL) for fluoride

Age Group	UL (mg/day)
Infants 0-6 months	0.7
Infants 7-12 months	0.9
Children 1-3 years	1.3
Children 4-8 years	2.2
Children 9-13 years	10.0
Adolescents 14-18 years	10.0
Adults 19 years and older	10.0

30.9 Research Recommendations

Fluoride may play an important role in maintaining the bone mass. In view of the high incidence of osteoporosis and its complications, and in view of the fact that fluoride is beneficial for the treatment of osteoporosis, studies have been carried out in this research unit to determine the retention and excretion of fluoride both during a normal dietary intake of fluoride and during fluoride supplementation. Such studies are lacking in the Malaysian population. Studies in estimated fluoride intake are outdated. Foo and Chong (1975) estimated that the average daily fluoride intakes by adults in the fluoridated and non-fluoridated areas were about 1.5 mg and 1 mg respectively, based on testing urine and food samples.

In children, excessive ingestion of fluoride from different sources including bottled drinking water and flavoured beverages can lead to dental fluorosis. In addition, low pH level of beverages can cause dental erosion. The mean pH of bottled-drinking water was near neutral (6.96±0.17), but found to be acidic for drinks sold in supermarkets (4.78.00±0.49) and hawkers (5.73±0.24), with lychee packet drink having the lowest pH level (2.97±0.03) (Zubaidah *et al.,* 2014). More studies should be undertaken to determine the fluoride and pH levels of drinks and beverages commonly available to children.

30.10 References

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Appendix 30.1: Comparison of recommended intake for Flouride: RNI Malaysia (2017) and IOM (2006)

Malaysia (2017)*		IOM (2006)	
Age group	**RNI (µg/day)**	**Age group**	**AI (µg/day)**
Infants		**Infants**	
0 - 6 months	0.01	0 - 6 months	0.01
7 -12 months	0.5	7 -12 months	0.5
Children		**Children**	
1 - 3 years	0.7	1 - 3 years	0.7
4 - 8 years	1.0	4 - 8 years	1.0
Boys		**Boys**	
9 - 13 years	2.0	9 - 13 years	2.0
14 -18 years	3.0	14 - 18 years	3.0
Girls		**Girls**	
9 - 13 years	2.0	9 - 13 years	2.0
14 - 18 years	3.0	14 - 18 years	3.0
Men		**Men**	
19 - 30 years	4.0	19 - 30 years	4.0
31 - 50 years	4.0	31 - 50 years	4.0
50 - 70 years	4.0	50 - 70 years	4.0
>70 years	4.0	>70 years	4.0
Women		**Women**	
19 - 30 years	3.0	19 - 30 years	3.0
31 - 50 years	3.0	31 - 50 years	3.0
50 - 70 years	3.0	50 - 70 years	3.0
>70 years	3.0	>70 years	3.0
Pregnancy		**Pregnancy**	
14-18 years old	3.0	14 - 18 years old	3.0
19-50 years old	3.0	19 - 50 years old	3.0
Lactation		**Lactation**	
14-18 years old	3.0	14-18 years old	3.0
19-50 years old	3.0	19-50 years old	3.0

*refer to AI

RECOMMENDED NUTRIENT INTAKE OF MALAYSIA CONSENSUS WORKSHOP 10 – 11 JANUARY 2017

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Malaysian Dietitians' Association

Malaysian Association for the Study of Obesity

Federation of Malaysian Manufacturers

Federation of Malaysian Consumers Association

Prime Minister Department

RECOMMENDED NUTRIENT INTAKES FOR MALAYSIA 2017

SUMMARY TABLES

NCCFN (2017). 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

Recommended Nutrient Intakes (RNI) for Malaysia 2017 Summary Tables 1. Energy Requirements (by physical activity level) and Protein Requirements

Age	Males					Females				
	Estimated Energy Requirements[1] kcal/day				Protein[2] g/day	Estimated Energy Requirements[1] kcal/day				Protein[2] g/day
Infants										
0 - 2 months	470				8	420				8
3 - 5 months	540				8	500				8
6 - 8 months	630				10	570				10
9 - 11 months	720				10	660				10
	PAL 1.4	**PAL 1.6**	**PAL 1.8**	**PAL 2.0**		**PAL 1.4**	**PAL 1.6**	**PAL 1.8**	**PAL 2.0**	
Children										
1 - 3 years	980				12	900				12
4 - 6 years	1300	1490	1670		16	1210	1380	1560		16
7 - 9 years	1530	1750	1970		23	1410	1610	1810		23
Adolescents										
10 - 12 years	1690	1930	2170	2420	30	1500	1710	1920	2140	31
13 - 15years	1930	2210	2480	2760	45	1580	1810	2040	2260	42
16 -<18 years	2050	2340	2640	2930	51	1660	1890	2130	2370	42
Adults										
≥ 18 - 29 years	1960	2240	2520	2800	62	1610	1840	2080	2310	53
30 - 59 years	1920	2190	2470	2740	61	1660	1900	2130	2370	52
≥ 60 years	1780	2030	2280	2540	58	1550	1770	1990	2220	50
Pregnancy										
1st trimester						+80				+0.5
2nd trimester						+280				+8
3rd trimester						+470				+25
Lactation										
1st six months						+ 500				+19
2nd six months										+ 13

Note: [1] For children aged 4 – 6 years, PAL 1.4 is recommended to be used for the general population. For children above 7 years, adolescents and adults, PAL of 1.6 (i.e. moderately active) is recommended to be used for the general population. For individuals, energy recommendation should be based on individual PAL.
[2] Protein calculated based on reference body weight.

Recommended Nutrient Intakes (RNI) for Malaysia 2017 Summary Tables 2: Vitamins

	Age	Thiamin mg/day	Riboflavin mg/day	Niacin mg NE/day	Pantothenic Acid mg/day	Pyridoxine mg/day	Folate µg/day	Cobalamin µg/day	Vitamin C mg/day	Vitamin A µg/day	Vitamin D µg/day	Vitamin E mg/day	Vitamin K µg/day
Infants (boys)	0 - 5 months	0.2	0.3	2	1.7	0.1	80	1.2	25	375	10	3	5
	6 - 11 months	0.3	0.4	4	1.8	0.3	80	1.5	30	400	10	3	10
Infants (girls)	0 - 5 months	0.2	0.3	2	1.7	0.1	80	1.2	25	375	10	3	5
	6 - 11 months	0.3	0.4	4	1.8	0.3	80	1.5	30	400	10	3	10
Children (boys)	1 - 3 years	0.5	0.5	6	2.0	0.5	160	1.5	30	400	15	5	15
	4 - 6 years	0.6	0.6	8	3.0	0.6	200	1.5	30	450	15	5	20
	7 - 9 years	0.9	0.9	12	4.0	1.0	300	2.5	35	500	15	7	25
Children (girls)	1 - 3 years	0.5	0.5	6	2.0	0.5	160	1.5	30	400	15	5	15
	4 - 6 years	0.6	0.6	8	3.0	0.6	200	1.5	30	450	15	5	20
	7 - 9 years	0.9	0.9	12	4.0	1.0	300	2.5	35	500	15	7	25
Adolescent (boys)	10 - 12 years	1.2	1.3	16	5.0	1.3	400	3.5	65	600	15	10	35-55
	13 - 14 years	1.2	1.3	16	5.0	1.3	400	4.0	65	600	15	10	35-55
	15 years	1.2	1.3	16	5.0	1.3	400	4.0	65	600	15	10	35-55
	16 - 18 years	1.2	1.3	16	5.0	1.3	400	4.0	65	600	15	10	35-55
Adolescent (girls)	10 - 12 years	1.1	1.0	16	5.0	1.2	400	3.5	65	600	15	7.5	35-55
	13 - 14 years	1.1	1.0	16	5.0	1.2	400	4.0	65	600	15	7.5	35-55
	15 years	1.1	1.0	16	5.0	1.2	400	4.0	65	600	15	7.5	35-55
	16 - 18 years	1.1	1.0	16	5.0	1.2	400	4.0	65	600	15	7.5	35-55
Men	19 - 29 years	1.2	1.3	16	5.0	1.3	400	4.0	70	600	15	10	65
	30 - 50 years	1.2	1.3	16	5.0	1.3	400	4.0	70	600	15	10	65
	51 - 59 years	1.2	1.3	16	5.0	1.7	400	4.0	70	600	15	10	65
	60 - 65 years	1.2	1.3	16	5.0	1.7	400	4.0	70	600	15	10	65
	> 65 years	1.2	1.3	16	5.0	1.7	400	4.0	70	600	20	10	65
Women	19 - 29 years	1.1	1.1	14	5.0	1.3	400	4.0	70	600	15	7.5	55
	30 - 50 years	1.1	1.1	14	5.0	1.3	400	4.0	70	600	15	7.5	55
	51 - 59 years	1.1	1.1	14	5.0	1.5	400	4.0	70	600	15	7.5	55
	60 - 65 years	1.1	1.1	14	5.0	1.5	400	4.0	70	600	15	7.5	55
	> 65 years	1.1	1.1	14	5.0	1.5	400	4.0	70	600	20	7.5	55
Pregnancy	1st trimester	1.4	1.4	18	6.0	1.9	600	4.5	80	800	15	7.5	55
	2nd trimester	1.4	1.4	18	6.0	1.9	600	4.5	80	800	15	7.5	55
	3rd trimester	1.4	1.4	18	6.0	1.9	600	4.5	80	800	15	7.5	55
Lactation	1st 6 months	1.5	1.6	17	7.0	2.0	500	5.0	95	850	15	7.5	55
	2nd 6 months	1.5	1.6	17	7.0	2.0	500	5.0	95	850	15	7.5	55

Recommended Nutrient Intakes (RNI) for Malaysia 2017 Summary Tables 3a: Minerals & Trace Elements

	Age	Calcium mg/day	Iodine µg/day	Selenium µg/day	Zinc mg/day	Iron mg/day	
						10%	15%
Infants (boys)	0 - 5 months	200 (bf) 250 (ff)	67.5 (0 - 2 months) 105.0 (3 - 5 months)	6	1.1 (bf) 2.8 (ff)	a	a
	6 - 11 months	260	124.5 (6 - 8 months) 138.0 (9 - 11 momths)	10	4.1	9	6
Infants (girls)	0 - 5 months	200 (bf) 250 (ff)	63.0 (0 - 2 months) 96.0 (3 - 5 months)	6	1.1 (bf) 2.8 (ff)	a	a
	6 - 11 months	260	114.0 (6 - 8 months) 127.5 (9 - 11 momths)	9	3.7	9	6
Children (boys)	1 - 3 years	700	73.2	17	4.2	6	4
	4 - 6 years	1000	109.8	21	5.2	6	4
	7 - 9 years	1000	101.6	22	5.7	9	6
Children (girls)	1 - 3 years	700	69.0	16	4.0	6	4
	4 - 6 years	1000	109.2	21	5.2	6	4
	7 - 9 years	1000	100.0	21	5.6	9	6
Adolescent (boys)	10 - 12 years	1300	133.6	21	7.0	15	10
	13 - 14 years	1300	99.2	31	9.3	15	10
	15 years	1300	99.2	31	9.3	19	12
	16 - 18 years	1300	118.4	37	9.9	19	12
Adolescent (girls)	10 - 12 years	1300	141.6	19	6.3	14 (nm) 33 (m)	9 (nm) 22 (m)
	13 - 14 years	1300	93.0	24	7.7	14 (nm) 33 (m)	9 (nm) 22 (m)
	15 years	1300	93.0	24	7.7	31	21
	16 - 18 years	1300	100.6	26	7.7	31	21
Men	19 - 29 years	1000	122.8	32	6.6	14	9
	30 - 50 years	1000	121.2	32	6.5	14	9
	51 - 59 years	1000	121.2	32	6.5	14	9
	60 - 65 years	1000	116.2	31	6.3	14	9
	> 65 years	1000	116.2	30	6.2	14	9
Women	19 - 29 years	1000	105.8	25	4.7	29	20
	30 - 50 years	1000	104.4	24	4.6	29	20
	51 - 59 years	1200	104.4	24	4.6	11	8
	60 - 65 years	1200	99.0	23	4.4	11	8
	> 65 years	1200	99.0	23	4.3	11	8
Pregnancy	13 - 19	1300					
	1st trimester	1000	200	25	5.5	b	b
	2nd trimester	1000	200	27	7.0	b	b
	3rd trimester	1000	200	29	10.0	b	b
Lactation	13 - 19	1300	200				
	1st 6 months	1000	200	34	9.5 (1 - 3mths) 8.8 (4 - 6 mths)	15	10
	2nd 6 months	1000	200	41	7.2 (7 - 12mths)	15 (nm) 32(m)	10 (nm) 21(m)

Note: a – no recommendations. Neonatal iron stores are sufficient to meet iron requirement for first 6 months in full-term intants. Premature infants and low birth weight infants require additional iron.
b – iron supplements in table form recommended for all pregnant women. In the non-anaemic pregnant woman, daily supplements of 100 mg iron given during second half of pregnancy are adequate. In anaemic women, higher doses are usually required. bf – breast fed ff – formula fed nm – non-menstruating m – menstruating

Recommended Nutrient Intakes (RNI) for Malaysia 2017 Summary Tables 3b: Minerals & Trace Elements

	Age	Phosphorus mg/day	Sodium mg/day	Potassium g/day	Magnesium mg/day	Chromium µg/day	Copper µg/day	Manganese mg/day	Molybdenum µg/day	Fluoride mg/day
Infants (boys)	0 - 6 months	100	120	0.4	30	0.2	200	0.003	2	0.01
	7 - 12 months	275	370	0.7	75	5.5	220	0.6	3	0.5
Infants (girls)	0 - 6 months	100	120	0.4	30	0.2	200	0.003	2	0.01
	7 - 12 months	275	370	0.7	75	5.5	220	0.6	3	0.5
Children (boys)	1 - 3 years	460	1000	3.0	80	11	340	1.2	17	0.7
	4 - 8 years	500	1200	3.8	130	15	440	1.5	22	1.0
Children (girls)	1 - 3 years	460	1000	3.0	80	11	340	1.2	17	0.7
	4 - 8 years	500	1200	3.8	130	15	440	1.5	22	1.0
Adolescent (boys)	9 - 13 years	1250	1500	4.5	240	25	700	1.9	34	2.0
	14 - 18 years	1250	1500	4.7	410	35	890	2.2	43	3.0
Adolescent (girls)	9 - 13 years	1250	1500	4.5	240	21	700	1.6	34	2.0
	14 - 18 years	1250	1500	4.7	360	24	890	1.6	43	3.0
Men	19 - 29 years	700	1500	4.7	400	35	900	2.3	45	4.0
	30 - 50 years	700	1500	4.7	420	35	900	2.3	45	4.0
	51 - 59 years	700	1500	4.7	420	30	900	2.3	45	4.0
	60 - 69 years	700	1500	4.7	420	30	900	2.3	45	4.0
	> 70 years	700	1200	4.7	420	30	900	2.3	45	4.0
Women	19 - 29 years	700	1500	4.7	310	25	900	1.8	45	3.0
	30 - 50 years	700	1500	4.7	320	25	900	1.8	45	3.0
	51 - 59 years	700	1500	4.7	420	20	900	1.8	45	3.0
	60 - 69 years	700	1500	4.7	420	20	900	1.8	45	3.0
	> 70 years	700	1200	4.7	320	20	900	1.8	45	3.0
Pregnancy	14 - 18 years	1250	1500	4.7	400	29	1000	2.0	50	3.0
	19 - 30 years	700	1500	4.7	350	30	1000	2.0	50	3.0
	31 - 50 years	700	1500	4.7	360	30	1000	2.0	50	3.0
Lactation	14 - 18 years	1250	1500	5.1	360	44	1300	2.6	50	3.0
	19 - 30 years	700	1500	5.1	310	45	1300	2.6	50	3.0
	31 - 50 years	700	1500	5.1	320	45	1300	2.6	50	3.0

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