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		