Chapter 1
Dietary Proteins: Functions, Health Benefits and Healthy Aging

Khetan Shevkani and Shivani Chourasia

Abstract Dietary proteins are the source of indispensable, dispensable and functional amino acids essential for the synthesis of body proteins and the regulation of physiological processes. They also are beneficial in alleviating lifestyle and/or age-related health problems (e.g. loss of muscle mass and strength, obesity/sarcopenic obesity, dyslipidaemia, bone mineral loss and type-2 diabetes) because of their roles in the promotion of muscle protein synthesis, satiation, optimisation of growth factors, inhibition of inflammatory substances and regulation of major pathways of metabolism, though protein-quantity and quality are major determinants of nutritional and disease-preventing effects. The intake of high quality proteins in adequate amounts together with healthy lifestyle can contribute to healthy aging through maintenance of muscle mass and/or enhanced ability of recovering from diseases, while proteins from legumes/pulses and milk (e.g. whey proteins) may benefit elderly people by reducing the risk of coronary artery diseases, obesity, bone density loss, type-2 diabetes and associated morbidities/mortalities. This chapter discusses health benefits of increased intake of dietary proteins in elderly people and provides an overview of protein quality and methods for evaluating the same. The chapter also outlines functions of dietary proteins and compares animal and plant proteins for their quality and health benefitting effects.

Keywords Dietary proteins · Health benefits · Health-span · Healthy aging · Plant vs animal proteins

1.1 Introduction

Proteins are complex nitrogenous compounds that are made up of amino acids linked through peptide bonds. At the elemental level, they contain 50–55% carbon, 20–25% oxygen, 12–19% nitrogen, 6–7% hydrogen and 0.2–3.0% sulphur (Damodaran 2017). Proteins are considered as the most fundamental component of body. They are the second most abundant constituent in human body, next only to water. A healthy

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man weighing 70 kg contains around 11 kg (approximately 16%) proteins in the body against around 10 kg (approximately 14%) in a woman of the same weight. About 45–50% of total body protein is present in muscles, 15–20% in bones, 10–15% in skin and the remaining portion in other tissues. Thousands different types of proteins occur in human body, which are involved in almost all metabolic and physiological processes. However, human body does not have mechanisms to store proteins. Therefore, a constant supply of a certain minimum amount of proteins through diet is essential for maintaining growth and other metabolic processes. Normal healthy adults are recommended to take 0.8 g quality proteins per kg body weight per day to meet protein requirements (Wolfe et al. 2017). Although body proteins differ considerably from proteins present in foods for chemical/biochemical and structural characteristics, dietary proteins provide amino acids and nitrogen that are utilized for synthesis of body proteins and other compounds vital for survival.

Milk, meat, fish, eggs, pulses/legumes and cereals are major sources of dietary proteins. These foods differ widely for protein content and characteristics. Protein content of selected foods are shown in Table 1.1. In general, animal foods (milk, egg, fish and meat) are considered as a source of high-quality proteins due to their ‘completeness’ in the context of amino acid composition and the absence of anti-nutritional constituents. However, plant foods, nowadays, are being promoted because of 1) the absence of cholesterol, 2) the presence of health-beneficial bioactive compounds (e.g. lectins, enzyme inhibitors, phytosterols, saponins, phenolic compounds, dietary fibre, etc.), and 3) their roles in ensuring sustainable agriculture and food security (Shevkani et al. 2015; Singh et al. 2017; Lonnie et al. 2018; Magrini et al. 2018; Bessada et al. 2019; Shevkani et al. 2021).

Dietary proteins also can be beneficial in alleviating lifestyle and age-related disorders/diseases and reducing morbidity/mortality in elderly people. Increased intake of dietary proteins was reported beneficial in the maintenance of muscle mass and the prevention of sarcopenia (Houstan et al. 2008; Zhang et al. 2020), obesity (Weigle et al. 2005; Millward et al. 2008; Zhou et al. 2014), bone demineralisation/osteoporosis (Thorpe et al. 2008; Shams-White et al. 2017; Groenendijk et al. 2019) and dyslipidaemia (Santesso et al. 2012; Hosomi et al. 2013). Also, a high protein diet caused about 28% reduction in mortality in elderly people of more than 65 years of age (Levine et al. 2014). This chapter discusses the health-benefitting effects of increased intake of dietary proteins in elderly people and provides an overview of protein quality and methods for evaluating the same. In addition, the chapter also outlines functions of dietary proteins and compares animal and plant proteins for their quality and health benefits.

Table 1.1 Protein content of selected foods

Food	Protein content (g/100 g)
Cereals and pseudocereals	
Wheat	8.0–18.0[a]
Brown rice	7.92–8.20[b]
Milled rice	7.46–8.02[b]
Maize	8.80[c]
Barley	10.94[c]
Ragi millet	7.16[c]
Sorghum	9.97[c]
Amaranth grains	12.5–15.2[d]
Quinoa	14.1–15.4[e]
Legumes/pulses	
Pinto bean	22.80[f]
Lima bean	23.92[f]
Small red bean	25.68[f]
Red kidney bean	25.60[f]
Black bean	25.37[f]
Navy bean	25.73[f]
Black eye bean	24.58[f]
Mung bean	27.10[f]
Lentils	28.05[f]
Chickpea	22.37[f]
Horse gram	21.73[c]
Urad bean	21.97[c]
Moth bean	19.75[c]
Pigeon pea/red gram	20.47[c]
Lupine	36.17[g]
Soybean	36.49[g]
Soybean, white	37.8[c]
Soybean, brown	35.58[c]
Soybean, split dehulled	37.5[h]
Lentils, split dehulled	27.6[h]
Mung bean, split dehulled	25.0[h]
Urad bean, split dehulled	31.4[h]

(continued)

Table 1.1 (continued)

Food	Protein content (g/100 g)
Chickpea, split dehulled	21.3[h]
Peas, split dehulled	31.1[h]
Cowpea, split dehulled	29.0[h]
Oilseeds	
Mustard seeds	19.51[c]
Groundnut	23.65[c]
Coconut kernel	7.27[c]
Sunflower seeds	23.53[c]
Safflower seeds	17.66[c]
Sesame seeds, whole, dried	17.73[g]
Flaxseed	19.35[g]
Tree nuts	
Almonds	18.41[c]
Cashew nuts	18.78[c]
Walnuts	14.92[c]
Pistachio nuts	23.35[c]
Fruits and vegetables	
Pomegranate, pulp	2.47[i]
Kinnow, pulp	1.76[i]
Mango, pulp	1.69[i]
Banana, pulp	1.82[i]
Jambolan	1.75[i]
Grapes	1.66[i]
Sapodila, pulp	1.54[i]
Beetroot	1.12[i]
Brinjal	1.68[i]
Carrot, orange	1.75[i]
Bitter gourd	1.64[i]
Mentha	3.30[i]
Carrot, black	1.75[i]
Spinach	1.71[i]

(continued)

Table 1.1 (continued)

Food	Protein content (g/100 g)
Potato	1.35–1.54[c]
Milk and Milk products	
Cow milk, whole	3.26[c]
Buffalo milk, whole	3.68[c]
Cheddar cheese	23.3[g]
Paneer	18.86[c]
Khoa	16.34[c]
Egg, meat and fish	
Egg	12.4[g]
Lamb, ground	20.33[g]
Chicken	16.07[g]
Ham, minced	16.28[g]
Fish, salmon, raw	20.5[g]
Fish, tuna, raw	24.4[g]
Fish, mackerel, raw	19.08[g]
Fish, porgy, raw	18.88[g]
Crab	10.23[c]
Oyster	9.51[c]
Tiger prawns, brown	14.85[c]
Lobster, brown	15.96[c]

References:
[a]Posner (2000).
[b]Sandhu et al. (2018).
[c]Longvah et al. (2017).
[d]Shevkani et al. (2014).
[e]Ghumman et al. (2021).
[f]Du et al. (2014).
[g]United States Department of Agriculture (available online at https://fdc.nal.usda.gov/).
[h]Shevkani et al. (2021).
[i]Singh et al. (2016).

1.2 Proteins as a Source of Amino Acids

Dietary proteins are the source of amino acids and nitrogen required in body for synthesis/formation of tissues/organs, enzymes, hormones and cell-specific physiologically important low molecular weight substances e.g. nitric oxide, glutathione, carnitine, carnosine, serotonin, melanin, melatonin, etc. (Blachier et al. 2011; Kong et al. 2012; Wu 2013). Although hundreds of amino acids occur in nature, only 20–21 are involved in synthesis of proteins in human body and in most other forms of life. Structurally, amino acids are composed of a carbon atom linked covalently with a hydrogen atom, an amine group, a carboxyl group and an alkyl group (also referred to as amino acid side chain). Different amino acids differ for the side chain, the characteristics of which decide physicochemical properties and reactivity of amino acid as well as properties of proteins. Based on the characteristics of side chains, amino acids are categorized in to acidic (aspartic acid and glutamic acid), basic (arginine, histidine and lysine), polar uncharged (serine, threonine, asparagine, glutamine and cysteine), aliphatic and nonpolar (alanine, isoleucine, leucine, valine, glycine, proline and methionine) and aromatic and nonpolar amino acids (phenylalanine, tyrosine and tryptophan), though a few (e.g. glycine, histidine and cysteine) do not fit perfectly into a particular group and their assignments to a particular group is not considered absolute (Nelson and Cox 2013; Ustunol 2015).

On the basis of nutritional properties, amino acids are grouped as essential (indispensable), non-essential (dispensable) and conditionally-essential (semi-essential/conditionally-indispensable) amino acids (Fig. 1.1). Although human body needs all 21 amino acids for proper functioning, growth and maintenance, only 9 have been classified as indispensable amino acids as these cannot be synthesized endogenously in human body. Some amino acids (arginine, cysteine, glutamine, glycine, proline and tyrosine) are conditionally-indispensable, the human body is unable to synthesize them endogenously under certain physiological conditions (e.g. infancy, pregnancy, lactation, injury, burn, infections or diseased conditions). Amino acids have also been classified as functional amino acids based on their specific functions in the body. Functional amino acids are defined as the amino acids that participate in regulation of key metabolic pathways involved in growth, development, lactation, reproduction and overall health of organisms (Wu 2010, 2013). Functional amino acids contribute to and/or play crucial roles in 1) intestine health, 2) foetal survival/development, 3) immunity, 4) neurological functions and synthesis of neurotransmitters, 5) secretion of insulin, 6) activation of taste receptors and 7) recovery from injury (Li et al. 2007; Brosnan and Brosnan 2013; Rezaei et al. 2013; Wu et al. 2017). For example, dietary glutamate, glutamine and aspartate serve as major metabolic fuels for enterocytes and small intestine (Rezaei et al. 2013). Glutamate, glutamine, arginine and glycine also can contribute to intestinal health through enhancing villus-height, width and surface area, mucosal protein synthesis, activity of antioxidant enzymes (e.g. catalase, superoxide dismutase and glutathione peroxidase) in small intestine and health of enterocytes (Jiao et al. 2015; Yi et al. 2018). In addition, glutamine is also involved in providing ATPs to macrophages and lymphocytes and serves as an exclusive source of energy for arteries in post-absorptive state (Li et al. 2007). Further, arginine and glutamine play important functions in placental metabolism and supplementation of these amino acids can help enhance foetal survival and growth (Wu et al. 2017). Selenocystein, a more recently discovered 21st proteinogenic amino acid, also performs important functions in human body. It is involved in the regulation of thyroid metabolism, removal of reactive oxygen species and protection of cells from oxidative damage, maintenance of cellular redox balance, regulation of signalling cascades, promotion of protein folding and maintenance of selenium homeostasis (Schmidt and Simonović 2012).

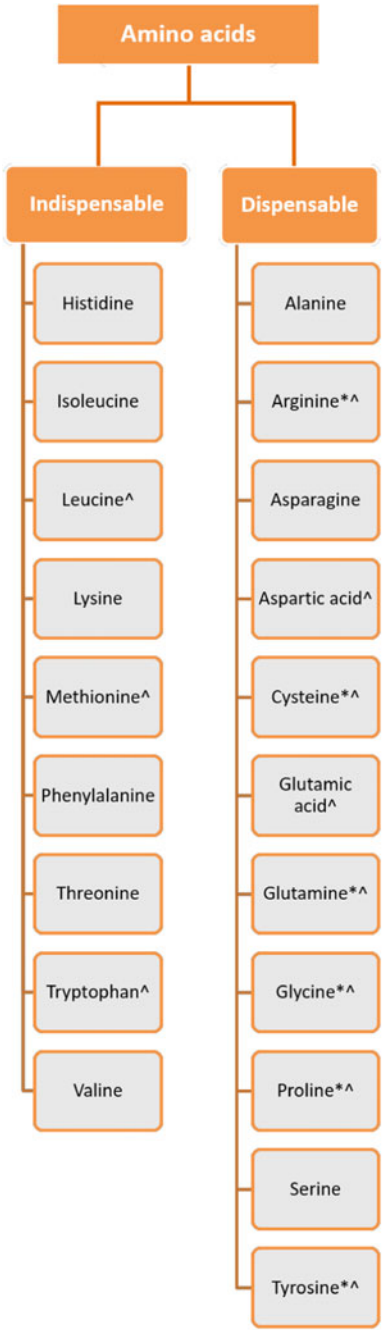

Fig. 1.1 Classification of amino acids. * Conditionally essential amino acids. ˆ Functional amino acids (Wu 2013)

Amino acids also have roles in healthy aging and longevity. Amongst different indispensable amino acids, branched chain amino acids (leucine, isoleucine and valine) have contributory roles in healthy aging and longevity. They do not undergo metabolism in liver, hence get circulated almost immediately after absorption and become available to the body (Holeček 2018; Dato et al. 2019). D'Antona et al. (2010) reported increased average lifespan and improved performance/endurance for male mice fed on branched chain amino acids diets. The effect was observed in association with an increased mitochondrial biogenesis, sirtuin-1 expression and reduced oxidative damage in cardiac and skeletal muscles (D'Antona et al. 2010). In another study on 24 elderly men (average age 74.3 years), co-ingestion of 2.5 g leucine with 20 g casein proteins led to 22% higher muscle protein synthesis rate compared with ingestion of casein alone (Wall et al. 2013). Improvements in nutritional status, cognitive performance, general health, muscle mass, strength and performance were also reported as a result of improved mitochondria functions in malnourished elderly people given branched chain amino acids (Buondonno et al. 2020). Fujita and Volpi (2006) attributed stimulatory effect of these amino acids on muscle protein synthesis in older individuals to the initiation of mRNA translation. Also, mammalian target of rapamycin (mTOR) Complex 1 (a key pathway in amino acid induced anabolic responses) was reported to be sensitive for leucine, while muscles in older adults required increasing concentrations of this amino acids for maintaining anabolic responses through the mTOR pathway (Dillon 2013). Furthermore, leucine, also contributed to health-span by improving glucose metabolism through promoting glucose uptake in muscles via phosphatidylinositol 3-kinase and protein kinase C pathways (Nishitani et al. 2002) and reducing body weight by increasing leptin secretion and decreasing food intake (Valerio et al. 2011). However, branched chain amino acids may also have negative health implications owing to their involvement in the promotion of oxidative stress and mitochondrial dysfunction in human peripheral blood mononuclear cells (Zhenyukh et al. 2017) as well as the ability to increase excitotoxicity in cortical neurons in brain (Contrusciere et al. 2010). Conditionally indispensable amino acids contributing to health-span include glutamine and arginine. Glutamine has important roles in the maintenance of normal skeletal muscle function and neuronal physiology owing to its ability of controlling heat-shock responses (Leite et al. 2016), while arginine can contribute to reduced risk of endothelial dysfunction-associated cardiovascular risk with aging (Heffernan et al. 2010; Dato et al. 2019).

1.3 Digestion and Absorption of Proteins

Proteins ingested through food are required to be digested before being absorbed and utilized in the body. Digestion involves breakdown of protein molecules to constituting amino acids. Proteins in liquid foods pass through the mouth almost unaltered (Loveday 2019). Semisolid and solid foods, however, undergo physicochemical changes during mastication, though most of protein digestion occurs in stomach and small intestine. In the stomach, proteins get mixed with gastric acid and pepsinogen (inactive form of pepsin also called as zymogen). The acid in the stomach transforms pepsinogen to active form (pepsin) and makes proteins susceptible to pepsinolysis by partially denaturating (unravelling) them. Pepsin acts on acid denatured proteins and catalyse hydrolysis of peptide bond next to phenylalanine, tryptophan and tyrosine residues. The partially digested proteins/polypeptides in *chyme* (stomach contents) then enters the duodenum, where they trigger the release of hormone cholecystokinin. The hormone reaches pancreas through blood stream and makes the organ to release alkaline secretions with proteolytic enzymes including trypsin, chymotrypsin and pancreatin in their inactive forms that get activated under specific conditions. The enzymes in pancreatic secretions selectively hydrolyse bonds in polypeptides, shorter peptides or oligopeptides to liberate amino acids and small peptides. These amino acids and small peptides are absorbed by the absorptive cells of the small intestine which then break down small peptides (dipeptides and tripeptides) to amino acids and transfer them into blood capillaries. Eventually, absorbed amino acids are metabolized by the liver for synthesising body proteins, to be used as an energy source, converting to sugars or fats and/or releasing into the blood stream (Byrd-Bredbenner et al. 2009).

Human digestive system is very effective in achieving breakdown of proteins to amino acids and small peptides, though some proteins and peptides may resist complete digestion in the small intestine. Such proteins and peptides eventually reach the large intestine where gut microflora ferment them to short chain fatty acids (SCFA) or amines through deamination or decarboxylation, respectively (Fan et al. 2015; Rios-Covian et al. 2020). Acetates, propionates and butyrates are major SCFA produced in the gut. These SCFA exert beneficial effects on host through maintaining acidic pH in the gut, fuelling epithelial cells for mucin production, increasing colonic blood flow and preventing the growth of abnormal colonic cells (Rios-Covian et al. 2016). The SCFA that are not utilized in colon may exert beneficial effects against diet-induced obesity and hyperlipidaemia by interfering with carbohydrate and lipid metabolism after reaching liver via hepatic vein (Rios-Covian et al. 2016). In addition to acetates, propionates and butyrates, branched-SCFA e.g. isobutyric, isovaleric, and 2-methylbutyric acids are also produced in gut as a result of fermentation of amino acids (e.g. valine, leucine and isoleucine) by *Bacteroides* and *Clostridium* spp. (Aguirre et al. 2016; Rios-Covian et al. 2020). Therefore, the presence of these SCFA in stools is considered as the indication of the fermentation of proteins in gut. However, the fermentation of indigested proteins also may have some negative effect on gut health as some products with potential harmful effects on colon epithelium (e.g. biogenic amines, sulphides, ammonia, cresols, phenol, etc.) are also produced during fermentation (Nie et al. 2018; Rios-Covian et al. 2020).

1.4 Functions of Dietary Proteins

Proteins are involved in nearly each physiological processes in the body. They function as transport proteins (e.g. haemoglobin and ferritin that are involved in transport of oxygen and Fe, respectively), structural proteins (e.g. elastin and collagen function to support the body), contractile proteins (e.g. actin and myosin required for movement), enzymes (required for catalysing several biochemical reactions), hormones (required for bodily functions), receptors (e.g. proteins involved in nerve cell transmission) or transcription factors (Fig. 1.2). Generalized functions of dietary proteins in human body are outlined below.

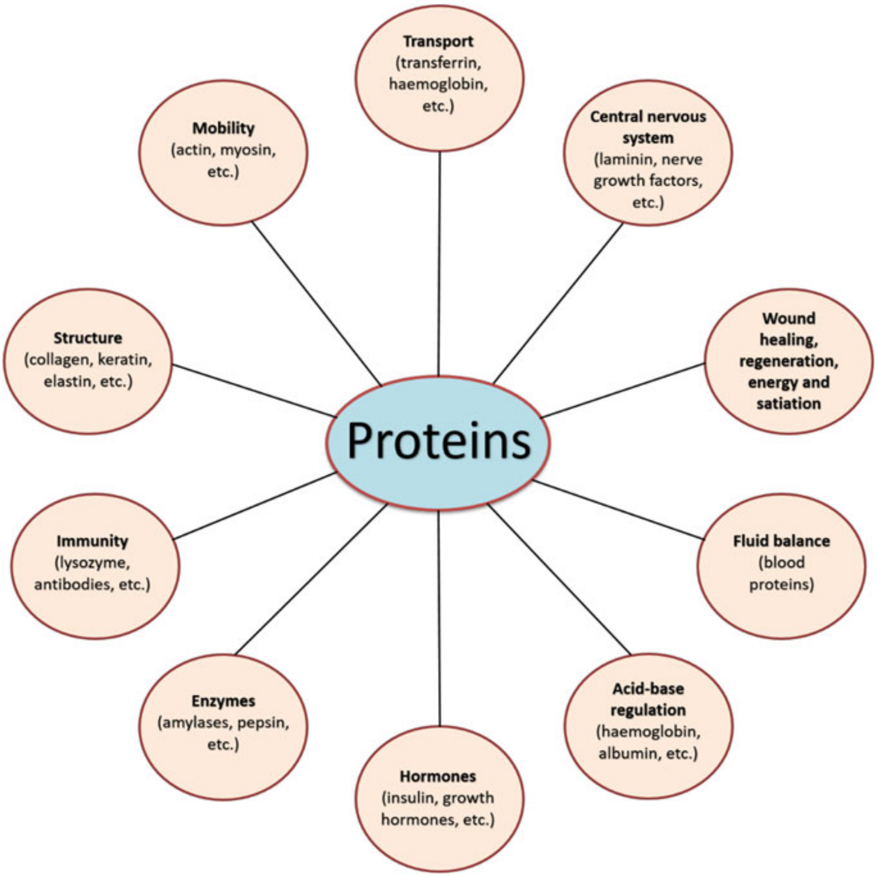

Fig. 1.2 Major functions of proteins in human body

1.4.1 Building Blocks

Dietary proteins are fundamentally the source of amino acids and nitrogen that serve as raw materials in the synthesis of body proteins required for formation/synthesis of vital organs, muscles, bones, biological fluids, hormones, enzymes, neurotransmitters, nucleic acids, etc. (Henley et al. 2010; Wu 2013; Wu et al. 2017). A large portion of proteins in animal body is involved in providing structural stability and mobility. These proteins include collagen, actin and myosin that are essential in muscle structure and functioning (e.g. contraction and relaxation). Collagen proteins also function to make a large portion of the organic structural matrix of bones. Dietary proteins provide amino acids for the synthesis of such structural and contractile proteins. As the human body is incapable of maintaining reserves of proteins, a constant supply of dietary protein is essential for maintaining tissue repairing/growth and other physiological functions. Insufficient intake of proteins especially during development and growth years can affect all tissues/organs of the body (Boye et al. 2012), while adverse protein deficiency in adults results in breakdown of lean tissues and muscle mass for providing amino acids and other nitrogenous precursors required for synthesis of compounds critical for survival e.g. insulin and haemoglobin (Hoffer 1994).

1.4.2 Biological Catalysts

Proteins are involved in biochemical reactions as biological catalysts or enzymes that accelerate the rate of reactions. All processes in nature require enzymes to occur at required rates, while metabolic pathways in cells are said to be determined by the type and amount of enzymes present (van Oort 2010). Enzymes are essential in signal transduction, cell regulation, mobility/movement, digestion, and so one (Hunter 1995; Berg et al. 2001). Their functioning in specific order is essential for creating metabolic pathways, whereas their absence will cause metabolism to neither progress nor be fast enough to serve the requirements of cells. For example, glucose can react directly with ATP to get phosphorylated during glycolysis. However, in the absence of enzymes, phosphorylation is insignificant, while the presence of enzyme hexokinase speeds up the phosphorylation of glucose to glucose-6-phosphate which gets finally converted to pyruvate in a series of reactions involving activities of a number of enzymes.

1.4.3 Immune Function

Proteins are key component in immune system. Amino acids play important roles in immune responses by regulating 1) the activation of T-lymphocytes, B-lymphocytes, natural killer cells and macrophages, 2) cellular redox state, gene expression and lymphocyte proliferation and 3) the production of antibodies, cytokines and other cytotoxic substances (Li et al. 2007). Glutamine serves as a major energy substrate for immunocytes (Wu 2013). During conditions of infection, amino acid requirement of immune cells increases rapidly to meet synthesis of immunological proteins (Le Floch et al. 2004). The deficiency of dietary proteins can make the body severely prone to infections and diseases owing to the unavailability/lesser availability of materials required by immune system, while dietary supplementation of specific amino acids (e.g. arginine, glutamine and cysteine) to individuals with malnutrition/infections could contribute to enhanced immunity and reduced morbidity/mortality (Li et al. 2007).

1.4.4 Transporting Nutrients

Proteins in body also function as carriers for transporting nutrients between different organs/tissues/cells (Byrd-Bredbenner et al. 2009). Haemoglobin and myoglobin (involved in transportation of oxygen from lungs to organs/tissues/cells) are the most common examples of transport proteins. Lipoproteins function to transport lipid molecules from the small intestine to various locations. Apart from these, important transfer proteins involved in nutrient metabolism in human body include ferritin (a protein that can store Fe and release the same in a controlled manner), transferrin (plasma protein responsible for transporting Fe in body), retinol-binding protein (a protein for carrying Vit-A) and ceruloplasmin (a carrier protein for copper).

1.4.5 Maintenance of Fluid Balance and Regulation of pH

Proteins are involved in maintenance of fluid balance in the body (Byrd-Bredbenner et al. 2009). Blood pressure in arteries makes blood to enter minute blood vessels (also called as capillary beds), from where blood fluids pass to extracellular spaces for providing nutrients to cells. As proteins in the blood (e.g. albumins) are too large to enter extracellular spaces, they stay there and contribute to the maintenance of fluid balance by attracting proper amount of fluid back from cellular spaces to the blood stream. Accumulation of excessive amount of fluids in tissues in the presence of lesser amounts of proteins in bloodstream caused by inadequate intake of dietary proteins results in swelling of tissues, a clinical condition called as *oedema*. Proteins in the membrane also contribute to the regulation of acid-base balance and maintenance of pH in human body by acting as buffers for the body (Byrd-Bredbenner et al. 2009).

1.4.6 Providing Glucose During Fasting or Starvation

Human body needs to maintain a certain minimum level of glucose in the blood stream to keep organs/tissues/cells functioning. During the period of fasting or starvation, body uses proteins for synthesizing glucose through gluconeogenesis. Body proteins provide most of the glucose (about 90%) needed during the first few days of fasting for functioning of body (Byrd-Bredbenner et al. 2009).

1.4.7 Satiation

Dietary proteins also contribute to the satiety and suppression of apatite. They provide greater satiety than carbohydrates and fats (Weigle et al. 2005). Protein induced satiety is attributed to the combined effects of elevated plasma amino acid and anorexigenic hormone levels, prolonged suppression of hunger hormone (ghrelin), increased diet-induced thermogenesis (increased dissipation of energy at rest) and ketogenic state (Drummen et al. 2018). Satiation effect of dietary proteins is discussed in detail in the Sect. 1.5.2 of the chapter.

1.4.8 Providing Energy

Similar as carbohydrates and lipids, proteins can provide energy to humans. The liver is able to break amino acids to the carbon skeleton, which can then be used for energy. *In vivo* oxidation of proteins to water and carbon dioxide releases 4.1 kcal/g, which is equal to starch (4.1 kcal/g) but lesser than that released from lipids (9.4 kcal/g) (Wu 2016). However, as these nutrients are not digested completely and because protein oxidation in the body is incomplete, the energy values of 4, 4 and 9 kcal/g, respectively are taken for dietary proteins, available carbohydrates (e.g. starches, sugars, etc.) and fats/oils (Wu 2016). Usually 10–20% of energy is derived from dietary proteins. However, amino acids are not preferred as a source of energy by body. They exhibit lower energetic efficiency than fatty acids and glucose (Wu 2013). Also, they burden kidneys and liver with excessive amount of processing and metabolism for being utilized as a source of energy (Byrd-Bredbenner et al. 2009).

1.5 Health Benefits and Healthy Aging

In addition of performing essential functions in metabolic and physiological processes, dietary proteins also contribute to disease prevention and healthy aging. High protein intake has been associated with reduced overall mortality and morbidity in elderly people owing to the positive effect of protein consumption towards reduced risk of age/lifestyle-related diseases including sarcopenia, cardiovascular diseases, osteoporosis, obesity and diabetes (Gaffney-Stomberg et al. 2009; Vikøren et al. 2013; Levine et al. 2014; Wu 2016). This section discusses health-benefitting effects of dietary proteins in elderly people.

1.5.1 Maintenance of Muscle Mass

Progressive muscle loss and associated reduction in lean body mass with aging is termed as sarcopenia. It is considered as an insidious process characterized by 3–8% reduction in lean muscle mass per decade after the age of 30 years (Paddon-Jones and Rasmussen 2009). Sarcopenia is one of the major causes of functional impairment/disability and mortality in elderly people (Houston et al. 2008). The condition is quite prevalent in elderly people affecting about 30% of population of over 60 years of age and more than half of those above 80 years (Baumgartner et al. 1998). Sarcopenia can develop because of a number of reasons including sedentary lifestyle, inadequate protein intake, impaired turnover rate of proteins, neurodegeneration, reduced anabolic hormone production, dysregulation of cytokines and inflammation (Gaffney-Stomberg et al. 2009; Paddon-jones and Rasmussen 2009; Fukagawa 2013). However, low protein intake is considered as a key factor in the age-related muscle loss as amino acids are a prerequisite for the synthesis of muscle proteins while many elderly people do not take dietary proteins in adequate amounts owing to several reasons including 1) high cost, 2) tooth decay and difficulties in chewing, 3) perceived intolerance to certain foods and 4) avoidance of animal foods to minimize cholesterol and lipids intake (Chernoff 2004; Houston et al. 2008). Moreover, reduction in anabolic signals for muscle protein synthesis with ageing further accelerates muscle loss and development of sarcopenia. Guillet et al. (2004) reported lower increase in muscle protein synthesis (0.023% per hour) with infusion of amino acids and insulin in older people than adult subjects (0.041% per hour).

Physical activity and intake of dietary proteins are main anabolic stimuli for synthesis of muscle proteins. Regular exercise with protein supplementation not only can reduce the progression of sarcopenia but also treat the same by increasing muscle-strength, size and mass (Campbell 2007; Strasser et al. 2018). However, as older adults require to take greater amount of proteins for achieving maximum stimulation of muscle protein synthesis than younger ones (Moore et al. 2015), they may be advised to take proteins in excess of the recommended dietary allowance (0.8–1.0 g proteins/kg body weight/day) for prevention of sarcopenia through maintenance of positive nitrogen balance and preservation of muscle mass (Campbell 2007; Gaffney-Stomberg et al. 2009; Kerstetter et al. 2015; Baum and Wolfe 2015; Lonnie et al. 2018). Genaro et al. (2015) while evaluating differences in protein intake in women with or without sarcopenia reported higher muscle mass for group consuming >1.2 g proteins/kg body weight/day. Rizzoli (2015) and Gaffney-Stomberg et al. (2009) also recommended protein intake of 1.0–1.2 g/kg body weight/day with repartition for preventing sarcopenia and maintaining nitrogen balance without having any harmful health effects. However, in addition to protein quantity, protein quality (amino acid composition and bioavailability) also is relevant in muscle protein synthesis and prevention of sarcopenia. Indispensable amino acids, particularly branched-chain amino acids, appear to be highly effective in stimulating muscle protein synthesis and enhancing muscle strength. The supplementation of branched-chain amino acids together with L-glutamine reversed muscle loss in total gastrectomized rats (Haba et al. 2019). Also, leucine supplementation improved condition of patients with muscle wasting diseases (Wandrag et al. 2015). In this regard, proteins from animal sources may be considered effective in stimulating muscle protein synthesis owing to high content of essential amino acids including leucine (Baum and Wolfe 2015; Shang et al. 2018). However, some recent studies highlight that proteins from plants (e.g. soybean) alone or in combination with animal proteins can be equally beneficial in minimizing muscle wasting and preventing sarcopenia. For example, Jarzaguet et al. (2018) reported increased anabolic response of skeletal muscles in aged rats with meals comprising of >25% soy/whey proteins. In another clinical study on bedridden patients, soybean proteins in diet were found to be superior to milk proteins (casein) in enhancing muscle strength, though casein supplementation resulted in greater enlargement of muscle volume (Hashimoto et al. 2015). However, more studies are required for evaluating the effectiveness of proteins from different plant sources in the prevention of sarcopenia.

1.5.2 Weight Management

Obesity is characterized by excessive deposition of fat in body. A person is said to be obese when exhibits body mass index (a measure of the healthy weight of an adult for her/his height, which is calculated by dividing body mass to squared height; BMI) value of $\geq$30 kg/m^2. Obesity prevails in all age groups and affects a significant portion of the population of the world. In 2016, about 13% of adults (11% men and 15% women of >18 years of age), while 6% of girls and 8% of boys of 5–19 years of age were obese (WHO 2020a). Moreover, its prevalence is increasing in many parts of the world including middle and low-income countries. In Africa, the number of overweight children has increased by approximately 24% since 2000, while almost 50% of children less than 5 years of age who were overweight or obese in 2019 lived in Asia (WHO 2020a).

Medically, obesity is classified as a complex condition that not only impairs physical ability but also can detrimentally affect longevity, health-span and quality of life by increasing the risk of many diseases including diabetes, cardiac diseases (including congestive heart failure, hypertension, atherosclerosis, etc.), non-alcoholic fatty liver disease, some types of cancers, psoriasis, inflammatory bowel disease, musculoskeletal disorders, chronic limb pains, osteoarthritis, Alzheimer's disease, dementia, impaired respiratory function, obstructive sleep apnoea and increased susceptibility to infections (Elia 2001; Coggon et al. 2001; Frasca and McElhaney

2019). In the elderly, obesity can lead to early onset of chronic diseases, respiratory tract infections, functional impairment and premature mortality (Lorenzo et al. 2006; Amarya et al. 2014; Frasca and McElhaney 2019). Causes of obesity include hereditary/genetics, hormonal imbalances, metabolic factors and life-style related conditions, though it is mostly because of life-style related factors that include chronic positive energy balance (regular intake of calories in excess of expenditure) and lack of physical activity (Paddon-Jones et al. 2008). The treatment of obesity requires a negative energy balance which may be achieved by indulging in physical activities and/or sticking to energy restricted diets. Further, increased satiety and suppressed apatite can also contribute to weight loss and obesity prevention. However, the weight loss strategies for overweight/obese elderly people are required to be considered carefully in order to achieve weight loss with minimum loss of lean muscles as a specific condition, termed as sarcopenic obesity (a medical condition characterized by an excessive fat deposition in combination with a detrimental loss of lean body mass), is more common in elderly people (Mathus-Vliegen 2012).

Protein intake has been associated with weight management/obesity prevention. Protein intake at levels of requirement through energy restricted diets contributes to weight loss, while increased intakes are effective in maintaining fat-free mass (Drummen et al. 2018). Soenen et al. (2013) reported reduction in body and fat-mass in overweight and obese men and women of 18–80 years of age with BMI >25 kg/m^2 given dietary proteins at and above the levels of requirements (0.8 g/kg body weight/day and 1.2 g/kg body weight/day, respectively). In comparison, the dietary protein intake below requirement levels could result in lesser weight loss and higher risk of weight regain (Acheson 2013). Weigle et al. (2005) reported positive outcomes of increased protein and reduced fat intake (from 15 to 30% and from 35 to 20% of energy, respectively) at constant carbohydrate intake on weight loss. Protein induced weight loss can be attributed to satiation and increased energy expenditure. Protein-rich diets were more satiating than that high in fats and carbohydrates. Marmonier et al. (2000) while investigating the effect of protein, fat or carbohydrates-rich snack (each providing 250 kcal) after *ad libitum* lunch on apatite (requests for next meal) observed the longest delay in requests for next meal (60 min) for protein-rich snack. High-carbohydrate and high-fat snacks, in comparison, delayed dinner requests by 35 and 25 min, respectively (Marmonier et al. 2000). The contribution of dietary proteins to satiety and weight loss has been attributed to their effect on hormones involved in apatite regulation, e.g. ghrelin, leptin, insulin, amylin, adiponectin, glucagon-like peptides, cholecystokinin, etc. (Lonnie et al. 2018), as well as to specific amino acids, e.g. tryptophan, tyrosine and/or histidine, which are used for synthesis of neurotransmitters (e.g. serotonin, dopamine, norepinephrine and histamine) involved in regulation of food intake and apatite (Keller 2011). Moreover, the satiation effect of a few food proteins (e.g. casein proteins of milk) also can be associated with acid-induced coagulation in stomach, which may inhibit gastric digestion by slowing diffusion of pepsin into coagulated proteins (Thévenot et al. 2017). This affects release of partially digested proteins/peptides into small intestine and contributes to appetite control by creating a prolonged feeling of fullness (Loveday 2019).

In addition, protein-induced weight loss also is attributed to high energy expenditure as protein-rich foods require greater amount of energy for digestion, absorption and metabolism (thermic effect of food; TEF) in comparison to carbohydrates and fats. The TEF value of protein-rich foods range from 20 to 30%, whereas carbohydrates and lipids-rich foods show TEF values of 5–10% and 0–3%, respectively (Byrd-Bredbenner et al. 2009). Increased energy expenditure for protein-rich foods is attributed to metabolic inefficiency of protein oxidation in comparison to glucose (more energy is required for producing ATP from amino acids than from glucose), gluconeogenesis and urea synthesis (Westerterp-Plantenga et al. 2009; Keller 2011). Furthermore, the energy expenditure of high protein intake has also been attributed to its effect on thyroid hormones, androgens, catecholamines and growth hormone (Mikkelsen et al. 2000).

Plant proteins also demonstrate effects similar to that of animal proteins in the context of the ability to increase satiety and delay hunger. Neacsu et al. (2014) while investigating appetite responses to high-protein weight-loss diets in obese men of 34–71 years of age reported statistically similar hunger, desire-to-eat and weight loss for meat and soybean-based diets. Veldhorst et al. (2009) compared the effect of casein, soybean and whey-protein breakfasts on appetite regulating hormones (glucagon-like peptide-1 and insulin). Although the strongest effect was shown by whey, mediating effects on these hormones were also observed for soybean proteins and casein (Veldhorst et al. 2009). In another report, Scully et al. (2017) also reported no significant differences in appetite and food intake for participants of 23–63 years of age with BMI of 19.3–38.9 kg/m^2 given buckwheat and fava bean based diets. In addition, legumes/pulses also have an edge over animal products for weight loss strategies in elderly people because of high protein to calorie ratio which is attributable to low lipids content (for most legumes/pulses with exception of dried seeds/kernels of soybean, lupine and groundnut, though lipids in these legumes are rich in health benefitting unsaturated fatty acids) and high content of unavailable complex carbohydrates including oligosaccharides, resistant starch and soluble and insoluble dietary fibres.

1.5.3 Cardiac Health

Cardiac diseases are responsible for approximately 18 million deaths annually. High levels of serum cholesterol, triacylglycerols, low-density lipoprotein cholesterol (LDL-C), high LDL-C to high-density lipoprotein cholesterol (HDL-C) ratio, smoking, stress/depression, obesity and alcohol abuse are major risk factors in cardiac diseases. Although the development of cardiac diseases is associated with many aspects of lifestyle, besides heredity, the most significant risk factors are related to food habits (Cam and de Mejia 2012). Dietary proteins are considered an important source of bioactive peptides that can contribute to lower the risk of cardiac diseases owing to anti-hypercholesterolemic, anti-hypertensive, anti-thrombotic and anti-inflammatory activities (Nasri 2017; Tapal et al. 2019). However, proteins from plant sources, in general, have an edge over animal proteins in the context of their effect on cardiac health. Virtanen et al. (2019) reported high mortality risk associated with ischaemic heart diseases amongst Finnish males of 42–60 years of age consuming greater amounts of animal proteins than plant proteins. Li et al. (2017) based on a meta-analysis highlighted reduction in blood LDL-C, non-HDL-C and apolipoprotein-B levels after substitution of animal proteins with plant proteins. Similarly, Bernstein et al. (2010) and Bernstein et al. (2012) while investigating the effect of protein sources on cardiac diseases also highlighted reduction in the risk of strokes and other coronary heart diseases with replacement of red meat as protein source with poultry, nuts, fish and low-fat milk products.

Amongst different plant proteins, legume proteins (particularly soybean) have been investigated extensively for their effects on cardiac health. Intake of soybean proteins was found to upregulate LDL receptors in the liver (Baum et al. 1998) and reduce levels of total cholesterol, LDL-C and triacylglycerol (Torres et al. 2006; Borodin et al. 2009). Sacks et al. (2006) based on 22 randomized trials recommended a minimum daily intake of 50 g of soybean proteins for achieving reduction in serum lipids levels. The lipid lowering effects of soybean proteins have been attributed to the presence of isoflavones (Anthony et al. 1996) and release of small peptides (<15 amino acids) after digestion/hydrolysis (Sirtori et al. 2007). In addition, the beneficial effect of soybean proteins on cardiac health may also be due to the lower ratio of leucine to arginine than animal proteins as high concentration of arginine in plasma was associated with hypocholesterolemic effect, whereas leucine acted as a cholesterol precursor having a strong insulinotropic effect (Chalvon-Demersay et al. 2017). Proteins from other legumes, e.g., lupine, chickpeas, cowpea and peas, also exert beneficial effects on cardiac health. In studies by Bettzieche et al. (2009) and Fontanari et al. (2012), lupine proteins were shown to increase plasma HDL-C level while reducing levels of triacylglycerol and LDL-C in hypercholesterolemic rats and hamsters. Similarly, the study by Frota et al. (2015) also reported 2.7% increase in HDL-C while 12% reduction in total cholesterol, 18.9% in LDL-C and 16% non-HDL-C in hypercholesterolemic subjects (30–70 years of age) with consumption of 25 g cowpea protein isolate per day. In relatively recent works, chickpea peptides lowered lipids level and reversed liver damage in hyperlipidaemic mice (Xue et al. 2018), while hydrolysed pea proteins inhibited lipid accumulation in adipose tissue cells (3T3-L1 cells) in a concentration dependent manner (Flores-Medellín et al. 2021).

Milk proteins (e.g. whey proteins) also exert beneficial effects on cardiac health owing to their ability to improve vascular functions and reduce LDL-C and total cholesterol. Ballard et al. (2013) reported improvements in vascular function in overweight individuals after ingestion of whey peptides, while Pal et al. (2010) showed reduction in triacylglycerol, total cholesterol and LDL-C levels by 22, 11 and 7%, respectively in overweight to obese subjects of 18–65 years of age and 25–40 kg/m^2 BMI with ingestion of whey protein isolate (27 g twice a day) for 12 weeks. The beneficial effects of milk proteins have been attributed to increased lipolysis, reduced lipid accretion, reduced absorption of dietary cholesterol/fatty acids and increased insulin resistance (Chen and Reimer 2009; Lillefosse et al. 2014; Fekete et al. 2016).

Proteins from fish also have hypocholesterolemic and hypolipidimic effects in animal models (Shukla et al. 2006; Kawabata et al. 2015; Drotningsvik et al. 2016). The effect of fish protein intake on blood lipid profile was investigated by Shukla et al. (2006). Rats fed on fish proteins showed lower levels of triacylglycerol and cholesterol while higher levels of liver LDL-receptors and sterol regulatory-binding proteins than casein fed rats. However, unlike legume proteins, fish proteins also decreased levels of HDL-C (Shukla et al. 2006). Reduced serum and liver cholesterol levels have also been reported for fish protein-fed rats by Hosomi et al. (2011) and Hosomi et al. (2013). The hypolipidimic effects of fish proteins were attributed to the inhibition of the absorption of bile acid in the small intestine and enhanced excretion of cholesterol in faeces as a result of increased expression of cholesterol 7α-hydroxylase and carnitine palmitoyltransferase-2 enzymes involved in lipid metabolism (Hosomi et al. 2011; Hosomi et al. 2013).

1.5.4 Bone Health

Proteins make up about half of the bone volume and about one-third of the bone weight (Heaney 2007) as they (mainly collagen and various other non-collagen proteins) are primarily responsible for the organic structural matrix of bones. The loss of bone mass/density with aging results in enhanced bone fragility and increased risks of fractures which not only causes people to become bedridden but also can cause life threatening complications in elderly individuals. Besides Ca, P and Vit-D, dietary proteins and physical activities are considered critical in achieving optimal bone mass and preventing bone loss in elderly people (Rizzoli 2014; Gaffney-Stomberg et al. 2014; Morris-Naumann and Wark 2015). However, high dietary protein intake has been shown to have positive as well as negative effects on bone health. For example, high protein intake was associated with demineralisation of bones and development of osteoporosis because of stimulation of urinary Ca excretion and acidification of blood (Heaney and Layman 2008; Wu 2016). Zwart et al. (2005) reported negative Ca balance and increased urinary excretion of this mineral in male subjects on diet high in essential amino acids. They attributed this response to the increased intake of sulphur containing amino acids that increase Ca excretion by increasing endogenous sulphuric acid production. On the other hand, many studies regarded dietary proteins as an essential nutrient in bone health owing to their involvement in 1) providing organic structural matrix, 2) optimising the level of growth factors (e.g. insulin-like growth factors which can increase bone mass by increasing osteoblasts activity), 3) increasing absorption of calcium in intestine, 4) stimulation of bone-mineralisation and 5) inhibition of bone resorption and inflammatory cytokines that could activate bone degradation (Fernandes et al. 2003; Heaney and Layman 2008; Millward et al. 2008; Gaffney-Stomberg et al. 2009; Hardy and Cooper 2009; Zhang et al. 2011; Gaffney-Stomberg et al. 2011; Kerstetter et al. 2015; Wolfe 2015; Shang et al. 2018). In addition, high protein diets have also been associated with higher Ca intake, which can, therefore, compensate for any moderate increase in urinary excretion of the mineral (Wu 2016). Shams-White et al. (2017) based on a meta-analysis evaluation concluded no adverse effect of high protein intake on bone health and highlighted that a higher protein intake may reduce bone mineral density loss in older adults in comparison to a lower protein intake. Similarly, Groenendijk et al. (2019) while performing a systematic review and meta-analysis on the effect of high/low protein intake on bone health in elderly people also highlighted a positive association of higher protein intakes with bone (femoral neck and hip) mineral density and reduced hip bone fracture risks. However, the effect of dietary protein intake on bone health relates with the intake of other nutrients. Dawson-Hughes et al. (2002) showed crucial roles of Ca and Vit-D on the impact of dietary protein on bone health as greater protein intake was associated with increased bone mineral density in older adults during a 3-year supplementation period of these nutrients, though such improvement required sufficient dietary Ca and Vit-D intake as relationships between protein intake and bone density were not observed for placebo group (Dawson-Hughes and Harris 2002).

1.5.5 Type-2 Diabetes

Diabetes is a chronic disease of metabolism characterized by high blood glucose levels occurring due to the lack of insulin production or ineffective use of insulin by cells (Roglic 2016). The disease affects about 8.5% population of the world and is considered as a major cause of kidney diseases, blindness, heart diseases and limb amputation (WHO 2020b). Obesity is one of the most important risk factors in type-2 diabetes. Moreover, reduced muscle mass as a result of sarcopenia/sarcopenic obesity may also result in reduced insulin sensitivity and decreased glucose uptake which in turn can increase the risk for hyperglycemia and insulin-resistance syndrome in elderly people with diabetes (Solerte et al. 2008). Healthy lifestyle (regular physical activity and consumption of healthy diets for maintenance of normal body weight) can, therefore, be useful in delaying or managing this chronic disease. However, the association of dietary protein intake with type-2 diabetes varies with proteins sources. Pal et al. (2010) reported favourable effects of whey protein intake towards reduced risk of diabetes in obese men and women of BMI 25–40 kg/m^2 and 18–65 years in age, while Sluijs et al. (2010) based on a prospective investigation highlighted increased risk of type-2 diabetes with increased intake of total and animal proteins. Based on a systematic review and meta-analysis study, Tian et al. (2017) also reported varying effect of protein source on the risk of diabetes. Red and processed meats increased risk of diabetes, soybean and milk had protective effect, while fish and egg intake had no association with decreased risk of diabetes (Tian et al. 2017). In comparison, Vikøren et al. (2013) in a randomised study on fish protein (3–6 g/day for 8 weeks) effect on glucose metabolism in adults of 20–70 years of age with BMI $\geq$27 kg/m^2 reported favourable effects of the protein supplementation on glucose metabolism (lower fasting and postprandial glucose levels in comparison to placebo).

1.6 Protein Quality and Its Evaluation

Protein quality is an index of how well proteins meet requirements of amino acids and physiological needs of organisms (Shivakumar et al. 2018). Protein quality depends on amino acid composition and digestibility. The presence of all nine indispensable amino acids in adequate amounts reflects completeness of proteins, while high digestibility indicates the bioavailability of those amino acids. Therefore, highly digestible proteins that provide indispensable amino acids in amounts equal to or greater than that required for growth and maintenance are said to be of high quality. As elderly people generally consume foods (including proteins) in lesser amounts than younger adults, protein quality becomes critical in meeting enhanced requirements of indispensable and functional amino acids. Positive associations between the consumption of foods with high protein quality and healthy aging have been reported (Mathus-Vliegen 2012; Paddon-Jones et al. 2015; Hidayat et al. 2018).

1.6.1 Evaluation of Protein Quality

Different methods, ranging from chemical and biochemical (enzymatic) to microbiological and biological in nature, have been developed and employed for evaluation of protein quality (Damodaran 2017). Chemical methods, e.g. *amino acid score* (AAS) also referred to as *chemical score* measure protein quality based on the amount of most limiting indispensable amino acid in test protein and the content of the same in a reference protein (usually egg white). It is calculated as:

$$\text{Amino acid score or chemical score} = \frac{\text{mg of limiting amino acid in 1 g of test protein}}{\text{mg of the same amino acid per g in reference protein}}$$

Enzymatic methods use proteolytic enzymes (pepsin, trypsin, chymotrypsin, peptidases and/or proteases) under specific conditions of the test to determine in vitro digestibility of proteins (Bodwell et al. 1980; Calsamiglia and Stern 1995). Microbiological methods, in comparison, measure quality of proteins by determining their ability to support growth of microorganisms, including bacteria (e.g. *Streptococcus zymogenes*, *S. faecalis*, *Leuconostoc mesenteroides* and *Clostridium perfringens*) and protozoa (e.g. *Tetrahymena pyriformis*) exhibiting amino acid requirements similar to humans (Ford 1981).

Chemical, enzymatic and microbiological methods have some advantages. Chemical methods are simple and less time consuming and involve comparison of amino acid composition of test and reference proteins. Enzymatic methods are specifically useful in comparing different proteins for digestibility, while microbiological assays can provide useful information on protein quality depending on the amino acid requirements of organisms. However, these methods are subjected to several drawbacks, e.g., these assays do not take into account the effect of toxins, antinutritional constituents, food additives and/or ingredients (e.g. common salt, nitrates, spices, etc.) affecting protein quality. Moreover, enzymatic and chemical assays provide information only about in vitro digestibility/bioavailability and amino acid composition, respectively, while microbiological assays are useful for foods with known composition (Satterlee et al. 1979; Pellett and Young 1980).

Biological methods, in comparison, measure protein quality based on nitrogen retention and the ability of proteins to support growth (weight gain) in test animals (Damodaran 2017). The protocols involve feeding test animals (usually rats) with a test diet containing proteins in limited amounts (10% on dry basis) for a specific period of time (9 days). Protein-free diet is given to the control group. The test diet is formulated to provide adequate amount of energy so that proteins can be utilized in the animal body to the maximum possible extent. Animal weight is noted daily and faeces and urine are collected for nitrogen/protein content determination (FAO/WHO 1991). The data obtained is used in different ways to express protein quality as *protein efficiency ratio*, *net protein ratio*, *true digestibility*, *biological value* and *net protein utilisation* (Damodaran 2017).

Protein efficiency ratio is the simplest and most commonly used expression which is defined as weight gained per gram of test protein consumed. It is expressed as:

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain in test animal on test diet}}{\text{Amount of protein ingested}}$$

The *protein efficiency ratio* is directly related to protein quality as the gain in weight and growth achieved are dependent on the incorporation of dietary proteins in body tissue. However, this expression is criticized for not taking into account the dietary protein utilized for maintenance as only gain in weight is taken in the calculation. *Net protein ratio*, in comparison, takes into account the weight lost in protein-free group, hence provide information on the ability of protein to support both maintenance and growth. This expression is calculated as:

$$\text{Net protein ratio} = \frac{(\text{Weight gain}) - (\text{Weight loss for animals on protein free diet})}{\text{Amount of protein ingested}}$$

True digestibility and *biological value* determine protein quality by measuring nitrogen uptake and nitrogen lost by test animals. These expressions involve faecal nitrogen content determination to take in to account digestibility/nitrogen retention as well as the metabolic/endogenous nitrogen. Therefore, these expressions are the measure of the ability of dietary proteins to convert into body proteins. However, in comparison to *true digestibility*, *biological value* also involves analysis of urine for nitrogen content. *True digestibility* and *biological value* are calculated as:

$$\text{True digestibility } (\%) = \frac{\text{Protein ingested} - (\text{FN} - \text{FKN})}{\text{Protein ingested}} \times 100.$$

$$\text{Biological value} = \frac{\text{Protein ingested} - (\text{FN} - \text{FKN}) - (\text{UN} - \text{UKN})}{\text{Protein ingested} - (\text{FN} - \text{FKN})} \times 100.$$

where, FN and UN are nitrogen in faeces and urine of test animal, respectively, while FKN and UKN represent metabolic/endogenous nitrogen lost through faeces and urine, respectively of test animals on protein-free diet.

Another useful expression of protein quality is *net protein utilisation*. This expression provides the information on percentage of diary proteins retained in animal body. It is a product of *true digestibility* and *biological value* and is calculated as:

$$\text{Net protein utilisation} = \frac{\text{Protein ingested} - (\text{FN} - \text{FKN}) - (\text{UN} - \text{UKN})}{\text{Protein ingested}} \times 100$$

Protein digestibility corrected amino acid score (PDCAAS) and *digestible indispensable amino acid score* (DIAAS) are relatively recent developments in protein quality evaluation. PDCAAS is obtained as the product of the *true digestibility* and AAS as:

$$\text{PDCAAS} = \text{Amino acid score} \times \text{True digestibility}$$

The PDCAAS is one of the recommended methods of protein quality evaluation in human nutrition. This method has been widely adapted for protein quality evaluation (Schaafsma 2000), though it has also been criticised for some shortcomings. For example, 1) the expression does not provide information of individual indispensable amino acids bioavailability, 2) there may be overestimation of protein quality, specifically, of products containing known antinutritional constituents and 3) the method is considered inappropriate for regulatory uses as there is overestimation of quality for poorly digestible proteins supplemented with limiting amino acids (FAO 2013; Rutherfurd et al. 2015; Mathai et al. 2017). The DIAAS is an alternative to PDCAAS. In calculation of this score, the digestibility values of each indispensable amino acid is taken into calculation. The DIAAS is calculated as:

$$\text{DIAAS} = \frac{\text{mg of dietary digestible indispensable amino acid in 1 g dietary protein}}{\text{mg of the same dietary indispensable amino acid in 1 g of the reference protein}} \times 100$$

FAO now recommends DIAAS method for protein quality evaluation instead of PDCAAS (FAO 2013). Also, it is now considered necessary to determine the digestibility of individual indispensable amino acids at the end of the small intestine (*ileum*) in order to avoid any change in amino acid composition made by microorganisms in the large intestine (FAO 2013; Mathai et al. 2017) as the large microbial load and long transit time in the large intestine can either promote microbial synthesis of amino acids or their utilization by the microorganisms (Sauer et al. 1975; Trottier and Walker 2015). Moreover, growing pig is now recognised as more appropriate animal model for protein quality analysis because of its resemblance with humans for rate of protein synthesis, 2) physiology of stomach (glandular-type stomach lined with cardiac, gastric and pyloric mucosa) and small intestine, 3) topography of portal vein, mesenteric vessels and duodenum and 4) functionality of liver and pancreas (Kararli 1995; Swindle and Smith 1998; Deglaire et al. 2009; FAO 2013).

1.7 Animal vs Plant Proteins

Proteins from different sources differ for protein quality. Animal products (milk, egg, meat and fish) contribute about 35% of total proteins globally (Wu et al. 2014). They are generally considered as a source of high quality proteins because of the presence of all nine indispensable amino acids in amounts adequate or more than adequate for supporting growth (with the exception of gelatin/partially hydrolysed collagen that lacks tryptophan and contains threonine, isoleucine and methionine in low amounts) and high digestibility. Proteins of animal origin are highly digestible (94–97%) and show higher PDCAAS (1.0 against 0.91, 0.67, 0.57, and 0.45 for soybeans, peas, oats and wheat, respectively) than plant foods (FAO/WHO/UNU 1985; van Vliet et al. 2015; Damodaran 2017). However, as proteins do not occur in foods in isolation, the entire food matrix should to be taken into account in order to evaluate health benefits of a particular diet (Millward et al. 2008). While milk and fish are sources of high quality proteins, healthy lipids (e.g. omega-3 oils in fish or conjugated linoleic acids in milk), bioavailable haem-Fe and Vit-B12, the consumption of red and processed meats has been associated with increased risk of chronic diseases (e.g. coronary artery diseases, dyslipidaemia and some cancers) and all-cause mortality (Song et al. 2004; Vang et al. 2008; Bernstein et al. 2010; Chan et al. 2011; Allen et al. 2013; Mirzaei et al. 2014; Song et al. 2016; Sacks et al. 2017). The diets high in animal proteins also can increase the risk of type-2 diabetes (Sluijs et al. 2010; Tian et al. 2017). A high consumption of animal proteins brought about 75% increase in overall mortality and about four-fold increase in the risk of developing cancer in the individuals of 50–65 years in age (Levine et al. 2014).

Plant proteins (particularly those from staple foods viz. cereals and legumes/pulses), in comparison, are deficient in one or more essential amino acids. Histidine, isoleucine, leucine, phenylalanine, tryptophan and valine are present in adequate amounts in plant foods, though lysine, threonine, tryptophan and/or methionine occur as limiting amino acids (indispensable amino acids present in amounts less than that recommended for ensuring optimal growth and maintenance) in plant proteins. For example, lysine is the most limiting amino acid in all cereals followed by tryptophan in maize and threonine in most other cereals (Serna-Saldivar 2010). Legumes/pulses, in comparison, are rich in lysine but lack methionine and cysteine (Shevkani et al. 2019a; Ge et al. 2021). Therefore, individuals on diets containing either cereal or legume proteins face difficulties in maintaining health/growth, though a mixed diet containing proteins from both sources in adequate amounts can provide all amino acids for supporting maintenance and growth. In addition, plant-based foods also contain dietary fibre and phytochemicals, e.g. polyphenols, enzyme inhibitors, phytates/phytic acid, saponins, etc. (Shevkani and Singh 2015; Singh et al. 2017; Shevkani et al. 2019b), which, nowadays, are regarded as bioactive compounds owing to several beneficial effects on health, e.g., regulation of blood glucose level, improvement in lipid profile and reduced risk of some cancers and coronary artery diseases (Schlemmer et al. 2009; Singh et al. 2017). Moreover, plant protein sources (e.g. pulses/legumes, nuts, pseudocereals, etc.) also can contribute to reduced risks of lifestyle associated chronic diseases (e.g. diabetes, dyslipidaemia, hypertension and some cancers) owing to the presence of active constituents e.g. lectins/hemagglutinins, enzyme inhibitors, peptides and amino acids with antioxidative effects (Duranti 2006; Mendonca et al. 2009; Carbonaro et al. 2015; De Souza et al. 2017; Shevkani et al. 2019a; Singh et al. 2019; Tovar-Pérez et al. 2019). Song et al. (2016) highlighted association of high plant protein intake and replacement of animal protein sources with plant sources with reduced all-cause and cardiovascular-mortality. Ginter (2008) also reported lower ischemic heart disease mortality in vegetarians. This association was attributed to high antioxidant status, lower prevalence of obesity and low cholesterol and blood pressure levels. World Cancer Research Fund (2018) also recommends to increase consumption of a plant-based diets (high in whole grains, vegetables, fruits and legumes/pulses) while limiting the consumption of red meats (beef, veal, pork, lamb, mutton, horse, and goat) to 3 servings (each of 350–500 g) per week and that of processed meats (i.e. meats processed by salting, curing, fermentation, smoking, etc.) to very little amounts. Furthermore, a few relatively recent studies on the effect of plant-based diet on physical performance show little or no differences in endurance capacity and performance (Lynch et al. 2016; Craddock et al. 2016; Nebl et al. 2019). However, in spite of the nutritional and nutraceutical advantages of plant proteins, a complete shift to vegan/vegetarian diets may not be recommended for elderly people considering the risk of Vit-B12 deficiency, elevating homocysteine levels (Obersby et al. 2013; Lonnie et al. 2018) and the association of protein quality with reduced morbidity or mortality. A mixed diet, e.g. lacto-vegetarian diet, may be regarded adequate taking in account high indispensable amino acids to calorie ratio of dairy foods (particularly low and reduced-fat milk and milk products) and considering dairy products as an excellent source of vitamins and minerals (e.g. Ca, P and Vit-D that are essential for optimal bone health), bioactive proteins/peptides (e.g. lactoferrin), probiotics (specifically fermented milk products) and high quality proteins. Song et al. (2016) while comparing major protein sources associated dairy product intake with lower mortality than the consumption of processed meats and eggs. New dietary guidelines (e.g. The Eatwell Guide) also recommend to increase intake of plant foods (fruits, vegetables, pulses and whole grain cereals) and include low fat dairy products/alternatives (e.g. low fat milk, reduced-fat cheese, plain low-fat yoghurt, soy milk, etc.) in daily diet while reducing the intake of red meats, processed meats and foods high in fat, sugar and salt-content (Public Health England 2016).

1.8 Conclusion

Proteins are essential for survival and perform critical functions in the growth and maintenance of human body. Increased intake of dietary proteins can contribute to healthy aging and longevity by preventing/delaying chronic age-related diseases, enhancing ability of recovering from the diseases and reducing morbidity/mortality. Consumption of plant proteins should be encouraged because of the associated health-benefitting effects, though a mixed diet providing both animal and plant proteins in adequate amounts may be recommended for elderly people for ensuring balanced intake of amino acids and other essential nutrients.

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Compliance with Ethical Standards

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