3. Protein

Definition

The physiological requirement for protein of an individual is the lowest level of dietary protein intake that will balance the losses of nitrogen from the body in adults maintaining energy balance at modest levels of physical activity [1].

Physiology of protein metabolism

The body protein amounts to about 12 kg in adult man. The proteins are formed by chains of the 20 amino acids; the structural integrity of the body and its metabolism are wholly dependent on specific proteins. The provision of enough dietary amino acids to maintain these proteins is crucial to survival.

The body's proteins are constantly being synthesized and degraded; in an adult man eating 70g of protein daily the total protein turnover of the body amounts to about 250g protein per day. This in turn means that about 180g protein is being resynthesized without the involvement of new dietary sources of amino acids. The turnover of proteins is controlled, thereby allowing the mass of specific enzymes or structural proteins to be altered. The inflow of amino acids from the cells' cytoplasm into the bloodstream enables the body to reroute the amino acid supply from one organ to another.

The body's proteins contain 96% of the total body nitrogen so a study of nitrogen metabolism usually reflects protein metabolism. Other nitrogenous compounds, e.g., creatine, purine and pyrimidine bases and the porphyrins, are all in part derived from amino acids. Not all the 20 amino acids used to provide these nitrogen-containing compounds can be synthesized in sufficient quantity to meet the body's needs. Nine amino acids are classified as essential because they have to be provided as such in the diet (see Table 3.2). Methionine and phenylalanine are required as such and also as sources of cysteine and tyrosine. Histidine cannot be made in sufficient quantity for children's needs so histidine is also specified as an essential amino acid (and may be required for adults). It is suggested that other amino acids, e.g. glycine, may be needed in the diet to boost amino acid availability when the body's demand exceeds its capacity to synthesize the amino acid. Thus the distinction between essential and some non-essential amino acids is becoming blurred [2].

Protein digestion and amino acid absorption

Dietary proteins are digested by a complex of enzymes secreted by the stomach and pancreas. Amino acids and peptides are released by selective enzymic splitting of the protein chains. Peptides are hydrolysed further by intestinal enzymes so amino acids and small peptides are transferred across the intestinal wall.

The intestine itself is rapidly turning over with protein-rich cells continually sloughing into the lumen. The pancreatic, biliary, and intestinal secretions of proteins, together with sloughed cells, contribute a mass of "endogenous" protein to the intestinal pool. This is thought to amount to about 20-80g or more per day [3,4]. Not all this endogenous protein is readily digestible so the residuum, together with any undigested dietary protein and the proteins incorporated into the bacterial population within the small intestine, will pass into the colon where they are fermented by the bacterial flora. Some of the nitrogen released from fermentation is reabsorbed as ammonia which can be re-used by the liver to synthesize non-essential amino acids. Bacterially produced nutrients including amino acids may also contribute to the body's needs. Thus the assessment of protein digestibility by simply monitoring dietary protein (or N) intake and faecal nitrogen excretion neglects a multiplicity of events within the intestine. The differences between intake and faecal excretion may bear little relationship to the true amino acid supply if considerable amounts of essential amino acids are lost into the colon to be reabsorbed as energy sources such as volatile fatty acids and as ammonia. The true digestibility of dietary proteins in man is therefore uncertain.

Nevertheless, proteins from hen's eggs, cow's milk, meat and fish are usually considered to be 100% absorbed in adults. Protein-containing foods from plant sources are not so readily digested and the true digestibility is more difficult to calculate because of non-protein N-containing compounds in the plant food. The fall in apparent digestibility reflects both an increase in faecal N output, which stems from increased transfer of sloughed cells and protein into the colon, and the additional proliferation of colonic bacteria once a greater supply of undigested polysaccharides enters the colon to provide energy for bacterial proliferation.

Large intakes of fibre-rich foods, especially those containing cereal bran, reduce the apparent digestibility of protein by about 10%. Diets based on coarse whole-grain cereals and vegetables may be given a digestibility value of 85% and those diets based on refined cereals a correction value of 95%.

Amino acid metabolism

Amino acids are transported from the intestine in both the red cells and plasma, and are extracted first by the liver and then by the other tissues. Specific transport systems under hormonal control determine the distribution of amino acids, which themselves not only provide the building blocks for new protein synthesis but may also stimulate an "anabolic drive" of hormonal secretion and tissue responses to amplify the formation of specific proteins [5]. The "anabolic drive" may explain the selective effect of dietary proteins, and particularly those of animal origin rich in essential amino acids, in stimulating the longitudinal growth of children. In addition to protein synthesis individual amino acids are needed for other metabolic functions, including the synthesis of peptide hormones, nucleic acids, neurotransmitters to control brain cell communication, and other hormones.

The use of the amino acids for protein synthesis depends upon the balance of need between the fast and slow turnover proteins [6] and on the controlled changes in synthesis and breakdown occurring in response to dietary changes [7,8]. The amino acid composition of each protein is fixed so the controlled synthesis of a particular array of proteins determines the amino acids needed. If some amino acids are not available in adequate amounts then the synthetic machinery competes for the limiting amino acids and cannot synthesize the whole range of proteins in the intended quantities. Thus amino acid intake may be too limited for the requirements of the growing child or when an adult needs to produce a greater mass of specific proteins, e.g. during pregnancy or lactation or when responding to an infection with the production of antibodies.

Each amino acid has its own pathway for oxidation. They are metabolised and excreted as carbon dioxide, water and as urea formed by the liver. The enzymatic oxidative pathway of each amino acid is controlled in part by the inflow of the amino acid so an excess supply leads to its preferential oxidation. The oxidation of one amino acid may, however, be affected by the inflow of other dietary amino acids so the rate of oxidation of an amino acid may not be solely dependent on its accumulation in the cellular pools of the tissues.

The concept that oxidation reflects an "overflow" of an excess intake has been used to assess amino acid requirements by monitoring the oxidative loss of isotopically labelled carbon or nitrogen in the amino acid at different levels of amino acid intake. The total amount of amino acid catabolized to carbon dioxide and urea is not equivalent to the requirement for absorbed amino acid because of other irretrievable losses. Proteins are lost as hair, in skin and intestinal cells, in bronchial and other secretions and in lactating women as milk protein. New protein formation during growth, pregnancy and early lactation also has to be estimated. Amino acids are also being lost from the body, as other metabolic non-reutilizeable products such as creatinine and some hormones. It is the total utilisation of each amino acid which determines its turnover ; the requirement is that minimum dietary intake needed to supplement the body's synthesis of the amino acid and meet minimum synthetic and catabolic processes. This approach neglects the potential need for a greater supply than the minimum to stimulate the "anabolic drive" or to sustain protein synthesis rates at a higher "optimum" level than the minimum.

Adults in N balance are assumed to be eating enough protein once an allowance is made for the irretrievable loss of body proteins. Egg or milk protein is used since these proteins are readily digested with a presumed 100 % small intestinal recovery and the protein's amino acid composition is well balanced. The amino acid needs of children and adults are also usually determined by N balance. Faecal and urinary nitrogen output are determined and the net loss of body proteins and amino acids estimated as nitrogen.

If the intake of an essential amino acid falls below the body's needs then the other available amino acids cannot be used to sustain body protein metabolism; they are therefore present in relative excess and have to be oxidised with further urea synthesis and urinary N loss. Thus the N output can be monitored at different carefully defined intakes to find the level which induces the lowest urinary N output. This gives a value for the minimum dietary requirement and includes provision for other losses in skin hair and secretions [9].

Responses to a low protein intake

The body adjusts to a low protein intake by adapting over a period of up to a week during which there is a net loss of body protein amounting to about 1.5% of the total protein mass [10]. The reduced inflow of amino acids into the body fails to meet the body's needs for protein synthesis and amino acids are initially oxidised by the highly active catabolic enzymes, which were set at their prevailing level by the previous protein intake. Protein breakdown continues despite the fall in protein synthesis so there is a net loss of protein. However the amino acid catabolic enzymes begin adjusting within hours so the experimental evolution of isotope from ^{14}C or ^{15}N labelled amino acids is rapidly reduced. As adaptation occurs there is an immediate fall in urinary nitrogen excretion as urea synthesis falls and amino acid catabolism declines. Thus amino acids derived from both the diet and from protein breakdown are conserved and channelled preferentially into protein synthesis; activating enzymes for protein synthesis are stimulated and the enzyme activities involved in the urea cycle and in amino acid catabolism decline [11]. The conservation of the essential amino acids, which is a fundamental feature of the metabolism of animals, including man, is accentuated. The rates of breakdown of body proteins and of amino acid exchange across cellular membranes are further reduced so internal recycling of amino acids within the body's cells is enhanced. The residual amino acids entering the blood are also preferentially channelled into protein synthesis rather than oxidation. The factors controlling these events are largely unknown.

If no dietary protein is given but energy intake is maintained then urinary N falls even further as the catabolic amino acid enzymes adjust to the complete absence of dietary protein. This obligatory nitrogen loss (ONL) on a protein-free diet reflects the progressive loss of body protein in the faeces, as urea in the urine and as sloughed and secreted proteins. In a series of 11 studies involving more than 200 adults aged 22-77 years from many different countries the observed ONL did not differ greatly (mean, 53 mg N/kg bodyweight /d; range, 41-69 mg) and corresponded to a mean milk protein intake of 0.33 g/kg bodyweight /day [1].

Excessive intakes of protein and amino acids

Excessive protein intake may be associated with health risks, but the level of intake at which a risk is induced and the precise role of protein in the pathophysiological processes remain uncertain. The principal concerns relate to the maintenance of renal function and bone mass. High protein intakes seem to accelerate loss of renal function in some kidney diseases, but whether these effects of dietary protein are relevant to the general population remains uncertain [12]. Similarly the potential for excess dietary protein to mobilise bone calcium and accelerate bone loss needs further study [13].

Animal studies indicate that an unusually high intake of a single amino acid may induce not only a loss of appetite but also secondary metabolic changes in metabolism which are disadvantageous. Recent reports of toxic effects associated with the ingestion of unusual amounts of tryptophan may relate to toxic contaminants but the use of supplements of selected amino acids in individuals on European diets is unwise.

Physiological requirements for protein

Early attempts to estimate protein requirements in adults depended on determining the obligatory nitrogen loss (ONL) of volunteers and then adding each component of the N or protein loss or extra factors. It was assumed that 100% of the milk protein fed was efficiently utilised. In practice the provision of milk protein in amounts corresponding to the ONL is inadequate to induce N balance because the observed loss of N increases above the ONL value. This is because the input of dietary amino acids necessarily stimulates a modest increase in amino acid oxidation and therefore a 25-50 % rise in urinary N output. The minimum requirement for totally digestible protein of high amino acid quality is therefore greater than the ONL and it is this higher intake to maintain N balance which is taken as the minimum dietary requirement under practical everyday conditions [1]. Measuring N balance is not easy because analytical losses of dietary or urinary nitrogen give the false impression of better N accumulation in the body. Repeated studies with short-term balances measured over about 2 weeks provide estimated mean protein requirements of 0.63 g milk or egg protein per kg bodyweight per day. Longer term balance studies over 1-3 months on 34 adults fed egg or milk protein suggested an average minimum protein requirement of 0.58 g/kg bodyweight/d so the WHO/FAO/UNU Committee [1] chose an average of 0.6 g protein/kg bodyweight/d as a reasonable minimum figure for both adult men and women.

This figure is considered to be the average minimal value of dietary protein compatible with sustained nitrogen balance in health. The intakes needed to achieve N balance have a coefficient of variation of 12.5 %. Thus the minimum on which an individual may sustain N balance can vary from about 0.45 g/kg/d to 0.75 g/kg/d. This upper figure is considered likely to cover the needs of all subjects and has therefore been designated a "safe" protein intake for healthy young adults. The various values for protein intake are included in Table 3.1.

Protein quality and essential amino acid requirements

Table 3.2 provides estimates of the essential amino acid intakes based on the classic studies of Rose [14] as adjusted by FAO/WHO [9]. Rose undertook highly controlled feeding studies on volunteers fed adequate intakes of all the amino acids except the amino acid under test. Therefore the studies were not conducted under physiological conditions and little is known of the potential impact of the concentration of the other amino acids on the catabolism of the tested amino acid.

Several authors have suggested that in adults these estimates of essential amino acid requirements are too low. In short term balance studies, purified amino acid mixtures containing more than the current requirement level have failed to allow balance to occur. From a series of recent experiments on the kinetics of essential amino acid metabolism in which the minimal physiological requirement might be obtained by estimating the obligatory rates of oxidation of essential amino acids, Young *et al* [15] have suggested new requirement values which are about two or three times higher than current requirement figures. However these high estimates have been challenged [5,16] on methodological and theoretical grounds. The relatively low requirements estimated for adults have been confirmed by a series of nitrogen balance studies on normal volunteers fed normal diets rather than purified amino acid mixtures [17]. There seems to be an appreciable need for non-essential amino acids or N for their synthesis at low protein intakes but detailed studies are still awaited.

Children

The assessment of needs made by the FAO/WHO/UNU Consultation [1] has not been superseded by new information suggesting the need for any changes in the protein requirement of children. The values are based on the amount of high quality egg or milk protein needed for achieving N balance plus the additional need for growth. Table 3.3 shows requirements for children, with a 50% increase being added to the average requirement to take account of the day to day variability in growth. The efficiency of utilisation was assumed to be 70% with all children receiving adequate energy intakes. Breast milk N, although containing appreciable quantities of non-amino N, is extremely well utilised for reasons which are unclear. It is therefore unwise to rely on breast milk data in producing recommendations on protein need for bottle-fed babies. Table 3.3 is derived from the FAO/WHO/UNU report [1] but taking account of European growth patterns. The values all refer to milk protein, which is assumed to be completely absorbed. Digestibility of dietary protein will vary from 80 to 100% of the reference protein depending on the type of diet consumed; cereal diets rich in fibre have digestibility values between 80 and 90%. Adjustments for lower quality protein will need to be made in the manner suggested by FAO/WHO/UNU [1], where the total protein intake is increased to ensure that the intake of each essential amino acid is equivalent to that which would have been obtained from milk protein.

The essential amino acid requirement as a proportion of the protein requirement falls markedly with age from 54% in infants to 14% in adults. The reason for this is not completely explained by the high essential amino acid requirements for growth. Examples of the essential amino acid requirements of children aged about two years and 10-12 years are shown in Table 3.2.

Pregnancy

The extra protein needs in pregnancy are usually based on the FAO/WHO/UNU report [1], these values having been accepted by the National Academy of Sciences [18]. The estimates of protein used are based on the original Hytten and Leitch [19] estimates of body compositional changes and their likely protein content, a total of 925 g accumulated protein being accepted by both groups. It is increasingly recognised however that pregnancy is associated with changes in protein metabolism which may well increase the efficiency of utilisation of amino acids [20,21], so it may be unwise to consider that the accumulated protein gain has to be superimposed on the normal protein requirement of the non-pregnant woman. Given these uncertainties the recommendation of the US RDA committee to increase protein intakes by 10 g reference protein throughout pregnancy seems appropriate.

Lactation

The need for lactation will depend on the amount of breast milk provided but, with suitable allowances for individual variation, an extra protein intake of 16 g/d in the first 6 months is reasonable, with 12 g being needed for the second six months of breast feeding when the child is beginning to derive additional nutrition from foods.

The elderly

There are no grounds for reducing the figure for the protein requirements of the elderly. Data collated by FAO/WHO/UNU [1] suggest that there may be a somewhat greater need, but a safe adult level of 0.75 g/kg bodyweight/d will provide a higher intake per kg of lean body mass in the elderly because of their having lost lean tissue.

Table 3.1 Levels of intake of high quality protein for achieving nitrogen balance in adults.

(g/kg bodyweight/d)

Average minimum protein Requirement	0.6
Population Reference Intake	0.75
Lowest Threshold Intake	0.45

(expressed as g/d)

	Males	*Females*
Average Requirement	45	37
Population Reference Intake	56	47

(increases, g/d)

Pregnancy		10
Lactation	First 6 months	16
	Second 6 months	12

Table 3.2 Estimates of essential amino acid requirements [1]

(mg/kg bodyweight/d)

	Children about 2 y	***Children 10-12 y***	***Adults***	
Amino acid	Mean requirement	Mean requirement	Mean requirement	Population Reference Intake
Histidine	?	?	[8 - 12]*	[16]*
Isoleucine	31	28	10	13
Leucine	73	42	14	19
Lysine	64	44	12	16
Methionine + Cysteine	27	22	13	17
Phenylalanine + Tyrosine	69	22	14	19
Threonine	37	28	7	9
Tryptophan	12.5	3.3	3.5	5
Valine	38	25	10	13
Total without histidine	**352**	**214**	**84**	**111**

* This figure based on work with children [22] remains uncertain with only limited evidence yet available on the essentiality of histidine for adults.

*Table 3.3 Recommended intakes of protein in children, based on milk protein**

Age**	Bodyweight (kg)	Safe level (g protein/ kg bodyweight/d)	Total intake (g/d)***
4-6 m	7.5	1.86	14.0
7-9 m	9.0	1.65	15.0
10-12 m	10.0	1.48	15.0
1.0-1.5 y	11.0	1.26	14.0
1.5-2.0 y	12.5	1.17	14.5
2-3 y	13.5	1.13	15.5
3-4 y	15.5	1.09	17.0
4-5 y	17.5	1.06	18.5
5-6 y	19.5	1.02	20.0
6-7 y	22.0	1.01	22.0
7-8 y	24.5	1.01	24.5
8-9 y	27.0	1.01	27.5
9-10 y	30.0	0.99	29.5
Males 10 y	33.0	0.99	32.5
11 y	36.5	0.98	36.0
12 y	41.0	1.00	41.0
13 y	47.0	0.97	45.5
14 y	53.0	0.96	51.0
15 y	58.0	0.92	53.5
16 y	62.5	0.90	56.5
17 y	64.5	0.86	55.5
Females 10 y	34.0	1.00	34.0
11 y	37.5	0.98	37.0
12 y	43.0	0.96	41.5
13 y	48.0	0.94	45.0
14 y	50.5	0.90	45.5
15 y	52.5	0.87	45.5
16 y	54.0	0.83	45.0
17 y	54.5	0.80	43.5

• Calculated as in Tables 33 and 34 in FAO/WHO/UNU report [1].

** The midpoint of each age group was used, except for those below 2 years, where the ages taken were 6 months, 9 months, 12 months, 1.5 years (for 1.0-1.5 y) and 2.0 years (for 1.5 to 2.0 y).

*** Rounded to nearest 0.5 g.

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