DRAFT SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for protein[1]

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA)[2, 3]

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ABSTRACT

This opinion of the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) deals with the setting of Dietary Reference Values (DRVs) for protein. The Panel concludes that a Population Reference Intake (PRI) for protein can be derived for adults, infants and children, and pregnant and lactating women based on nitrogen balance studies. The Panel also considered several health outcomes that may be associated with protein intake, but the available data were considered insufficient to establish DRVs. For adults, the Panel accepted the value of 0.66 g protein/kg body weight per day based on a meta-analysis of nitrogen balance data as the average requirement (AR). In healthy adults, the protein requirement per kg body weight is considered to be the same for both sexes and for all body weights. Considering the 97.5th percentile of the population distribution of the requirement and assuming an efficiency of utilisation of dietary protein for maintenance of body protein of 47 %, the PRI for adults of all ages was estimated to be 0.83 g protein/kg body weight per day. This PRI is applicable both to high quality protein and to protein in mixed diets. For infants from six months, children and adolescents a factorial approach as proposed by WHO/FAO/UNU (2007) was accepted. For this, protein requirements for growth were estimated from average daily rates of protein deposition, assuming an efficiency of utilisation of dietary protein for growth of 58 %. To these age-dependent protein requirements for growth the protein requirement for maintenance of 0.66 g protein/kg body weight per day was added. For pregnant women, a protein intake of 1, 9 and 28 g/d in the first, second and third trimesters, respectively, is proposed in addition to the PRI for non-pregnant women. For lactating women, a protein intake of 19 g/d during the first six months of lactation, and of 13 g/d after six months, is proposed in addition to the PRI for non-lactating women. The available data are not sufficient to establish a Tolerable Upper Intake Level (UL) for protein. Intakes up to twice the PRI are regularly consumed from mixed diets by some physically active and healthy adults in Europe and are considered safe.

KEY WORDS

Protein, amino acids, nitrogen balance, maintenance, growth, factorial method, efficiency of utilisation, digestibility, muscle mass, body weight, obesity, insulin sensitivity, bone mineral density, kidney function, urea cycle.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Population Reference Intakes for the European population on energy and macronutrients, including protein.

Dietary proteins are the source of the nitrogen and indispensable amino acids which the body requires for tissue growth and maintenance. The main pathway of amino acid metabolism is protein synthesis. In this opinion, "protein" is total N x 6.25, and protein requirements are based on nitrogen content. Protein digestion takes place in the stomach and in the small intestine. In healthy humans, the absorption and transport of amino acids is usually not limited by the availability of digestive enzymes or transport mechanisms, but some protein escapes digestion in the small intestine and is degraded in the colon through bacterial proteolysis and amino acid catabolism. By the time digesta are excreted as faeces, they consist largely of microbial protein. Therefore, when assessing protein digestibility, it is important to distinguish between faecal and ileal digestibility, as well as apparent, and true, nitrogen and amino acid digestibility.

The concept of protein requirement includes both total nitrogen and indispensable amino acid requirements. The quantity and utilisation of indispensable amino acids is considered to be an indicator of dietary protein quality, which is usually assessed using the Protein Digestibility-Corrected Amino Acid Score (PD-CAAS). It is important to determine to what extent the nitrogen from dietary protein is retained in the body. Different values for the efficiency of protein utilisation have been observed for maintenance of body protein and for tissue deposition/growth; at maintenance, the efficiency of nitrogen utilisation for retention is about 47 % in healthy adults who are in nitrogen balance and on mixed diets.

The main dietary sources of proteins of animal origin are meat, fish, eggs, milk and milk products. Cereal grains, leguminous vegetables, and nuts are the main dietary sources of plant proteins. Most of the animal sources are considered high quality protein since they are high in indispensable amino acids, whereas the indispensable amino acid content of plant proteins is usually lower.

Data from dietary surveys show that the average protein intake in European countries varies between 72 to 108 g/d in adult men and 56 to 82 g/d in adult women, or about 13 to 20 % of total energy intake (E %) for both sexes. Few data are available for the mean protein intake on a body weight basis, which varies from 0.8 to 1.2 g/kg bw per day for adults.

In order to derive Dietary Reference Values (DRVs) for protein the Panel decided to use the nitrogen balance approach to determine protein requirements. Nitrogen balance is the difference between nitrogen intake and the amount lost in urine, faeces, skin and other routes. In healthy adults who are in energy balance the protein requirement (maintenance requirement) is defined as the amount of dietary protein which is sufficient to achieve zero nitrogen balance. The dietary protein requirement is considered to be the amount needed to replace obligatory nitrogen losses, after adjustment for the efficiency of dietary protein utilisation and the quality of the dietary protein. The factorial method is used to calculate protein requirements for physiological conditions 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 (i.e. milk).

According to a meta-analysis of available nitrogen balance data as a function of nitrogen intake in healthy adults, the best estimate of average requirement for healthy adults was 105 mg N/kg body weight per day (0.66 g high quality protein/kg per day). The 97.5th percentile was estimated as 133 mg N/kg body weight per day (0.83 g high quality protein/kg per day) from the distribution of the log of the requirement, with a CV of about 12 %. The Panel considers that the value of 0.66 g/kg body weight per day can be accepted as the Average Requirement (AR), and the value of 0.83 g/kg body weight per day as the Population Reference Intake (PRI), derived for proteins with a PD-CAAS value of 1.0. This value can be applied to usual mixed diets in Europe which are unlikely to be limiting in their content of indispensable amino acids. For older adults, the protein requirement is equal to that for

adults. The lower energy requirement of sedentary elderly people means that the protein to energy ratio of their requirement may be higher than for younger age groups.

For infants, children and adolescents, the Panel accepted the approach of WHO/FAO/UNU (2007) in which estimates of the protein requirements from six months to adulthood were derived factorially as the sum of requirements for maintenance and growth corrected for efficiency of utilisation. An average maintenance value of 0.66 g protein/kg body weight per day was applied. Average daily needs for dietary protein for growth were estimated from average daily rates of protein deposition, calculated from studies on whole-body potassium deposition, and considering an efficiency of utilisation of dietary protein for growth of 58 %. The PRI was estimated based on the average requirement plus 1.96 SD using a combined SD for growth and maintenance.

For pregnant women, the Panel accepted the factorial approach for deriving protein requirements during pregnancy, which was based on the newly deposited protein in the foetus and maternal tissue, and the maintenance requirement associated with the increased body weight. Because of the paucity of data in pregnant women, and because it is unlikely that the efficiency of protein utilisation decreases during pregnancy, the efficiency of protein utilisation was taken to be 47 % as in non-pregnant women. Thus, for pregnant women, a PRI for protein of 1, 9 and 28 g/d in the first, second and third trimesters, respectively, is proposed in addition to the PRI for non-pregnant women.

For lactation, the Panel accepted the factorial approach which requires assessing milk volume produced and its content of both protein nitrogen and non-protein nitrogen, and calculating the amount of dietary protein needed for milk protein production. As the efficiency of protein utilisation for milk protein production is unknown, the same efficiency as in the non-lactating adult (47 %) was assumed. The PRI was estimated by adding 1.96 SD to give an additional 19 g protein/d during the first six months of lactation (exclusive breastfeeding), and 13 g protein/d after six months (partial breastfeeding).

The Panel also considered several health outcomes that may be associated with protein intake. The available data on the effects of an additional dietary protein intake beyond the PRI on muscle mass and function, on body weight control and obesity (risk) in children and adults, and on insulin sensitivity and glucose homeostasis do not provide evidence that can be considered as a criterion for determining DRVs for protein. Likewise, the available evidence does not permit the conclusion that an additional protein intake might affect bone mineral density and could be used as a criterion for the setting of DRVs for protein.

Data from food consumption surveys show that actual mean protein intakes of adults in Europe are at, or more often above, the PRI of 0.83 g/kg body weight per day. In Europe, adult protein intakes at the upper end (90-97.5th percentile) of the intake distributions have been reported to be between 17 and 25 E%. The available data are not sufficient to establish a Tolerable Upper Intake Level (UL) for protein. In adults an intake of twice the PRI is considered safe.

DRVs have not been derived for indispensable amino acids, since amino acids are not provided as individual nutrients but in the form of protein. In addition, the Panel notes that more data are needed to obtain sufficiently precise values for indispensable amino acid requirements.

TABLE OF CONTENTS

Abstract 1
Summary 2
Table of contents 4
Background as provided by the European Commission 6
Terms of reference as provided by European Commission 6
Assessment 8
1. Introduction 8
2. Definition/category 8
2.1. Definition 8
2.2. Protein digestion and metabolism 9
2.2.1. Intestinal protein digestion and amino acid absorption 9
2.2.2. Protein turnover, amino acid metabolism and amino acid losses 10
2.3. Protein quality from digestibility and indispensable amino acid composition 10
2.3.1. Measurement of protein digestibility 10
2.3.2. The indispensable amino acid scoring method 11
2.4. Nitrogen retention and efficiency of dietary protein utilisation 11
3. Dietary protein sources and intake data 12
3.1. Nitrogen and protein content in foodstuffs – the nitrogen conversion factor 12
3.2. Dietary sources 13
3.3. Dietary intake 14
4. Overview of dietary reference values and recommendations 14
4.1. Dietary reference values for protein for adults 14
4.1.1. Older adults 15
4.2. Dietary reference values for protein for infants and children 16
4.3. Dietary reference values for protein during pregnancy 18
4.4. Dietary reference values for protein during lactation 18
4.5. Requirements for indispensable amino acids 19
5. Criteria (endpoints) on which to base dietary reference values (DRVs) 20
5.1. Protein intake and protein and nitrogen homeostasis 20
5.1.1. Methods for the determination of protein requirement 20
5.1.1.1. Nitrogen balance 20
5.1.1.2. The factorial method 21
5.1.1.3. Protein quality and reference pattern for indispensable amino acids 22
5.1.2. Protein requirement of adults 22
5.1.2.1. Older adults 23
5.1.3. Protein requirement of infants and children 23
5.1.4. Protein requirement during pregnancy 24
5.1.5. Protein requirement during lactation 25
5.2. Protein intake and health consequences 25
5.2.1. Muscle mass 25
5.2.2. Body weight control and obesity 26
5.2.2.1. Infants 26
5.2.2.2. Adults 27
5.2.3. Insulin sensitivity and glucose control 27
5.2.4. Bone health 27
5.2.5. Kidney function 28
5.2.6. Capacity of the urea cycle 28
5.2.7. Tolerance of protein 29
6. Data on which to base dietary reference values (DRVs) 29
6.1. Protein requirement of adults 29
6.1.1. Protein requirement of older adults 29
6.2. Protein requirement of infants and children 29
6.3. Protein requirement during pregnancy 30

6.4. Protein requirement during lactation....30
6.5. Safety of protein intakes above the PRI....30
Conclusions....31
References....32
Appendices....45
Appendix 1a: Population, methods and period of dietary assessment in children and adolescents in European countries....45
Appendix 1b: Intake of protein among children aged ~1-3 years in European countries....47
Appendix 1c: Intake of protein among children aged ~4-6 years in European countries....48
Appendix 1d: Intake of protein among children aged ~7-9 years in European countries....49
Appendix 1e: Intake of protein among children aged ~10-14 years and over in European countries....50
Appendix 1f: Intake of protein among adolescents aged ~15-18 years and over in European countries....51
Appendix 2a: Population, methods and period of dietary assessment in adults in European countries....52
Appendix 2b: Intake of protein among adults aged ~19-65 years in European countries....55
Appendix 2c: Intake of protein among adults aged ~19-34 years in European countries....56
Appendix 2d: Intake of protein among adults aged ~35-64 years in European countries....57
Appendix 2e: Intake of protein among adults aged ~65 years and over in European countries....58
Appendix 3: Calculation of PRI for infants, children and adolescents....59
Glossary/Abbreviations....60

BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community[4]. The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context the EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context the EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002, the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on Population Reference Intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance the EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, poly-unsaturated fatty acids and mono-unsaturated fatty acids, *trans* fatty acids;
- Protein;
- Dietary fibre.

[4] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

Following on from the first part of the task, the EFSA is asked to advise on Population Reference Intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, the EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

1. Introduction

Dietary proteins are an essential component of the diet by virtue of supplying the body with nitrogen (N) and amino acids which are used to synthesise and maintain the around 25,000 proteins encoded within the human genome as well as other non protein metabolically active nitrogenous substances like peptide hormones, neurotransmitters, nucleic acids, glutathione or creatine. In addition, amino acids are also subjected to deamination, and their carbon skeleton is used in different metabolic pathways or as energy substrate.

2. Definition/category

2.1. Definition

Proteins are built from amino acids joined together by peptide bonds between the carboxyl group and the amino (or imino in the case of proline) group of the next amino acid in line. These polypeptide chains are folded into a three dimensional structure to form the protein. The primary structure or sequence of amino acids in proteins is pre-determined in the genetic code. Twenty of the naturally occurring amino acids are so-called proteinogenic amino acids, which build proteins in living organisms. With few exceptions, only L-isomers are incorporated into proteins.

Dietary proteins are the source of nitrogen and indispensable amino acids for the body. Both in the diet and in the body, 95 % of the nitrogen is found in the form of proteins and 5 % is found in the form of other nitrogenous compounds, i.e. free amino acids, urea or nucleotides. A conversion factor of 6.25 for the conversion of nitrogen to protein is usually used for labelling purposes, assessment of protein intake and for protein reference values. Total N x 6.25 is called crude protein and [total minus non-protein-N] x 6.25 is called true protein. For other purposes, protein specific nitrogen conversion factors can be used (see section 3.1). In this opinion, unless specifically mentioned, "protein" is total N x 6.25, and protein requirements are calculated from nitrogen content.

The 20 proteinogenic amino acids are classified as indispensable or dispensable amino acids. Nine amino acids are classified as indispensable in humans (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) as they cannot be synthesised in the human body from naturally occurring precursors at a rate to meet the metabolic requirement. The remaining dietary amino acids are dispensable (alanine, arginine, cysteine, glutamine, glycine, proline, tyrosine, aspartic acid, asparagine, glutamic acid and serine). Among the nine indispensable amino acids, lysine and threonine are strictly indispensable since they are not transaminated and their deamination is irreversible. In contrast, the seven other indispensable amino acids can participate in transamination reactions. In addition, some of the dispensable amino acids which under normal physiological conditions can be synthesised in the body, can become limiting under special physiological or pathological conditions, such as in premature neonates when the metabolic requirement cannot be met unless these amino acids are supplied in adequate amounts with the diet; they are then called conditionally indispensable amino acids (arginine, cysteine, glutamine, glycine, proline, tyrosine) (IoM, 2005; NNR, 2004).

Besides being a building block for protein synthesis, each amino acid has its own non-proteogenic metabolic pathways. Some amino acids are used as precursors for nitrogenous compounds such as glutathione, various neurotransmitters, nitrogen monoxide, creatine, carnitine, taurine or niacin. Glutamine, aspartate and glycine are used for the synthesis of ribo- and desoxyribonucleotides, precursors for the synthesis of the nucleic acids RNA and DNA. Arginine and glutamine are precursors of non-proteinogenic amino acids including ornithine and citrulline that play a role in inter-organ exchange of nitrogen. Glutamine and glutamate are precursors of Krebs cycle components and are also important energy substrates for various cells. Amino acids are used after deamination as energy substrates, and in gluconeogenesis and ketogenesis. Some of the amino acids can also act directly or indirectly as intracellular signal molecules. Glutamate is a well known neurotransmitter, tryptophan is the precursor of serotonin, tyrosine is the precursor of catecholamines and dopamine, as well as of thyroid hormones, and histidine is the precursor of histamine. Arginine is an activator of the first step of NH_4^+/NH_3 elimination in the hepatic urea cycle, acts as a secretagogue for β-cells of pancreatic Langerhans islets, and is - via nitric oxide synthase activity - the precursor of nitrogen monoxide that regulates blood pressure. Lastly, leucine has been subjected to numerous studies for its role as a signal for protein synthesis via the mTOR (mammalian target of rapamycin) signalling pathway. These non-proteogenic metabolic pathways and signalling activities are included in the concept of protein requirement when nitrogen balance is achieved and indispensable amino acid requirements are met. As a consequence, they are not used as additional markers for the determination of protein requirement.

2.2. Protein digestion and 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 dispensable and indispensable dietary amino acids, the *de novo* synthesis of dispensable amino acids, protein hydrolysis, protein synthesis and amino acid utilisation in catabolic pathways or as precursors for nitrogenous components.

2.2.1. Intestinal protein digestion and amino acid absorption

The fluxes of nitrogen, amino acids and protein in the gut exhibit a relatively complex pattern. In humans, ingested dietary proteins (about 40–110 g/d), endogenous protein secreted into the gut (20–50 g/d), and molecules containing non-protein nitrogen (urea and other molecules) secreted into the gut are mixed in the lumen of the stomach and the small intestine, and are subjected to transit, digestion and absorption (Gaudichon et al., 2002). The majority is transferred into the body by absorption across the intestinal mucosa whereas a smaller part remains in the lumen and reaches the terminal ileum. This, along with other undigested luminal components, passes from the terminal ileum into the large intestine, where it is all subjected to fermentation by the microflora.

Protein digestion starts in the stomach and is continued in the small intestine. In healthy humans, digestive enzymes and transport across the brush border membrane via a variety of transporters are not a limiting factor for amino acid absorption (Johnson et al., 2006). The metabolic activity of the small intestine is high, and the small intestinal mucosa metabolises a significant proportion of both dispensable and indispensable amino acids in the course of absorption. In the absorptive state, dietary rather than systemic amino acids are the major precursors for mucosal protein synthesis. Glutamine and glutamate, which are the most important fuels for intestinal tissue, are mostly used by the intestine, and their appearance in the portal circulation is usually very low. Fifty to sixty percent of threonine is used by the intestine mainly for mucin synthesis by goblet cells. Of the amino acids lysine, leucine or phenylalanine, 15-30 % is used by the intestine whereas the other fraction appears in the portal circulation. Catabolism dominates the intestinal utilisation of dietary amino acids, since only 12 % of the amino acids extracted by the intestine are used for mucosal protein synthesis.

Approximately 15 g protein/d remains in the intestinal lumen and enters the colon. There it is degraded into peptides and amino acids through bacterial proteolysis, and amino acids are further deaminated and decarboxylated. This process is considered to be a major pathway for amino acid losses at maintenance intake of dietary protein (Gaudichon et al., 2002). The microflora possesses ureolytic activity so that urea nitrogen secreted into the intestine can be recycled both by microbial amino acid synthesis and by the uptake of ammonia from the gut. The ammonia is predominantly incorporated into alanine, aspartate/asparagine and glutamate/glutamine from which it may be incorporated into most of the amino acids by transamination. This mechanism of urea recycling might be of value in conserving nitrogen (Fouillet et al., 2008; Jackson, 1995).

As a consequence of the activities of the intestinal microbiota, by the time digesta are excreted as faeces their protein content is largely of microbial origin. Therefore, faecal or ileal digestibility measurements, as well as apparent and true nitrogen and amino acid digestibility measurements (see section 2.3.1.), have very different significance and can be used for different objectives. Measurements at the ileal level are critical for determining amino acid losses of both dietary and endogenous origin, whereas measurements at the faecal level are critical in assessing whole-body nitrogen losses (Fuller and Tome, 2005). The impact of the recycling of intestinal nitrogen, and of amino acids synthesised by bacteria, on whole body requirement of nitrogen, amino acids and protein is not clear. Other bacteria-derived amino acid metabolites include short chain fatty acids, sulphides, ammonia, phenols or indoles. The health consequences of changes in the luminal concentration of these products have not been extensively studied.

2.2.2. Protein turnover, amino acid metabolism and amino acid losses

The main pathway of amino acid metabolism is protein synthesis. In a 70 kg adult man, the body protein pool represents 10-12 kg, of which 42 % is in skeletal muscle, 15 % each in skin and blood, and 10 % in visceral organs. Four proteins (collagen, myosin, actin and haemoglobin) account for half of the body protein pool, and 25 % of the proteins of the body are present as collagen. The 12 kg body protein pool is in continuous turnover and exchanges with the free amino acid pool, which is approximately 100 g, via the proteosynthesis and proteolysis pathways at a rate of 250-300 g/d in a 70 kg adult man (Waterlow, 1995, 1996). This protein turnover is 2-3 times higher than the usual dietary protein intake (NNR, 2004). Moreover, the synthesis and turnover rates vary between the different body proteins. Visceral tissues have a fast protein turnover whereas peripheral tissues have a lower rate.

Amino acids are irreversibly lost in the faeces (25-30 % of total amino acid losses), by metabolic oxidation (70-75 % of total amino acid losses) and as miscellaneous losses in urine (about 0.6 g amino acids or 40 mg nitrogen in male adults), hair, skin, bronchial and other secretions, and in lactating women as milk (SCF, 1993). These amino acid losses need to be balanced by the supply of dietary protein-derived amino acids (50-100 g/d). When protein intake is increased the metabolic oxidative losses are also increased in order to achieve amino acid and nitrogen balance (Forslund et al., 1998; Morens et al., 2003; Pacy et al., 1994; Price et al., 1994).

2.3. Protein quality from digestibility and indispensable amino acid composition

The nutritional value of dietary proteins is related to their ability to satisfy nitrogen and amino acid requirements for tissue growth and maintenance. According to current knowledge this ability mainly depends on the digestibility of protein and amino acids, and on the dispensable and indispensable amino acid composition of the proteins.

2.3.1. Measurement of protein digestibility

The aim of measuring protein digestibility is to predict the quantity of absorbed nitrogen or amino acids following protein consumption. Though several *in vitro* methods requiring enzymatic hydrolysis have been proposed, the classical approach uses *in vivo* digestibility in an animal model or in humans. The classical *in vivo* procedure is based on faecal collection and determination of the nitrogen output over several days. *Apparent digestibility* of protein is measured from the difference between nitrogen ingested and nitrogen excreted in the faeces. It does not take into account the presence of endogenous nitrogen secretion and colonic metabolism. Apparent digestibility is one component in the assessment of whole-body nitrogen losses. For the determination of *true* (or *real*) *digestibility*, discrimination between exogenous nitrogen (food) and endogenous nitrogen losses (secretions, desquamations, etc.) is needed. Individual amino acid digestibility is usually related to whole protein nitrogen digestibility. Alternatively, individual amino acid digestibility can be determined.

Both direct and indirect methods have been proposed to distinguish and quantify the endogenous and dietary components of nitrogen and amino acids in ileal chyme or faeces. These approaches include the administration of a protein-free diet, the enzyme-hydrolysed protein method, different levels of protein intake, or multiple regression methods, in which it is assumed that the quantity and amino acid composition of endogenous losses is constant and independent of diet (Baglieri et al., 1995; Fuller and Reeds, 1998; Fuller and Tome, 2005). Substantial advances in the ability to discriminate between exogenous (dietary) and endogenous nitrogen have been achieved using stable isotopes (Fouillet et al., 2002). By giving diets containing isotopically-labelled amino acids (usually at the carbon or nitrogen atom) the endogenous flow is estimated from the dilution of the isotopic enrichment in the digesta (Fouillet et al., 2002; Gaudichon et al., 1999; Tome and Bos, 2000). Regarding the dietary amino acid fraction, it is also questionable whether protein (overall nitrogen) digestibility is a good proxy for individual ileal amino acid digestibility because some studies have reported modest ranges of variation of individual amino acid digestibility around the value for nitrogen digestibility (Fuller and Tome, 2005). It appeared that in some cases there are substantial differences in true digestibility among amino acids (Fouillet et al., 2002; Gaudichon et al., 2002; Tome and Bos, 2000).

The unabsorbed amino acids are mostly metabolised by colonic bacteria. Therefore, the apparent digestibility measured in ileal effluent should be considered as a critical biological parameter for dietary amino acid digestibility (Fuller and Tome, 2005). Digestibility values obtained by the faecal analysis method usually overestimate those obtained by the ileal analysis method. In humans, intestinal effluents for the estimation of apparent digestibility are obtained either from ileostomy patients or, preferably, in healthy volunteers by using naso-intestinal tubes. These approaches are not, however, straightforward, and are too demanding for routine evaluation of food, but can be used as reference methods (Fouillet et al., 2002; Fuller et al., 1994). An alternative is the use of animal models, most commonly the rat and the pig. The rat is used for the determination of protein quality in human diets (FAO/WHO, 1991). However, some differences in protein digestibility have been observed between rats, pigs and humans (Fuller and Tome, 2005).

The usefulness of the values obtained by digestibility measurements depends on the objective. *In vitro* digestibility measurements can only be used to compare products with one another, and can never serve as independent reference values. Measurement of apparent and real digestibility is critical for determining amino acid losses of both dietary and endogenous origin. Data in humans are preferred whenever possible. The determination of individual amino acid digestibility is also preferred whenever possible. A complementary and still unresolved aspect of digestibility assessments is how to take into account the recycling of intestinal nitrogen and bacterial amino acids in the body.

2.3.2. The indispensable amino acid scoring method

The concept of protein requirement includes both total nitrogen and indispensable amino acids requirements. Therefore, the content and utilisation of indispensable amino acids can be considered as valuable criteria for the evaluation of dietary protein quality (WHO/FAO/UNU, 2007). This idea leads to the use of the amino acid scoring approach in which the indispensable amino acid composition of the dietary protein is compared to a reference pattern of indispensable amino acids which is assumed to meet requirements for indispensable amino acids at a protein supply which corresponds to the average protein requirement. The reference pattern of indispensable amino acids is derived from measurements of the indispensable amino acid requirements (WHO/FAO/UNU, 2007) (see section 4.5). Originally, the chemical score was based on the complete analysis of the food amino acid content and its comparison to the amino acid pattern of a chosen reference protein (e.g. egg or milk protein).

In the traditional scoring method, the ratio between the content in a protein and the content in the reference pattern is determined for each indispensable amino acid. The lowest value is used as the score. The Protein Digestibility-Corrected Amino Acid Score (PD-CAAS) corrects the amino acid score by the digestibility of the protein (FAO/WHO, 1991) or of each individual amino acid. The accuracy of the scoring approach depends on the precision of amino acid analysis and on the measurement of protein digestibility. A more precise approach is to use the specific ileal digestibility of individual amino acids. The PD-CAAS can be used as a criterion for the protein quality of both foods and diets. A PD-CAAS <1 indicates that at least one amino acid is limiting, whereas a score ≥ 1 indicates that there is no limiting amino acid in the food or diet.

2.4. Nitrogen retention and efficiency of dietary protein utilisation

A traditional approach for evaluating the efficiency of protein utilisation has been to consider the interaction with a physiological process such as growth. The Protein Efficiency Ratio (PER) that relates the average animal (rat) weight gain to the amount of ingested protein over 28 days (AOAC, art. 43.253 to 43.257) is simple, but presents several shortcomings and inaccuracies. The main difficulty lies in the significance of extrapolation to humans.

Determination of the nutritional efficiency of protein in the diet is in most cases based on estimating the extent to which dietary protein nitrogen is absorbed and retained by the organism, and is able to balance daily nitrogen losses. It is determined by measuring faecal, urinary and miscellaneous nitrogen losses. Net Protein Utilisation (NPU) is the percentage of ingested nitrogen that is retained in the body, and the Biological Value (BV) gives the percentage of absorbed nitrogen that is retained. BV is the product of NPU and digestibility. Similar to digestibility, NPU values are true or apparent depending on whether the loss of endogenous nitrogen is taken into account or not, and this is critical to precisely determining the efficiency of dietary protein utilisation and the quality of the different dietary protein sources. The true NPU can be calculated as follows:

$$\text{True NPU} = \text{total } N_{ingested} - [(\text{total } N_{faeces} - \text{endogenous } N_{faeces}) + (\text{total } N_{urine} - \text{endogenous } N_{urine})]/\text{total } N_{ingested}$$

Endogenous intestinal (faecal) and metabolic (urinary) nitrogen losses can be obtained with a protein free diet, derived from the y-intercept of the regression line relating nitrogen intake to retention at different levels of protein intake, or directly determined from experiments using isotopically-labelled dietary proteins.

As the post-prandial phase is critical for dietary protein utilisation, the measurement of the immediate retention of dietary nitrogen following meal ingestion represents a reliable approach for the assessment of protein nutritional efficiency. In the net protein postprandial utilisation (NPPU) approach, true dietary protein nitrogen retention is directly measured in the post-prandial phase from experiments using ^{15}N-labelled dietary proteins (Fouillet et al., 2002). A mean value of 70 % can be considered for the NPPU of dietary proteins (Bos et al., 2005). This NPPU approach represents the maximal potential NPU efficiency of the dietary protein sources when determined in optimised controlled conditions in healthy adults, and it can be modified by different factors including food matrix, diet and physiological conditions.

From nitrogen balance studies, an NPU value of 47 % (median value, 95 % CI 44–50 %) was derived from the slope of the regression line relating nitrogen intake to retention for healthy adults at maintenance, and no differences were found between the results when the data were grouped by sex, diet or climate (Rand et al., 2003; WHO/FAO/UNU, 2007). The results suggested a possible age difference in nitrogen utilisation with a lower efficiency in individuals above 55 years (31 % compared with 48 % for adults up to 55 years, p=0.003), but because of the apparent interaction between age and sex in the data, the extreme variability in the younger men, and the fact that the lower values for the older adults came from a single study, these results were not accepted as conclusive (Rand et al., 2003). There are different values used for efficiency of protein utilisation for maintenance (47 %) and for tissue deposition/growth in different populations and age groups including infants and pregnant or lactating women (IoM, 2005; King et al., 1973; WHO/FAO/UNU, 2007).

The Panel considers that methods related to growth in rats (protein efficiency ratio, PER) are not reliable for humans. Methods related to nitrogen retention (NPPU, NPU, BV) are preferable as they reflect more accurately the protein nutritional value, and can be used as reference methods. From available data in healthy adults at maintenance the mean optimal NPU value defined as NPPU is 70 %, and the usual NPU value as determined from nitrogen balance studies is approximately 47 %.

3. Dietary protein sources and intake data

3.1. Nitrogen and protein content in foodstuffs – the nitrogen conversion factor

Assuming an average nitrogen content of 160 mg/g protein, a conversion factor of 6.25 is used for the calculation of the (crude) protein content of a food from the total nitrogen content. Specific conversion factors for different proteins have been proposed (Jones, 1941; Leung et al., 1968; Pellett and Young, 1980), including for instance milk and milk products (6.38), other animal products (6.25), wheat (5.83) or soy protein (5.71). Besides variations in the nitrogen content of different proteins, the presence or absence of a non-protein fraction of the total nitrogen content of a food will influence the calculated crude protein content (SCF, 2003).

Conversion factors based on the amino acid composition of a protein have been proposed to define more accurately the true protein content of different foodstuffs (AFSSA, 2007; SCF, 2003). The choice of one or several conversion factors depends on the objective, and if the aim is to indicate a product’s capacity to supply nitrogen, a single coefficient is enough. However, if the objective is to indicate a product’s potential to supply amino acids, the use of specific coefficients based on amino acid-derived nitrogen content is more relevant. Such protein amino acid composition-derived conversion factors have been determined for different protein sources: milk and milk products (5.85), meat, fish and eggs (5.6), wheat and legumes (5.4), and a default conversion factor (5.6) (AFSSA, 2007).

3.2. Dietary sources

Dietary proteins are found in variable proportions in different foods, resulting in variability of dietary protein intake within and between populations. Proteins differ in their amino acid composition and indispensable amino acid content. The main dietary sources of proteins of animal origin are meat, fish, eggs, milk and milk products. Most of these animal dietary protein sources have a high content in protein and indispensable amino acids. Main dietary sources of plant proteins are cereal grains, leguminous vegetables and nuts. The protein content differs from one plant source to another accounting for 20-30 % (w/w) for uncooked legume seeds or around 10 % for cereal seeds. The indispensable amino acid content of plant proteins is usually lower than that of animal proteins. In addition to the PD-CAAS, technological treatments applied to proteins during extraction processes and during the production of foodstuffs may modify the characteristics and properties of food proteins.

Examples of the range of protein content of some animal- and plant-derived foods are provided in Table 1. The water and energy contents of these foods can differ greatly.

Table 1: Protein content (N x 6.25, g/100 g of edible food) of some animal- and plant-derived food products.

Animal-derived foods	Protein content (N x 6.25, g/100 g)	Plant-derived foods	Protein content (N x 6.25, g/100 g)
Red meat (raw and cooked)	20-33	Vegetables	1-5
Poultry (raw and cooked)	22-37	Legumes	4-14
Fish	15-25	Fruits	0.3-2
Eggs	11-13	Nuts and seeds	8-29
Cheese, hard	27-34	Pasta and rice (cooked)	2-6
Cheese, soft	12-28	Breads and rolls	6-13
Milk products	2-6	Breakfast cereals	5-13

Data adapted from the ANSES/CIQUAL French food composition table version 2008, available from: http://www.afssa.fr/TableCIQUAL/index.htm (ANSES/CIQUAL, 2008)

Several methods exist for assessing protein quality, for example the content of indispensable amino acids. One of the food composition tables providing the most detailed amino acid profiles of various foodstuffs is the table of the United States Department of Agriculture (USDA/ARS, 2009). High quality protein has an optimal indispensable amino acid composition for human needs and a high digestibility. Most dietary protein of animal origin (meat, fish, milk and egg) can be considered as such high quality protein. In contrast, some dietary proteins of plant origin can be regarded as being of lower nutritional quality due to their low content in one or several indispensable amino acids, or due to their lower digestibility. It is well established that lysine is limiting in cereal protein, and that sulphur-containing amino acids (cysteine and methionine) are limiting in legumes. Most of the Western diets have a PD-CAAS equal or higher than 1 because high quality proteins dominate over low quality proteins. Although proteins limited in one amino acid can complement in the diet proteins which are limited in another amino acid, a high level of cereal in the diet in some countries can lead to a PD-CAAS lower than 1, mainly because of a low content of lysine.

Due to the high content of indispensable amino acids in animal proteins, a diet rich in animal protein usually has a content of each indispensable amino acid above the requirement. It is widely accepted that a balance between dispensable and indispensable amino acids is a more favourable metabolic situation than a predominance of indispensable amino acids, since indispensable amino acids consumed in excess of requirement are either converted to dispensable amino acids or directly oxidised.

3.3. Dietary intake

Typical intakes of (crude) protein of children and adolescents from 19 countries (Appendix 1) and of adults from 22 countries in Europe are presented (Appendix 2). The data refer to individual-based food consumption surveys, conducted from 1989 onwards. Most studies comprise nationally representative population samples.

As demonstrated in the annexes, there is a large diversity in the methodology used to assess the individual intakes of children, adolescents and adults. Because the different methods apply to different time frames, this inevitably results in variability in both the quality and quantity of available data, which makes direct comparison difficult. Moreover, age classifications are in general not uniform. Comparability is also hampered by differences in the food composition tables used for the conversion of food consumption data to estimated nutrient intakes (Deharveng et al., 1999).

Although these differences may have an impact on the accuracy of between country comparisons, the presented data give a rough overview of the protein intake in a number of European countries. Most studies reported mean intakes and standard deviations (SD), or mean intakes and intake distributions. In most studies the contribution of protein to energy intake is based on total energy intake (including energy from alcohol).

In adults, average protein intake in absolute amounts ranges from approximately 72 to 108 g/d in men and from 56 to 82 g/d in women. Available data suggest an average intake of 0.8 to 1.25 g/kg body weight per day for adults. Average protein intake varies in infants and young children from 25 to 63 g/d. Average daily intake increases with age to about 55 to 116 g/d in adolescents aged 15-18 years. In general, males have higher intakes than females. Only a few countries present data per kg body weight. However, when related to reported mean body weights (SCF, 1993), the estimated mean intake varies from ≥3 g/kg body weight per day in the youngest age groups to approximately 1.2 to 2.0 g/kg body weight per day in children aged 10-18 years.

When expressed as % of energy intake (E%), average total protein intake ranges from about 13 to 20 E% in adults, with within population ranges varying from 10-14 E% at the lower (2.5–10th percentile) to 17-25 E% at the upper (90-97.5th percentile) end of the intake distributions. Average intakes of 17 E% and higher are observed, for example in France, Romania, Portugal and Spain. Available data show that average protein intake in children and adolescents in European countries varies from 11 to 18 E%. Within population ranges vary from about 6-13 E% (2.5-10th percentile) to 16-22 E% (90-97.5th percentile).

4. Overview of dietary reference values and recommendations

A number of national and international organisations and authorities have set dietary reference values (DRVs) for protein and other energy-providing nutrients, as well as for dietary fibre. Generally, reference intakes for protein are expressed as g/kg per day and g/d (adjusting for reference body weights), and as a percentage of total energy intake (E%), and refer to high quality protein (e.g. milk and egg protein).

4.1. Dietary reference values for protein for adults

Table 2 lists the reference intakes set by various organisations for adult humans.

In their report, FAO/WHO/UNU (1985) used nitrogen balance to derive a population average requirement of 0.6 g/kg body weight per day and, adding two standard deviations (2x12.5 %) to allow for individual variability, a “safe level of intake” of 0.75 g/kg body weight per day. The UK COMA (DoH, 1991) and the SCF (1993) accepted the values adopted by FAO/WHO/UNU (1985). The Netherlands (Health Council of the Netherlands, 2001) also used the approach of FAO/WHO/UNU (1985) but applied a CV of 15 % to allow for individual variability, and derived a recommended intake of 0.8 g/kg body weight per day. The Nordic Nutrition recommendations (NNR, 2004), taking account of the fact that diets in industrialised countries have high protein contents, set a desirable protein intake of 15 E% for food planning purposes, with a range of 10-20 E% for adults. This translates into protein intakes of well above 0.8 g/kg body weight per day. The US Institute of Medicine recommended 0.8 g/kg body weight per day of good quality protein for adults (IoM, 2005). The criterion of adequacy used for the estimated average requirement (EAR) of protein is based on the lowest continuing intake of dietary protein that is sufficient to achieve body nitrogen equilibrium (zero balance).

WHO/FAO/UNU (2007) re-evaluated their recommendations from 1985. Based on a meta-analysis of nitrogen balance studies in humans by Rand et al. (2003) which involved studies stratified for a number of subpopulations, settings in different climates, sex, age and protein source, a population average requirement of 0.66 g/kg body weight per day resulted as the best estimate. The "safe level of intake" was identified as the 97.5th percentile of the population distribution of requirement, which was equivalent to 0.83 g/kg body weight per day of high quality proteins (WHO/FAO/UNU, 2007). The French recommendations (AFSSA, 2007) established a PRI of 0.83 g/kg body weight per day for adults based on the WHO/FAO/UNU (2007) report. The German speaking countries (D-A-CH, 2008) used the average requirement for high quality protein of 0.6 g/kg body weight per day (estimated by FAO/WHO (1985)), included an allowance for individual variability (value increased to 0.75 g/kg body weight per day), and took account of frequently reduced protein digestibility in mixed diets to establish a recommended intake of 0.8 g/kg body weight per day for adults.

Table 2: Overview of dietary reference values for protein for adults.

	FAO/ WHO/UNU (1985)	**DoH (1991)**	**SCF (1993)**	**Health Council of the Netherlands (2001)**	**NNR (2004)**	**IoM (2005)**	**WHO/ FAO/UNU (2007)**	**AFSSA (2007)**	**D-A-CH (2008)**
AR - Adults (g/kg bw x d^{-1})	0.60	0.60	0.60	0.60	-	0.66	0.66	0.66	0.60
PRI - Adults (g/kg bw x d^{-1})	0.75[1]	0.75	0.75	0.80	-	0.80[2]	0.83[1]	0.83	0.80
PRI - Adult Males (g/d)	-	56	56	59	-	56	-	-	59
PRI - Adult Females (g/d)	-	45	47	50	-	46	-	-	47
Recommended intake range – Adults (E%)	-	-	-	-	10-20	10-35[3]	-	-	-

[1]Safe level of intake; [2] Recommended dietary allowance (RDA); [3]Acceptable Macronutrient Distribution Range

4.1.1. Older adults

In 1985, FAO/WHO/UNU recommended an intake of 0.75 g/kg body weight per day of good quality protein for adults and the same recommendation was made for adults over the age of 60 years because, although efficiency of protein utilisation is assumed to be lower in older adults, the smaller amount of lean body mass per kg body weight will result in a higher figure per unit lean body mass than in younger adults (FAO/WHO/UNU, 1985).

The recommended intake for adults in the Netherlands (Health Council of the Netherlands, 2001) is 0.8 g/kg body weight per day and no additional allowance was considered necessary for adults aged >70 years. The US Institute of Medicine recommended 0.8 g/kg body weight per day of good quality protein for adults (IoM, 2005). For adults aged 51-70 years and >70 years, no additional protein allowance beyond that of younger adults was considered necessary since no significant effect of age on protein requirement expressed per kg body weight was observed in the analysis by Rand et al. (2003), recognising that lean body mass as % body weight, and protein content of the body, both decrease with age.

Also WHO/FAO/UNU (2007) concluded that the available data did not provide convincing evidence that the protein requirement of elderly people (per kg body weight, no age range given) differs from the protein requirement of younger adults. The conclusion is partly supported by data on nitrogen balance (Campbell et al., 2008) which showed that the mean protein requirement was not different between younger (21–46 years) and older (63–81 years) healthy adults: 0.61 (SD 0.14) compared with 0.58 (SD 0.12) g protein/kg body weight per day. However, the low energy requirement of sedentary elderly people means that the protein to energy ratio of their requirement is higher than for younger age groups. Thus, unless the elderly people are physically active they may need a more protein-dense diet.

In France, an intake of 1.0 g/kg body weight per day has been recommended for people ≥75 years based on considerations about protein metabolism regulation in the elderly (AFSSA, 2007). The German speaking countries (D-A-CH, 2008) recommended an intake of 0.8 g protein/kg body weight per day for adults, and the same recommendation was made for adults aged 65 years and older since it was considered that the available evidence was insufficient to prove a higher requirement for the elderly.

4.2. Dietary reference values for protein for infants and children

Table 3 lists reference intakes set by various organisations for infants and children.

In their report, FAO/WHO/UNU (1985) calculated protein requirements of children from six months onwards by a modified factorial method. Maintenance requirements were interpolated between the values from nitrogen balance studies for children aged one year and for young adults aged 20 years. A coefficient of variation of 12.5 % was used to allow for individual variability. The growth component of the protein requirement was set at 50 % above that based on the theoretical daily amount of nitrogen laid down, corrected for an efficiency of protein utilisation of 70 %. The average requirement was then estimated as the sum of maintenance and growth requirement. The “safe level of intake” was estimated based on the average requirement plus two standard deviations corresponding to a CV of 12-16 %.

In its re-evaluation, WHO/FAO/UNU (2007) calculated a maintenance value of 0.66 g protein/kg body weight per day for children and infants from 6 months to 18 years. The maintenance level was derived from a regression analysis of nitrogen balance studies on children from 6 months to 12 years. Protein deposition needs were calculated from combined data of two studies, and assuming an efficiency of utilisation for growth of 58 %. The average requirement was then estimated as the sum of maintenance and growth requirement. The “safe level of intake” was estimated based on the average level plus 1.96 SD. Requirements fall very rapidly in the first two years of life (safe level at six months of age: 1.31 g/kg body weight per day; at two years of age: 0.97 g/kg body weight per day). Thereafter, the decrease towards the adult level is very slow (WHO/FAO/UNU, 2007).

Dewey et al. (1996) reviewed the approach by FAO/WHO/UNU (1985) and suggested revised estimates for protein requirements for infants and children. The German speaking countries (D-A-CH, 2008) followed the proposal of Dewey et al. (1996). For infants aged from 6 to <12 months the maintenance requirement was estimated from nitrogen balance studies at 0.56 g/kg body weight per day. Age dependent additions of between 35 % and 31 % for the increase in body protein were made to take into account inter-individual variability of maintenance and growth requirements (Dewey et al., 1996). A recommended intake of 1.1 g/kg body weight per day (10 g/d) of high quality protein was established between 6 and <12 months. Recommended intakes were established for children aged 1 to <4 years (1.0 g/kg body weight per day) and 4 to <15 years (0.9 g/kg body weight per day), and for boys aged 15 to <19 years (0.9 g/kg body weight per day) and girls aged 15 to <19 years (0.8 g/kg body weight per day). Maintenance requirement was estimated at 0.63 g/kg body weight per day (Dewey et al., 1996) and total requirement, allowing for the decreasing growth requirement with age, was estimated to range from 0.63 to 0.7 g/kg body weight per day. An additional 30 % allowance was made to account for inter-individual variability in protein utilisation and digestibility.

The Nordic Nutrition recommendations (NNR, 2004) also followed the approach by Dewey et al. (1996) to establish recommended intakes of 1.1 and 1.0 g/kg body weight per day for infants aged 6-11 months and children aged 12-23 months, respectively. For children aged 2-17 years a recommended intake of 0.9 g/kg body weight per day was established, in agreement with the values in other recommendations (D-A-CH, 2008; Health Council of the Netherlands, 2001; IoM, 2005). The French recommendations (AFSSA, 2007) also followed the approach of Dewey et al. (1996).

The Health Council of the Netherlands (2001) used a factorial method derived from nitrogen balance experiments to estimate the protein requirements of infants over six months, children and adolescents. For infants aged 6-11 months a recommended intake of 1.2 g/kg body weight per day (10 g/d) of high quality protein was established. This was based on an average requirement for maintenance and growth of 0.9 g/kg body weight per day, with a CV of 15 % to allow for individual variability, and assuming an efficiency of protein utilisation of 70 %. Recommended intakes were established for children aged 1 to 13 years (0.9 g/kg body weight per day) and 14 to 18 years (0.8 g/kg body weight per day), on the same basis but using an average requirement for maintenance and growth of 0.8 g/kg body weight per day for children aged 1 to 3 years, and 0.7 g/kg body weight per day for children aged 4 to 18 years (Health Council of the Netherlands, 2001).

Table 3: Overview of dietary reference values for protein for children.

	FAO/ WHO/ UNU (1985)[1]	SCF (1993)[1]	Health Council of the Netherlands (2001)	NNR (2004)	IoM (2005)[2]	WHO/ FAO/ UNU (2007)[1]	AFSSA (2007)	D-A-CH (2008)
Age	6–9 months	7-9 months	6-11 months	6-11 months	7-12 months	6 months	6-12 months	6-<12 months
PRI (g/kg bw x d^{-1})	1.65 (m + f)	1.65 (m + f)	1.2 (m + f)	1.1 (m + f)	1.2 (m + f)	1.31 (m + f)	1.1 (m + f)	1.1 (m + f)
Age	9-12 months	10-12 months	1-13 y	1-1.9 y	1-3 y	1 y	12-24 months	1- <4 y
PRI (g/kg bw x d^{-1})	1.50 (m + f)	1.48 (m + f)	0.9 (m + f)	1.0 (m + f)	1.05 (m + f)	1.14 (m + f)	1.0 (m+f)	1.0 (m + f)
Age	1-2 y	1-1.5 y				1.5 y	24-36 months	
PRI (g/kg bw x d^{-1})	1.20 (m + f)	1.26 (m + f)				1.03 (m + f)	0.9 (m+f)	
Age	2-3 y	2-3 y		2-17 y		2 y	3-10 y	
PRI (g/kg bw x d^{-1})	1.15 (m + f)	1.13 (m + f)		0.9 (m + f)		0.97 (m + f)	0.9 (m+f)	
Age	3-5 y	4-5 y				3 y	10-12 y (m), 10-11 y (f)	
PRI (g/kg bw x d^{-1})	1.10 (m + f)	1.06 (m + f)				0.90 (m + f)	0.85 (m), 0.9 (f)	
Age	5-12 y	6-9 y			4-13 y	4-6 y	12-13 y (m), 11-14 y (f)	4-<15 y
PRI (g/kg bw x d^{-1})	1.0 (m + f)	1.01 (m + f)			0.95 (m + f)	0.87 (m + f)	0.9 (m), 0.85 (f)	0.9 (m + f)
Age	12-14 y	12 y				7-10 y	13-17 y (m), 14-16 y (f)	
PRI (g/kg bw x d^{-1})	1.0 (m) 0.95 (f)	1.0 (m) 0.96 (f)				0.92 (m + f)	0.85 (m), 0.8 (f)	
Age	14-16 y	14 y	14-18 y		14-18 y	11-14 y		
PRI (g/kg bw x d^{-1})	0.95 (m) 0.9 (f)	0.96 (m) 0.90 (f)	0.8 (m + f)		0.85 (m + f)	0.90 (m) 0.89 (f)		
Age	16-18 y	16 y				15-18 y	17-18 y (m), 16-18 y (f)	15-<19 y
PRI (g/kg bw x d^{-1})	0.9 (m) 0.8 (f)	0.90 (m) 0.83 (f)				0.87 (m) 0.84 (f)	0.8 (m+f)	0.9 (m) 0.8 (f)

[1] Safe level of intake; [2] RDA

The US Institute of Medicine recommended intakes ranging from 1.2 g/kg body weight per day of high quality protein for infants aged 6-12 months to 0.85 g/kg body weight per day for 14 to 18 year-old boys and girls based on estimates of requirements for maintenance, with additions for growth (IoM, 2005). Maintenance requirements were estimated from short-term nitrogen balance studies in older infants and children as 110 mg N/kg body weight per day for ages 7 months through 13 years, and as 105 mg N/kg body weight per day (estimated from short-term nitrogen balance studies in adults and based on a meta-analysis by

Rand et al. (2003)) for ages 14 through 18 years. Growth requirements were estimated in infants and children from estimated rates of nitrogen accretion calculated from rates of weight gain and from estimates of the nitrogen content of tissues. The efficiency of dietary protein utilisation was assumed to be 58 % for ages 7 months through 13 years and 47 % for ages 14 through 18 years, estimated from the slopes of the nitrogen balance data. The EAR was thus estimated as 1.0 g/kg body weight per day for infants aged 7-12 months, 0.87 and 0.76 for boys and girls aged 1-3 and 4-13 years, respectively, and 0.73 and 0.71 for boys and girls aged 14-18 years, respectively. A CV of 12 % for maintenance and 43 % for growth was used to allow for individual variability in the calculation of the RDA (IoM, 2005).

4.3. Dietary reference values for protein during pregnancy

FAO/WHO/UNU (1985) recommended an average additional intake of 6 g/d throughout pregnancy, based on derived additional levels of protein intake of 1.2 g/d, 6.1 g/d and 10.7 g/d for the first, second and third trimester, respectively. This was based on the calculated average increment of 925 g protein during a pregnancy, plus 30 % (2 SD of birth weight), adjusted for the efficiency with which dietary protein is converted to foetal, placental and maternal tissues (estimated as 70 %) (FAO/WHO/UNU, 1985). WHO/FAO/UNU (2007) revised this value and recommended 1, 9 and 31 g of additional protein/d in the first, second and third trimester, respectively, as “safe intake levels”. Based on a theoretical model (Hytten and Chamberlain, 1991), the total deposition of protein in the foetus and maternal tissue has been estimated to be 925 g (assuming a 12.5 kg gestational weight gain), of which 42 % is deposited in the foetus, 17 % in the uterus, 14 % in the blood, 10 % in the placenta and 8 % in the breasts. Protein deposition has also been estimated indirectly from measurements of total body potassium accretion, measured by whole body counting in a number of studies with pregnant women (Butte et al., 2003; Forsum et al., 1988; King et al., 1973; Pipe et al., 1979). From these studies, mean protein deposition during pregnancy was estimated as 686 g (WHO/FAO/UNU, 2007). Based on the study by Butte et al. (2003), protein deposition per trimester was then calculated for well-nourished women achieving a gestational weight gain of 13.8 kg (the mid-point of the recommended weight gain range for women with normal pre-pregnancy weight) (IoM, 1990). The efficiency of protein utilisation was taken to be 42 % in pregnant women (in comparison to 47 % in non-pregnant adults) (WHO/FAO/UNU, 2007).

In Europe, the UK COMA (DoH, 1991) accepted the value proposed by FAO/WHO/UNU (1985). The SCF (1993) used the approach of FAO/WHO/UNU (1985) but recommended an additional intake of 10 g/d throughout pregnancy because of uncertainty about changes in protein metabolism associated with pregnancy (SCF, 1993). The Dutch (Health Council of the Netherlands, 2001) recommended an additional intake of 0.1 g/kg body weight per day throughout pregnancy. AFSSA (2007) followed the approach of FAO/WHO/UNU (1985) and recommended an intake between about 0.82 and 1 g/kg body weight per day for a woman of 60 kg (calculated from 50, 55 and 60 g/d for each trimester of pregnancy). The German speaking countries (D-A-CH, 2008) recommended an additional intake of 10 g/d (for the second and third trimesters).

The US Institute of Medicine set the EAR at 21 g/d above the average protein requirements of non-pregnant women, averaging the overall protein needs over the last two trimesters of pregnancy (IoM, 2005). It recommended an additional intake of 25 g/d (RDA for the second and third trimesters), assuming a CV of 12 % (26 g protein) and rounding to the nearest 5 g/d. The EAR for additional protein needs was based upon an estimated average protein deposition of 12.6 g/d over the second and third trimesters (calculated from potassium retention studies for accretion of 5.4 g protein/d), assuming an efficiency of dietary protein utilisation of 43 %, plus an additional 8.4 g/d for maintenance of the increased body tissue.

4.4. Dietary reference values for protein during lactation

FAO/WHO/UNU (1985) recommended an additional intake of 16 g/d of high quality protein during the first six months of lactation, 12 g/d during the second six months, and 11 g/d thereafter. This is based on the average protein content of breast milk, an efficiency factor of 70 % to adjust for the conversion of dietary protein to milk protein, and a CV of breast-milk volume of 12.5 % (FAO/WHO/UNU, 1985). WHO/FAO/UNU (2007) revised this value and recommended an average value of additional protein intake of 19 g/d in the first six months of lactation and 12.5 g/d after six months. This is based on the increased nitrogen needs of lactating women to synthesise milk proteins, with the assumption that the efficiency of milk protein production is the same as the efficiency of protein synthesis in non-lactating adults, i.e. 47 %. Therefore, the additional "safe intake" of dietary protein was calculated using an amount of dietary protein equal to milk protein, taking into account an efficiency of 47 %, and adding 1.96 SD corresponding to a CV of 12 % (WHO/FAO/UNU, 2007).

In Europe, the UK COMA (DoH, 1991) recommended an additional intake of 11 g/d for the first six months and an additional intake of 8 g/d thereafter. The approach used was similar to that of FAO/WHO/UNU (1985), except that the values used for breast milk protein content were lower because of correction for the amount (up to 25 %) of non-protein nitrogen present. The SCF (1993) accepted the values proposed by FAO/WHO/UNU (1985), i.e. an additional intake of 16 g/d of high quality protein during the first six months of lactation and 12 g/d during the second six months. The Netherlands (Health Council of the Netherlands, 2001) recommended an additional intake of 0.2 g/kg body weight per day during lactation to allow for the additional protein loss of about 7 g/d in breast milk. AFSSA considered the quantity of protein and non-protein nitrogen excreted in milk and its change during lactation, and recommended an additional intake of 16 g/d for the first six months, resulting in a recommended intake of about 1.1 g/kg body weight per day for a woman of 60 kg (AFSSA, 2007). The German speaking countries (D-A-CH, 2008) recommended an additional intake of 15 g/d during lactation based on a mean protein loss of 7-9 g/d in breast milk, assuming an efficiency of utilisation of 70 % and adding 2 SD to account for inter-individual variability.

The US Institute of Medicine (IoM, 2005) calculated the EAR of additional protein during lactation (21 g/d) from the average protein equivalent of milk nitrogen output and an assumed efficiency of utilisation of 47 %. Adding 2 SD (24 %) to account for inter-individual variability yielded an RDA of +25 g/d, or a recommended protein intake of 1.3 g/kg body weight per day during lactation.

4.5. Requirements for indispensable amino acids

Different approaches have been used to determine indispensable amino acid requirements. These requirements were first determined in adults using a nitrogen balance approach (Rose, 1957). The values obtained by this approach are usually considered to underestimate the requirements (Rand and Young, 1999; WHO/FAO/UNU, 2007; Young and Marchini, 1990). More recent data in adults have been obtained using amino acids labelled with stable isotopes, and are based on the measurement of amino acid oxidation as a function of intake (Bos et al., 2002). This includes the indicator amino acid balance method (Young and Borgonha, 2000), the indicator amino acid oxidation method (Elango et al., 2008a, 2008b; Pencharz and Ball, 2003), the 24 h-indicator amino acid oxidation method (Kurpad et al., 2001) or the protein post-prandial retention method (Bos et al., 2005; Millward et al., 2000).

The rationale for deriving DRVs for each indispensable amino acid remains questionable since as a rule amino acids are not provided as individual nutrients in the diet, but in the form of protein. Moreover, the values obtained for indispensable amino acid requirements are not yet sufficiently precise, and require further investigation (AFSSA, 2007; WHO/FAO/UNU, 2007). Only the US has introduced specific RDAs for indispensable amino acids, derived from the average values of requirements deduced from amino acid oxidation methods and adding 2 CV (of 12 %) (IoM, 2005).

Average indispensable amino acid requirements are used to calculate the indispensable amino acid reference pattern, which is used in the assessment of protein quality according to the chemical score approach and the PD-CAAS. The mean values for indispensable amino acid requirements were provided in the WHO/FAO/UNU (2007) report.

Table 4: Mean requirements for indispensable amino acids in adults (WHO/FAO/UNU, 2007).

	mg/kg x d^{-1}		mg/kg x d^{-1}
Histidine	10	**Phenylalanine+tyrosine**	25
Isoleucine	20	**Threonine**	15
Leucine	39	**Tryptophan**	4
Lysine	30	**Valine**	26
Methionine+cysteine	15[1]	**Total**	184
methionine	10.4		
cysteine	4.1		

[1] resulting from rounding

The amino acid requirements of infants and children have been derived using a factorial method, based on the estimated protein requirements for maintenance and growth (Dewey et al., 1996; WHO/FAO/UNU, 2007) (Table 5). It is assumed that the required amino acid pattern for maintenance is the same as that for adults, and that the amino acid pattern required for growth is given by the amino acid composition of whole-body tissue protein (Davis et al., 1993; Dewey et al., 1996; Widdowson et al., 1979).

Table 5: Mean requirements for indispensable amino acids in infants, children and adolescents (WHO/FAO/UNU, 2007).

	Mean amino acid requirement at different ages (mg/kg x d^{-1})				
	0.5 years	**1-2 years**	**3-10 years**	**11-14 years**	**15-18 years**
Histidine	22	15	12	12	11
Isoleucine	36	27	23	22	21
Leucine	73	54	44	44	42
Lysine	64	45	35	35	33
Methionine+cysteine	31	22	18	17	16
Phenylalanine+tyrosine	59	40	30	30	28
Threonine	34	23	18	18	17
Tryptophan	9.5	6.4	4.8	4.8	4.5
Valine	49	36	29	29	28

5. Criteria (endpoints) on which to base dietary reference values (DRVs)

Current DRVs for protein are based on protein homeostasis measured as nitrogen balance. DRVs also take into account protein quality, which is related to the capacity of a protein source to meet both the requirement for nitrogen and the requirement for indispensable amino acids as limiting precursors for body protein synthesis. Other criteria taking into account functional and health consequences of protein intake may also be considered in order to derive reference values for protein intake.

5.1. Protein intake and protein and nitrogen homeostasis

5.1.1. Methods for the determination of protein requirement

5.1.1.1. Nitrogen balance

Nitrogen balance is the classical approach for the determination of protein requirement, and in the initial studies of indispensable amino acid requirements (FAO/WHO/UNU, 1985). Nitrogen balance is the difference between nitrogen intake and the amount lost in urine, faeces, and via the skin and other routes such as nasal secretions, menstrual losses or seminal fluid (IoM, 2005). In healthy adults at energy balance the protein requirement (maintenance requirement) is defined as that amount of dietary protein sufficient to achieve zero nitrogen balance. It is assumed that nitrogen balance will be negative when protein intakes are inadequate. In infants and children, nitrogen balance has to be positive to allow for growth. While the method has substantial practical limitations, mainly related to the accuracy of the measurements and the interpretation of the results (WHO/FAO/UNU, 2007), it remains the method of choice for determining protein requirement in adults (Rand et al., 2003).

5.1.1.2. The factorial method

The factorial approach is based on the assessment of the extent to which dietary protein nitrogen is absorbed and retained by the organism, and is able to balance daily nitrogen losses and to allow additional protein deposition in newly formed tissue for growth and in specific physiological conditions such as pregnancy or lactation. Obligatory nitrogen losses are estimated from subjects fed a diet that meets energy needs but is essentially protein-free, or more reliably are derived from the y-intercept of the slope of the regression line relating nitrogen intake to nitrogen retention. The requirement for dietary protein is considered to be the amount needed to replace nitrogen losses and to allow additional protein deposition, after adjustment for the efficiency of dietary protein utilisation (see section 2.4) and the quality of the dietary protein. The factorial method is used to calculate protein requirements in physiological conditions such as growth, pregnancy or lactation. A critical factor is the value used for efficiency of dietary protein utilisation.

Table 6: Previously used values for protein efficiency in different population groups and values used by EFSA.

Population group	Previously used values (%)	Values used by EFSA (%)
Adults	70[1], 47[2]	47
Infants and children (for maintenance and growth, respectively)	70[1], 47/58[2,3]	47/58
Pregnancy (for protein deposition)	70[1], 42[2], 43[3]	47
Lactation	70[1], 47[2]	47

[1]FAO/WHO/UNU (1985); [2]WHO/FAO/UNU (2007); [3]IoM (2005)

In healthy adults, the mean post-prandial protein efficiency in controlled optimal conditions is considered to be 70 %, and this value was first used in FAO/WHO/UNU (1985) as a reference for the different population groups including infants and women during pregnancy and lactation. However, the NPU value can be modified by various factors including the food matrix, the diet and certain physiological conditions. More recently, a value of 47 % was derived from nitrogen balance studies in healthy adults under maintenance conditions (Rand et al., 2003). For children, WHO/FAO/UNU (2007) estimated the NPU for protein deposition with growth to be 58 % from 6 months to 18 years, whereas IoM (2005) estimated it to be 58 % from 7 months to 13 years, and 47 % from 14 to 18 years. During lactation the NPU was estimated to be 47 %, and not to be different from that in non-lactating healthy adults (WHO/FAO/UNU, 2007). For ten pregnant adolescents, King et al. (1973) derived a relatively low value of nitrogen retention of 30 %. From different nitrogen balance studies, Calloway (1974) calculated a nitrogen retention of 25-30 %. However, in healthy pregnant women nitrogen efficiency was found to be increased in comparison with non-pregnant women receiving the same nitrogen intake above the requirement (Mojtahedi et al., 2002). From the study by King et al. (1973), IoM (2005) recalculated an NPU value of 43 % based on those six adolescents who demonstrated a positive efficiency at multiple levels of protein intake, and WHO/FAO/UNU (2007) recalculated the efficiency of utilisation of dietary protein to be 42 % after omitting the two subjects who gave negative gradients. Eight pregnant Indian women utilised 47 % of the dietary nitrogen when 60-118 g/d of mixed protein was consumed. The nitrogen intake of the Indian women was unrelated to nitrogen retention unless intakes above 0.45 g N/kg body weight per day were omitted (Jayalakshmi et al., 1959). A similar range of values has been observed in pregnant sows (Dunn and Speer, 1991; Jones and Maxwell, 1982; King and Brown, 1993; Renteria-Flores et al., 2008; Theil et al., 2002).

The Panel considers that for healthy adults a protein efficiency value of 47 % is reasonable since it is the value derived from the nitrogen balance studies used to define nitrogen requirement in adults. There is no convincing scientific evidence that protein efficiency for maintenance of body protein and for protein deposition is lower during pregnancy or lactation. As a consequence, the same value can be considered as that determined for healthy adults (47 %). For infants and children, a value of 58 % for growth is justified because of an increased efficiency of dietary protein utilisation, whilst for maintenance the same protein efficiency as for adults is applied.

5.1.1.3. Protein quality and reference pattern for indispensable amino acids

The protein requirement is dependent on the dietary protein quality, which is mainly determined by the pattern of indispensable amino acids in the protein. The reference pattern of amino acids for infants <0.5 years is the amino acid pattern of human milk. The reference pattern of amino acids (mg/g protein) for the assessment of protein quality for adults is derived from proposed data on the requirement for individual indispensable amino acids (WHO/FAO/UNU, 2007) by dividing the requirement (mg amino acid/kg body weight per day) by the average requirement for protein (g/kg body weight per day). Age specific scoring patterns for dietary proteins can be derived by dividing the requirement of each indispensable amino acid by the protein requirement of the selected age group (WHO/FAO/UNU, 2007) (Table 7).

In practice, three reference patterns are used: the amino acid pattern of human milk for infants <0.5 years, the 3-10 years reference pattern for infants and children, and the adult reference pattern.

Table 7: Scoring pattern (indispensable amino acid reference profiles) for infants, children, adolescents and adults (WHO/FAO/UNU, 2007).

	Infants, children, adolescents (mg/g protein)					Adults
	0.5 years	**1-2 years**	**3-10 years**	**11-14 years**	**15-18 years**	**(mg/g protein)**
Histidine	20	18	16	16	16	15
Isoleucine	32	31	31	30	30	30
Leucine	66	63	61	60	60	59
Lysine	57	52	48	48	47	45
Methionine+cysteine	28	26	24	23	23	22
Phenylalanine+tyrosine	52	46	41	41	40	30
Threonine	31	27	25	25	24	23
Tryptophan	8.5	7.4	6.6	6.5	6.3	6
Valine	43	42	40	40	40	39

5.1.2. Protein requirement of adults

In a meta-analysis by Rand et al. (2003), available nitrogen balance data as a function of nitrogen intake among healthy persons were analysed. Data obtained from 235 individuals, each studied at ≥3 test protein intakes, were gathered from 19 primary and secondary studies, and used for estimating the average requirement. Subjects were classified by sex and age (≤55 (n=221) and >55 years of age (n=14)), diets were classified by the main source of protein (animal (>90 % of total protein intake from animal sources), vegetable (>90 % of total protein intake from vegetable sources) or mixed), and climate was classified as temperate or tropical. As the distribution of individual requirements was significantly skewed and kurtotic, the mean was not a robust estimate of the centre of the population, and the median was taken as the average requirement.

The Panel notes that the study by Rand et al. (2003) concluded that the best estimate of average requirement for 235 healthy adults from 19 studies was 105 mg N/kg body weight per day (0.66 g high quality protein/kg per day). The 97.5th percentile of the population distribution of the requirement was estimated from the log median plus 1.96 times the SD of 0.12, and found to be 133 mg N/kg body weight per day (0.83 g high quality protein/kg body weight per day). Thus, 0.83 g protein/kg body weight per day can be expected to meet the requirements of most (97.5 %) of the healthy adult population. This value can be considered to fulfil the function of a PRI even though derived differently. The data did not provide sufficient statistical power to establish different requirements for different adult groups based on age, sex or dietary protein source (animal or vegetable proteins) (Rand et al., 2003). The Panel notes that considering only the primary studies based on 32 data points the requirement would be 101.5 mg/kg body weight per day, but that the statistical power is greatly reduced and that this value is not significantly different to the value of 105 mg N/kg body weight per day.

The Panel considers that the value of 0.66 g/kg body weight per day can be accepted as the AR and the value of 0.83 g/kg body weight per day as the PRI derived for proteins with a PD-CAAS value of 1.0. This value can be applied to usual mixed diets in Europe which are unlikely to be limiting in their content of indispensable amino acids (WHO/FAO/UNU, 2007).

5.1.2.1. Older adults

Few data are available on the protein requirement of older adults compared to young and middle aged adults. A negative nitrogen balance was observed in six elderly females (69 ± 5 years) consuming a diet providing 0.8 g protein/kg body weight per day for two weeks (Pannemans et al., 1997), and the same level of intake was associated with a decrease in the mid-thigh muscle area in ten men and women (aged 55-77 years) during 14 weeks, although whole body leucine metabolism and whole body composition were not affected (Campbell et al., 2001). Several studies concluded that the PRI for older adults may be greater than that for younger adults (0.83 g/kg body weight per day) (Gaffney-Stomberg et al., 2009; Thalacker-Mercer et al., 2010; Wolfe et al., 2008). This was particularly deduced from an assumed, although not significantly, lower efficiency of protein utilisation in the elderly (AFSSA, 2007; Rand et al., 2003). However, one study did not show differences between younger (21-46 years) and older (63-81 years) subjects after short-term assessment of nitrogen balance (Campbell et al., 2008). Some authors (Campbell and Leidy, 2007; Iglay et al., 2009) found that an increase in dietary protein alone does not change body composition or improve lean body mass unless accompanied by physical training programmes.

The Panel concludes that the available data are insufficient to specifically determine the protein requirement in the elderly, and that at least the same level of protein intake as for young adults has to be proposed for older adults.

5.1.3. Protein requirement of infants and children

The protein requirement of infants and children includes two components, i.e. maintenance requirement and growth requirement. This protein requirement can be defined as the minimum intake that will permit a positive nitrogen equilibrium to allow for growth in normally growing subjects who have an appropriate body composition, are in energy balance and are moderately physically active (WHO/FAO/UNU, 2007).

In the report by WHO/FAO/UNU (2007), estimates of the protein requirement from 6 months to 18 years were derived factorially as the sum of requirements for maintenance and growth corrected for efficiency of dietary protein utilisation. An average maintenance requirement of 0.66 g/kg body weight per day protein was applied to infants and children from 6 months to 18 years (Tables 8 and 9). Regression analysis of nitrogen balance studies on children from 6 months to 12 years resulted in a maintenance level of 110 mg N/kg body weight per day. Because this value was close to the adult maintenance value of 105 mg N/kg body weight per day and it could not be determined with certainty that maintenance values for infants and children differ from those for adults, the latter value was selected for the maintenance value for ages from six months onwards. Average daily needs for dietary protein for growth were estimated from average daily rates of protein deposition, calculated from studies on whole-body potassium deposition, and adjusted by an efficiency of utilisation of dietary protein of 58 %. The total average requirement for protein was adjusted according to the expected variability of maintenance and growth to give a value equivalent to the 97.5th percentile of the distribution as a measure of the PRI, based on the average requirement plus 1.96 SD.

The Panel agrees with the analysis of the data.

Table 8: Average protein requirement of infants from 6 months onwards and children up to 10 years of age derived by WHO/FAO/UNU (2007).

Age (years)	0.5	1	1.5	2	3	4	5	6	7	8	9	10
Maintenance requirement (g/kg bw x d^{-1})	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Growth requirement (g/kg bw x d^{-1})	0.46	0.29	0.19	0.13	0.07	0.03	0.03	0.06	0.08	0.09	0.09	0.09
Average requirement (g/kg bw x d^{-1})	1.12	0.95	0.85	0.79	0.73	0.69	0.69	0.72	0.74	0.75	0.75	0.75

Table 9: Average protein requirement of adolescents derived by WHO/FAO/UNU (2007).

Age (years)	11	12	13	14	15	16	17	18
Maintenance requirement (g/kg bw x d^{-1})	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
Growth requirement (g/kg bw x d^{-1})	0.09 (m) 0.07 (f)	0.08 (m) 0.06 (f)	0.07 (m) 0.05 (f)	0.06 (m) 0.04 (f)	0.06 (m) 0.03 (f)	0.05 (m) 0.02 (f)	0.04 (m) 0.01 (f)	0.03 (m) 0.00 (f)
Average requirement (g/kg bw x d^{-1})	0.75 (m) 0.73 (f)	0.74 (m) 0.72 (f)	0.73 (m) 0.71 (f)	0.72 (m) 0.70 (f)	0.72 (m) 0.69 (f)	0.71 (m) 0.68 (f)	0.70 (m) 0.67 (f)	0.69 (m) 0.66 (f)

5.1.4. Protein requirement during pregnancy

The protein requirement during pregnancy has to take into account the requirements for the deposition of new protein and the requirement for the maintenance of the weight gained, in addition to the requirement in the non-pregnant state. It can be determined by using either the nitrogen balance approach or the factorial approach.

In the nitrogen balance approach, nitrogen requirement is derived from nitrogen balance studies. This requires balance measurements in women at different levels of protein intake in order to determine the maximal nitrogen deposition potential, and to derive the nitrogen requirement from this maximal nitrogen deposition (Calloway, 1974). However, it appears from the available studies that there is a linear increase in apparent nitrogen deposition with increasing protein intake in pregnant women. The linear relationship between nitrogen intake and deposition towards the end of pregnancy is statistically significant[5] (Calloway, 1974; Jayalakshmi et al., 1959; Johnstone et al., 1981; King et al., 1973).

According to the slope of these equations, the average nitrogen efficiency is very low, i.e. between 21 and 47 %. The linear nature of the relation between nitrogen intake and retention does not permit the determination of a maximal nitrogen deposition potential, nor to derive a nitrogen requirement related to this maximal nitrogen deposition. The cause for this linear relationship remains unclear. This linear relation and the low level of nitrogen efficiency derived from the slopes indicate uncertainties and errors in the measurement of nitrogen balance, and implicate important limitations for the use of this approach to determine the nitrogen requirement in pregnant women.

The alternative approach is the factorial approach used by IoM (2005) and WHO/FAO/UNU (2007). The maintenance costs were based upon the mid-trimester increase in maternal body weight, and the maintenance value of 0.66 g/kg body weight per day was derived from the average requirement in healthy adults, assuming a CV of 12 %. Protein deposition in the foetus and maternal tissue has been estimated indirectly

[5] In the study by Jayalakshmi et al. (1959), a linear relationship was only obtained after exclusion of four values indicating nitrogen retention for intakes >0.45 g N/kg body weight per day.

from measurements of total body potassium accretion. However, studies show that protein is not deposited equally throughout pregnancy. For well-nourished women with a gestational weight gain of 13.8 kg, total protein deposition was estimated as 1.9 g/d in the second trimester and 7.4 g/d in the third trimester (Butte et al., 2003; WHO/FAO/UNU, 2007). For protein deposition towards the end of pregnancy, IoM (2005) derived a mean value of 7.2 g/d based on six studies estimating the increase in whole body potassium during pregnancy in 120 women. They then assumed that nitrogen accretion during the second trimester is only about half of that observed in the third trimester, leading to an estimated value for protein deposition of 3.6 g/d for the second trimester.

Based on an efficiency of protein utilisation of 42 %, WHO/FAO/UNU (2007) estimated that an additional 1, 9 and 31 g protein/d in the first, second and third trimesters, respectively, are required to support a gestational weight gain of 13.8 kg.

The Panel notes that a 42 % efficiency of protein utilisation is low, and cannot see a plausible reason to depart from the value of 47 % derived for adults for maintenance of body protein (see also section 5.1.1.2).

5.1.5. Protein requirement during lactation

The additional protein requirement for milk production can be estimated factorially from milk protein output and the efficiency of dietary protein utilisation for milk protein production. The efficiency of protein utilisation for milk protein production is unknown and was taken to be the same as for protein deposition in the non-lactating adult (47 %). In the report by WHO/FAO/UNU (2007), mean rates of milk production by well-nourished women exclusively breastfeeding their infants during the first six months postpartum and partially breastfeeding in the second six months postpartum, together with the mean concentrations of protein and non-protein nitrogen in human milk, were used to calculate mean milk protein output. The factor of 6.25 was used to convert milk nitrogen to protein. Thus, the additional dietary protein requirement during lactation will be an amount of dietary protein equal to milk protein output, taking into account an efficiency of protein utilisation of 47 %. Assuming a CV of 12 %, the additional protein intakes during the first six months of lactation were estimated as 19 g protein/d, falling to 13 g protein/d after six months.

The Panel accepts the approach of WHO/FAO/UNU (2007).

5.2. Protein intake and health consequences

Protein requirement and PRI are derived from nitrogen balance but several health outcomes associated with protein intake could also be considered as criteria for setting DRVs for protein. It is conceivable that, in case of sufficient evidence for a positive effect on health, a PRI for protein above the PRI derived from nitrogen balances and factorial estimates would result. In addition, potentially adverse effects on health should be taken into account when assessing a protein intake above the PRI derived from nitrogen balance.

5.2.1. Muscle mass

The major anabolic influences on muscle are contractile activity and feeding. Ingestion of sufficient dietary energy and protein is a prerequisite for muscle protein synthesis and maintenance of muscle mass and function.

As a result of feeding, anabolism occurs chiefly by an increase in protein synthesis. Insulin has a permissive role in increasing synthesis, and the availability of amino acids is crucial for net anabolism. *In vivo*, amino acids display an anabolic effect (Giordano et al., 1996; Volpi et al., 1996) and were shown to stimulate muscle protein synthesis (Bohe et al., 2003; Liu et al., 2002; Nair and Short, 2005; Nygren and Nair, 2003). There was no effect of a dietary protein level above the PRI on muscle mass and protein content, and a high protein diet of around 2 g/kg body weight per day has not been demonstrated to modulate skeletal protein synthesis in both exercising and non-exercising human subjects (Bolster et al., 2005; IoM, 2005; Juillet et al., 2008) or in animals (Almurshed and Grunewald, 2000; Chevalier et al., 2009; Masanés et al., 1999; Morens et al., 2001; Taillandier et al., 2003). However, increasing protein intake above the individual requirement increases amino acid oxidation and modifies protein turnover. When protein intake is increased from around 1 g/kg body weight per day to 2 g/kg body weight per day, the increase of amino acid oxidation is associated with stimulation of protein breakdown rates in the fasted state and a strong inhibition in the fed state, whereas whole-body protein synthesis rates are little affected (Forslund et al., 1998; Fouillet et al., 2008; Harber et al., 2005; Morens et al., 2003; Pacy et al., 1994; Price et al., 1994).

The branched chain amino acids (BCAA) (leucine, valine, isoleucine), particularly leucine, have been demonstrated to act as a signal for muscle protein synthesis *in vitro* (Buse and Reid, 1975; Busquets et al., 2002; Dardevet et al., 2000; Fulks et al., 1975; Hong and Layman, 1984; Kimball et al., 1998; Kimball et al., 1999; Li and Jefferson, 1978; Mitch and Clark, 1984; Mordier et al., 2000; Tischler et al., 1982). *In vivo* experiments in animal models have been less consistent, but confirm *in vitro* results that leucine acts as a signal that up-regulates muscle protein synthesis and/or down-regulates muscle protein degradation (Anthony et al., 2000; Dardevet et al., 2002; Funabiki et al., 1992; Guillet et al., 2004; Layman and Grogan, 1986; McNurlan et al., 1982; Nagasawa et al., 2002; Rieu et al., 2003). In contrast, there is limited information available on the influence of leucine alone on muscle protein synthesis in humans (Koopman et al., 2005; Nair et al., 1992; Schwenk and Haymond, 1987; Sherwin, 1978; Tessari et al., 1985). At present, there is no convincing evidence that chronic leucine supplementation above the requirement as previously defined at 39 mg/kg body weight per day is efficient in promoting an increase in muscle mass (Balage and Dardevet, 2010; Leenders et al., 2011). Thus, when the intake of protein is at the PRI based on nitrogen balance, and when amino acid requirements are met, an additional intake of leucine has no further effect on muscle mass.

The Panel considers that in healthy adults the available data on the effects of dietary protein intake on muscle mass and function do not provide evidence that it can be considered as a criterion to set a PRI for protein. There are no data showing that an additional intake of protein would increase muscle mass in different age groups who are in nitrogen balance, including subjects undertaking endurance or resistance exercise. There are also no data showing that an additional protein intake would increase muscle growth in children.

5.2.2. Body weight control and obesity

5.2.2.1. Infants

It has been proposed that the well-known difference in growth observed between formula-fed and breast-fed infants may be related to differences in protein intake estimated to be 1.4-1.8 times higher per kg body weight in formula-fed infants compared to breast-fed infants (Alexy et al., 1999). In addition, it has been suggested that a higher protein intake may contribute to an enhanced insulin secretion and release of insulin-like growth factor (IGF)-1 and IGF-binding protein (IGFBP)-1, which was observed in prospective feeding studies with infant formulae of different protein content (13, 15 or 18 g protein/L) and a breast-fed control group (Axelsson, 2006).

In a double-blind, randomised controlled manner the European Childhood Obesity Project explored whether two types of infant formulae (standard infant formula and follow-on formula) with either lower or higher protein content (1.8 vs. 2.9 g/100 kcal for infant formula and 2.2 vs. 4.4 g/100 kcal for follow-on formula, all complying with European regulatory standards) fed during the first year of life resulted in different growth in the first two years of life (Grote et al., 2010; Koletzko et al., 2009). A reference group of breast-fed infants was also studied. The mean weight attained at 24 months was 12.4 kg and 12.6 kg for the lower- and higher-protein groups, respectively; the adjusted z-score for weight-for-length was 0.20 (95% CI 0.06–0.34; $p=0.005$) higher in the higher-protein formula group than in the lower-protein formula group. Children fed lower-protein formula did not differ from breast-fed children with respect to weight-for-length and BMI, but weight and length were higher. Whether this statistically significant but small difference in growth observed in infants fed higher-protein formula persists and is related to obesity risk in later life is the subject of ongoing investigations. Currently, these preliminary results do not allow conclusions to be made on the effects of protein intake with regard to obesity development.

The Panel considers that the results from these studies are not suitable for the derivation of a PRI or a UL for protein for infants and children.

5.2.2.2. Adults

Controlled studies in humans have investigated whether an increase in protein intake (as E%) *ad libitum* induces a decrease in body weight and adiposity. However, these studies are difficult to interpret with respect to whether the effects observed are due to an increase in dietary protein intake or to the concomitant modification of carbohydrate and/or fat intakes, and whether any observed effect of an increase in dietary protein intake would be sustainable (Brehm et al., 2003; Foster et al., 2003; Larsen et al., 2010; Samaha et al., 2003; Skov et al., 1999b; Weigle et al., 2005; Westerterp-Plantenga et al., 2004; Yancy et al., 2004). A recent review of the literature concluded that there is strong and consistent evidence that when calorie intake is controlled, the macronutrient proportion of the diet is not directly related to weight loss (DGAC, 2010).

The Panel considers that these data cannot be used to derive a PRI for protein for adults.

5.2.3. Insulin sensitivity and glucose control

Contradictory results have been obtained for the effects of an increase in protein intake above the PRI on insulin sensitivity and glucose tolerance. Some human studies showed no effects of a high protein intake on insulin sensitivity and glucose tolerance (Kitagawa et al., 1998; Tsunehara et al., 1990), but a high protein intake was found to be accompanied by an increased insulin secretion and demand (Linn et al., 2000). In other studies, a high protein intake was shown to improve insulin sensitivity and glucose tolerance in humans (Baba et al., 1999; Gannon et al., 2003; Layman et al., 2003; Piatti et al., 1994; Sharman et al., 2002; Volek et al., 2002) and animals (Karabatas et al., 1992; Lacroix et al., 2004; Wang et al., 1998). A beneficial effect of a high-protein diet on insulin resistance and glucose homeostasis has also been reported with a reduced calorie diet, regardless of weight loss (Farnsworth et al., 2003). In contrast, studies conducted *in vitro* or in animal models suggested that exposure to high levels of branched chain amino acids could have a deleterious effect on insulin signalling, leading to impaired insulin sensitivity and impaired glucose tolerance (Nair and Short, 2005; Patti et al., 1998; Tremblay and Marette, 2001). This was also suggested by a human cohort study with a follow-up of 12 years showing that high blood levels of five branched-chain and aromatic amino acids (isoleucine, leucine, valine, tyrosine and phenylalanine), which are known to be modified by amino acid profiles of the diet, were significantly associated with future diabetes (Wang et al., 2011). In contrast, prolonged leucine supplementation (7.5 g/d) in elderly type 2 diabetics habitually consuming an adequate amount of dietary protein did not modulate their glycaemic control (Leenders et al., 2011).

The Panel considers that these data cannot be used to derive a PRI or a UL for protein for healthy subjects.

5.2.4. Bone health

Protein and calcium are main components of bone structure, and it is widely accepted that protein deficiency increases the risk of bone fragility and fracture (Dawson-Hughes, 2003; Hannan et al., 2000; Kerstetter et al., 2000; Munger et al., 1999; Promislow et al., 2002; Skov et al., 2002; Zernicke et al., 1995). In several epidemiological studies, bone mineral density is positively related to protein intake (Chiu et al., 1997; Cooper et al., 1996; Devine et al., 2005; Geinoz et al., 1993; Hannan et al., 2000; Lau et al., 1998; Promislow et al., 2002; Teegarden et al., 1998; Tucker et al., 2001).

Although protein is essential for bone health, it has been observed that an increase in protein intake could also be associated with an increase in urinary calcium excretion. It was first hypothesised that this could originate from an activation of bone resorption in order to provide calcium for the neutralisation of the acid load produced by the oxidation of sulphur amino acids (Barzel and Massey, 1998). However, an increase in protein intake is often associated with an increase in calcium intake (Heaney, 1998), and also induces an increase in calcium absorption (Kerstetter et al., 1998, 2003) that can be related to the increased urinary calcium. In addition, the regulation of body acid load is a complex process in which urinary acidity is not directly related to blood acidity; moreover, the theory that considers bone mineral mobilisation as the main physiological system involved in the regulation of extracellular hydrogen ion concentration is questionable since it does not take into account the major role of both the respiratory and the renal tubular systems in this regulation (Fenton et al., 2009). Some studies have shown a positive relationship between protein intake and the risk of bone fracture (Abelow et al., 1992; Frassetto et al., 2000; Hegsted, 1986), whereas others have found no clear association (Meyer et al., 1997; Mussolino et al., 1998) or have shown an inverse association

(Munger et al., 1999). Intervention studies did not show clear results of a protein intake above the PRI on markers of bone formation or resorption (Cao et al., 2011; Darling et al., 2009; Fenton et al., 2009).

The Panel considers that the available evidence is insufficient to be taken into consideration when deriving a PRI or a UL for protein.

5.2.5. Kidney function

Protein intake is a modulator of renal function and increases the glomerular filtration rate (GFR) (Brändle et al., 1996). An increase in amino acid catabolism induced by an increase in protein intake increases the production of amino acid-derived metabolites such as bicarbonate**,** ammonia and urea which require elimination from the body, for example via the kidneys.

High protein diets have been found to be associated with increases in blood urea levels and urinary urea excretion, to promote plasma vasopressin, to increase creatinine clearance, and to result in a transient increase in kidney size in humans (Brändle et al., 1996; Diamond, 1990; Gin et al., 2000; Jenkins et al., 2001; Lentine and Wrone, 2004; Zeller, 1991) and animals (Dunger et al., 1997; Hammond and Janes, 1998; Lacroix et al., 2004; Schoknecht and Pond, 1993). High intakes of protein by patients with renal disease contribute to the deterioration of kidney function, and a reduction of protein intake is usually beneficial to subjects with renal insufficiency (Klahr et al., 1994; Knight et al., 2003; Maroni and Mitch, 1997), and possibly also to subjects with microalbuminuria (Friedman, 2004). In contrast, protein intake at the PRI based on nitrogen balance is not a risk factor for renal insufficiency in healthy subjects (Locatelli et al., 1991; Skov et al., 1999a; Wiegmann et al., 1990). According to the available evidence (WHO/FAO/UNU, 2007), the decline of GFR that occurs with advancing age in healthy subjects cannot be attenuated by reducing dietary protein intake below the PRI based on nitrogen balance.

As reported in the DRVs for water (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2010), urine osmolarity is physiologically limited between about 50 and 1,400 mOsm/L, and dehydration of more than 10 % at high ambient temperatures is a serious risk for a life-threatening heat stroke with elevated body temperature, inadequate cardiac output leading to reduced perfusion of tissues and eventually to rhabdomyolysis (i.e. rapid breakdown of skeletal muscle) and organ failure (Bouchama and Knochel, 2002). This risk is particularly high in infants with gastro-enteritis receiving a formula with a high potential renal solute load (Fomon, 1993). Water required for the excretion of solutes is determined by the composition of the diet, and by the concentrating capacity of the kidneys. Because the protein content of the diet is, as a rule, the main determinant of the potential renal solute load which needs water for excretion, a very high protein intake (±20 E%, e.g. cow's milk) with a consecutive increased production of urea can severely impair the water balance of infants, particularly when no other liquids are consumed and/or extrarenal water losses, for example through diarrhoea, are increased.

The Panel considers that the available evidence is insufficient to be taken into consideration when deriving a UL for protein.

5.2.6. Capacity of the urea cycle

It is established that there is adequate capacity in the human metabolism to adapt to a large range of protein intakes above the PRI based on nitrogen balance. This is mainly due to the adaptation of amino acid catabolic pathways, and it is established that amino acid oxidation varies at a rate dependent on the habitual protein intake. The level of protein intake has been evaluated in relation to the capacity of the urea cycle to control the transfer to urea of ammonia released from amino acid deamination (AFSSA, 2007). They concluded that for a healthy human adult male, protein intake in the range of 0.83 to 2.2 g/kg body weight per day (around 10 to 27 E%) is considered as safe, whilst IoM (2005) concluded that the maximum rate of urea production of a 70 kg male not commonly consuming a high-protein diet corresponds to a protein intake of 250 g/d or about 40 E%.

The Panel considers that the available evidence is insufficient to be taken into consideration when deriving a UL for protein.

5.2.7. Tolerance of protein

IoM (2005) quotes some reports on very high protein intakes up to 35 E% without adverse effects, whereas acute adverse effects were reported for intakes ≥45 E% and lethal outcomes occurred when such a diet was consumed by adults for several weeks. In Europe, adult protein intakes at the upper end of the intake distributions (90-97.5th percentile) have been reported to be between 17 and 25 E% (Appendix 2B).

The available data on the tolerance of dietary protein are not sufficient to derive a UL for protein.

6. Data on which to base dietary reference values (DRVs)

6.1. Protein requirement of adults

The criterion of adequacy for protein intake is the lowest intake that is sufficient to achieve body nitrogen equilibrium (zero balance), during energy balance. The analysis of available nitrogen balance data performed by Rand et al. (2003) concluded that the best estimate of average requirement for healthy adults was the median requirement of 105 mg N/kg body weight per day or 0.66 g protein/kg body weight per day (N x 6.25). The 97.5th percentile of the population distribution of requirement was estimated as 133 mg N/kg body weight per day, or 0.83 g protein/kg body weight per day. This quantity should meet the requirement of most (97.5 %) of the healthy adult population, and is therefore proposed as the PRI for protein for adults. The protein requirement per kg body weight is considered to be the same for both sexes and for all body weights. The PRI of 0.83 g/kg body weight per day is applicable both to high quality proteins and to protein in mixed diets.

6.1.1. Protein requirement of older adults

There is some evidence that protein efficiency and the anabolic response of muscle and bone to dietary protein is attenuated in elderly people, and can result in loss of bone and muscle, and in significant morbidity, osteoporosis and sarcopenia, which are degenerative diseases frequently associated with ageing. As a result, the amount of protein needed to achieve anabolism could be greater than for younger adults, particularly as a percentage of energy intake. However, no precise data are available for defining a PRI for older adults.

6.2. Protein requirement of infants and children

The protein requirement of infants and children can be defined as the minimum intake that will allow nitrogen equilibrium at an appropriate body composition during energy balance at moderate physical activity, plus the needs associated with the deposition of tissues consistent with growth and good health (WHO/FAO/UNU, 2007).

The Panel accepted the approach of WHO/FAO/UNU (2007) in which estimates of the protein requirement from 6 months to adulthood were derived from a factorial model. In selecting values for maintenance and growth efficiency for ages greater than 6 months, the likelihood that mixed diets consumed after weaning are utilised less efficiently is taken into account.

An average maintenance value of 0.66 g protein/kg body weight per day was applied to children and infants aged from 6 months to 18 years. Average daily needs for dietary protein for growth were estimated from average daily rates of protein deposition, and an efficiency of utilisation of dietary protein for growth of 58 % was assumed. The average requirement was then estimated as the sum of the maintenance and growth requirements.

The PRI was estimated based on the average requirement plus 1.96 SD; for this, a combined SD was calculated from the SD for growth for the respective age (see Appendix 3), which was adjusted for efficiency of utilisation of dietary protein (58 %), and the SD for maintenance (based on a CV of 12 % for all ages).

6.3. Protein requirement during pregnancy

The Panel follows the approach (WHO/FAO/UNU, 2007) in which the additional protein intake needed during pregnancy was derived from the newly deposited protein, taking into account the efficiency of protein utilisation and the maintenance costs associated with increased body weight. Mean total protein deposition and daily protein deposition in each trimester were estimated indirectly from measurements of total body potassium accretion and calculated for an average weight gain of 13.8 kg (the mid-point of the recommended weight gain range for women with normal pre-pregnancy weight) (IoM and NRC, 2009; WHO/FAO/UNU, 2007). The efficiency of protein utilisation was taken by the Panel to be 47 %. The maintenance costs were based upon the mid-trimester gain in maternal body weight and the adult maintenance value of 0.66 g/kg body weight per day. The PRI was estimated by adding 1.96 SD (with 1 SD calculated on the basis of a CV of 12 %) to give an additional 1, 9 and 28 g protein/d in the first, second and third trimesters, respectively (Table 10).

Table 10: Derivation of dietary reference values for protein during pregnancy.

Trimester	Mid-trimester weight gain (kg)	Additional protein for maintenance (g/d)[1]	Protein deposition (g/d)	Protein deposition, adjusted for efficiency[2] (g/d)	Additional protein requirement (g/d)	PRI, additional intake[3] (g/d)
1	0.8	0.5	0	0	0.5	1
2	4.8	3.2	1.9	4.0	7.2	9
3	11	7.3	7.4	15.7	23	28

[1] Mid-trimester increase in weight x average requirement (AR) for maintenance of protein for adults of 0.66 g/kg body weight per day.
[2] Protein deposition adjusted for the efficiency of protein utilisation during pregnancy: 47 %.
[3] Calculated as the average requirement plus allowance for estimated coefficient of variation of 12 %.

6.4. Protein requirement during lactation

The Panel accepted the factorial approach based on milk protein output assessment (from milk volumes and the content of both protein nitrogen and NPN) and calculation of the amount of dietary protein needed for milk protein production with an efficiency of utilisation of 47 %. The factor 6.25 was used to convert nitrogen to protein. The PRI was estimated by adding 1.96 SD (with 1 SD calculated on the basis of a CV of 12 %) to give an additional 19 g protein/d during the first six months of lactation, and 13 g protein/d after six months.

6.5. Safety of protein intakes above the PRI

A UL cannot be derived. Concerns about potential detrimental effects of very high protein intake remain controversial. Acute adverse effects have been reported for protein intakes ≥45 E%, but very high protein intakes up to 35 E% have not been associated with adverse effects in some reports. It can be concluded that in adults an intake of twice the PRI is safe. Such intakes from mixed diets are regularly consumed in Europe by some physically active and healthy individuals. Intakes of 3–4 times the PRI have been observed without apparent adverse effects or benefits.

Data from food consumption surveys show that actual mean protein intakes of adults in Europe are at, or more often above, the PRI of 0.83 g/kg body weight per day. Protein intakes as high as 1.7 g/kg body weight per day (95th percentile of the protein intake of Dutch men aged ≥65 years) or 25 E% have been observed (see Appendix 2B).

In infants, a very high protein intake (±20 E%) can severely impair the water balance, particularly when no other liquids are consumed and/or extrarenal water losses are increased. Consequently, such high protein intakes should be avoided in the first year of life.

CONCLUSIONS

The Panel concludes that an Average Requirement (AR) and a Population Reference Intake (PRI) for protein can be derived for adults, infants and children and pregnant and lactating women based on nitrogen balance studies and on factorial estimates of the nitrogen needed for deposition of newly formed tissue and for milk output. The Panel also considered several health outcomes that may be associated with protein intake, but the available data were considered insufficient to contribute to the setting of Dietary Reference Values (DRVs).

The Panel concludes that the available data are not sufficient to establish a Tolerable Upper Intake Level (UL) for protein.

Table 11: Summary of dietary reference values for protein.

Age (years)	AR (g/kg bw x d^{-1})	PRI (g/kg bw x d^{-1})	Reference weight (kg)[1]		PRI (g/d)	
			males (m)	females (f)	m	f
0.5	1.12	1.31	8.0	7.5	10	10
1	0.95	1.14	10.0	9.5	11	11
1.5	0.85	1.03	11.5	11.0	12	11
2	0.79	0.97	12.5	12.0	12	12
3	0.73	0.90	15.0	14.0	14	13
4	0.69	0.86	17.5	17.0	15	15
5	0.69	0.85	19.5	19.5	17	17
6	0.72	0.89	22.0	21.5	20	19
7	0.74	0.91	24.5	24.0	22	22
8	0.75	0.92	27.0	27.0	25	25
9	0.75	0.92	30.0	30.5	28	28
10	0.75	0.91	33.0	34.0	30	31
11	0.75 (m), 0.73 (f)	0.91 (m), 0.90 (f)	36.5	37.5	33	34
12	0.74 (m), 0.72 (f)	0.90 (m), 0.89 (f)	41.0	43.0	37	38
13	0.73 (m), 0.71 (f)	0.90 (m), 0.88 (f)	47.0	48.0	42	42
14	0.72 (m), 0.70 (f)	0.89 (m), 0.87 (f)	53.0	50.5	47	44
15	0.72 (m), 0.69 (f)	0.88 (m), 0.85 (f)	58.0	52.5	51	45
16	0.71 (m), 0.68 (f)	0.87 (m), 0.84 (f)	62.5	54.0	54	45
17	0.70 (m), 0.67 (f)	0.86 (m), 0.83 (f)	64.5	54.5	55	45
18-59	0.66	0.83	74.6	62.1	62	52
≥ 60	0.66	0.83	73.5	66.1	61	55
Pregnant women[2]						
1st trimester						+1
2nd trimester						+9
3rd trimester						+28
Lactating women[2]						
0-6 months *post partum*						+19
>6 months *post partum*						+13

[1] For infants and children, based upon weighted mean body weights (kg) of European children. For children aged 4-17 years, the body weights given in the table refer to children actually aged 0.5 years older than the age stated, i.e. 4.5 years, 5.5 years, etc. (SCF, 1993). For adults, based upon weighted median body weights (kg) of European men and women (SCF, 1993).
[2] In addition to the PRI for non-pregnant women.

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Appendices

Appendix 1A: Population, methods and period of dietary assessment in children and adolescents in European countries

Country	Population	Dietary method	Year of survey	Reference
Austria	Boys and girls aged 7-9 years	3-day record	2007	(Elmadfa et al., 2009b)
	Boys and girls aged 10-14 years	3-day record	2007	(Elmadfa et al., 2009b)
	Boys and girls aged 14-19 years	24-hour recall	2003-2004	(Elmadfa et al., 2009b)
Belgium	Boys and girls aged 2.5-3 years	3-day record	2002-2003	(Huybrechts and De Henauw, 2007)
	Boys and girls aged 4-6.5 years	3-day record	2002-2003	(Huybrechts and De Henauw, 2007)
	Boys and girls aged 13-15 years	7-day record	1997	(Matthys et al., 2003)
	Boys and girls aged 15-18	2 x 24-hour recall	2004	(De Vriese et al., 2006)
Czech Republic	Boys and girls aged 4-6 years	2 x 24-hour recall	2007	(In: Elmadfa et al. (2009a))
	Boys and girls aged 7-9 years	2 x 24-hour recall	2007	(In: Elmadfa et al. (2009a))
Denmark	Boys and girls aged 1-3 years	7-day record	1995	(Andersen et al., 1996)
	Boys and girls aged 4-5 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Boys and girls aged 6-9 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Boys and girls aged 10-13 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Boys and girls aged 14-17 years	7-day record	2003-2008	(Pedersen et al., 2010)
Finland	Infants aged 8 months	3-day record	1999	(Lägstrom, 1999)
	Children aged 3 years	4-day record	1999	(Lägstrom, 1999)
	Children aged 4 years	4 day record	1999	(Lägstrom, 1999)
	Children aged 4 years	3-day record	2008	(Kyttälä et al., 2008)
	Children aged 6 years	3-day record	2008	(Kyttälä et al., 2008)
France	Boys and girls aged 4-6 years	3 x 24-hour recall	2006-2007	(In: Elmadfa et al., (2009a))
	Boys and girls aged 7-9 years	3 x 24-hour recall	2006-2007	(In: Elmadfa et al., (2009a))
	Boys and girls aged 10-14 years	3 x 24-hour recall	2006-2007	(In: Elmadfa et al., (2009a))
	Boys and girls aged 15-18 years	3 x 24-hour recall	2006-2007	(In: Elmadfa et al., (2009a))
Germany	Infants aged 12 months	3-day record	1989-2003	(Hilbig and Kersting, 2006)
	Children aged 18 months	3-day record	1989-2003	(Hilbig and Kersting, 2006)
	Children aged 2 years	3-day record	1989-2003	(Hilbig and Kersting, 2006)
	Children aged 3 years	3-day record	1989-2003	(Hilbig and Kersting, 2006)
	Boys and girls aged 6 years	3-day record	2006	(Mensink et al., 2007)
	Boys and girls aged 7-9 years	3-day record	2006	(Mensink et al., 2007)
	Boys and girls aged 10-11 years	3-day record	2006	(Mensink et al., 2007)
	Boys and girls aged 12 years	Dietary history (over the last 4 weeks)	2006	(Mensink et al., 2007)
	Boys and girls aged 13-14 years	Dietary history (over the last 4 weeks)	2006	(Mensink et al., 2007)
	Boys and girls aged 15-17 years	Dietary history (over the last 4 weeks)	2006	(Mensink et al., 2007)
Greece	Boys and girls aged 4-5 years	3-day record+24-hour recall / 3-day record	2003-2004	(Manios et al., 2008)

Country	Population	Dietary method	Year of survey	Reference
Hungary	Boys and girls aged 11-14 years	3 x 24-hour recall	2005-2006	(Biro et al., 2007; Elmadfa et al., 2009a)
Ireland	Boys and girls 5-8 years	7-day record	2003-2004	Irish Universities Nutrition Alliance, (IUNA) www.iuna.net
	Boys and girls 9-12 years	7-day record	2003-2004	Irish Universities Nutrition Alliance, (IUNA) www.iuna.net
Italy	Boys and girls 0-<3 years	consecutive 3-day food records	2005-2006	(Sette et al., 2010)
	Boys and girls 3-<10 years	consecutive 3-day food records	2005-2006	(Sette et al., 2010)
	Boys and girls 10-<18 years	consecutive 3-day food records	2005-2006	(Sette et al., 2010)
The Netherlands	Infants aged 9 month	2-day record (independent days)	2002	(de Boer et al., 2006)
	Infants aged 12 monts	2-day record (independent days)	2002	(de Boer et al., 2006)
	Children aged 18 months	2-day record (independent days)	2002	(de Boer et al., 2006)
	Boys and girls aged 2-3 years	2-day record (independent days)	2005-2006	(Ocke et al., 2008)
	Boys and girls aged 4-6 years	2-day record (independent days)	2005-2006	(Ocke et al., 2008)
	Boys and girls aged 7-9 years	2-day record	1997-1998	(Hulshof et al., 1998)
	Boys and girls aged 10-12 years	2-day record	1997-1998	(Hulshof et al., 1998)
	Boys and girls aged 13-15 years	2-day record	1997-1998	(Hulshof et al., 1998)
	Boys and girls aged 16-19 years	2-day record	1997-1998	(Hulshof et al., 1998)
Norway	Children aged 2 years	Food Frequency Questionnaire	1998-1999	(Lande and Andersen, 2005)
	Boys and girls aged 4 years	4-day record	2000	(Øverby and Andersen, 2002)
	Boys and girls aged 9 years	4-day record	2000	(Øverby and Andersen, 2002)
	Boys and girls aged 13	4-day record	2000	(Øverby and Andersen, 2002)
	Boys and girls aged 16-19 years	Food Frequency Questionnaire	1997	(Johansson and Sovoll, 1999)
Poland	Boys and girls aged 4-6 years	24-hour recall	2000	(In: Elmadfa et al., (2009a))
	Boys and girls aged 7-9 years	24-hour recall	2000	(In: Elmadfa et al., (2009a))
	Boys and girls aged 10-14 years	24-hour recall	2000	(In: Elmadfa et al., (2009a))
	Boys and girls aged 15-18 years	24-hour recall	2000	(In: Elmadfa et al., (2009a))
Portugal	Boys and girls aged 7-9 years	24-hour recall	2000-2002	(Moreira et al., 2005)
	Boys and girls aged 13 years	24-hour recall	2000-2002	(Moreira et al., 2005)
Slovenia	Boys and girls aged 14-17 years	Food Frequency Questionnaire	2003-2005.	(In: Elmadfa et al., (2009a))
Sweden	Boys and girls aged 4 years	4-day record	2003	(Enghardt-Barbieri et al., 2006)
	Boys and girls aged 8-9 years	4-day record	2003	(Enghardt-Barbieri et al., 2006)
	Boys and girls aged 11-12 years	4-day record	2003	(Enghardt-Barbieri et al., 2006)
Spain	Boys and girls aged 10-14 years	2 x 24-hour recall	2002-2003	(Elmadfa et al., 2009a; Serra-Majem et al., 2007)
	Boys and girls aged 15-18 years	2 x 24-hour recall	2002-2003	(Elmadfa et al., 2009a; Serra-Majem et al., 2007)
United Kingdom	Boys and girls aged 4-6 years	7-day record	1997	(Gregory et al., 2000)
	Boys and girls aged 7-10 years	7-day record	1997	(Gregory et al., 2000)
	Boys and girls aged 11-14 years	7-day record	1997	(Gregory et al., 2000)
	Boys and girls aged 15-18 years	7-day record	1997	(Gregory et al., 2000)

APPENDIX 1B: INTAKE OF PROTEIN AMONG CHILDREN AGED ~1-3 YEARS IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d^{-1})		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Infants and young children (both sexes)											
Finland	8 mo	215	12	3		25	6				
	13 mo	449	17	4		42	10				
	2 years	398	16	3		45	10				
	3 years	359	15	4		47	12				
Germany	12 mo	432	13.2	2.2							
	18 mo	478	13.9	2.1							
	2 years	458	13.6	2.2							
	3 years	427	12.9	2.0							
Italy	0-<3 years	52	14.7	4.4	5.7-21.6	41.5	18.0	7.7-71.3	3.64	1.24	1.46-5.58
The Netherlands	9 mo	333	11.8	1.4	10.2-13.7[1]	28.8	6.2	21.4-27.0[1]			
	12 mo	306	13.7	2.5	10.8-17.0[1]	36.5	8.3	26.8-47.6[1]			
	18 mo	302	15.0	2.1	12.4-17.7[1]	43.1	6.5	34.9-51.5[1]			
Norway	2 years	172	13.4	1.8		47.2	14.2				
Young children											
Males											
Belgium	2.5-3	102	16.2	2.4		62.5	11.3				
Denmark	1-3	129	13			52					
The Netherlands	2-3	313	13		11-16	44		31-60			
Females											
Belgium	2.5-3	95	16.7	1.6		57.7	11.3				
Denmark	1-3	149	14			54					
The Netherlands	2-3	313	13		11-16	43		31-57			

[1]P10-P90

APPENDIX 1C: INTAKE OF PROTEIN AMONG CHILDREN AGED ~4-6 YEARS IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d^{-1})		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males											
Belgium	4-6.5	236	15.4	2.2		58.5	10.0				
Czech Republic	4-6	641	14.0	2.2							
Denmark	4-5	81	14	2.0	11-18	63	13	44-85			
Finland	4	307	15								
	6	364	16								
France	4-6	164	15.5	0.1[1]							
Germany	6	106	13.3	1.9	10.3-17.1	55.3	10.8	39.5-76.9			
Greece	4-5	356	16.4	2.5							
The Netherlands	4-6	327	13		10-16	51		33-70			
Norway	4	206	14.2	2.3		52.4	14.5				
Poland	4-6	82	11.1	2.3							
Sweden	4	302	14.4	2.2	10.9-18.1	55	13	35-77			
United Kingdom	4-6	184	12.9	1.8	9.6-16.3[2]	49.0	13.4	25.4-76.8[2]			
Females											
Belgium	4-6.5	228	15.1	2.0		52.9	10.5				
Czech Republic	4-6	446	14.0	2.2							
Denmark	4-5	78	14	2.0	12-18	58	14	35-80			
Finland	4	307	15								
	6	349	15								
France	4-6	162	15.0	0.2[1]							
Germany	6	102	13.6	2.0	11.0-18.5	50.6	12.4	32.1-68.1			
Greece	4-5	389	16.3	2.3							
The Netherlands	4-6	312	13		10-16	46		32-60			
Norway	4	185	14.0	2.2		49.5	11.9				
Poland	4-6	84	12.0	2.8							
Sweden	4	288	14.4	2.1	11.3-18.1	51	11	34-71			
United Kingdom	4-6	171	12.7	2.0	9.4-17.1[2]	44.5	11.1	26.3-66.8[2]			
Both sexes											
Italy	3-<10	193	15.7	2.3	12.5-19.5	74.1	18.5	46.9-109.4	3.05	1.02	1.57-4.73

[1]SE; [2]P2.5-P97.5

APPENDIX 1D: INTAKE OF PROTEIN AMONG CHILDREN AGED ~7-9 YEARS IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d[-1])		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males											
Austria	7-9	146	14.4	2.7							
Czech Republic	7-9	940	14.5	2.4							
Denmark	6-9	172	14	2.1	10-18	73	19	48-102			
France	7-9	160	14.7	0.2[1]							
Germany	7-9	321	13.5	2.1	10.4-17.4	62.0	14.0	40.6-87.0			
Ireland	5-8	145	13.6	2.0	10.6-17.1	55.3	15.8	33.8-82.8			
The Netherlands	7-9	104	13.5	2.7	9.8-18.8	66	15	44-94	2.3	0.7	1.4-3.2
Norway	9	402	14	2		73	21				
Poland	7-9	101	11.7	2.8							
Portugal	7-9	1541	16.6	3.8							
Sweden	8-9	444	15.4	2.3	11.9-19.6	72	17	48-101			
United Kingdom	7-10	256	12.4	1.9	9.0-17.1[2]	54.8	12.3	34.5-79.5[2]			
Females											
Austria	7-9	134	13.5	2.7							
Czech Republic	7-9	765	14.5	2.4							
Denmark	6-9	151	14	2.0	11-17	63	14	43-90			
France	7-9	144	15.0	0.3[1]							
Germany	7-9	308	13.6	2.7	9.5-18.5	55.5	14.9	35.6-81.3			
Ireland	5-8	151	13.7	2.1	10.3-17.1	51.9	12.8	34.7-73.0			
The Netherlands	7-9	134	13.5	2.6	9.9-17.6	61	16	36-90	2.2	0.6	1.4-3.3
Norway	9	408	14	3		63	20				
Poland	7-9	103	11.3	2.5							
Portugal	7-9	1503	16.6	3.7							
Sweden	8-9	445	15.4	2.2	12.1-19.2	65	15	43-92			
United Kingdom	7-10	226	12.8	1.9	9.5-16.7[2]	51.2	11.1	29.5-75.2[2]			

[1]SE ;[2]P2.5-P97.5

APPENDIX 1E: INTAKE OF PROTEIN AMONG CHILDREN AGED ~10-14 YEARS AND OVER IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d^{-1})		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males											
Austria	10-14	248	14.6	3.2							
Belgium	13-15	74	14.7	2.1							
Denmark	10-13	164	15	2.3	11-18	79	20	49-109			
France	10-14	160	15.5	0.2[1]							
Germany	10-11	199	13.8	2.3	10.3-18.1	64.4	16.2	43.1-94.5			
	12	114	13.3	1.9	10.5-16.5	82.5	29.1	46.2-135.5			
	13-14	214	13.7	2.3	10.3-17.4	94.0	33.9	47.5-159.6			
Hungary	11-14	124	14.6	2.0		89.7	18.9		1.99	0.59	
Ireland	9-12	148	13.6	2.4	9.5-18.0	64.2	15.8	40.9-90.9			
Italy	10-<18	108	15.6	1.9	12.9-19.2	99.3	26.2	62.8-147.1	1.82	0.59	1.02-3.22
The Netherlands	10-12	112	13.4	2.4	9.2-17.6	74	20	45-110	1.9	0.6	1.0-3.1
	13-15	137	13.1	2.4	9.0-17.4	84	22	51-126	1.6	0.5	0.9-2.5
Norway	13	590	15.0	3.0							
Poland	10-14	202	11.5	2.8							
Portugal	13	987	17.3	2.6							
Sweden	11-12	517	15.9	2.7	11.8-20.5	72	19	44-106			
Spain	10-14	66	16.9	2.1							
United Kingdom	11-14	237	13.1	2.2	8.9-17.6[2]	64	15.4	30.9-93.9[2]			
Females											
Austria	10-14	239	14.1	3.0							
Belgium	13-15	89	15.3	2.5							
Denmark	10-13	196	14	2.2	11-18	65	18	36-91			
France	10-14	144	15.6	0.2[1]							
Germany	10-11	198	13.7	2.4	10.3-18.0	60.7	15.3	32.2-86.4			
	12	103	13.1	1.9	9.6-16.3	70.4	23.7	36.4-112.0			
	13-14	230	13.1	2.2	9.7-17.0	73.0	21.7	40.5-115.3			
Hungary	11-14	111	13.9	1.9		75.4	15.3		1.73	0.60	
Ireland	9-12	150	13.5	2.2	9.8-17.2	55.6	13.4	35.8-80.5			
Italy	10-<18	139	15.8	2.2	12.2-19.8	81.8	20.1	49.4-118.7	1.74	0.56	0.97-2.94
The Netherlands	10-12	124	13.0	2.3	9.3-16.6	66	15	45-97	1.7	0.5	1.1-2.4
	13-15	117	13.7	2.5	10.1-17.9	70	17	44-101	1.3	0.4	0.8-1.9
Norway	13	515	14.0	3.0							
Poland	10-14	202	11.7	2.7							
Portugal	13	1053	17.1	2.9							
Sweden	11-12	499	15.4	2.7	11.1-20.2	62	17	37-91			
Spain	10-14	53	17.6	1.9							
United Kingdom	11-14	238	12.7	2.2	9.2-17.9[2]	52.9	13.2	26.9-78.4[2]			

[1]SE; [2]P2.5-P97.5

APPENDIX 1F: INTAKE OF PROTEIN AMONG ADOLESCENTS AGED ~15-18 YEARS AND OVER IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d^{-1})		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males											
Austria	14-19	1527	16.1	4.0							
Belgium	15-18	405	13.8	2.1							
Denmark	14-17	101	15	2.3	11-19	88	28	46-135			
France	15-18	181	15.7	0.3[1]							
Germany	15-17	294	13.9	2.5	10.6-17.1	116.1	48.2	62.3-201.0			
The Netherlands	16-18	142	13.3	2.6	9.0-18.4	90	26	51-134	1.3	0.4	0.7-1.9
Norway	16-19	92	14			114					
Poland	15-18	174	12.4	3.0							
Slovenia	15-18	1010	15.0	3.0							
Spain	15-18	61	17.8	2.6							
United Kingdom	15-18	179	13.9	2.5	9.4-19.6[2]	76.5	19.6	45.4-112.2[2]			
Females											
Austria	14-19	1422	14.7	4.1							
Belgium	15-18	401	13.7	2.1							
Denmark	14-17	134	14	2.2	11-18	61	21	28-98			
France	15-18	222	15.6	0.2[1]							
Germany	15-17	317	12.9	2.3	9.6-16.7	75.0	32.3	37.8-125.6			
The Netherlands	16-18	139	13.4	2.6	9.0-18.4	72	20	39-108	1.2	0.4	0.7-1.8
Norway	16-19	62	15			80					
Poland	15-18	175	12.0	2.9							
Slovenia	15-18	1214	14.0	3.0							
Spain	15-18	57	18.0	2.5							
United Kingdom	15-18	210	13.9	2.5	9.9-18.9[2]	54.8	15.2	26.4-87.4[2]			

[1]SE; [2]P2.5-P97.5

APPENDIX 2A: POPULATION, METHODS AND PERIOD OF DIETARY ASSESSMENT IN ADULTS IN EUROPEAN COUNTRIES

Country	Population	Dietary method	Year of survey	Reference
Austria	Males and females aged 19-64 years	24-hour recall	2007	(Elmadfa et al., 2009b)
	Males and females aged 65 and over	3-day record	2007	(Elmadfa et al., 2009b)
Belgium	Males and females aged 19-59 years	2 x 24-hour recall	2004	(De Vriese et al., 2006)
	Males and females aged 60-75 years	2 x 24-hour recall	2004	(De Vriese et al., 2006)
	Males and females aged 75+ years	2 x 24-hour recall	2004	(De Vriese et al., 2006)
Czech Republic	Males and females aged 19-64 years	24-hour recalls	2000-2001	(Cifkova and Skodova, 2004; Elmadfa et al., 2009a)
Denmark	Males and females aged 18-74 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Males and females aged 18-24 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Males and females aged 25-34 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Males and females aged 35-44 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Males and females aged 45-54 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Males and females aged 55-64 years	7-day record	2003-2008	(Pedersen et al., 2010)
	Males and females aged 65-75 years	7-day record	2003-2008	(Pedersen et al., 2010)
Estonia	Males and females aged 19-64 years	24-hour recall	1997	(Pomerleau et al., 2001)
	Males and females aged 19-34 years	24-hour recall	1997	(Pomerleau et al., 2001)
	Males and females aged 35-49 years	24-hour recall	1997	(Pomerleau et al., 2001)
	Males and females aged 50 -64	24-hour recall	1997	(Pomerleau et al., 2001)
Finland	Males and females aged 25-64 years	3-day record	2002	(Paturi et al., 2008)
	Males and females aged 65-74 years	4-day record	2002	(Paturi et al., 2008)
France	Males and females aged 19-64 years	3 x 24-hour recall	2006-2007	(In: Elmadfa et al., (2009a))
	Males and females aged 65-75 years	3 x 24-hour recall	2006-2007	(In: Elmadfa et al., (2009a))
Germany	Males and females aged 35-64 years	24-hour recall	1996-1998	(Linseisen et al., 2003)
	Males and females aged 19-80 years	24-hour recall + Dietary History	2005-2006	(Anonymous, 2008)
	Males and females aged 19-24 years	24-hour recall + Dietary History	2005-2006	(Anonymous, 2008)
	Males and females aged 25-34 years	24-hour recall + Dietary History	2005-2006	(Anonymous, 2008)
	Males and females aged 35-50 years	24-hour recall + Dietary History	2005-2006	(Anonymous, 2008)
	Males and females aged 51-64 years	24-hour recall + Dietary History	2005-2006	(Anonymous, 2008)
	Males and females aged 65-80 years	24-hour recall + Dietary History	2005-2006	(Anonymous, 2008)
Greece	Males and females aged 19-64 years	FFQ + 24-hour recall in sub group	1994-1999	(In: Elmadfa et al., (2009a))
	Males and females aged 65 and over	FFQ	1994-1999	(In: Elmadfa et al., (2009a))
Hungary	Males and females aged 18-59	3-day record	2003-2004	(Elmadfa et al., 2009a; Rodler et al., 2005)
	Males and females aged 60 and over	3-day record	2003-2004	(Elmadfa et al., 2009a; Rodler et al., 2005)

Country	Population	Dietary method	Year of survey	Reference
Ireland	Males and females 18-64 years	7-day record	1997-1999	Irish Universities Nutrition Alliance (IUNA)
	Males and females 18-35 years	7-day record	1997-1999	Irish Universities Nutrition Alliance (IUNA)
	Males and females 36-50 years	7-day record	1997-1999	Irish Universities Nutrition Alliance (IUNA)
	Males and females 51-64 years	7-day record	1997-1999	Irish Universities Nutrition Alliance (IUNA)
Italy	Males and females 18-<65years	consecutive 3-day food record	2005-2006	(Sette et al., 2010)
	Males and females aged 65 and over	consecutive 3-day food record	2005-2006	(Sette et al., 2010)
Latvia	Males and females 19-64 years	24-hour recall	1997	(Pomerleau et al., 2001)
	Males and females aged 19-34 years	24-hour recall	1997	(Pomerleau et al., 2001)
	Males and females aged 35-49 years	24-hour recall	1997	(Pomerleau et al., 2001)
	Males and females aged 50-64 yearsr	24-hour recall	1997	(Pomerleau et al., 2001)
Lithuania	Males and females 19-65 years	24-hour recall	2007	(In: Elmadfa et al., (2009a))
The Netherlands	Males and Females aged 19-64 years	2-day record	1997-1998	(Hulshof et al., 1998)
	Males and Females aged 65 and over	2-day record	1997-1998	(Hulshof et al., 1998)
	Males and females aged 19-30 years	2 x 24-hour recall	2003	(Hulshof and Ocke, 2005)
Norway	Males and females aged 19-64 years	FFQ	1997	(Johansson and Sovoll, 1999)
	Males and females aged 65 and over	FFQ	1997	(Johansson and Sovoll, 1999)
Poland	Males and females aged 19-64 years	24-hour recall	2000	(In: Elmadfa et al., (2009a))
	Males and females aged 65 and over	24-hour recall	2000	(In: Elmadfa et al., (2009a))
Portugal	Males and females aged 18-64 years	FFQ	1999-2003.	(Elmadfa et al., 2009a; Lopes et al., 2006)
	Males and females aged 65 and over	FFQ	1999-2003	(Elmadfa et al., 2009a; Lopes et al., 2006)
Romania	Males and females aged 19-64 years	personal interview	2006	(In: Elmadfa et al., (2009a))
	Males and females aged 65 and over	personal interview	2006	(In: Elmadfa et al., (2009a))
Spain	Males and females aged 18-64 years	24-hour recall	2002-2003	(Elmadfa et al., 2009a; Serra-Majem et al., 2007)
	Males and females aged 65-75 years	24-hour recall	2002-2003	(Elmadfa et al., 2009a; Serra-Majem et al., 2007)
Sweden	Males and females aged 17-74 years	7-day record	1997-1998	(Becker and Pearson, 2002)
	Males and females aged 17-24 years	7-day record	1997-1998	(Becker and Pearson, 2002)
	Males and females aged 25-34 years	7-day record	1997-1998	(Becker and Pearson, 2002)
	Males and females aged 35-44 years	7-day record	1997-1998	(Becker and Pearson, 2002)
	Males and females aged 45-54 years	7-day record	1997-1998	(Becker and Pearson, 2002)
	Males and females aged 55-64 years	7-day record	1997-1998	(Becker and Pearson, 2002)
	Males and females aged 65 -74	7-day record	1997-1998	(Becker and Pearson, 2002)

Country	Population	Dietary method	Year of survey	Reference
United Kingdom	Males and females aged 19-64 years	7-day record	2000-2001	(Hendersen et al., 2003)
	Males and females aged 19-24 years	7-day record	2000-2001	(Hendersen et al., 2003)
	Males and females aged 25-34 years	7-day record	2000-2001	(Hendersen et al., 2003)
	Males and females aged 35-49 years	7-day record	2000-2001	(Hendersen et al., 2003)
	Males and females aged 50-64 years	7-day record	2000-2001	(Hendersen et al., 2003)
	Males and females aged 65+ years	4-day record	1994-1995	(Finch et al., 1998)

APPENDIX 2B: INTAKE OF PROTEIN AMONG ADULTS AGED ~19-65 YEARS IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d^{-1})		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males											
Austria	19-64	778	16.8	4.9							
Belgium	19-59	413	16.0	3.1							
Czech Republic	19-64	1046	14.1	4.0							
Denmark	18-75	1569	14	2.3	11-17[2]	87	25	57-118[2]			
Estonia	19-64	900	14.7	4.7					1.0	0.6	
Finland	25-64	730	16.3	3.5		86	29				
France	19-64	852	16.3	0.1[3]							
Germany[1]	19-64	4912	14.6	3.2							
Greece	19-64	8365	14.1	1.7							
Hungary	>18	473	14.7	2.0		102.0	23.6				
Ireland	18-64	662	15.5	2.7	11.3-20.4	100.2	26.6	60.6-149.5			
Italy	18-<65	1068	16.3	2.2	13.2-20.2	92.6	25.3	56.2-136.1	1.20	0.36	0.71-1.83
Latvia	19-64	1065	13.7	4.2					1.1	0.6	
Lithuania	19-65	849	16.5	5.2							
The Netherlands[4]	19-64	1836	14.7	3.1							
Norway	19-64	1050	16.0	2.0							
Poland	19-64	1106	13.5	3.1							
Portugal	18-64	917	17.6	2.4							
Romania	19-64	177	17.8	3.8							
Spain	18-64	718	19.1			99.6					
Sweden	17-74	589	16	2	13-19	90	23	55-130			
United Kingdom	19-64	833	16.5	3.6	11.3-23.4[5]	88.2	32.7	47.1-135[5]			
Females											
Austria	19-64	1345	15.4	2.8							
Belgium	19-59	460	16.7	3.4							
Czech Republic	19-64	1094	14.7	7.7							
Denmark	18-75	1785	15	2.4	12-18[2]	67	18	46-91[2]			
Estonia	19-64	1115	15.0	4.4					0.9	0.5	
Finland	25-64	846	16.5	3.6		63	20				
France	19-64	1499	17.0	0.1[3]							
Germany[1]	19-64	6016	14.4	2.6							
Greece	19-64	12034	14.4	1.7							
Hungary	>18	706	14.6	1.9		79.7	18.0				
Ireland	18-64	717	15.6	2.9	11.2-20.6	69.8	17.2	43.4-99.0			
Italy	18-<65	1245	15.9	2.3	12.4-19.9	76.0	19.5	45.4-108.6	1.25	0.36	0.71-1.90
Latvia	19-64	1234	13.7	4.8							
Lithuania	19-65	1087	16.7	6.2					0.9	0.5	
The Netherlands[4]	19-64	2112	15.6	3.8							
Norway	19-64	1146	16.0	3.0							
Poland	19-64	1334	13.1	3.5							
Portugal	18-64	1472	19.0	2.4							
Romania	19-64	341	17.1	3.6							
Spain	18-64	895	19.5			79.7					
Sweden	17-74	626	16	2	13-20	73	17	47-102			
United Kingdom	19-64	891	16.6	3.5	10.6-24.2[5]	63.7	16.6	29.9-96.0[5]			

[1](Anonymous, 2008); [2]P10-P90; [3]SE; [4](Hulshof et al., 1998); [5]P2.5-P97.5

APPENDIX 2C: INTAKE OF PROTEIN AMONG ADULTS AGED ~19-34 YEARS IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d^{-1})		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males											
Denmark	18-24	105	15	2.4	11-19	96	28	50-147			
	25-34	234	14	2.3	11-19	93	25	58-137			
Estonia	19-34	396	14.3	4.6					1.0	0.5	
Germany	19-24	510				101.8	1.84[1]	51.4-189.0			
	25-34	690				99.0	1.50[1]	53.2-168.0			
Hungary	18-34	136	14.8	2.0		108	23.6				
Ireland	18-35	253	14.8	2.6	10.6-19.3	100.8	26.8	58.6-149.4			
Latvia	19-34	337	13.5	4.1					1.0	0.5	
The Netherlands[2]	19-30	352	14.2		11.5-17.2	98		72-127	1.2	0.39	
Sweden	17-24	67	15	2	12-19	92	27	48-144			
	25-34	128	15	2	12-18	91	21	58-129			
United Kingdom	19-24	108	14.9	2.6	10.2-22.2[3]	77.8	18.9	34.0-111.3[3]			
	25-34	219	16.5	4.7	10.8-24.2[3]	90.6	51.0	53.8-156.2[3]			
Females											
Denmark	18-24	150	14	2.2	11-18	66	18	41-98			
	25-34	340	15	2.4	11-18	70	18	42-99			
Estonia	19-34	459	14.6	4.5					1.0	0.5	
Germany	19-24	510				65.2	1.00[1]	35.8-106.5			
	25-34	972				69.6	0.73[1]	40.4-108.5			
Hungary	18-34	176	14.4	1.9		81.5	17.4				
Ireland	18-35	269	14.7	3.0	10.7-19.9	66.5	17.5	39.0-95.1			
Latvia	19-34	342	13.3	5.0					1.0	0.5	
The Netherlands[2]	19-30	398	14.8		10.9-19.2	70		49-93	0.98	0.31	
Sweden	17-24	70	15	2	12-20	70	19	35-103			
	25-34	132	16	2	12-20	73	16	49-103			
United Kingdom	19-24	104	15.4	2.5	10.3-23.4[3]	59.9	16.3	22.8-90.0[3]			
	25-34	210	15.9	3.6	10.4-24.4[3]	58.7	15.7	30.5-90.2[3]			

[1]SE; [2](Hulshof and Ocke, 2005); [3]P2.5-P97.5

Appendix 2D: Intake of protein among adults aged ~35-64 years in European countries

Country	Age (years)	N	Protein (E%) mean	SD	P5 – P95	Protein (g/d) mean	SD	P5 - P95	Protein (g/kg bw x d⁻¹) mean	SD	P5 - P95
Males											
Denmark	35-44	318	14	2.0	11-18	93	27	55-134			
	45-54	336	14	2.2	11-18	86	23	50-125			
	55-64	336	14	2.4	11-19	82	24	49-129			
Estonia	35-49	319	14.7	4.8					1.0	0.5	
	50-64	185	15.4	4.7					1.0	0.5	
Germany	35-64[1]	1013	14.8	4.1		89.9	39,7				
	35-64[2]	1032	14.0	3.9		88.0	36.8				
	35-50[3]	2079				93.9	0.74[4]	51.0-151.5			
	51-64[3]	1633				85.7	0.69[4]	47.5-136.6			
Hungary	35-59	199	14.7	2.0		104.5	22.6				
Ireland	36-50	236	15.9	2.6	12.3-20.8	102.8	28.8	59.6-156.4			
	51-64	173	16.2	2.7	11.8-21.6	95.8	22.2	65.4-140.6			
Latvia	35-49	372	13.8	4.5					1.1	0.6	
	50-64	356	13.8	4.0					1.0	0.5	
Spain	45-64	265	20.0			95.4					
Sweden	35-44	143	16	2	13-19	91	22	57-133			
	45-54	18	16	2	12-20	91	23	63-129			
	55-64	68	16	2	13-20	85	20	49-118			
United Kingdom	35-49	253	16.7	2.9	12.2-23.1[5]	90.1	23.3	47.7-139.9[5]			
	50-64	253	17.0	3.4	11.9-25.1[5]	88.8	22.9	41.5-132.3[5]			
Females											
Denmark	35-44	412	15	2.4	11-19	71	18	44-104			
	45-54	359	15	2.4	11-19	65	16	39-92			
	55-64	326	15	2.5	11-19	63	16	40-94			
Estonia	35-49	376	15.2	4.5					0.9	0.5	
	50-64	280	15.3	4.3					0.8	0.4	
Germany	35-64[1]	1078	14.5	4.2		65.6	25.6				
	35-64[2]	898	13.9	4.2		60.9	24.6				
	35-50[3]	2694				68.9	0.41[4]	39.3-106.7			
	51-64[3]	1840				67.3	0.49[4]	38.7-105.2			
Hungary	35-59	295	14.7	2.0		81.6	17.5				
Ireland	36-50	286	15.9	2.6	11.8-20.5[5]	72.4	16.6	48.9-102.2[5]			
	51-64	162	16.7	2.8	12.3-21.6[5]	70.7	16.9	40.8-99.7[5]			
Latvia	35-49	396	13.7	4.4					0.9	0.5	
	50-64	496	14.0	4.9					0.8	0.4	
Spain	45-64	337	20.2			76.4					
Sweden	35-44	132	16	2	13-20	71	15	47-98			
	45-54	153	16	2	13-21	73	17	49-102			
	55-64	81	17	2	14-21	75	16	49-99			
United Kingdom	35-49	318	16.7	3.5	11.1-24.5[5]	65.1	16.9	29.7-100.3[5]			
	50-64	259	17.4	3.2	11.6-24.4[5]	67.4	15.9	37.3-99.4[5]			

[1]Cohort Heidelberg (Linseisen et al., 2003); [2]Cohort Potsdam (Linseisen et al., 2003); [3](Anonymous, 2008); [4]SE ; [5]P2.5-P97.5

APPENDIX 2E: INTAKE OF PROTEIN AMONG ADULTS AGED ~65 YEARS AND OVER IN EUROPEAN COUNTRIES

Country	Age (years)	N	Protein (E%)			Protein (g/d)			Protein (g/kg bw x d^{-1})		
			mean	*SD*	P5 – P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males											
Austria	65+	147	14.9	3.1							
Belgium	60-74	416	16.9	2.7							
	>75	389	16.0	3.2							
Denmark	65-75	240	14	2.6	10-18	76	22	44-113			
Finland	65-74	229	17.4	3.8							
France	65-75	130	16.5	0.2[1]							
Germany	65-80	1469	14.5	2.6		77.8	0.59[1]	45.0-119.7			
Greece	65+	2508	14.1	1.7							
Hungary	60+	138	14.8	2.1		91.9	21.7				
Italy	65+	202	15.5	2.0	12.2-18.8	88.2	21.4	55.6-124.5	1.15	0.30	0.70-1.67
The Netherlands	65+	185	15.5	3,3	10.6-22.0	86	24	48-124	1.1	0.3	0.6-1.7
Norway	65+	176	16.0	2.0							
Poland	65+	176	13.6	3.3							
Portugal	65+	246	17.5	2.4							
Romania	65+	177	17.2	3.4							
Spain	65-75	122	19.5			77.6					
Sweden	65-74	65	16	2	13-19	87	24	53-131			
United Kingdom	65+	540	16.1	3.0	10.8-23.0[2]	71.5	17.0	38.5-105.3[2]			
Females											
Austria	65+	202	15.0	2.5							
Belgium	60-74	406	16.7	2.8							
	>75	355	17.0	3.8							
Denmark	65-75	198	14	2.6	11-19	62	17	36-95			
Finland	65-74	234	17.6	3.4							
France	65-75	219	17.5	0.3[1]							
Germany	65-80	1562	14.4	2.7		61.6	0.45[1]	34.9-91.6			
Greece	65+	3600	14.4	1.8							
Hungary	60+	235	14.5	1.8		76.0	18.5				
Italy	65+	316	15.7	2.4	12.4-19.9	71.4	18.8	41.0-100.7	1.12	0.32	0.63-1.69
The Netherlands	65+	236	16.7	3.9	11.1-23.4	73	18	44-101	1.0	0.3	0.6-1.6
Norway	65+	166	17.0	3.0							
Poland	65+	277	13.2	3.5							
Portugal	65+	339	18.7	2.3							
Romania	65+	341	16.3	3.0							
Spain	65-75	122	20.2			68					
Sweden	65-74	57	16	3	12-22	75	20	41-119			
United Kingdom	65+	735	16.5	3.7	10.7-24.8[2]	56.0	13.4	30.1-84.3[2]			

[1]SE; [2]P2.5-P97.5

APPENDIX 3: CALCULATION OF PRI FOR INFANTS, CHILDREN AND ADOLESCENTS

The PRI for infants from 6 months onwards and children is calculated as follows:

$PRI = AR + 1.96\ SD_{combined}$, with the $SD_{combined}$ calculated from the formula:

$SD_{combined} = (\sqrt{[CV_{maintenance} \text{ x maintenance requirement}]^2 + [CV_{growth} \text{ x growth requirement}]^2}),$

Where $CV_{maintenance}$ is 0.12, the maintenance requirement is given in Tables 8 and 9, the CV_{growth} can be calculated from the SD for growth given by WHO/FAO/UNU (2007) in Table 29, and the growth requirement is the rate of protein deposition (see Tables 8 and 9) divided by the efficiency of dietary protein utilisation.

GLOSSARY/ABBREVIATIONS

AFSSA	Agence Française de Sécurité Sanitaire des Aliments
ANSES	Agence Nationale de Sécurité Sanitaire de l'alimentation, de l' environnement et du travail
AOAC	American Organization of Analytical Chemists
AR	Average requirement
BCAA	Branched chain amino acid
BMI	Body mass index
BV	Biological value
bw	Body weight
CI	Confidence interval
CIQUAL	Centre d'information sur la qualité des aliments (French data centre on food quality)
COMA	Committee on Medical Aspects of Food Policy
CV	Coefficient of variation
d	day
D-A-CH	Deutschland-Austria-Confoederatio Helvetica
DGAC	Dietary Guidelines Advisory Committee
DNA	Deoxyribonucleic acid
DoH	Department of Health
DRV	Dietary reference value
EAR	Estimated average requirement
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
f	female
FAO	Food and Agriculture Organisation
GFR	Glomerular filtration rate
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor-binding protein

IoM	U.S. Institute of Medicine of the National Academy of Sciences
K	Potassium
m	male
mTOR	Mammalian target of rapamycin
N	Nitrogen
NNR	Nordic Nutrition recommendations
NPN	Non-protein nitrogen
NPPU	Net protein postprandial utilisation
NPU	Net protein utilisation
PD-CAAS	Protein digestibility-corrected amino acid score
PER	Protein efficiency ratio
PRI	Population reference intake
RDA	Recommended dietary allowances
RNA	Ribonucleic acid
SACN	Scientific Advisory Committee on Nutrition
SCF	Scientific Committee for Food
SD	Standard deviation
UL	Tolerable upper intake level
UNU	United Nations University
USDA	United States Department of Agriculture
WHO	World Health Organisation
y	year