PROTEIN NUTRITION and METABOLISM

9.1 *Nitrogen balance and protein requirements*

Figure 9.1 shows an overview of protein metabolism; in addition to the dietary intake of about 80 g of protein, almost the same amount of endogenous protein is secreted into the intestinal lumen. There is a small faecal loss equivalent to about 10 g of protein per day; the remainder is hydrolysed to free amino acids and small peptides, and absorbed . The faecal loss of nitrogen is partly composed of undigested dietary protein, but the main contributors are intestinal bacteria and shed mucosal cells, which are only partially broken down, and the protective mucus secreted by intestinal mucosal goblet cells . Mucus is especially resistant to enzymic hydrolysis, and contributes a considerable proportion of inevitable losses of nitrogen, even on a protein-free diet.

There is only a small pool of free amino acids in the body, in equilibrium with proteins that are being catabolized and synthesized. A small proportion of the amino acid pool is used for synthesis of a wide variety of specialized metabolites (including hormones and neurotransmitters, purines and pyrimidines). An amount of amino acids equivalent to that absorbed is oxidized, with the carbon skeletons being used for gluconeogenesis or as metabolic fuels, and the nitrogen being excreted mainly as urea .

The state of protein nutrition, and the overall state of body protein metabolism, can be determined by measuring the dietary intake of nitrogenous compounds and

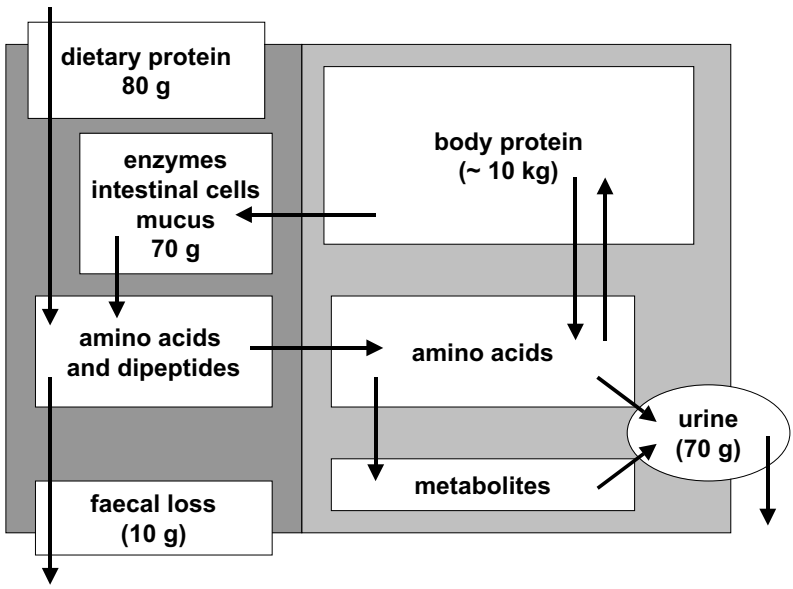

FIGURE 9.1 *An overview of protein metabolism.*

the output of nitrogenous compounds from the body. Although nucleic acids also contain nitrogen (section 9.2.1), protein is the major dietary source of nitrogenous compounds, and measurement of total nitrogen intake gives a good estimate of protein intake. Nitrogen constitutes 16% of most proteins, and therefore the protein content of foods is calculated on the basis of mg N × 6.25, although for some foods with an unusual amino acid composition other factors are used.

The output of N from the body is largely in the urine and faeces, but significant amounts may also be lost in sweat and shed skin cells – and in longer-term studies the growth of hair and nails must be taken into account. Obviously, any loss of blood or tissue will also involve a loss of protein. Although the intake of nitrogenous compounds is mainly protein, the output is mainly urea (section 9.3.1.4), though small amounts of a number of other products of amino acid metabolism are also excreted, as shown in Table 9.1.

The difference between intake and output of nitrogenous compounds is known as nitrogen balance. Three states can be defined:

- An adult in good health and with an adequate intake of protein excretes the same amount of nitrogen each day as is taken in from the diet. This is nitrogen balance or nitrogen equilibrium: intake = output and there is no change in the total body content of protein.
- In a growing child, a pregnant woman or someone recovering from protein loss, the excretion of nitrogenous compounds is less than the dietary intake – there is a net retention of nitrogen in the body and an increase in the body content of protein. This is positive nitrogen balance: intake > output and there is a net gain in total body protein.
- In response to trauma or infection (section 9.1.2.2) or if the intake of protein is inadequate to meet requirements, there is net a loss of nitrogen from the body – the output is greater than the intake. This is negative nitrogen balance: intake < output and there is a loss of body protein.

TABLE 9.1 *Average daily excretion of nitrogenous compounds in the urine*

Urea	10–35 g	150–600 mol	Depends on the intake of protein
Ammonium	340–1200 mg	20–70 mmol	Depends on the state of acid–base balance
Amino acids, peptides and conjugates	1.3–3.2 g	–	
Protein	< 60 mg	–	Significant proteinuria indicates kidney damage
Uric acid	250–750 mg	1.5–4.5 mmol	Major product of purine metabolism
Creatinine	Male 1.8 g Female 1.2 g	Male 16 mmol Female 10 mmol	Depends on muscle mass
Creatine	< 50 mg	< 400 mmol	Higher levels indicate muscle catabolism

9.1.1 Dynamic equilibrium

The proteins of the body are continually being broken down and replaced. As shown in Table 9.2, some proteins (especially enzymes that have a role in controlling metabolic pathways) may turn over within a matter of minutes or hours; others last for longer before they are broken down, perhaps days or weeks. Some proteins only turn over very slowly – for example the connective tissue protein collagen is broken down and replaced so slowly that it is almost impossible to measure the rate – perhaps half of the body's collagen is replaced in a year.

This continual breakdown and replacement is dynamic equilibrium. Superficially, there is no change in body protein. In an adult there is no detectable change in the amount of protein in the body from one month to the next. Nevertheless, if an isotopically labelled amino acid is given, the process of turnover can be followed. As shown in Figure 9.2, the label rapidly becomes incorporated into newly synthesized proteins, and is gradually lost as the proteins are broken down. The rate at which the label is lost from any one protein depends on the rate at which that protein is broken down and replaced; the time for the labelling to fall to half its peak is the half-life of that protein.

Protein breakdown occurs at a more or less constant rate throughout the day, and an adult catabolizes and replaces some 3–6 g of protein per kilogram body weight per day. Turnover is also important in growing children, who synthesize considerably more protein per day than the net increase in body protein. Even children recovering from severe protein–energy malnutrition (see Chapter 8), and increasing their body protein rapidly, still synthesize 2–3 times more protein per day than the net increase.

Although an adult may be in overall nitrogen balance, this is the average of periods of negative balance in the fasting state and positive balance in the fed state. As discussed

Table 9.2 *Half-lives of some proteins*

Protein	Half-life
Ornithine decarboxylase	11 minutes
Lipoprotein lipase	1 hours
Tyrosine transaminase	1.5 hours
Phosphoenolpyruvate carboxykinase	2 hours
Tryptophan oxygenase	2 hours
HMG CoA reductase	3 hours
Glucokinase	12 hours
Alanine transaminase	0.7–1 days
Glucokinase	1.25 days
Serum albumin	3.5 days
Arginase	4–5 days
Lactate dehydrogenase	16 days
Adult collagen	300 days
Infant collagen	1–2 days and 150 days

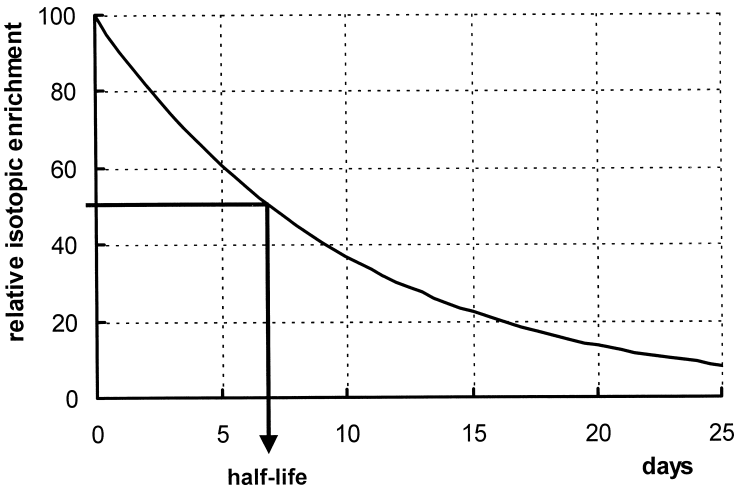

FIGURE 9.2 *Determination of the half-life of body proteins using ^{15}N-labelled amino acids.*

in section 9.2.3.3, protein synthesis is energy expensive, and in the fasting state the rate of synthesis is lower than that of protein catabolism. There is a loss of tissue protein, which provides amino acids for gluconeogenesis (section 5.7). In the fed state, when there is an abundant supply of metabolic fuel, the rate of protein synthesis increases and exceeds that of breakdown, so that what is observed is an increase in tissue protein, replacing that which was lost in the fasting state.

As discussed in section 8.3, even in severe undernutrition, the rate of protein breakdown remains more or less constant, while the rate of replacement synthesis falls, as a result of the low availability of metabolic fuels. It is only in cachexia (section 8.4) that there is increased protein catabolism as well as reduced replacement synthesis.

9.1.1.1 Mechanisms involved in tissue protein catabolism

The catabolism of tissue proteins is obviously a highly regulated process; as shown in Table 9.1, different proteins are catabolized (and replaced) at very different rates. Three different mechanisms are involved in the process:

- *Lysosomal cathepsins* are proteases with a broad range of specificity, leading to complete hydrolysis of proteins to free amino acids. They hydrolyse proteins that have entered the cell by phagocytosis and are also involved in the hydrolysis of cell proteins after cell death, when they are released into the cytosol. In addition, a number of intracellular proteins contain the sequence Lys-Phe-Glu-Arg-Gly, which targets them for uptake into the lysosomes, where they undergo hydrolysis.
- *Calpain* is a protease with a broad specificity for hydrophobic amino acids, resulting in partial proteolysis. It has a calcium-dependent regulatory subunit and is inhibited by a second protein, calstatin, so its activity in the cell is regulated. Both proteins

turn over relatively rapidly, and there is an increase in the amount of mRNA for both proteins in the cell during fasting and starvation – this seems to be the result of increased gene expression in order to maintain a constant amount of both proteins despite the reduction in overall protein synthesis.

- *The ubiquitin–proteasome system* catalyses ATP-dependent proteolysis. It is important in both protein turnover and antigen processing.
  - Ubiquitin is a small peptide (M_r 8,500) that forms a covalent bond from the carboxy terminus to the ε-amino of lysine residues in target proteins – this is an ATP-dependent process, and multiple molecules of ubiquitin are attached to target proteins. It is not known what targets proteins for ubiquitination; at least four different ubiquitin-transferring enzymes are known.
  - The proteasome (also known as the multifunctional protease) is a multi-subunit complex that accounts for about 1% of the total soluble protein of cells. There are at least five types of subunit with specificity for esters of hydrophobic, basic and acidic amino acids.

9.1.2 Protein requirements

It is the continual catabolism of tissue proteins that creates the requirement for dietary protein. Although some of the amino acids released by breakdown of tissue proteins can be re-used, most are metabolized, by pathways which are discussed in section 9.3, yielding intermediates that can be used as metabolic fuels and for gluconeogenesis (section 5.7) and urea (section 9.3.1.4), which is excreted. This means that there is a need for dietary protein to replace losses even in an adult who is not growing. In addition, relatively large amounts of protein are lost from the body in mucus, enzymes and other proteins, which are secreted into the gastrointestinal tract and are not completely digested and reabsorbed.

Current estimates of protein requirements are based on studies of the amount required to maintain nitrogen balance. If the intake is not adequate to replace the protein that has been broken down, then there is negative nitrogen balance – a greater output of nitrogen from the body than the dietary intake. Once the intake is adequate to meet requirements, nitrogen balance is restored. The proteins that have been broken down can be replaced, and any surplus intake of protein can be used as a metabolic fuel.

Such studies show that for adults the average daily requirement is 0.6 g of protein per kilogram body weight. Allowing for individual variation, the reference intake (section 11.1.1) is 0.75 g/kg body weight, or 50 g/day for a 65 kg adult. Average intakes of protein by adults in developed countries are considerably greater than requirements, of the order of 80–100 g/day. The reference intake of protein is sometimes called the safe level of intake, meaning that it is safe and (more than) adequate to meet requirements, not implying that there is any hazard from higher levels of intake.

Protein requirements can also be expressed as a proportion of energy intake. The energy yield of protein is 17 kJ/g, and the reference intake of protein represents some 7–8% of energy intake. In Western countries protein provides 14–15% of energy intake.

It is unlikely that adults in any country will suffer from protein deficiency if they are eating enough food to meet their energy requirements. As shown in Figure 9.3, the major dietary staples that are generally considered as sources of carbohydrate also provide significant amounts of protein. Even among people in Western countries who eat meat, fish and eggs (which are generally regarded as rich protein sources) about 25% of protein intake comes from cereals and cereal products, with an additional 10% from fruit and vegetables.

Only cassava, yam and possibly rice provide insufficient protein (as a percentage of energy) to meet adult requirements. The shortfall in protein provided by a diet based on yam or rice would be made up by small amounts of other foods that are sources of protein – this may be either small amounts of meat and fish or legumes and nuts, which are rich vegetable sources of protein. With diets based largely on cassava there is a more serious problem in meeting protein requirements.

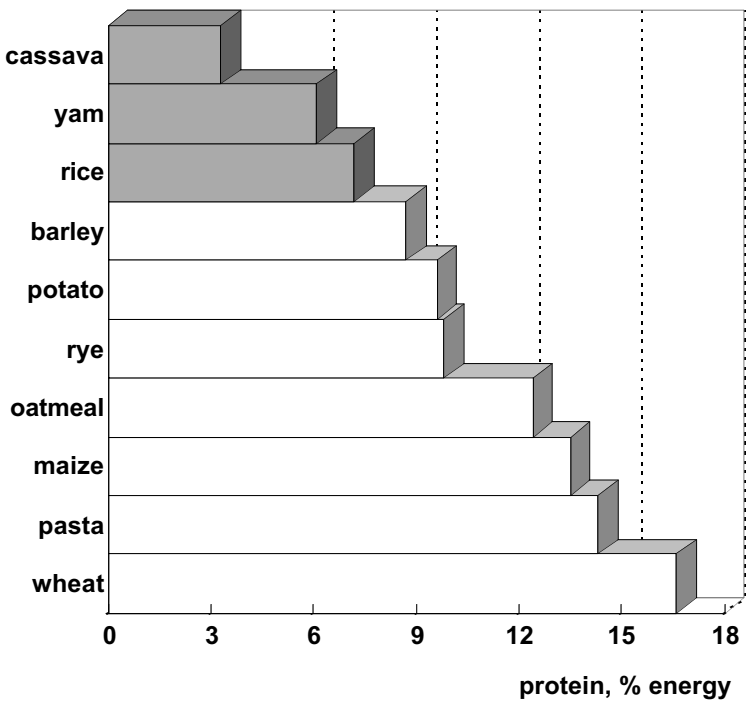

FIGURE 9.3 *Protein as percentage of energy in dietary staples. Protein requirements of an adult are met when the diet provides 7–8% of energy from protein. Of the major dietary staples, only cassava, yam and (marginally) rice fail to provide this much protein.*

9.1.2.1 Protein requirements of children

Because children are growing, and increasing the total amount of protein in the body, they have a proportionally greater requirement than adults. A child should be in positive nitrogen balance while he or she is growing. Even so, the need for protein for growth is relatively small compared with the requirement to replace proteins which are turning over. Table 9.3 shows protein requirements at different ages. Children in Western countries consume more protein than is needed to meet their requirements, but in developing countries protein intake may well be inadequate to meet the requirement for growth.

A protein-deficient child will grow more slowly than one receiving an adequate intake of protein – this is stunting of growth. As discussed in section 8.2, the protein–energy deficiency diseases, marasmus and kwashiorkor, result from a general lack of food (and hence metabolic fuels), not a specific deficiency of protein.

9.1.2.2 Protein losses in trauma and infection – requirements for convalescence

One of the metabolic reactions to a major trauma, such as a burn, a broken limb or surgery, is an increase in the net catabolism of tissue proteins. As shown in Table 9.4, apart from the loss of blood associated with injury, as much as 750 g of protein (about 6–7% of the total body content) may be lost over 10 days. Even prolonged bed rest results in a considerable loss of protein, because there is atrophy of muscles that are not used. Muscle protein is catabolized as normal, but without the stimulus of exercise it is not completely replaced.

This protein loss is mediated by the hormone cortisol, which is secreted in response to stress, and the cytokines that are secreted in response to trauma; four mechanisms are involved:

- Tryptophan dioxygenase and tyrosine transaminase. This results in depletion of the tissue pools of these two amino acids, leaving an unbalanced mixture of amino acids that cannot be used for protein synthesis (section 9.2.3).
- In response to cytokine action there is an increase in metabolic rate, leading to an increased rate of oxidation of amino acids as metabolic fuel, so reducing the amount available for protein synthesis.
- Cytokines cause an increase in the rate of protein catabolism, as occurs in cachexia (section 8.4).
- A variety of plasma proteins synthesized in increased amount in response to cytokine action (the so-called acute-phase proteins) are richer in two amino acids, cysteine and threonine, than most tissue proteins. This leads to depletion of tissue pools of these two amino acids, again leaving an unbalanced mixture of amino acids that cannot be used for protein synthesis.

The lost protein has to be replaced during recovery, and patients who are

TABLE 9.3 *Reference nutrient intakes for protein*

Age	Recommended protein intake (g/day)
4–6 months	1.85
7–9 months	1.65
10–12 months	1.50
1–1.5 years	1.20
1.5–2 years	1.20
2–3 years	1.15
3–4 years	1.10
4–5 years	1.10
5–6 years	1.00
6–7 years	1.00
7–8 years	1.00
8–9 years	1.00
9–10 years	1.00
Males	
10 years	1.00
11 years	1.00
12 years	1.00
13 years	1.00
14 years	0.95
15 years	0.95
16 years	0.90
17 years	0.90
Adult	0.75
Females	
10 years	1.00
11 years	1.00
12 years	0.95
13 years	0.95
14 years	0.90
15 years	0.90
16 years	0.80
17 years	0.80
Adult	0.75

Source: FAO/WHO/UNU (1985) Energy and protein requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. *WHO Technical Report Series* 724, Geneva.

convalescing will be in positive nitrogen balance. However, this does not mean that a convalescent patient requires a diet that is richer in protein than usual. As discussed in section 9.1.2, average protein intakes are twice requirements; a normal diet will provide adequate protein to permit replacement of the losses due to illness and hospitalization.

TABLE 9.4 *Protein losses (g) over 10 days after trauma or infection*

	Tissue loss	Blood loss	Catabolism	Total
Fracture of femur	–	200	700	900
Muscle wound	500–750	150–400	750	1350–1900
35% burns	500	150–400	750	1400–1650
Gastrectomy	20–180	20–10	625–750	645–850
Typhoid fever	–	–	675	685

From data reported by Cuthbertson DP (1964), in *Human Protein Metabolism*, Vol. II, Munro HN and Allison JB (eds), New York, Academic Press, pp. 373–414.

9.1.3 Essential amino acids

Early studies of nitrogen balance showed that not all proteins are nutritionally equivalent. More of some is needed to maintain nitrogen balance than others. This is because different proteins contain different amounts of the various amino acids (section 4.4.1). The body's requirement is not simply for protein, but for the amino acids which make up proteins, in the correct proportions to replace the body proteins.

As shown in Table 9.5, the amino acids can be divided into two main groups, with each group further subdivided:

- The nine essential or indispensable amino acids, which cannot be synthesized in the body. If one of these is lacking or provided in inadequate amount, then regardless of the total intake of protein it will not be possible to maintain nitrogen balance, as there will not be an adequate amount of the amino acid for protein synthesis.
  - Two amino acids, cysteine and tyrosine, can be synthesized in the body, but only from essential amino acid precursors – cysteine from methionine and tyrosine from phenylalanine. The dietary intakes of cysteine and tyrosine thus affect the requirements for methionine and phenylalanine – if more of either is provided in the diet, then less will have to be synthesized from the essential precursor.
  - For premature infants, and possibly also for full-term infants, a tenth amino acid is essential – arginine. Although adults can synthesize adequate amounts of arginine to meet their requirements, the capacity for arginine synthesis is low in infants and may not be adequate to meet the requirements for growth.
- The non-essential or dispensable amino acids, which can be synthesized from metabolic intermediates, as long as there is enough total protein in the diet. If one of these amino acids is omitted from the diet, nitrogen balance can still be maintained.
- Only three amino acids, alanine, aspartate and glutamate, can be considered to be truly dispensable; they are synthesized from common metabolic intermediates (pyruvate, oxaloacetate and α-ketoglutarate respectively; section 9.3.1.2).

TABLE 9.5 *Essential and non-essential amino acids*

Essential	Essential precursor	Non-essential	Semi-essential
Histidine		Alanine	Arginine
Isoleucine		Aspartate	Asparagine
Leucine		Glutamate	Glutamine
Lysine			Glycine
Methionine	Cysteine		Proline
Phenylalanine	Tyrosine		Serine
Threonine			
Tryptophan			
Valine			

- The remaining amino acids are generally considered as non-essential, but under some circumstances the requirement may outstrip the capacity for synthesis:
  - A high intake of compounds that are excreted as glycine conjugates will increase the requirement for glycine considerably.
  - In response to severe trauma there is an increased requirement for proline for collagen synthesis for healing,
  - In surgical trauma and sepsis the requirement for glutamine increases significantly – a number of studies have shown considerably improved healing after major surgery if additional glutamine is provided.

The requirements for essential amino acids for growth (expressed as proportion of total protein intake) are higher than the requirement to maintain N balance in adults, and younger children, with a faster growth rate, have a higher requirement for essential amino acids as a proportion of total protein than do older children with a lower rate of growth. Table 9.6 shows various estimates of essential amino acid requirements and the 'reference pattern' of the amount of each amino acid that should ideally be present per gram of dietary protein.

Early studies of essential amino acid requirements were based on the amounts required to maintain nitrogen balance in young adults. Interestingly, for reasons that are not clear, these relatively short-term studies did not show any requirement for histidine. More recent studies have measured the rate of whole-body protein turnover using isotopically labelled amino acids. These have shown that the maximum rate of protein turnover is achieved with intakes of the essential amino acids some threefold higher than are required to maintain nitrogen balance. What is not clear is whether the maximum rate of protein turnover is essential, or even desirable.

9.1.3.1 Protein quality and complementation

A protein that contains at least as much of each of the essential amino acids as is required will be completely useable for tissue protein synthesis, whereas one that is

TABLE 9.6 *Estimates of essential amino acids requirements and reference pattern for adults*

	WHO/FAO (1973)		FAO/WHO/ UNU (1985)		Protein turnover requirement (mg/kg bw)	Amino acid oxidation requirement (mg/kg bw)
	Requirement (mg/kg bw)	Pattern (mg/g protein)	Requirement (mg/kg bw)	Pattern (mg/g protein)		
Histidine	0	0	8–12	16	–	–
Isoleucine	10	18	10	13	38	–
Leucine	14	25	14	19	65	66
Lysine	12	22	12	16	70	50
Methionine + cysteine	13	24	13	17	27	22
Phenylalanine + tyrosine	14	25			65	–
Threonine	7	13	7	9	35	25
Tryptophan	3.5	6.5	3.5	5	10	–
Valine	10	18	10	13	40	33

Sources: WHO/FAO (1973) Energy and protein requirements: Report of a joint FAO/WHO ad hoc expert committee. *WHO Technical Reports Series* 522, WHO, Geneva. FAO/WHO/UNU (1985) Energy and protein requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. *WHO Technical Report Series* 724, Geneva. Young VR, Bier DM and Pellett PL (1989) *American Journal of Clinical Nutrition* 50: 80–92. Young VR (1994) *Journal of Nutrition* 124: 1517–1523S.

relatively deficient in one or more of the essential amino acids will not. More of such a protein will be required to maintain nitrogen balance or growth.

The limiting amino acid of a protein is that essential amino acid which is present in lowest amount relative to the requirement. In cereal proteins the limiting amino acid is lysine, while in animal and most other vegetable proteins it is methionine. (Correctly, the sum of methionine plus cysteine, as cysteine is synthesized from methionine and the presence of cysteine lowers the requirement for methionine.)

The nutritional value or quality of individual proteins depends on whether or not they contain the essential amino acids in the amounts that are required. A number of different ways of determining protein quality have been developed:

- Biological value (BV) is the proportion of absorbed protein retained in the body. A protein that is completely useable (e.g. egg and human milk) has a BV of 0.9–1; meat and fish have a BV of 0.75–0.8, wheat protein 0.5 and gelatine (which completely lacks tryptophan) a BV of 0.
- Net protein utilization (NPU) is the proportion of dietary protein that is retained in the body (i.e. it takes account of the digestibility of the protein). By convention it is measured at 10% dietary protein, at which level the protein synthetic mechanism of the animal can utilize all of the protein so long as the balance of essential amino acids is correct.

- Protein efficiency ratio (PER) is the gain in weight of growing animals per gram of protein eaten.
- Relative protein value (RPV) is the ability of a test protein, fed at various levels of intake, to support nitrogen balance, compared with a standard protein.
- Chemical score is based on chemical analysis of the protein; it is the amount of the limiting amino acid compared with the amount of the same amino acid in egg protein (which is completely useable for tissue protein synthesis). Protein score (or amino acid score) uses a reference pattern of amino acid requirements as the standard.

Although protein quality is important when considering individual dietary proteins, it is not particularly relevant when considering total diets, because different proteins are limited by different amino acids, and have a relative excess of other essential amino acids. This means that the result of mixing different proteins in a diet is to give an unexpected increase in the nutritional value of the mixture. Wheat protein is limited by lysine and has a protein score of 0.6; pea protein is limited by methionine and cysteine and has a protein score of 0.4. A mixture of equal amounts of these two individually poor-quality proteins has a protein score of 0.82 – as high as that of meat.

The result of this complementation between proteins that might individually be of low quality means that most diets have very nearly the same protein quality, regardless of the quality of individual protein sources. The average Western diet has a protein score of 0.73, while the poorest diets in developing countries, with a restricted range of foods, and very little milk, meat or fish, have a protein score of 0.6.

9.2 *Protein synthesis*

The information for the amino acid sequence of each of the 30–50,000 different proteins in the body is contained in the DNA in the nucleus of each cell. As required, a working copy of the information for an individual protein (the gene for that protein) is transcribed, as messenger RNA (mRNA), and this is then translated during protein synthesis on the ribosomes. Both DNA and RNA are linear polymers of nucleotides. In RNA the sugar is ribose, whereas in DNA it is deoxyribose.

9.2.1 The structure and information content of DNA

As shown in Figure 9.4, DNA is a linear polymer of nucleotides. It consists of a backbone of alternating deoxyribose and phosphate units, with the phosphate groups forming links from carbon-3 of one sugar to carbon-5 of the next. The bases of the nucleotides project from this sugar–phosphate backbone. There are of two strands of

else in mRNA it binds the normal methionine tRNA. It is only immediately adjacent to the cap that AUG binds the initiator methionine tRNA.

After the ribosome has been assembled, with the initiator tRNA bound at the P site and occupying the AUG initiator codon, the next amino acyl tRNA binds to the A site of the ribosome, with its anticodon bound to the next codon in the sequence.

The methionine is released from the initiator tRNA at the P site, and forms a peptide bond to the amino group of the amino acyl tRNA at the A site of the ribosome. The initiator tRNA is then released from the P site, and the growing peptide chain, attached to its tRNA, moves from the A site to the P site. As the peptide chain is attached to tRNA, which occupies a codon on the mRNA, this means that as the peptide chain moves from the A site to the P site, so the whole assembly moves one codon along the mRNA.

As the growing peptide chain moves from the A site to the P site, and the ribosome moves along the mRNA chain, so the next amino acyl tRNA occupies the A site, covering its codon. The growing peptide chain is transferred from the tRNA at the P site, forming a peptide bond to the amino acid at the A site. Again the free tRNA at the P site is released, and the growing peptide, attached to tRNA, moves from the A site to the P site, moving one codon along the mRNA as it does so.

The stop codons (UAA, UAG and UGA) are read not by tRNA but by protein release factors. These occupy the A site of the ribosome and hydrolyse the peptide–tRNA bond. This releases the finished protein from the ribosome. As the protein leaves, so the two subunits of the ribosome separate, and leave the mRNA; they are now available to bind another initiator tRNA and begin the process of translation over again.

Just as several molecules of RNA polymerase can transcribe the same gene at the same time, so several ribosomes translate the same molecule of mRNA at the same time. As the ribosomes travel along the ribosome, so each has a longer growing peptide chain than the one following. Such assemblies of ribosomes on a molecule of mRNA are called polysomes.

Termination and release of the protein from the ribosome requires the presence of a stop codon and the protein release factors. However, protein synthesis can also come to a halt if there is not enough of one of the amino acids bound to tRNA. In this case, the growing peptide chain is not released from the ribosome, but remains, in arrested development, until the required amino acyl tRNA is available. This means that if the intake of one of the essential amino acids is inadequate then, once supplies are exhausted, protein synthesis will come to a halt.

9.2.3.3 The energy cost of protein synthesis

The minimum estimate of the energy cost of protein synthesis is four ATP equivalents per peptide bond formed, or 2.8 kJ per gram of protein synthesized:

- Formation of the amino acyl tRNA requires the formation of amino acyl AMP,

with the release of pyrophosphate, which again breaks down to yield phosphate. Hence, for each amino acid attached to tRNA there is a cost equivalent to 2 mol of ATP being hydrolysed to ADP plus phosphate.
- The binding of each amino acyl tRNA to the A site of the ribosome involves the hydrolysis of GTP to GDP plus phosphate, which is equivalent to the hydrolysis of ATP to ADP plus phosphate.
- Movement of the growing peptide chain from the A site of the ribosome to the P site again involves the hydrolysis of ATP to ADP plus phosphate.

If allowance is made for the energy cost of active transport of amino acids into cells, the cost of protein synthesis is increased to 3.6 kJ/g. Allowing for the nucleoside triphosphates required for mRNA synthesis gives a total cost of 4.2 kJ per gram of protein synthesized.

In the fasting state, when the rate of protein synthesis is relatively low, about 8% of total energy expenditure (i.e. about 12% of the basal metabolic rate) is accounted for by protein synthesis. After a meal, when the rate of protein synthesis increases, it may account for 12–20% of total energy expenditure.

9.2.3.4 Post-translational modification of proteins

Proteins that are to be exported from the cell, or are to be targeted into mitochondria, are synthesized with a hydrophobic signal sequence of amino acids at the amino terminus to direct them through the membrane. This is removed in the process of post-translational modification. Many other proteins have regions removed from the amino or carboxy terminus during post-translational modification, and the initial (amino-terminal) methionine is removed from most newly synthesized proteins.

Many proteins contain carbohydrates and lipids, covalently bound to amino acid side-chains. Others contain covalently bound cofactors and prosthetic groups, such as vitamins and their derivatives, metal ions or haem. Again the attachment of these non-amino acid parts of the protein is part of the process of post-translational modification to form the active protein.

Some proteins contain unusual amino acids for which there is no codon and no tRNA. These are formed by modification of the protein after translation is complete. Such amino acids include:

- Methylhistidine in the contractile proteins of muscle.
- Hydroxyproline and hydroxylysine in the connective tissue proteins. The formation of hydroxyproline and hydroxylysine requires vitamin C as a cofactor. This explains why wound healing, which requires new synthesis of connective tissue, is impaired in vitamin C deficiency (section 11.14.3). See Problem 9.3 for the role of vitamin C in synthesis of hydroxyproline and hydroxylysine.
- Interchain links in collagen and elastin, formed by the oxidation of lysine residues. This reaction is catalysed by a copper-dependent enzyme, and copper deficiency

leads to fragility of bones and loss of the elasticity of connective tissues (section 11.15.2.2).

- γ-Carboxyglutamate in several of the blood clotting proteins, and in osteocalcin in bone. The formation of γ-carboxyglutamate requires vitamin K (section 11.15.2). See Problem 9.2 for the role of vitamin K in synthesis of γ-carboxyglutamate.

9.3 *The metabolism of amino acids*

An adult has a requirement for a dietary intake of protein because there is continual oxidation of amino acids as a source of metabolic fuel and for gluconeogenesis in the fasting state. In the fed state, amino acids in excess of immediate requirements for protein synthesis are oxidized. Overall, for an adult in nitrogen balance, the total amount of amino acids being metabolized will be equal to the total intake of amino acids in dietary proteins.

Amino acids are also required for the synthesis of a variety of metabolic products, including:

- purines and pyrimidines for nucleic acid synthesis;
- haem, synthesized from glycine;
- the catecholamine neurotransmitters, dopamine, noradrenaline and adrenaline, synthesized from tyrosine;
- the thyroid hormones thyroxine and tri-iodothyronine, synthesized from tyrosine (section 11.15.3.3);
- melanin, the pigment of skin and hair, synthesized from tyrosine;
- the nicotinamide ring of the coenzymes NAD and NADP, synthesized from tryptophan (section 11.8.2);
- the neurotransmitter serotonin (5-hydroxytryptamine), synthesized from tryptophan.
- The neurotransmitter histamine, synthesized from histidine;
- the neurotransmitter GABA (γ-aminobutyrate) synthesized from glutamate (see Figure 5.19);
- carnitine (section 5.5.1), synthesized from lysine and methionine;
- creatine (section 3.2.3.1), synthesized from arginine, glycine and methionine;
- the phospholipid bases ethanolamine and choline (section 4.2.1.3), synthesized from serine and methionine. Acetyl choline functions as a neurotransmitter;
- taurine, synthesized from cysteine.

In general, the amounts of amino acids required for synthesis of these products are small compared with the requirement for maintenance of nitrogen balance and protein turnover.

9.3.1 Metabolism of the amino nitrogen

The initial step in the metabolism of amino acids is the removal of the amino group ($-NH_2$), leaving the carbon skeleton of the amino acid. Chemically, these carbon skeletons are ketoacids (more correctly, they are oxo-acids). A ketoacid has a –C=O group in place of the HC–NH_2 group of an amino acid; the metabolism of ketoacids is discussed in section 9.3.2.

9.3.1.1 Deamination

Some amino acids can be directly oxidized to their corresponding ketoacids, releasing ammonia: the process of deamination (Figure 9.7). There is a general amino acid oxidase which catalyses this reaction, but it has a low activity.

There is an active D-amino acid oxidase in the kidneys, which acts to deaminate, and hence detoxify, the small amounts of D-amino acids that arise from bacterial proteins. The ketoacids resulting from the action of D-amino acid oxidase on D-amino acids can undergo transamination (section 9.3.1.2) to yield the L-isomers. This means that, at least to a limited extent, D-amino acids can be isomerized and used for protein synthesis. Although there is evidence from experimental animals that D-isomers of (some of) the essential amino acids can be used to maintain nitrogen balance, there is little information on utilization of D-amino acids in human beings.

Four amino acids are deaminated by specific enzymes:

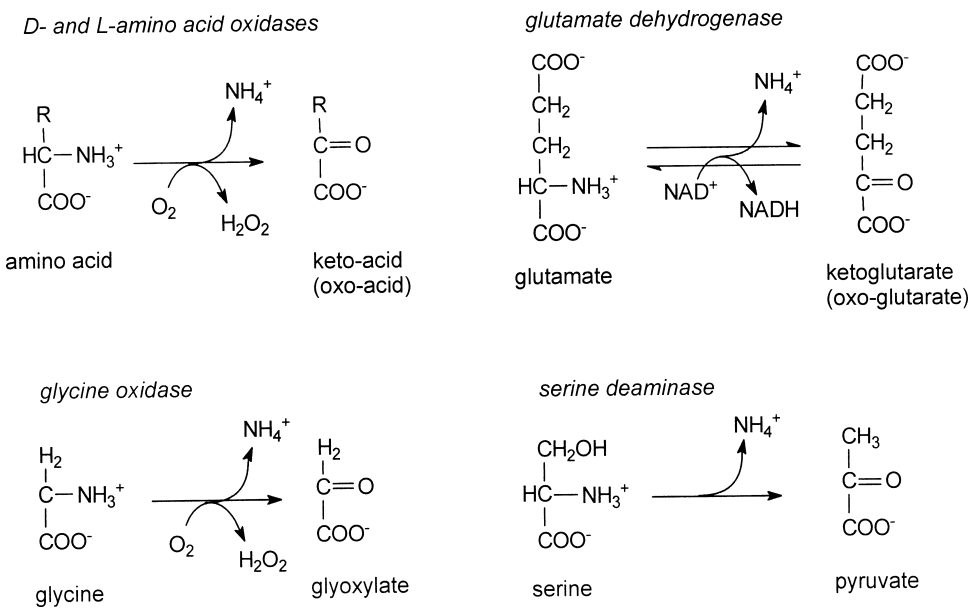

FIGURE 9.7 *Deamination of amino acids.*

- Glycine is deaminated to its ketoacid, glyoxylic acid, and ammonium ions by glycine oxidase.
- Glutamate is deaminated to ketoglutarate and ammonium ions by glutamate dehydrogenase.
- Serine is deaminated and dehydrated to pyruvate by serine deaminase (sometimes called serine dehydratase).
- Threonine is deaminated and dehydrated to oxobutyrate by threonine deaminase.

9.3.1.3 The metabolism of ammonia

The deamination of amino acids (and a number of other reactions in the body) results in the formation of ammonium ions. Ammonium is highly toxic. The normal plasma concentration is less than 50 μmol/L; an increase to 80–100 μmol/L (far too little to have any detectable effect on plasma pH) results in disturbance of consciousness, and in patients whose blood ammonium rises above about 200 μmol/L ammonia intoxication leads to coma and convulsions, and may be fatal.

TABLE 9.9 *Transamination products of the amino acids*

Amino acid	Ketoacid
Alanine	Pyruvate
Arginine	α-Keto-γ-guanidoacetate
Aspartic acid	Oxaloacetate
Cysteine	β-Mercaptopyruvate
Glutamic acid	α-Ketoglutarate
Glutamine	α-Ketoglutaramic acid
Glycine	Glyoxylate
Histidine	Imidazolepyruvate
Isoleucine	α-Keto-β-methylvalerate
Leucine	α-Ketoisocaproate
[Lysine*	α-Keto-ε-aminocaproate → pipecolic acid]
Methionine	*S*-Methyl-β-thiol 1α-oxopropionate
Ornithine	Glutamic-γ-semialdehyde
Phenylalanine	Phenylpyruvate
Proline	γ-Hydroxypyruvate
Serine	Hydroxypyruvate
Threonine	α-Keto-β-hydroxybutyrate
Tryptophan	Indolepyruvate
Tyrosine	*p*-Hydroxyphenylpyruvate
Valine	α-Ketoisovalerate

*The ketoacid formed by transamination of lysine undergoes spontaneous cyclization.

At any time, the total amount of ammonium to be transported around the body, and eventually excreted, is greatly in excess of the toxic level. What happens is that, as it is formed, ammonium is metabolized, mainly by the formation of glutamate from α-ketoglutarate, and then glutamine from glutamate, in a reaction catalysed by glutamine synthetase, as shown in Figure 9.10. Glutamine is transported in the bloodstream to the liver and kidneys.

It is the formation of glutamate from α-ketoglutarate that explains the neurotoxicity of ammonium; as ammonium concentrations in the nervous system rise, the reaction of glutamate dehydrogenase depletes the mitochondrial pool of α-ketoglutarate, resulting in impairment of the activity of the citric acid cycle (section 5.4.4), and so impairing energy-yielding metabolism.

In the kidneys, some glutamine is hydrolysed to glutamate (which remains in the body) and ammonium, which is excreted in the urine to neutralize excess acid excretion.

9.3.2 The metabolism of amino acid carbon skeletons

Acetyl CoA and acetoacetate arising from the carbon skeletons of amino acids may be used for fatty acid synthesis (section 5.6.1) or be oxidized as metabolic fuel, but cannot

be utilized for the synthesis of glucose (gluconeogenesis; section 5.7). Amino acids that yield acetyl CoA or acetoacetate are termed ketogenic.

By contrast, those amino acids that yield intermediates that can be used for gluconeogenesis are termed glucogenic. As shown in Table 9.10, only two amino acids are purely ketogenic: leucine and lysine. Three others yield both glucogenic fragments and either acetyl CoA or acetoacetate: tryptophan, isoleucine and phenylalanine.

The principal substrate for gluconeogenesis is oxaloacetate, which undergoes the reaction catalysed by phosphoenolpyruvate carboxykinase to yield phosphoenolpyruvate, as shown in Figure 5.31. The onward metabolism of phosphoenolpyruvate to glucose is essentially the reverse of glycolysis shown in Figure 5.10.

The points of entry of amino acid carbon skeletons into central metabolic pathways are shown in Figure 5.20. Those that give rise to ketoglutarate, succinyl CoA, fumarate or oxaloacetate can be regarded as directly increasing the tissue pool of citric acid cycle intermediates, and hence permitting the withdrawal of oxaloacetate for gluconeogenesis.

Those amino acids that give rise to pyruvate also increase the tissue pool of oxaloacetate, as pyruvate is carboxylated to oxaloacetate in the reaction catalysed by pyruvate carboxylase (section 5.7).

Gluconeogenesis is an important fate of amino acid carbon skeletons in the fasting state, when the metabolic imperative is to maintain a supply of glucose for the central nervous system and red blood cells. However, in the fed state the carbon skeletons of

TABLE 9.10 *Metabolic fates of the carbon skeletons of amino acids*

	Glucogenic intermediates	**Ketogenic intermediates**
Alanine	Pyruvate	–
Glycine → serine	Pyruvate	–
Cysteine	Pyruvate	–
Tryptophan	Pyruvate	Acetyl CoA
Arginine → ornithine	α-Ketoglutarate	–
Glutamine → glutamate	α-Ketoglutarate	–
Proline → glutamate	α-Ketoglutarate	–
Histidine → glutamate	α-Ketoglutarate	–
Methionine	Propionyl CoA	–
Isoleucine	Propionyl CoA	Acetyl CoA
Valine	Succinyl CoA	–
Asparagine → aspartate	Oxaloacetate	–
Aspartate	Oxaloacetate *or* fumarate	–
Phenylalanine → tyrosine	Fumarate	Acetoacetate
Leucine	–	Acetoacetate *and* acetyl CoA
Lysine		Acetyl CoA