10
Protein and Amino Acids

SUMMARY

Protein is the major structural component of all cells in the body. Proteins also function as enzymes, in membranes, as transport carriers, and as hormones; and their component amino acids serve as precursors for nucleic acids, hormones, vitamins, and other important molecules. The Recommended Dietary Allowance (RDA) for both men and women is 0.80 g of good quality protein/kg body weight/d and is based on careful analyses of available nitrogen balance studies. For amino acids, isotopic tracer methods and linear regression analysis were used whenever possible to determine the requirements. The estimated average requirements for amino acids were used to develop amino acid scoring patterns for various age groups based on the recommended intake of dietary protein. The recommended protein digestibility corrected amino acid scoring pattern (PDCAAS) for proteins for children 1 year of age and older and all other age groups is as follows (in mg/g of protein): isoleucine, 25; leucine, 55; lysine, 51, methionine + cysteine (SAA), 25; phenylalanine + tyrosine, 47; threonine, 27; tryptophan, 7; valine, 32; and histidine, 18. While an upper range for total protein in the diet as a percent of total energy intake was set at no more than 35 percent to decrease risk of chronic disease (see Chapter 11), there were insufficient data to provide dose–response relationships to establish a Tolerable Upper Intake Level (UL) for total protein or for any of the amino acids. However, the absence of a UL means that caution is warranted in using any single amino acid at levels significantly above that normally found in food.

BACKGROUND INFORMATION

Chemistry of Proteins and Amino Acids

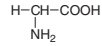
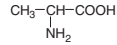
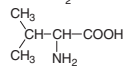
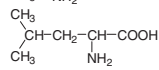
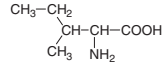
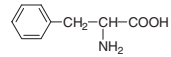
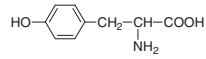
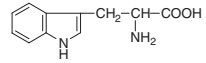
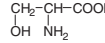
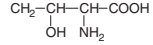
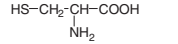
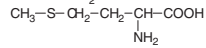
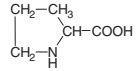
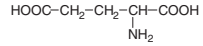
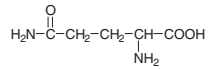
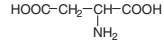
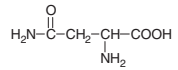
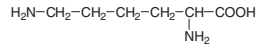
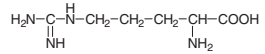
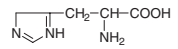
Protein

Protein is the major functional and structural component of all the cells of the body; for example, all enzymes, membrane carriers, blood transport molecules, the intracellular matrices, hair, fingernails, serum albumin, keratin, and collagen are proteins, as are many hormones and a large part of membranes. Moreover, the constituent amino acids of protein act as precursors of many coenzymes, hormones, nucleic acids, and other molecules essential for life. Thus an adequate supply of dietary protein is essential to maintain cellular integrity and function, and for health and reproduction.

Proteins in both the diet and body are more complex and variable than the other energy sources, carbohydrates and fats. The defining characteristic of protein is its requisite amino (or imino) nitrogen group. The average content of nitrogen in dietary protein is about 16 percent by weight, so nitrogen metabolism is often considered to be synonymous with protein metabolism. Carbon, oxygen, and hydrogen are also abundant elements in proteins, and there is a smaller proportion of sulfur.

Proteins are macromolecules consisting of long chains of amino acid subunits. The structures for the common L-amino acids found in typical dietary proteins are shown in Figure 10-1. In the protein molecule, the amino acids are joined together by peptide bonds, which result from the elimination of water between the carboxyl group of one amino acid and the α-amino (or imino in the case of proline) group of the next in line. In biological systems, the chains formed might be anything from a few amino acid units (di, tri, or oligopeptide) to thousands of units long (polypeptide), corresponding to molecular weights ranging from hundreds to hundreds of thousands of Daltons. The sequence of amino acids in the chain is known as the primary structure.

A critical feature of proteins is the complexity of their physical structures. Polypeptide chains do not exist as long straight chains, nor do they curl up into random shapes, but instead fold into a definite three-dimensional structure. The chains of amino acids tend to coil into helices (secondary structure) due to hydrogen bonding between side chain residues, and sections of the helices may fold on each other due to hydrophobic interactions between nonpolar side chains and, in some proteins, to disulfide bonds so that the overall molecule might be globular or rod-like (tertiary structure). Their exact shape depends on their function and for some proteins, their interaction with other molecules (quaternary structure).

Name	Abbreviation	Form
Aliphatic side chains		
Glycine	Gly	
Alanine	Ala	
Valine[a]	Val	
Leucine[a]	Leu	
Isoleucine[a]	Ile	
Aromatic side chains		
Phenylalanine	Phe	
Tyrosine	Tyr	
Tryptophan	Trp	
Hydroxyl groups in side chains		
Serine	Ser	
Threonine	Thr	
Sulfur-containing side chains		
Cysteine[b]	Cys	
Methionine	Met	
Imino Acids		
Proline[c]	Pro	
Acidic side chains and their amides		
Glutam	Glu	
Glutamine	Gln	
Aspartic acid	Asp	
Asparagione	Asn	
Basic side chains		
Lysine	Lys	
Arginine	Arg	
Histidine	His	

Amino acids in italics are classed as nutritionally indispensable to humans.
[a] Leucine, valine, and isoleucine are known as the branched-chain amino acids.
[b] Cysteine is often found as a dimer (cysteine), linked through sulfur atoms (-S-S-) by oxidation.
[c] Proline is, strictly speaking, an imino acid rather than an amino acid.

FIGURE 10-1 L-amino acids of nutritional significance.

Many proteins are composed of several separate peptide chains held together by ionic or covalent links, an example being hemoglobin, in which each active unit consists of two pairs of dissimilar subunits (the α and β chains).

The most important aspect of a protein from a nutritional point of view is its amino acid composition, but the protein's structure may also influence its digestibility. Some proteins, such as keratin, are highly insoluble in water and hence are resistant to digestion, while highly glycosylated proteins, such as the intestinal mucins, are resistant to attack by the proteolytic enzymes of the intestine.

Amino Acids

The amino acids that are incorporated into mammalian protein are α-amino acids, with the exception of proline, which is an α-imino acid. This means that they have a carboxyl group, an amino nitrogen group, and a side chain attached to a central α-carbon (Figure 10-1). Functional differences among the amino acids lie in the structure of their side chains. In addition to differences in size, these side groups carry different charges at physiological pH (e.g., nonpolar, uncharged but polar, negatively charged, positively charged); some groups are hydrophobic (e.g., branched chain and aromatic amino acids) and some hydrophilic (most others).

These side chains have an important bearing on the ways in which the higher orders of protein structure are stabilized and are intimate parts of many other aspects of protein function. Attractions between positive and negative charges pull different parts of the molecule together. Hydrophobic groups tend to cluster together in the center of globular proteins, while hydrophilic groups remain in contact with water on the periphery. The ease with which the sulfhydryl group in cysteine forms a disulfide bond with the sulfhydryl group of another cysteine in a polypeptide chain is an important factor in the stabilization of folded structures within the polypeptide and is a crucial element in the formation of inter-polypeptide bonds. The hydroxyl and amide groups of amino acids provide the sites for the attachment of the complex oligosaccharide side chains that are a feature of many mammalian proteins such as lactase, sucrase, and the mucins. Histidine and amino acids with the carboxyl side chains (glutamic acid and aspartic acid) are critical features in ion-binding proteins, such as the calcium-binding proteins (e.g., troponin C), critical for muscular contraction, and the iron-binding proteins (e.g., hemoglobin) responsible for oxygen transport.

Some amino acids in protein only achieve their final structure after their precursors have been incorporated into the polypeptide. Notable examples of such post-translational modifications are the hydroxyproline

and hydroxylysine residues found in collagen (proline and lysine are converted to these after they have been incorporated into procollagen) and 3-methylhistidine present in actin and myosin. The former hydroxylated amino acids are critical parts of the cross-linking of collagen chains that lead to rigid and stable structures. The role of methylated histidine in contractile protein function is unknown.

Nutritional and Metabolic Classification of Amino Acids

Older views of the nutritional classification of amino acids categorized them into two groups: indispensable (essential) and dispensable (nonessential). The nine indispensable amino acids (Table 10-1) are those that have carbon skeletons that cannot be synthesized to meet body needs from simpler molecules in animals, and therefore must be provided in the diet. Although the classification of the indispensable amino acids and their assignment into a single category has been maintained in this report, the definition of dispensable amino acids has become blurred as more information on the intermediary metabolism and nutritional characteristics of these compounds has accumulated. Laidlaw and Kopple (1987) divided dispensable amino acids into two classes: truly dispensable and conditionally indispensable. Five of the amino acids in Table 10-1 are termed dispensable as they can be synthesized in the body from either other amino

TABLE 10-1 Indispensable, Dispensable, and Conditionally Indispensable Amino Acids in the Human Diet

Indispensable	Dispensable	Conditionally Indispensable[a]	Precursors of Conditionally Indispensable
Histidine[b]	Alanine	Arginine	Glutamine/glutamate, asparate
Isoleucine	Aspartic acid	Cysteine	Methionine, serine
Leucine	Asparagine	Glutamine	Gl utamic acid/ammonia
Lysine	Glutamic acid	Glycine	Serine, choline
Methionine	Serine	Proline	Glutamate
Phenylalanine		Tyrosine	Phenylalanine
Threonine			
Tryptophan			
Valine			

[a] Conditionally indispensable is defined as requiring a dietary source when endogenous synthesis cannot meet metabolic need.
[b] Although histidine is considered indispensable, unlike the other eight indispensable amino acids, it does not fulfill the criteria used in this report of reducing protein deposition and inducing negative nitrogen balance promptly upon removal from the diet.
SOURCE: Laidlaw and Kopple (1987).

acids or other complex nitrogenous metabolites. In addition, six other amino acids, including cysteine and tyrosine, are conditionally indispensable as they are synthesized from other amino acids or their synthesis is limited under special pathophysiological conditions (Chipponi et al., 1982; Harper, 1983; Laidlaw and Kopple, 1987). This is even more of an issue in the neonate where it has been suggested that only alanine, aspartate, glutamate, serine, and probably asparagine are truly dietarily dispensable (Pencharz et al., 1996).

The term conditionally indispensable recognizes the fact that under most normal conditions the body can synthesize these amino acids to meet metabolic needs. However, there may be certain physiological circumstances: prematurity in the young infant where there is an inadequate rate at which cysteine can be produced from methionine; the newborn, where enzymes that are involved in quite complex synthetic pathways may be present in inadequate amounts as in the case of arginine (Brunton et al., 1999), which results in a dietary requirement for this amino acid; or pathological states, such as severe catabolic stress in an adult, where the limited tissue capacity to produce glutamine to meet increased needs and to balance increased catabolic rates makes a dietary source of these amino acids required to achieve body nitrogen homeostasis. The cells of the small intestine become important sites of conditionally indispensable amino acid, synthesis, with some amino acids (e.g., glutamine and arginine) becoming nutritionally indispensable under circumstances of intestinal metabolic dysfunction (Stechmiller et al., 1997). However, the quantitative requirement levels for conditionally indispensable amino acids have not been determined and these, presumably, vary greatly according to the specific condition.

There now appears to be a requirement for preformed α-amino nitrogen in the form of glutamate, alanine, or aspartate, for example (Katagiri and Nakamura, 2002). It was previously thought that, in addition to the indispensable amino acids, simple sources of nitrogen such as urea and diammonium citrate together with carbon sources would be sufficient to maintain nitrogen homeostasis (FAO/WHO, 1965). However, there are now good theoretical reasons to conclude that this is not likely in the human (Katagiri and Nakamura, 2002). The mixture of dispensable and conditionally indispensable amino acids as supplied by food proteins at adequate intakes of total nitrogen will assure that both the nitrogen and specific amino acid needs are met.

Protein and Amino Acid Homeostasis

Maintenance of Body Protein

Body Protein Reserve. The body of a 70-kg man contains about 11 kg of protein. Nearly half of this protein (about 43 percent) is present as skeletal muscle, while other structural tissues such as skin and blood each contain approximately 15 percent of the total protein (Lentner, 1981). The metabolically active visceral tissues (e.g., liver and kidney) contain comparatively small amounts of protein (together about 10 percent of the total). Other organs such as the brain, lung, heart, and bone contribute the remainder. The distribution among the organs varies with developmental age, as the newborn infant has proportionately less muscle and much more brain and visceral tissue than the adult. It is also notable that, despite the very wide variety of enzymes and proteins within a single organism, almost one half of the total protein content of the human is present in just four proteins (myosin, actin, collagen, and hemoglobin). Collagen in particular may comprise 25 percent of the total. Moreover, in induced malnutrition, this proportion can rise to 50 percent because of the substantial loss of noncollagen proteins, whereas collagen itself is retained (Picou et al., 1966).

Even in the adult, when the protein mass of the body has reached a plateau, it can be influenced by a variety of nutritional and pathological factors. Thus, when diets high or low in protein are given, there is a gain or loss of body protein over the first few days, before re-equilibration of protein intake with the rates of oxidation and excretion (Swick and Benevenga, 1977). This phenomenon has led to the concept of a "labile protein reserve," which can be gained or lost from the body as a short-term store for use in emergencies or to take account of day-to-day variations in dietary intake. Studies in animals have suggested that this immediate labile protein store is contained in the liver and visceral tissues, as their protein content decreases very rapidly during starvation or protein depletion (by as much as 40 percent), while skeletal muscle protein drops much more slowly (Swick and Benevenga, 1977). During this situation, protein breakdown becomes a source of indispensable amino acid needs for synthesis of proteins critical to maintaining essential body function (Reeds et al., 1994).

This labile protein reserve in humans is unlikely to account for more than about 1 percent of total body protein (Waterlow, 1969; Young et al., 1968). Thus, the immediately accessible stores of protein (which serve as the source of indispensable amino acids and amino nitrogen) cannot be considered in the same light as the huge energy stores in the form of body fat; the labile protein reserve is similar in weight to the glycogen store. However, it should be recognized that this protein reserve is unlike the fat

and glycogen stores, whose primary roles are for energy use. The protein lost during fasting is functional body protein and thus there is no evidence for a protein reserve that serves only as a store to meet future needs.

There is a wide range of variation in daily dietary protein intake, from the protein requirement and beyond, to which the body is able to adapt over a period of days, after which no further change in body protein content occurs. However, pathological conditions, such as severe disease states, can cause substantial rates of protein loss due to the increased demand for either amino acids or carbon skeletons to meet local energy demands. If these conditions go unchecked for more than a few days, there may be a serious depletion of the body's protein mass, which might eventually become life threatening. Although the evidence from short-term changes in diet suggests that the main loss of protein is from the viscera (de Blaauw et al., 1996), in chronic illness skeletal muscle, which comprises over 40 percent of the protein mass of a healthy individual, becomes the largest single contributor to protein loss (Hansen et al., 2000).

Free Amino Acids. Although the free amino acids dissolved in the body fluids are only a very small proportion of the body's total mass of amino acids, they are very important for the nutritional and metabolic control of the body's proteins.

The content of free and protein-bound amino acids in rat muscle is shown in Table 10-2. It can be seen that their ranges are considerable and that their concentrations in the free pool are in no way related to their concentrations in body proteins. In the human, free phenylalanine comprises less than 2 percent of its total body pool, and corresponds to only about 1.5 hour worth of protein synthesis, or 25 percent of the day's intake of protein (Waterlow et al., 1978). Free glutamate and alanine comprise a larger proportion of their respective body pools, but they could not be considered as reserves for more than a very short time. In human muscle, glutamine has an exceptionally large free pool, containing about 10 to 15 g of nitrogen. After trauma, this pool can become depleted by more than 50 percent (Labow and Souba, 2000); its loss may then make a significant contribution to the total loss of nitrogen.

Although the plasma compartment is most easily sampled, the concentration of most amino acids is higher in tissue intracellular pools. Typically, large neutral amino acids, such as leucine and phenylalanine, are essentially in equilibrium with the plasma. Others, notably glutamine, glutamic acid, and glycine, are 10- to 50-fold more concentrated in the intracellular pool. Dietary variations or pathological conditions can result in substantial changes in the concentrations of the individual free amino acids in both the plasma and tissue pools (Furst, 1989; Waterlow et al., 1978).

TABLE 10-2 Comparison of the Pool Sizes of Free and Protein-Bound Amino Acids in Rat Muscle

	μmol/g Wet Weight		
	Protein	Free	Protein: Free Ratio
Indispensable amino acids			
Histidine	26	0.39	67
Isoleucine	50	0.16	306
Leucine	109	0.20	556
Lysine	58	1.86	31
Methionine	36	0.16	225
Phenylalanine	45	0.07	646
Threonine	60	1.94	31
Valine	83	0.31	272
Dispensable and some conditionally indispensable amino acids			
Alanine	111	2.77	40
Arginine	67	0.25	269
Aspartic acid (+ amide)	110	1.13	97
Glutamic acid (+ amide)	148	9.91	15
Glycine	117	1.94	60
Serine	74	1.96	38
Tyrosine	36	0.14	266

SOURCE: Data of E.B. Fern, quoted by Waterlow et al. (1978).

Pathways of Amino Acid Metabolism

The exchange between body protein and the free amino acid pool is illustrated by the highly simplified scheme shown in Figure 10-2. Here, all the proteins in the tissues and circulation are grouped into one pool. Similarly, there is a second pool, consisting of the free amino acids dissolved in body fluids. The arrows into and out of the protein pool show the continual degradation and resynthesis of these macromolecules (i.e., protein turnover). The other major pathways that involve the free amino acid pool are the supply of amino acids by the gut from the absorbed amino acids derived from dietary proteins, the de novo synthesis in cells (including those of the gut, which are a source of dispensable amino acids), and the loss of amino acids by oxidation, excretion, or conversion to other metabolites. Although this scheme represents protein metabolism in the human as a whole, with minor modifications it can also be used to repre-

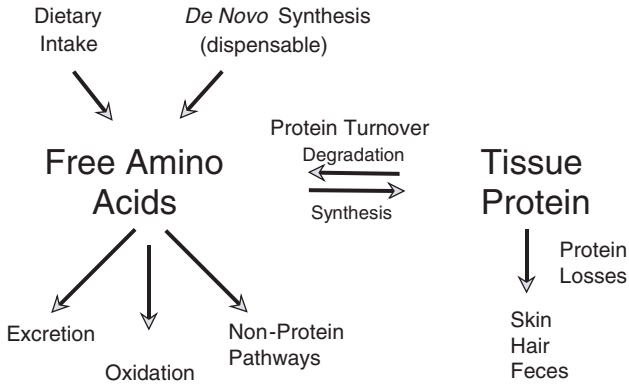


FIGURE 10-2 Exchange between body protein and free amino acid pools.

sent protein metabolism in individual organs, or indeed the metabolism of a single protein.

Amino Acid Utilization for Growth

Dietary protein is not only needed for maintaining protein turnover and the synthesis of physiologically important products of amino acid metabolism but is, of course, laid down as new tissue. Studies in animals show that the composition of amino acids needed for growth is very similar to the composition of body protein (Dewey et al., 1996). It is important to note, however, that the amino acid composition of human milk is not the same as that of body protein (Dewey et al., 1996), and although the present recommendations for the dietary amino acids for infants provided in this report continue to be based on human milk as the standard, recent authors (Dewey et al., 1996) have cautioned that the composition of human milk proteins is not necessarily a definition of the biological amino acid requirements of the growing neonate.

Maintenance Protein Needs

Even when mammals consume no protein, nitrogen continues to be lost. Provided that the energy intake is adequate, these "basal" losses are closely related to body weight and basal metabolic rate (Castaneda et al., 1995b; Scrimshaw et al., 1972). In man, normal growth is very slow and the dietary requirement to support growth is small in relation to maintenance needs except at very young ages. Moreover, the human being is a long-

lived species. It follows that maintenance needs are of particular importance to humans and account for a very large majority of lifetime needs for dietary protein.

It has been known for decades (Said and Hegsted, 1970) that the body's capacity to conserve individual amino acids at low intakes varies, so the pattern of amino acids needed in the diet to match their individual catabolic rates does not correspond precisely with the composition of body protein. For example, the indispensable amino acid requirements for adults may provide a quarter of their minimum total need for amino nitrogen, compared with the need for noncollagen body protein in which approximately half of the amino acids are indispensable (FAO/WHO/UNU, 1985). This implies that there is very effective recycling of indispensable amino acids released continuously from protein degradation back into protein synthesis. Under conditions where the diet is devoid of protein, the efficiency of amino acid recycling is over 90 percent for both indispensable and dispensable amino acids (Neale and Waterlow, 1974). While highly efficient, some amino acids are recycled at different rates than others.

Physiology of Absorption, Metabolism, and Excretion

Protein Digestion and Absorption

After ingestion, proteins are denatured by the acid in the stomach, where they are also cleaved into smaller peptides by the enzyme pepsin, which is activated by the increase in stomach acidity that occurs on feeding. The proteins and peptides then pass into the small intestine, where the peptide bonds are hydrolyzed by a variety of enzymes. These bond-specific enzymes originate in the pancreas and include trypsin, chymotrypsins, elastase, and carboxypeptidases. The resultant mixture of free amino acids and small peptides is then transported into the mucosal cells by a number of carrier systems for specific amino acids and for di- and tri-peptides, each specific for a limited range of peptide substrates. After intracellular hydrolysis of the absorbed peptides, the free amino acids are then secreted into the portal blood by other specific carrier systems in the mucosal cell or are further metabolized within the cell itself. Absorbed amino acids pass into the liver, where a portion of the amino acids are taken up and used; the remainder pass through into the systemic circulation and are utilized by the peripheral tissues.

Although there are good reasons to suppose that dietary protein digestion is incomplete and variable among different diets, recent studies using proteins intrinsically labeled with ^{15}N added to a diet suggest that many common dietary proteins, including proteins from casein, mixed whey, wheat, and legumes, are digested with an efficiency of greater than 90 per-

cent when fed as isolates, concentrates, or flours (Bergner et al., 1990; Gausserès et al., 1997). Thus, a significant portion (at least 50 percent) of fecal nitrogen losses represents the fixation by the colonic and cecal bacteria of nitrogenous substances (urea, ammonia, and protein secretions) that have been secreted into the intestinal lumen.

Some authors have argued that the host-colon nitrogen cycle, by which nitrogenous compounds that diffuse into the gut are converted to ammonia by the microflora and are reabsorbed, is a regulated function and serves as a mechanism of nitrogen conservation (Jackson, 1989). The theoretical basis of this proposition has been partly confirmed by the recent demonstration of the availability to the host of indispensable amino acids synthesized by intestinal microbes (Metges et al., 1999a, 1999b). However, not all investigators have obtained results indicative of regulated nitrogen cycling (Raguso et al., 1999; Young et al., 2000).

Although it seems clear that the efficiency of dietary protein digestion (in the sense of removal of amino acids from the small intestinal lumen) is high, there is now good evidence to show that nutritionally significant quantities of indispensable amino acids are metabolized by the tissues of the splanchnic bed, including the mucosal cells of the intestine (Fuller and Reeds, 1998). Thus, less than 100 percent of the amino acids removed from the intestinal lumen appear in the peripheral circulation, and the quantities that are metabolized by the splanchnic bed vary among the amino acids, with intestinal threonine metabolism being particularly high (Stoll et al., 1998). Currently, there is a lack of systematic information about the relationship between dietary amino acid intake and splanchnic metabolism, although there are indications that there is a nonlinear relationship between amino acid intake and appearance in the peripheral blood (van der Schoor et al., 2001).

Intestinal Protein Losses

Protein secretion into the intestine continues even under conditions of protein-free feeding, and fecal nitrogen losses (i.e., nitrogen lost as bacteria in the feces) may account for 25 percent of the obligatory loss of nitrogen (Fuller and Reeds, 1998). Under this dietary circumstance, the amino acids secreted into the intestine as components of proteolytic enzymes and from sloughed mucosal cells are the only sources of amino acids for the maintenance of the intestinal bacterial biomass. In those studies in which highly digestible protein-containing diets have been given to individuals previously ingesting protein-free diets, fecal nitrogen excretion increased by only a small amount. For highly digestible proteins, it also is likely that when humans consume diets that do not provide an excessive quantity of protein, a high proportion of the fecal nitrogen losses

originate from a combination of gastrointestinal secretions and the partial capture of the significant quantities of secreted urea that are hydrolyzed and subsequently used by the microflora in the large intestine (Jackson, 1989).

The following points support the view that the intestinal route of protein (amino acid) loss is of quantitative significance to maintenance protein needs. First, continued mucosal cell turnover and enzyme and mucin secretion are necessary for maintaining the integrity of the gastrointestinal tract and its normal digestive physiology. Second, animal studies show that the amino acid composition of the proteins leaving the ileum for bacterial fermentation in the colon is quite different from that of body protein (Taverner et al., 1981). In particular, the secretions are relatively rich in dispensable amino acids as well as threonine and cysteine (Dekker et al., 1991; Khatri et al., 1998; Taverner et al., 1981), probably because mucin secretions make a substantial contribution to the endogenous outflow. These two amino acids are of significance in meeting amino acid needs when intake is close to the requirement (Laidlaw and Kopple, 1987).

Other routes of loss of intact amino acids are via the urine and through skin and hair loss. These losses are small by comparison with those described above, but nonetheless may have a significant impact on estimates of requirements, especially in disease states (Matthews, 1999).

Protein Synthesis

Amino acids are selected for protein synthesis by binding with transfer RNA (tRNA) in the cell cytoplasm. The information on the amino acid sequence of each individual protein is contained in the sequence of nucleotides in the messenger RNA (mRNA) molecules, which are synthesized in the nucleus from regions of DNA by the process of transcription. The mRNA molecules then interact with various tRNA molecules attached to specific amino acids in the cytoplasm to synthesize the specific protein by linking together individual amino acids; this process, known as translation, is regulated by amino acids (e.g., leucine) (Jefferson and Kimball, 2001), and hormones. Which specific proteins are expressed in any particular cell and the relative rates at which the different cellular proteins are synthesized, are determined by the relative abundances of the different mRNAs and the availability of specific tRNA-amino acid combinations, and hence by the rate of transcription and the stability of the messages.

From a nutritional and metabolic point of view, it is important to recognize that protein synthesis is a continuing process that takes place in most cells of the body. In a steady state, when neither net growth nor protein loss is occurring, protein synthesis is balanced by an equal amount of protein degradation. The major consequence of inadequate protein

intakes, or diets low or lacking in specific indispensable amino acids relative to other amino acids (often termed limiting amino acids), is a shift in this balance so that rates of synthesis of some body proteins decrease while protein degradation continues, thus providing an endogenous source of those amino acids most in need.

Protein Degradation

The mechanism of intracellular protein degradation, by which protein is hydrolyzed to free amino acids, is more complex and is not as well characterized at the mechanistic level as that of synthesis (Kirschner, 1999). A wide variety of different enzymes that are capable of splitting peptide bonds are present in cells. However, the bulk of cellular proteolysis seems to be shared between two multienzyme systems: the lysosomal and proteasomal systems. The lysosome is a membrane-enclosed vesicle inside the cell that contains a variety of proteolytic enzymes and operates mostly at acid pH. Volumes of the cytoplasm are engulfed (autophagy) and are then subjected to the action of the protease enzymes at high concentration. This system is thought to be relatively unselective in most cases, although it can also degrade specific intracellular proteins (Cuervo and Dice, 1998). The system is highly regulated by hormones such as insulin and glucocorticoids, and by amino acids (Inubushi et al., 1996).

The second system is the ATP-dependent ubiquitin-proteasome system, which is present in the cytoplasm. The first step is to join molecules of ubiquitin, a basic 76-amino acid peptide, to lysine residues in the target protein. Several enzymes are involved in this process, which selectively targets proteins for degradation by a second component, the proteasome. This is a very large complex of proteins, possessing a range of different proteolytic activities. The ubiquitin-proteasome system is highly selective, so can account for the wide range of degradation rates (half-lives ranging from minutes to days) observed for different proteins. It is thought to be particularly responsible for degrading abnormal or damaged proteins, along with regulatory proteins that typically are synthesized and degraded very rapidly (Ciechanover et al., 1991; Goldberg and Rock, 1992; Hershko and Ciechanover, 1998).

Protein Turnover

The process by which all body proteins are being continuously broken down and resynthesized is known as protein turnover. In the adult human body, upward of 250 g/d of protein is synthesized and degraded (Waterlow, 1984). This compares with a median daily adult intake of about 55 to 100 g/d (Appendix Table E-16). The daily amount of protein turned

over is greater in infants and less in the elderly, when compared with young adults on a body-weight basis (Table 10-3). Some tissues are more active in protein turnover than others. Thus the liver and intestine, despite their rather small contribution to the total protein content of the body, are together believed to contribute as much as 50 percent of whole body protein turnover (McNurlan and Garlick, 1980; Waterlow, 1984). Conversely, skeletal muscle is the largest single component of body protein mass (43 percent), but contributes only about 25 percent to total body protein turnover (Reeds and Garlick, 1984; Waterlow, 1984).

At the tissue level, proteins are continually being synthesized and degraded as a sensitive means of regulating the amount of each separate enzyme or structural component. Other proteins may be secreted from the cell after synthesis and subsequently degraded at a distant site. Examples of such proteins are serum albumin synthesized in the liver, antibodies in the B-lymphocytes, digestive enzymes in the pancreas, and peptide hormones formed in the endocrine glands.

Amino Acid Catabolism

Nitrogen Metabolism

About 11 to 15 g of nitrogen are excreted each day in the urine of a healthy adult consuming 70 to 100 g of protein, mostly in the form of urea, with smaller contributions from ammonia, uric acid, creatinine, and some free amino acids (Table 10-4). These are the end products of protein metabolism, with urea and ammonia arising from the partial oxidation of amino acids. Uric acid and creatinine are indirectly derived from amino acids as well.

The removal of nitrogen from the individual amino acids and its conversion to a form that can be excreted by the kidney can be considered as a two-part process. The first step usually takes place by one of two types of

TABLE 10-3 Whole-Body Protein Synthesis in Humans at Different Life Stages

Life Stage	Protein Synthesis (g/kg/d)
Newborn (preterm)	17.4
Infant	6.9
Adult	3.0
Elderly	1.9

SOURCE: Young et al. (1975b).

TABLE 10-4 Approximate Distribution of Nitrogen in Urinary Constituents in Humans Consuming 100 g of Protein per Day (~16 g of Nitrogen)

Compound	Nitrogen (g/d)
Urea	12.8
Ammonia	0.7
Amino acids	0.7
Creatine/Creatinine	0.7
Uric acid	0.3
Hippuric acid	0.1
Total	15.3

SOURCE: Diem (1962).

enzymatic reactions: transamination or deamination. Transamination is a reversible reaction that uses ketoacid intermediates of glucose metabolism (e.g., pyruvate, oxaloacetate, and α-ketoglutarate) as recipients of the amino nitrogen. Most amino acids can take part in these reactions, with the result that their amino nitrogen is transferred to just three amino acids: alanine from pyruvate, aspartate from oxaloacetate, and glutamate from α-ketoglutarate.

Unlike many amino acids, branched-chain amino acid transamination occurs throughout the body, particularly in skeletal muscle. Here the main recipients of amino nitrogen are alanine and glutamine (from pyruvate and glutamate, respectively), which then pass into the circulation. These serve as important carriers of nitrogen from the periphery (skeletal muscle) to the intestine and liver. In the small intestine, glutamine is extracted and metabolized to ammonia, alanine, and citrulline, which are then conveyed to the liver via the portal circulation (Harper et al., 1984).

Nitrogen is also removed from amino acids by deamination reactions, which result in the formation of ammonia. A number of amino acids can be deaminated, either directly (histidine), by dehydration (serine, threonine), by way of the purine nucleotide cycle (aspartate), or by oxidative deamination (glutamate). These latter two processes are important because glutamate and aspartate are recipients of nitrogen by transamination from other amino acids, including alanine. Glutamate is also formed in the specific degradation pathways of arginine and lysine. Thus, nitrogen from any amino acid can be funneled into the two precursors of urea synthesis, ammonia and aspartate.

Urea synthesis takes place in the liver by the cyclic pathway known as the Krebs-Henseleit cycle. Among the essential reactions in this process is

the hydrolysis of the amino acid arginine by the enzyme arginase to yield urea and another amino acid, ornithine, which is not incorporated into body protein. The remaining part of the cycle involves the resynthesis of arginine using nitrogen from ammonia and aspartate. Thus, although arginine is the direct precursor of urea, it is not consumed in the process, as the nitrogen excreted as urea is all derived from ammonia and aspartate.

After synthesis, the urea is carried by the circulation from the liver to the kidney, where it is excreted into the urine. Although the excretion of urea dominates nitrogen excretion as a whole, significant quantities of ammonium ions are also excreted. There are some metabolic pathways, notably the purine nucleotide cycle, whereby purine nitrogen is converted to ammonium ions. It is generally believed that much of the ammonia produced by this cycle in skeletal muscle is transported in the blood as glutamine. Some of this glutamine is metabolized in the kidneys, where the enzyme glutaminase leads to the release of ammonium ions and glutamate. This glutamate, after losing its amino group, is then utilized in the synthesis of glucose in the kidney. The generation of ammonium ions from glutamine has a specific role in acid–base homeostasis, as ammonium ion excretion serves as the main vehicle for the excretion of excess hydrogen ions to prevent acidosis.

Carbon Metabolism

For most amino acids, removal of the amino nitrogen group generates their ketoacid analogues. Many of these are already in a form for entry into the pathways of oxidative metabolism (Figure 10-3). For example, both α-ketoglutarate (from glutamate) and pyruvate (from alanine) are intermediates of the glycolysis-tricarboxylic acid (TCA) pathway of glucose oxidation. All the others have specific degradation systems that give rise to intermediates that can be metabolized in these oxidative pathways. Thus, protein can make a significant contribution to the body's energy supply. This is particularly true in non-growing adults, who on average consume, and therefore oxidize, about 10 to 15 percent of their dietary energy as protein (Appendix Table E-17).

The contribution of protein to energy needs may be significant during periods of energy restriction or following the utilization of the body's limited endogenous carbohydrate stores. Protein oxidation also has been shown to rise considerably in highly traumatized or septic individuals, which results in large amounts of body protein loss; this loss can compromise recovery or even lead to death (see below) (Klein, 1990). It is much less in periods of chronic starvation because of various metabolic adaptations related to ketone utilization, or on protein-restricted diets.

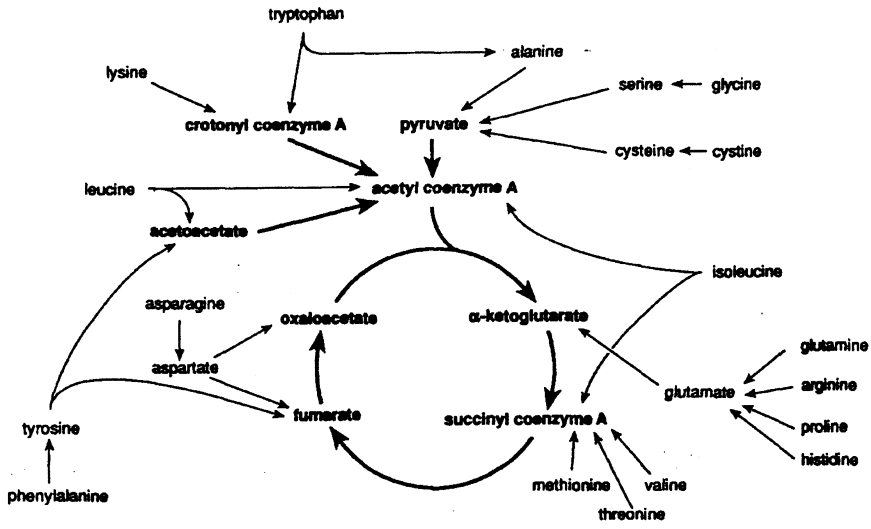


FIGURE 10-3 Metabolism of the carbon skeletons of the amino acid chains (light arrows) and their points of entry into the general pathways of metabolism of glucose and fat (bold arrows).
SOURCE: Garlick and Reeds (1993).

Once the amino acid deamination products enter the TCA cycle (also known as the citric acid cycle or Krebs cycle) or the glycolytic pathway, their carbon skeletons are also available for use in biosynthetic pathways, particularly for glucose and fat. Whether glucose or fat is formed from the carbon skeleton of an amino acid depends on its point of entry into these two pathways. If they enter as acetyl-CoA, then only fat or ketone bodies can be formed. The carbon skeletons of other amino acids can, however, enter the pathways in such a way that their carbons can be used for gluconeogenesis. This is the basis for the classical nutritional description of amino acids as either ketogenic or glucogenic (i.e., able to give rise to either ketones [or fat] or glucose).

Some amino acids produce both products upon degradation and so are considered both ketogenic and glucogenic (Figure 10-3). It has been argued that the majority of hepatic amino acid catabolism is directed in an obligatory fashion to glucose synthesis (Jungas et al., 1992). The synthesis of glucose in the liver from amino acids is dominated by alanine and glutamate, which derive much of their carbon from peripheral metabolism of glucose to lactate and TCA cycle intermediates. Thus, much of gluco-

neogenesis in metabolism is the result of a metabolic cycle of glucose carbon between the peripheral tissues (especially muscle) and the liver and kidney. This cycle also involves the peripheral synthesis of glutamine, an amino acid that is utilized in substantial quantities by the intestinal cells in which it is used for energy and for the synthesis of proline, citrulline, and nucleic acids.

A significant proportion of the glucose synthesized in the liver is due to recapture and recycling via the liver of 3-carbon units in the form of lactate derived from anaerobic glucose breakdown in muscle (the Cori cycle). Hepatic gluconeogenesis also occurs via the glucose–alanine cycle (a direct parallel of the Cori cycle) and the glucose–glutamine cycle. Since the nitrogen donors may be either glucogenic or ketogenic amino acids, these cycles function as mechanisms for transporting nitrogen from the periphery to the liver as well as for glucose production. The cycle involving glutamine transport from the periphery to the gastrointestinal tract is also vital to the synthesis of arginine and proline and is critical to the prevention of the build up of excessive ammonia in the circulation.

Nonprotein Pathways of Amino Acid Nitrogen Utilization

Although in general the utilization of dietary amino acids is dominated by their incorporation into protein and their role in energy metabolism, amino acids are also involved in the synthesis of other nitrogenous compounds important to physiological viability as shown in Table 10-5. Some pathways have the potential for exerting a substantial impact on the utilization of certain amino acids, and may be of potential significance for the requirements for these amino acids. This is particularly true for glycine, which is a precursor for six nitrogenous compounds, as shown in Table 10-5. Its utilization in the synthesis of creatine (muscle function), heme (oxygen transport and oxidative phosphorylation), and glutathione (protective reactions which are limited by the amount of available cysteine) is not only of physiological importance, but can also involve substantial quantities of the amino acid. For example, in the absence of a dietary source of creatine, adults require at least 1.1 g/d of glycine in order to sustain an adequate rate of creatine synthesis (calculated from a creatinine excretion of 1.8 g/d for a 70-kg man [Young et al., 1984] assuming 1 mole of glycine is used to synthesize 1 mole of creatine which gives rise to 1 mole of urinary creatinine). In premature infants, mainly fed human milk, there is evidence that the glycine supply may be a primary nutritional limitation to growth (Jackson, 1991). This so-called dispensable amino acid is then needed in the diet for optimum growth and may be termed “conditionally indispensable.”

Similarly, the synthesis of carnitine (involved in intracellular fatty acid transport) could, under some circumstances, become of quantitative sig-

TABLE 10-5 Amino Acid Precursors of Nonprotein Products

Precursor Amino Acids	End Product
Tryptophan	Serotonin
Tryptophan	Nicotinic acid
Tyrosine	Catecholamines
Tyrosine	Thyroid hormones
Tyrosine	Melanin
Lysine	Carnitine
Cysteine	Taurine
Arginine	Nitric oxide
Glycine	Heme
Glycine, arginine, methionine	Creatine
Methionine, glycine, serine	"Methyl group metabolism"
Glycine, taurine	Bile acids
Glutamate, cysteine, glycine	Glutathione
Glutamate, aspartate, glycine	Nucleic acid bases

nificance to lysine requirements. These may be important nutritional considerations in individuals consuming marginal amounts of proteins of plant origin and undoubtedly have an impact on overall amino acid utilization when protein intake is very low.

Clinical Effects of Inadequate Protein Intake

As outlined above, protein is the fundamental component necessary for cellular and organ function. Not only must sufficient protein be provided, but also sufficient nonprotein energy (i.e., carbohydrates, fats) must be available so that the carbon skeletons of amino acids are not used to meet energy needs (Duffy et al., 1981). Similarly, unless amino acids are present in the diet in the right balance (see later section, "Protein Quality"), protein utilization will be affected (Duffy et al., 1981). In the world as a whole, protein-energy malnutrition (PEM) is fairly common in both children and adults (Stephenson et al., 2000), and is associated with the deaths of about 6 million children each year (FAO, 2000). In the industrialized world, PEM is seen predominantly in hospitals (Bistrian, 1990; Willard et al., 1980), is associated with disease (Wilson and Pencharz, 1997), and often found in the elderly (Allison, 1995). Hypoalbuminemic malnutrition has been described in hospitalized adults (Bistrian, 1990) and has also been called adult kwashiorkor (Hill, 1992).

Clearly, protein deficiency has adverse effects on all organs (Corish and Kennedy, 2000). In infants and young children, it has been shown to have harmful effects on the brain and may have longer-term effects on

brain function (Pollitt, 2000). Furthermore, protein deficiency has been shown to have adverse effects on the immune system, resulting in a higher risk of infections (Bistrian, 1990). It also affects gut mucosal function and permeability, which, in turn, affects absorption and makes possible bacterial invasion from the gut, which can result in septicemia (Reynolds et al., 1996). Protein deficiency has also been shown to adversely affect kidney function, where it has adverse effects on both glomerular and tubular function (Benabe and Martinez-Moldonado, 1998).

Total starvation will result in death in initially normal-weight adults in 60 to 70 days (Allison, 1992). For comparison, protein and energy reserves are much smaller in premature infants, and survival of 1,000-g neonates is only about 5 days (Heird et al., 1972).

Clinical Assessment of Protein Nutritional Status

No single parameter is completely reliable to assess protein nutritional status. Borderline inadequate protein intakes in infants and children are reflected in failure to grow as estimated by length or height (Jelliffe, 1966; Pencharz, 1985). However, weight-height relationships can be distorted by edema and ascites (Corish and Kennedy, 2000). Mid-upper arm parameters such as arm muscle circumference have been used to measure protein status (Young et al., 1990). The triceps skinfold is reflective of energy nutritional status while the arm muscle circumference (or diameter) is reflective of protein nutritional status (unless a myopathy or neuropathy is present) (Patrick et al., 1994).

In addition, urinary creatinine excretion has been used as a reflection of muscle mass (Corish and Kennedy, 2000; Forbes, 1987; Young et al., 1990), but it is not very sensitive. The most commonly used methods to clinically evaluate protein status measure serum proteins; the strengths and weaknesses of these indicators are summarized in Table 10-6. In practical terms, acute protein depletion is not clinically important as it is rare, while chronic deficiency is important. Serum proteins as shown in Table 10-6 are useful, especially albumin and transferrin (an iron-binding protein). In a study from Nigeria, low transferrin levels were more predictive of risk of death in children with PEM than were albumin levels (Ramsey et al., 1992). Due to their very short half-lives, prealbumin and retinol binding protein (apart from their dependence on vitamin A status) may reflect more acute protein intake than risk of protein malnutrition (which is a process with an onset of period of 7 to 10 days (Ramsey et al., 1992). Hence, albumin and transferrin remain the best measures of protein malnutrition, but with all of the caveats listed in Table 10-6.

Physical examination related to protein malnutrition focuses attention on the skin and hair as they are rapidly growing protein-containing

TABLE 10-6 Use of Serum Proteins to Assess Protein Nutritional Status

	Half-Life	Clinical Use	Limitations
Albumin	18 d	Severe malnutrition	Affected by protein losing enteropathy, renal loss, burn, and by liver disease
Transferrin	8–9 d	Limited—chronic deficiency	Affected by iron deficiency and by infection
Pre-albumin	2 d	Acute depletion	Affected by vitamin A deficiency
Retinol-binding protein	12 h	Acute depletion	Affected by vitamin A deficiency

SOURCE: Adapted from Young et al. (1990).

tissues. In protein malnutrition, the skin becomes thinner and appears dull; the hair first does not grow, then it may fall out or show color changes (Pencharz, 1985). Over a longer period of time, assessment of changes in lean body mass reflects protein nutritional status. The clinical tools most available to assess lean mass are dual emission x-ray absorptiometry and bioelectrical impedance (Pencharz and Azcue, 1996).

SELECTION OF INDICATORS FOR ESTIMATING THE REQUIREMENT FOR PROTEIN (NITROGEN)

In the framework for Dietary Reference Intakes, as described in Chapter 1 and Appendix B, adequacy of requirements is defined as the lowest daily intake value for a nutrient that will meet the need, as defined by the specified indicator or criterion of adequacy, of apparently healthy individuals. This section reviews some of the possible indicators used or proposed for use in analyses estimating human protein requirements.

Factorial Method

The factorial method is based on estimating the nitrogen (obligatory) losses that occur when a person is fed a diet that meets energy needs but is essentially protein free and, when appropriate, also relies on estimates of the amount of nitrogen that is accreted during periods of growth or lost to mothers during lactation. The major losses of nitrogen under most conditions are in urine and feces, but also include sweat and miscellaneous losses, such as nasal secretions, menstrual losses, or seminal fluid. In this

method, the protein requirement is estimated by interpolating or extrapolating the obligatory losses to the zero balance point in which protein needs (as nitrogen) are assumed to equal the obligatory protein lost as nitrogen (nitrogen equilibrium).

This is where the factorial method has its greatest weakness, since the relationship between protein intake and nitrogen retention is somewhat curvilinear; the efficiency of nitrogen retention becomes less as the zero balance point is approached (Rand and Young, 1999; Young et al., 1973). Additionally, in order to utilize the factorial approach when determining the protein requirement for infants and children, their needs for protein accreted as a result of growth must be added to their maintenance needs.

Nitrogen Balance Method

This classical method has been viewed by many as theoretically the most satisfactory way of determining the protein requirement. Nitrogen balance is the difference between nitrogen intake and the amount excreted in urine, feces, skin, and miscellaneous losses. As discussed below, nitrogen balance remains the only method that has generated sufficient data for the determination of the total protein (nitrogen) requirement. It is assumed that when needs are met or exceeded adults come into nitrogen balance; when intakes are inadequate, negative nitrogen balance results. In determining total protein (nitrogen) needs, high-quality proteins are utilized as test proteins to prevent negative nitrogen balance resulting from the inadequate intake of a limiting indispensable amino acid. A significant literature exists regarding the methods and procedures to use in determining nitrogen balance amount (Manatt and Garcia, 1992; Rand et al., 1981).

Limitations of the Method

The nitrogen balance method does have substantial practical limitations and problems. First, the rate of urea turnover in adults is slow, so several days of adaptation are required for each level of dietary protein tested to attain a new steady state of nitrogen excretion (Meakins and Jackson, 1996; Rand et al., 1976). Second, the execution of accurate nitrogen balance measurements requires very careful attention to all the details of the procedures involved. Since it is easy to overestimate intake and underestimate excretion, falsely positive nitrogen balances may be obtained (Hegsted, 1976). Indeed, an overestimate of nitrogen balance seems consistent throughout the literature because there are many observations of quite considerable apparent retention of nitrogen in adults (Oddoye and Margen, 1979). This observation is biologically implausible because (a) adults

do not normally accrete body protein, and (b) the magnitude of the positive nitrogen balances is inconsistent with stability of body weight. A third limitation of the nitrogen balance method is that since the requirement is defined for the individual, and studies rarely provide exactly the amount of protein necessary to produce zero balance, individuals must be studied at several levels of protein intake in the region of the requirement so that estimates of individual requirements can be interpolated (Rand et al., 1976; Zello et al., 1990). Finally, dermal and miscellaneous losses of nitrogen must be included in the calculation. These are inordinately difficult to measure, and vary with the environmental conditions (e.g., ambient temperature). In fact, the literature indicates marked (at least twofold) differences between studies (Calloway et al., 1971). The inclusion of dermal and miscellaneous nitrogen losses can have a significant effect on estimates of amino acid requirements via nitrogen balance, especially in adults (Calloway et al., 1971; Millward, 1998; Rand and Young, 1999).

Statistical Analysis of Nitrogen Balance Data

In studies with healthy adults in presumably good nutritional status, it is generally assumed that the protein requirement is achieved when an individual is in zero nitrogen balance. To some extent, this assumption poses problems that may lead to underestimates of the true protein requirement. First, there are sufficient observations of paradoxically high positive nitrogen balances in the literature to imply that when individuals are in *measured* body nitrogen equilibrium, they are in fact in a small negative nitrogen balance (Kopple, 1987). The large majority of the studies have concentrated their measurements of protein adequacy at levels of intake below nitrogen balance and as a result, the intercept of protein intake at zero nitrogen balance is lower than the true intercept as the efficiency of protein utilization decreases as zero balance is reached (Young et al., 1973).

The empirical solution is to carry out measurements that span nitrogen equilibrium, ideally by using multiple levels of intake in the same individual and interpolating individual requirement levels. Three different interpolation schemes have been proposed, based on (1) a smooth nonlinear model (Hegsted, 1963; Rand and Young, 1999), (2) a two-phase linear model (also called bilinear or breakpoint) (Kurpad et al., 2001a; Zello et al., 1995), and (3) a linear model (Rand et al., 1976; Rand et al., 2003). Since the physiological response relationship between nitrogen intake and balance is theoretically expected not to be linear, the more complex models (1 and 2 above) would be appropriate bases for arriving at a requirement estimate. However, in order to set the Recommended Dietary Allowance (RDA), it is necessary to determine the variability of the

requirement between individuals, free from the considerable within-individual variability, and both these models require more data points on each individual than are currently available in published studies.

Thus, while it is recognized that the first two models above are more realistic biologically, because of the lack of available data the method adopted for this report is to use linear interpolation to estimate the individual requirements (the intakes predicted to result in zero balance) that in turn are used to estimate the distribution of protein requirements. The bilinear model was used to estimate requirements for some of the amino acids; however, estimates of population variability (between individuals) were derived from the analysis of protein requirements.

SELECTION OF INDICATORS FOR ESTIMATING THE REQUIRMENT FOR INDIVIDUAL AMINO ACIDS

Irrespective of whether a design involving multiple studies in a limited number of individuals or single studies in a larger number of subjects has been used, the uniform approach to the determination of the requirement for an individual indispensable amino acid involves measuring the relationship between the intake of the amino acid (in an otherwise adequate diet) and some predetermined marker of nutritional adequacy. The marker can be one that follows the state of protein metabolism or balance (e.g., nitrogen balance or whole body protein turnover) or the status of the metabolism and utilization of the amino acid under investigation (e.g., its concentration). These approaches give somewhat different information about the requirement for the amino acid. Moreover, each method has peculiar theoretical and practical disadvantages, thus the level of consistency of estimates based on different approaches should be examined. The following approaches have been proposed.

Nitrogen Balance Method

This classical method is discussed earlier in more detail under "Selection of Indicators for Estimating the Requirement for Protein (Nitrogen)." It has been apparent for at least 15 years that the nitrogen balance–derived values for amino acid requirements in adults are lower than values derived by the other methods described below, which provide results similar to each other (Millward et al., 1990; Young, 1987; Young et al., 1989). Many explanations have been put forward for the lower results using nitrogen balance methodology, including the fact that excess nonprotein energy may have been used in many nitrogen balance studies (Garza et al., 1976, 1977a, 1977b, 1978).

Rand and Young (1999) analyzed the data of Jones and coworkers (1956) in which young women were fed up to five different intake levels of lysine. The design of that study allowed for the determination of between-individual variance by studying each individual at several levels of lysine intake. In fact, within the large nitrogen balance and amino acid requirement literature, only one other study (Reynolds et al., 1958) was found in which adults were studied at four or more different intakes of amino acids with constant levels of total nitrogen (Reynolds et al., 1958). These investigators studied four different levels of methionine and cysteine.

With these two data sets, nonlinear regression can be utilized. The reanalysis of the 1956 Jones study produced an estimate of nitrogen equilibrium for lysine of 30 mg/kg/d, which is comparable to the values derived by the other methods described below (Rand and Young, 1999). In addition, most of the classic amino acid work using nitrogen balance (Leverton et al., 1956a, 1956b, 1956c, 1956d; Rose, 1957; Rose et al., 1955a, 1955b, 1955c, 1955d, 1955e, 1955f) did not include dermal and miscellaneous losses, which result in further underestimation of indispensable amino acid requirements.

Unfortunately, for infants and children the only data available are those based on nitrogen balance, and considerable uncertainty about the accuracy of the estimates remains. However, recent factorial estimates are in reasonable agreement with the nitrogen balance estimates (Dewey et al., 1996).

Plasma Amino Acid Response Method

This method was the first that focused on the physiology of the individual amino acid (Longnecker and Hause, 1959; Munro, 1970). The reasoning behind this approach is that when the intake of the test amino acid is below its dietary requirement, then its circulating concentration is not only low, but also is relatively insensitive to changes in intake. As intakes of the target amino acid approach the requirement level by increasing the intake of the limiting amino acid, the plasma level of the amino acid starts to increase progressively (see Figure 10-4). The point at which the "constant" portion of the relationship between intake and plasma concentration intersects the linear portion is considered to be an estimate of the requirement.

A variation on this method involves the examination of the changes in the plasma concentration of the test amino acid as the adult moves from the post absorptive to the fed state post-consumption (Longnecker and Hause, 1961). At intakes below the requirement, the plasma concentration of the test amino acid is expected to fall in the fed state and rise only when the dietary supply of the amino acid is greater than the individual's

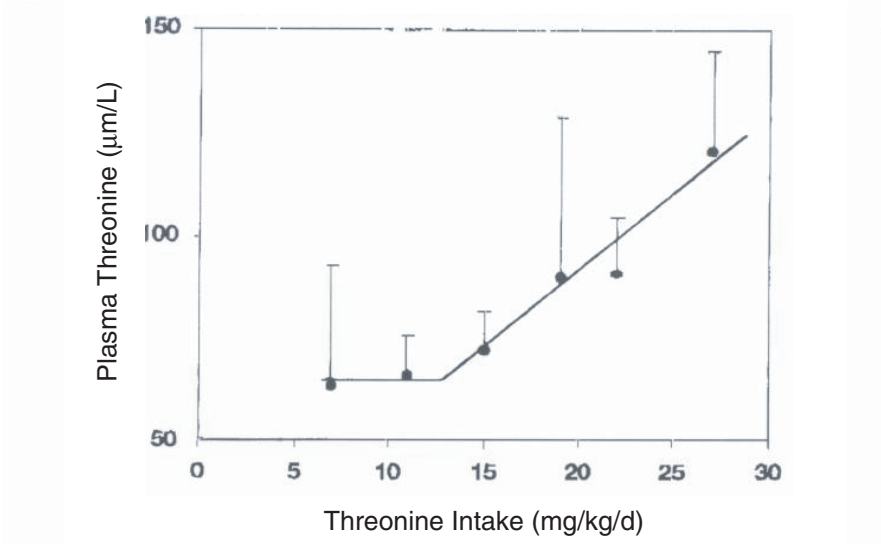


FIGURE 10-4 Plasma threonine levels in the fed state with increasing levels of threonine intake in well-nourished men. Two-phase regression revealed a breakpoint at about 13 mg/kg/d. Taken from Kurpad and Young (2001), with permission.

requirement. The theoretical basis of the approach is sound but the method has disadvantages. The main difficulty is that amino acid metabolism is so complex that factors other than the level of amino acid intake, such as gastric emptying time, can influence its concentration (Munro, 1970). Furthermore, the relationship between the intake of the amino acid and its circulating concentration is not necessarily bilinear, so it is difficult to determine a "breakpoint" (Young et al., 1972). Although in some regards this problem applies also to the oxidation methods discussed below, over the last 20 years these later methods have supplanted plasma amino acid concentration–based approaches.

Direct Amino Acid Oxidation (DAAO) Method

In the 1980s, Young and his coworkers introduced the use of measurements of the carbon oxidation of single indispensable amino acids as indicators of adequacy of the amino acids (Young et al., 1989). This marked a major theoretical advance over the nitrogen balance and plasma amino acid response methods. The theoretical basis of the direct amino acid oxidation (DAAO) method is that the nutritional indispensability of an amino acid is a function of its inability to synthesize its carbon skeleton.

Thus, when the test amino acid is labeled with ^{13}C, the production of breath $^{13}CO_2$ is assumed to be a good measure of the irreversible oxidative loss of the amino acid.

The method has been applied in a similar manner to the plasma amino acid response approach by designing experiments to determine a breakpoint in the relationship between the carbon catabolism of the amino acid (as measured as breath $^{13}CO_2$) and its intake. Thus by analogy to the concentration method, it is assumed that below the requirement the test amino acid is conserved and that there is a low constant oxidation rate, but once the requirement is reached, the oxidation of the test amino acid increases progressively.

Although the DAAO method was an important advance beyond nitrogen balance, it also presented limitations, which have been discussed in depth (Fuller and Garlick, 1994). The most salient problem arises from the reliance on the determination of a breakpoint in the oxidation of the test amino acid. This reliance requires studies at very low intakes of the test amino acid. However, at these low dietary intakes, the intake of the infused labeled amino acid becomes significant in relation to dietary intake. This can lead to errors in the estimation of amino acid oxidation based on the production of labeled CO_2. Thus, values of amino acid oxidation based on the production of $^{13}CO_2$ are likely to be overestimated. The second limitation is that the DAAO method can only be used with full accuracy for those amino acids whose carboxyl group is released directly to the body bicarbonate pools. This limits its use largely to the branched chain amino acids, phenylalanine, and lysine. Other amino acids, such as threonine and tryptophan, pose particular problems (Zhao et al., 1986).

A criticism of this method has been that measurements were only made during a short period during which food was given at regular hourly intervals. This period was therefore not representative of the day as a whole. A later modification of this approach was to infuse the labeled amino acid during a period of fasting followed by a period of hourly meals, thus acknowledging the discontinuous way in which food is normally taken (Young et al., 1987). However, although this was an advance on the earlier approach, assumptions still had to be made to extrapolate the results from the short periods to a full day.

Twenty-four Hour Amino Acid Balance Method

Over the last decade, the DAAO method has been adapted in such a way that it allows the carbon balance of the amino acid under investigation to be determined over a full 24-hour period (El-Khoury et al., 1994a, 1994b). In some respects, the development of the 24-hour amino acid balance method stemmed from the fact that the DAAO method had been

criticized because measurements were made only in the fed state. Thus the 24-hour amino acid balance method was developed to determine the balance of the test amino acid over a 24-hour period that encompassed periods of fasting and feeding. This marked a significant advance in determining amino acid requirements because it moved investigations away from the simple study of nitrogen metabolism and allowed, in principle at least, direct measurements of the quantities of the amino acid lost under different nutritional circumstances.

There also are drawbacks to this method. The first limitation arises from the unresolved questions related to the method's theoretical basis. Although the end point of the method is the measurement of the body's balance of the test amino acid, the base measurement is the proportion of the dose of ^{13}C-amino acid that is excreted as $^{13}CO_2$. In order to convert the measured rate of production of labeled CO_2 to a rate of oxidation of the amino acid, it is necessary to know the isotopic enrichment of the amino acid at the intracellular site of oxidation. This is difficult because amino acid metabolism is compartmentalized and measurements of plasma amino acid labeling likely underestimate true turnover, and hence true oxidative loss, of the amino acid. Although for some amino acids this problem can be circumvented by administering a labeled metabolic product of the amino acid (e.g., α-keto isocaproic acid in studies of leucine metabolism).

The second drawback is practical—measuring the oxidation of the test amino acid over a complete 24-hour period makes the method labor intensive. This probably underlies the fact that to date this method has been applied to only three amino acids: leucine (El-Khoury et al., 1994a, 1994b; Kurpad et al., 2001b), lysine (El-Khoury et al., 2000; Kurpad et al., 2001a), and phenylalanine with and without tyrosine (Basile-Filho et al., 1998), and only a limited number of different levels of amino acid intake have been investigated.

Indicator Amino Acid Oxidation (IAAO) Method

The indicator amino acid oxidation (IAAO) method arose from work on the amino acid requirements of neonatal pigs (Kim et al., 1983). Although the IAAO method is based on measurements of amino acid oxidation, it uses measurements of the carbon catabolism of a nonlimiting amino acid (called the indicator amino acid) as a carbon analogue of nitrogen balance. The reasoning is that when a single indispensable amino acid is provided below its requirement, it acts as the single and primary limitation to the ability to retain other nonlimiting amino acids in body protein. These other amino acids, including the indicator amino acid, are then in nutritional excess and are oxidized (Zello et al., 1995). When the

intake of the test amino acid is zero, then protein synthesis is minimal and oxidation of the indicator is maximal. As the intake of the test amino acid is increased, protein retention increases and the oxidation of the indicator amino acid falls until the requirement level of the test amino acid is reached, after which the oxidation of the indicator amino acid is lower and essentially constant. The data are then analyzed to obtain as estimate of the intersection of the constant and linear portions of the relationship (the breakpoint).

The IAAO method has some advantages over the direct oxidation and carbon balance methods and has been validated in growing piglets by comparing estimates based on growth and body composition (Kim et al., 1983). The first advantage is that the metabolic restrictions of carbon dioxide release apply only to the indicator amino acid. Thus amino acids such as threonine, whose peculiar metabolism makes them problematical in the DAAO method, can be studied. Second, the pool size of the indicator amino acid does not change radically as the intake of the test amino acid is varied. Thus to some extent, potential problems of compartmentation are minimized and, in principle, the method does not require estimates of the turnover of the indicator amino acid.

However, the IAAO method also has several limitations as it has been applied. First, like the DAAO approach, it has only been used in the fed state and the extent to which the fasting-state oxidation of the indicator amino acid is altered by the status of the limiting amino acid has not been determined. Second, the dependence of the result on the amount of total protein given during the isotope infusion has not been established. Third, the choice of the best indicator is still under study so that data obtained with the method are dependent on the assumption of the general applicability of the indicator amino acids (phenylalanine and lysine) that have been used most frequently.

Classical nitrogen balance studies in humans show that it takes 7 to 10 days for urinary nitrogen to equilibrate in adults put on a protein-free diet (Rand et al., 1976). On the other hand, it has been shown that most (about 90 percent) of the adaptation in leucine kinetics is complete in 24 hours (Motil et al., 1994). Zello and coworkers (1990) studied 2- to 8-day adaptation periods to either 4.2 or 14 mg/kg/d of phenylalanine on rates of phenylalanine oxidation at phenylalanine intakes of 5, 7, 10, 14, 21, 28, or 60 mg/kg/d. These investigators were unable to show any effect of prior adaptation to these two different phenylalanine intakes on the rates of phenylalanine oxidation at changing phenylalanine intakes, where the adaptation to the test level was about 4 hours. Clearly, from this study, adaptations in amino acid metabolism appear to take place much more quickly than do adaptations in urinary nitrogen excretion and are (at least for leucine [Motil et al., 1994]) virtually complete within 24 hours.

The most satisfactory statistical models for determining amino acid requirements use regression to define the population mean and variance. For the regression models to work, ranges of intake (particularly at the low end) have to be fed. In practical terms, this has greatly hampered studies in infants, children, and other vulnerable groups. On the other hand, if the individual only needs to be on a low or even zero intake of the test amino acid for a matter of 8 hours, then it becomes feasible to study indispensable amino acids in these and other vulnerable groups.

Such a minimally invasive indicator oxidation model has been developed (Bross et al., 1998) and applied to determine tyrosine requirements in children with phenylketonuria (Bross et al., 2000). In this model the oxidation study is conducted after only 6 hours of adaptation to the level of the test amino acid, which is administered every 30 minutes.

For amino acid oxidation measurements, two-phase linear crossover regression analysis was introduced during the validation of indicator amino acid oxidation in piglets (Kim et al., 1983). Later, this approach was transferred to humans in a direct oxidation study (Zello et al., 1990) and in indicator oxidation studies (Bross et al., 2000; Zello et al., 1993). This technique permits a precise determination of the breakpoint, which is used as the estimate of the requirement for the amino acid Estimated Average Requirement (EAR).

As pointed out above, the drawbacks of the indicator method are the short period of measurement in the fed state only, and the lack of a period of adaptation to the test diets. To avoid these drawbacks, a 24-hour indicator method has been developed (Kurpad et al., 2001a), which takes advantage of the strengths of the indicator approach, as well as the 24-hour period of measurements including feeding and fasting. On theoretical grounds, this method has advantages over other methods for estimating amino acid requirements, and is the chosen method for estimated amino acids requirements where data are available.

FINDINGS BY LIFE STAGE AND GENDER GROUP FOR TOTAL PROTEIN

Infants Ages 0 Through 6 Months

Method Used to Set the Adequate Intake

The recommended intakes of protein are based on an Adequate Intake (AI) that reflects the observed mean protein intake of infants fed principally with human milk.

Human Milk. Human milk is recognized as the optimal source of nutrients for infants throughout at least the first year of life and is recommended as the sole nutritional source for infants during the first 4 to 6 months of life (IOM, 1991). There are no reports of apparently healthy, full-term infants, exclusively fed human milk, who manifest any signs of protein deficiency (Heinig et al., 1993). Therefore, determination of the AI for protein for infants is based on data from infants fed human milk as the principal source of nutrients during the period 0 through 6 months of age. As is described in Chapter 2, the AI is set at the mean value calculated from studies in which the volume of human milk was measured by test weighing, and the average concentration of the protein content in human milk was determined using average values from several reported studies.

The protein content of human milk at various stages of lactation is shown in Table 10-7. In general, protein concentrations decline in the later stages of lactation. Nonprotein nitrogen contributes 20 to 27 percent of total milk nitrogen (Atkinson et al., 1980; Butte et al., 1984a, 1984b; Dewey et al., 1996). These nonprotein nitrogenous components include free amino acids, pyrimidine nucleotides, creatine, and glutathione, but the large majority is urea. Using data from 13 lactating mothers of term infants, Butte and coworkers (1984a) reported that the protein content of human milk was 1.29 g/dL at 2 weeks of lactation, 1.08 g/dL at 4 weeks, 1.01 g/dL at 6 weeks, 0.94 g/dL at 8 weeks, and 0.91 g/dL from 10 to 12 weeks. Similar results of 0.91 g/dL were reported by Lammi-Keefe and coworkers (1990) at 8 weeks of lactation in 6 mothers of term infants. Both of these studies analyzed nitrogen by the Kjeldahl method. However, higher human milk protein content has been reported by Nommsen and coworkers (1991): 1.21 g/dL at 3 months, 1.14 g/dL at 6 months, 1.16 g/dL at 9 months, and 1.24 g/dL at 12 months of lactation. Dewey and coworkers (1984) reported values of approximately 1.25 g/dL at 4 to 11 months of lactation. These latter investigators attribute the higher values to their utilization of the modified Lowry assay for total protein, which tends to result in slightly higher values (Nommsen et al., 1991).

Ages 0 Through 6 Months. The AI for infants 0 through 6 months is based on the estimated average volume of milk intake of 0.78 L/d (Allen et al., 1991; Heinig et al., 1993) for this age group, and an average protein content of human milk of 11.7 g/L. This is the average protein content of human milk during the first six months of lactation from studies (Butte et al., 1984a; Dewey and Lönnerdal, 1983; Dewey et al., 1984; Nommsen et al., 1991) in which the sample size was at least 10 and actual data were provided (see Table 10-7). This value is in the range of protein content reported in other studies (Table 10-7). Multiplying this amount by the estimated average volume of intake of human milk for infants 0 through

6 months, the AI would be 11.7 g/L × 0.78 L/d = 9.1 g/d or 1.52 g/kg/d based on the reference weight of 6 kg for the 2- through 6-month-old infant from Chapter 1 (Table 1-1).

Protein AI Summary, Ages 0 Through 6 Months

AI for Infants	
0–6 months	**1.52 g/kg/d**

Special Considerations

Although protein intakes have been reported to be 66 to 70 percent higher in infants fed formula compared with those fed human milk for up to 12 months of age, there is no evidence that the lower protein intakes in the breast-fed infants were associated with adverse outcomes (Heinig et al., 1993). In fact, despite their lower protein intakes, some studies have demonstrated that infants fed human milk have better immune function and behavioral development than formula-fed infants (IOM, 1991; Lucas et al., 1992; Rogan and Gladen, 1993). As expected, gains in weight and lean body mass are higher in the formula-fed than breast-fed infants, but when controlled for energy intake, protein intake is not associated with weight or length gain within the breast-fed infants (Heinig et al., 1993). Several studies have shown that infants fed formula with a true protein level ([total nitrogen – nonprotein nitrogen] multiplied by 6.25) of 15 g/L have higher urea nitrogen and plasma amino acid levels than those seen in breast-fed infants (Janas et al., 1985, 1987; Järvenpää et al., 1982a, 1982b; Räihä et al., 1986a, 1986b), a true protein intake of 13 g/L of infant formula based on cow milk has been shown to result in a similar plasma amino acid profile in formula-fed infants to that seen in breast-fed infants (Lönnerdal and Chen, 1990).

It is recognized that casein and whey in cow milk is not the same as human casein and whey and that the absorption and digestibility of amino acids from formula is different than that of human milk. The 1985 Joint FAO/WHO/UNU expert group (FAO/WHO/UNU, 1985) recommended a factor of 0.70 for protein in cow milk, finding that it is 70 percent as efficiently utilized as the protein in human milk based on studies in 1-year-old infants. Later Fomon (1991) recommended a conversion estimate of 90 percent for infants receiving infant formula as the only source of dietary protein and suggested that infant formula should contain a minimum of 1.6 g α-amino nitrogen/100 kcal. Thus in determining the level of protein to be included in infant formula based on various possible protein sources, it is important to evaluate the digestibility and comparative protein quality (see "Protein Quality") as indicated above.

TABLE 10-7 Protein Content of Human Milk in the United States and Canada

Reference	Country	*n*	Stage of Lactation	Protein Content in Milk (g/dL)[a]	Comments
Anderson et al., 1981	Canada	10	3–5 d	1.9	Milk protein content was approximated from study figure
			8–11 d	1.7	
			15–18 d	1.5	Nitrogen determined by Kjeldahl analysis
			25–29 d	1.3	
Lemons et al., 1982	United States	7	7 d	1.59 ± 0.08	Nitrogen determined by Kjeldahl analysis
			14 d	1.23 ± 0.09	Protein determined by multiplying milk protein nitrogen by 6.25
			21 d	1.18 ± 0.04	
			28 d	1.10 ± 0.05	
Anderson et al., 1983	United States	9	3 d	2.3 ± 0.6	Nitrogen determined by Kjeldahl analysis
			7 d	1.7 ± 0.2	
			14 d	1.3 ± 0.4	
Dewey and Lönnerdal, 1983	United States	13	1 mo	**1.44 ± 0.20**	Protein analyzed by dye-binding assay
		16	2 mo	**1.33 ± 0.16**	
		18	3 mo	**1.32 ± 0.16**	
		16	4 mo	**1.30 ± 0.24**	
		14	5 mo	**1.25 ± 0.17**	
		15	6 mo	**1.27 ± 0.36**	
Neville et al., 1984	United States	10	33–210 d (median 115 d)	1.41 ± 0.06 (SEM)	Protein analyzed by the Biuret reaction Mid-feed sample

Dewey et al., 1984	United States	40 27	4–6 mo 7–11 mo	**1.26 ± 0.27** *1.24 ± 0.22*	Protein analyzed by a modified Lowry assay
Butte et al., 1984a	United States	13	2 wk 4 wk 6 wk 8 wk 10 wk 12 wk	1.29 ± 0.18 **1.08 ± 0.16** **1.01 ± 0.10** **0.94 ± 0.15** **0.91 ± 0.16** **0.91 ± 0.16**	Nitrogen determined by Kjeldahl analysis Protein determined by multiplying milk protein nitrogen by 6.25
Lönnerdal et al., 1987	United States	3 4 7	1–3 d 7–20 d 32–166 d	2.70 ± 0.18 (SEM) 1.61 ± 0.10 1.02 ± 0.05	Nitrogen determined by Kjeldahl analysis Protein determined by multiplying milk protein nitrogen by 6.25
Lammi-Keefe et al., 1990	United States	6	8 wk	0.91	Nitrogen determined by Kjeldahl analysis Protein determined by multiplying milk protein nitrogen by 6.25
Nommsen et al., 1991	United States	58 45 28 21	3 mo 6 mo 9 mo 12 mo	**1.21 ± 0.15** **1.14 ± 0.15** *1.16 ± 0.18* *1.24 ± 0.15*	Protein analyzed by a modified Lowry assay

[a] Mean ± standard deviation, unless otherwise noted. Values in bold used to estimate average protein content of human milk as 11.7 g/L during months 1 through 6 of lactation. Values in italics used to estimate average protein content of human milk as 12.3 g/L during months 7 through 12 of lactation.

Infants Ages 7 Through 12 Months

Method Used to Estimate Average Intakes

During the second 6 months of life, solid foods become a more important part of the diet of infants and add a significant amount of protein to the diet. Although limited data are available for typical protein intakes from foods by infants fed human milk, mean protein intake from complementary foods for infants aged 7 through 12 months was estimated to be 7.1 g/d for human milk–fed infants based on data from the Third National Health and Nutrition Examination Survey. Heinig and coworkers (1993) reported slightly higher values for nonmilk protein intake during the second 6 months of life. Based on their data, the average volume of human milk consumed during the second 6 months of life would be about 0.6 L/d. Thus, protein intake from human milk with a protein content of about 12.1 g/L at 7 to 12 months of lactation from the data for this age group (Dewey et al., 1984; Nommsen et al., 1991) would be approximately 7.3 g/d (12.1 g/L × 0.6 L/d). It should be noted that this is greater than that derived from the studies of content of milk from earlier lactation periods, primarily due to the use of the Lowry methods by both of these reports and the small number of studies available from this lactation period.

Adding the intake from milk (7.3 g/d) and food (7.1 g/d), the total average protein intake is estimated to be 14.4 g/d or 1.6 g/kg/d based on the reference weight of 9 kg for the 7- through 12-month-old infant from Chapter 1 (Table 1-1).

Method Used to Estimate the Average Requirement

Published data on the relationship between protein (nitrogen) intake and nitrogen balance were utilized to estimate protein requirements by the factorial method for infants 7 through 12 months of age as well as for children and adolescents through 18 years of age. The factorial method includes: (1) estimates of the maintenance requirement, which is determined by regression analysis of the relationship between nitrogen intake and nitrogen balance, (2) measurement of the rates of protein deposition, which are derived from body composition analysis, and (3) estimates of the efficiency of protein utilization, which is derived from the slope of the line relating intake and balance from the available data on infants and children.

Several nitrogen balance studies that involved children in the age range of 9 months to about 14 years were identified and analyzed (Table 10-8). The studies fall into three groups: (1) studies designed to measure "basal" nitrogen loss at very low or zero protein intakes, (2) studies

involving children receiving only one of a variety of protein levels, and (3) studies involving a limited number of individuals but with each individual receiving a range of protein intakes. Included in the analysis were studies in which the children consumed diets containing milk/egg, legume/cereal, and mixed vegetable/animal protein sources. The results, summarized in Table 10-8, were obtained in mostly boys and include a number of different ethnic groups including European, African, Central American, and Chinese.

Miscellaneous Losses. A critical aspect of the analysis is the inclusion of an estimate for integumental and unaccounted losses that were based on direct measurements in children, mostly boys, aged 7 months through 14 years. On the basis of five reports (Howat et al., 1975; Huang et al., 1980; Korslund et al., 1976; Uauy et al., 1981; Viteri and Martinez, 1981), the mean miscellaneous nitrogen losses are estimated to be 6.5 (± 2.3) mg/kg/d with a range of 5 to 9 mg/kg/d. In deriving the protein requirement, this estimate of miscellaneous losses was included as an adjustment to the reported nitrogen balances for the studies included in Table 10-8. The miscellaneous losses from both boys and girls are assumed to be the same since data from girls were limited.

Maintenance Requirement. Individual maintenance protein requirements were estimated by first regressing nitrogen balance on nitrogen intake for the individuals studied at several different intake levels, and then using these individual regression equations to interpolate the intakes that would be expected to produce zero nitrogen balance (adjusting for 6.5 mg/kg/d for miscellaneous losses). Table 10-8 contains seven studies that permit estimation of individual requirements and three studies that were used to estimate pooled requirements. As shown in the table, the average individual maintenance requirement was estimated as the median of the individual nitrogen requirements (108 mg/kg/d). For each study, an estimate was calculated as the median of the individual studies or the study pooled nitrogen requirement for those studies without individual data, and was 110 mg/kg/d . Since data for girls were sparse and could not be separated from that for boys, the protein maintenance requirement for both boys and girls is set at the same level. In addition, the maintenance protein requirement was not adjusted for age, as the requirement per kg of body weight for children 8 years of age and above appeared to be similar to that of younger children ranging in age from 9 months to 5 years (Table 10-8). Supporting this decision are the data of Widdowson and Dickerson (1964), which demonstrated that around 4 years of age, body protein concentration reaches the adult value of 18 to 19 percent of body weight.

TABLE 10-8 Maintenance Protein Requirement for Children Based on Nitrogen Balance Data[a]

Reference	Country	Diet	Age
Huang et al., 1980	China	Milk	9–17 mo
Huang et al., 1980	China	Egg	9–17 mo
Intengan et al., 1981	Philippines	Rice and fish	18–26 mo
Torun and Viteri, 1981	Guatemala	Milk	17–31 mo
Torun et al., 1981	Guatemala	Soy	17–31 mo
Egana et al., 1984	Chile	Milk	34–62 mo
Egana et al., 1984	Chile	Soy	34–62 mo
Intengan, 1984	Philippines	Rice and beans	22–29 mo
Gattas et al., 1990	Chile	Mixed	8–10 y
Gattas et al., 1992	Chile	Mixed	12–14 y
Median of all individual estimates (n = 7 studies)			
Median of all studies (n = 10)			

[a] Entries are medians (mean ± standard deviation).
[b] Multiple data on each individual not available.
[c] Regression estimate of study requirement.

Protein Deposition. Estimates of rates of protein deposition for infants from 9 months through 3 years of age (Butte et al., 2000) and total body protein content from 4 through 18 years of age (Ellis et al., 2000) were utilized to estimate rates of body protein deposition and are shown in Table 10-9. This table contains longitudinal (Butte et al., 2000) and cross-

n	Intercept at 6.5 mg N/kg/d	Slope	Maintenance Requirement Including 6.5 mg N/kg/d
32 points[b] (24 boys)	–77.5[c]	0.69[c]	112[c]
29 points[b] (10 boys)	–81.6[c]	0.71[c]	116[c]
7 boys	–53.6 (–47.4 ± 26.0)	0.52 (0.49 ± 0.10)	102 (91 ± 37)
10 boys	–52.0 (–51.1 ± 22.1)	0.70 (0.71 ± 0.12)	66 (71 ± 28)
10 boys	–55.5 (–52.2 ± 14.5)	0.55 (0.58 ± 0.09)	90 (89 ± 18)
6 boys and girls	–35.4 (–40.1 ± 16.2)	0.52 (0.51 ± 0.08)	76 (79 ± 27)
7 boys and girls	–58.2 (–59.4 ± 9.9)	0.51 (0.49 ± 0.10)	127 (124 ± 19)
5 boys	–98.1 (–121.1 ± 43.7)	0.68 (0.77 ± 0.24)	149 (156 ± 15)
8 boys	–67.3 (–55.4 ± 39.2)	0.54 (0.43 ± 0.29)	126 (126 ± 11)
8 boys (pooled)[b]	–61.4[c]	0.57[c]	107[c]
53	–57.5 (–57.9 ± 32.3)	0.56 (0.57 ± 0.19)	108 (101 ± 35)
	–57.4	0.58	110

sectional (Ellis et al., 2000) data based on a combination of water dilution, whole body potassium, and dual-energy x-ray absorptiometry (DXA) scanning methods used to estimate body composition. To obtain protein deposition rates since the data in young children were longitudinal (Butte et al., 2000), and the data in older children were cross-sectional (Ellis et

TABLE 10-9 Mean Daily Rates of Protein Deposition and Factorial Model Calculations of Mean Requirements for Protein

	Girls		Boys	
Age (y)	Protein Deposition[a] (mg/kg/d)	Mean Requirement[b] (g/kg/d)	Protein Deposition[a] (mg/kg/d)	Mean Requirement[b] (g/kg/d)
0.75	183	1.00	180	1.00
1	150	0.94	150	0.94
1.5	112	0.88	116	0.89
2	91	0.84	96	0.85
3	57	0.78	54	0.78
1–3	103	0.86	104	0.87
4	48	0.77	44	0.76
5	44	0.76	40	0.76
6	48	0.77	42	0.76
7	46	0.76	46	0.76
8	42	0.76	51	0.77
4–8	46	0.77	45	0.77
9	48	0.77	55	0.78
10	36	0.74	51	0.77
11	35	0.75	48	0.77
12	39	0.75	48	0.77
13	29	0.74	41	0.76
9–13	37	0.75	49	0.77
14	23	0.73	38	0.75
15	19	0.72	34	0.74
16	8	0.70	28	0.73
17	0	0.69	19	0.72
18	0	0.69	6	0.70
14–18	10	0.71	25	0.71

[a] Deposition was derived from the data for protein accumulation in children (Butte et al., 2000; Ellis et al., 2000), which were fitted to the following polynomial equations. The gradients at specific ages in the range 4 through 17 years were determined by differentiation of the regression equation. The growth rates given by Butte et al. (2000) were employed for ages 0.75 through 2 years.

Girls protein content = $-0.00027 \times \text{age (y)}^4 + 0.00816 \times \text{age (y)}^3 - 0.0665 \times \text{age (y)}^2 + 0.51819 \times \text{age (y)} + 0.60856$ ($R^2 = 0.9946$).

Boys protein content = $-0.00047 \times \text{age (y)}^4 + 0.01663 \times \text{age (y)}^3 - 0.16613 \times \text{age (y)}^2 + 0.95166 \times \text{age (y)} + 0.36037$ ($R^2 = 0.9966$).

notes continue

al., 2000), the data for body protein content from the two studies were pooled and regressed on age, giving a smooth curve and yielding the polynomial equations that are shown in footnote *a* in Table 10-9. Inclusion of data from the young children (Butte et al., 2000) improved the fit over the range of ages 4 through 18 years, but the fit at the younger ages, near the tail of the curve, was not satisfactory. Hence, the gradients at specific ages in the age range 4 through 18 years were determined by differentiation of the regression equation, whereas for ages 9 months through 2 years, the growth rates given by Butte and coworkers (2000) were employed.

Protein EAR Summary, Ages 7 Through 12 Months

The Estimated Average Requirement (EAR) is estimated by the factorial method by taking the median (110 mg nitrogen/kg/d equivalent to 688 mg protein/kg/d) of the nitrogen intake for nitrogen equilibrium (thus measuring maintenance requirement only) derived from Table 10-8, plus the product of 1.72 (the reciprocal of the slope [0.58] of those data, which estimates the efficiency of protein utilization for growth) and the mean protein deposition (Table 10-9) for boys and for girls. The resulting mean protein requirement is estimated to be 1.0 g/kg/d for boys and for girls.

EAR for Older Infants

7–12 months	**1.0 g/kg/d**

Protein RDA Summary, Ages 7 Through 12 Months

The Recommended Dietary Allowance (RDA) is defined as covering 97.5 percent of the age group. Thus, the EAR must be increased by an amount equal to two times its standard deviation to cover the needs of almost all of this age group. The variation in requirements is based on both the variation in maintenance needs and the variation in the rate of protein deposition (protein for growth).

---

[b] Mean requirement = maintenance requirement from Table 10-8 or 10-12 + dietary amount needed from protein deposition by life stage and gender group.

Median requirement for ages 0.75 through 13 years = 688 mg protein/kg/d (110 mg N/kg/d [Table 10-8] × 6.25 mg protein/kg N) + (1.72 [efficiency of protein utilization derived from reciprocal of slope in Table 10-8] × mean protein deposition for life stage and gender group).

Median requirement for ages 14 through 18 years = 656 mg protein/kg/d (105 mg N/kg/d [Table 10-12] × 6.25 mg protein/kg N) + (2.13 [the efficiency of protein utilization derived from reciprocal of slope in Table 10-12 = 0.47] × mean protein deposition).

Due to lack of adequate data on this age group, the variation in maintenance requirement for protein was assumed to be the same in children of all ages as in adults. Thus, the coefficient of variation (CV) for maintenance for this age group is 12 percent, the same as the CV of the protein requirement for adults developed by Rand and coworkers (2003) (see "Adults Ages 19 Through 30 Years"). A coefficient of variation for growth of 43 percent was determined in a study of whole body potassium-40 content in children (Butte et al., 2002). The total variation from both sources combined is calculated from the formula:

$$SD_T = (\sqrt{} [CV_M \times \text{maintenance requirement}]^2 + [CV_G \times \text{growth requirement}]^2),$$

where CV_M is 0.12, the maintenance requirement is 0.688 g protein/kg/d, CV_G is 0.43, and the growth requirement is the rate of protein deposition divided by the efficiency of dietary protein utilization. This yields the following formula:

$$SD_T = (\sqrt{} [0.12 \times 0.688 \text{ g protein/kg/d}]^2 + [0.43 \times 1.72 \times Y \text{ g protein/kg/d}^2]),$$

where Y = 0.182 g/kg/d (average of value for boys and girls from Table 10-9).

The RDA is then calculated as the RDA = EAR + 2 × SD_T, yielding the formula:

$$\text{RDA} = \text{EAR} + 2 \times (\sqrt{} [0.12 \times 0.688 \text{ g protein/kg/d}]^2 + [0.43 \times 1.72 \times 0.182 \text{ g protein/kg/d}]^2)$$

The estimated amount by which to increase the EAR to cover 97.5 percent of older infants is thus the EAR + 2 (0.101 g protein/kg/d) = 1.0 + 0.2 g protein/kg/d for a total of 1.2 g/kg/d of protein. This value is slightly lower than the AI for protein based on mean protein content of human milk and the intake from complementary foods of 1.6 g/kg/d.

RDA for Older Infants

7–12 months	**1.2 g/kg/d or 11.0 g/d of protein**[1]

[1]Due to a calculation error in the prepublication copy, the value is changed from 1.5 g/kg/d to 1.2 g/kg/d and for the reference infant from 13.5 g/d to 11.0 g/d.

Children Ages 1 Through 13 Years

Protein EAR Summary, Ages 1 Through 13 Years

The Estimated Average Requirement (EAR) is estimated by the factorial method, which adds the amount needed for maintenance based on body weight to the amount estimated to be needed for protein deposition. The mean of the nitrogen intake for nitrogen equilibrium (thus measuring maintenance requirement only) is derived from all of the individual estimates for children and is 110 mg nitrogen/kg/d or 688 mg protein/kg/d (110 × 6.25) (Table 10-8). This is increased by the product of 1.72 (the reciprocal of the slope [0.58] of that data, which estimates the amount of protein utilization) and the efficiency of utilization as estimated by Rand and coworkers (2003) for adults (see "Adults Ages 19 Years and Older"). This is multiplied by the mean protein deposition (Table 10-9) for boys and for girls for each age group. Given the assumptions in this method and the few girls in the studies included in Tables 10-8 and 10-9, the EAR is set at the average for boys and girls in each age group.

EAR for Boys and Girls

1–3 years	**0.87 g/kg/d of protein**[2]
4–8 years	**0.76 g/kg/d of protein**
9–13 years	**0.76 g/kg/d of protein**

Protein RDA Summary, Ages 1 Through 13 Years

Assuming the variation of maintenance requirements for protein and protein deposition requirements vary, then the RDA is set as indicated at the 97.5th percentile, estimated as follows:

$$\text{RDA} = \text{EAR} + 2\,(\sqrt{\,[0.12 \times 0.688 \text{ g protein/kg/d}]^2 + [0.43 \times 1.72 \times \text{Y g protein/kg/d}]^2}),$$

where Y = 0.104 g for age group 1–3 years, 0.046 g for age group 4–8 years, and 0.043 g for age group 9–13 years. Numbers are rounded to nearest 0.05 g.

Using the reference values for body weight for each age group as shown in Table 1-1, the RDA for protein would be 13 g/d for ages 1–3 years, 19 g/d for 4–8 years, and 34 g/d for 9–13 years.

---

[2]Due to a calculation error in the prepublication copy, the value is changed from 0.88 g/kg/d to 0.87 g/kg/d.

RDA for Boys and Girls

1–3 years	**1.05 g/kg/d or 13 g/d of protein**[3]
4–8 years	**0.95 g/kg/d or 19 g/d of protein**
9–13 years	**0.95 g/kg/d of 34 g/d of protein**

Adolescents Ages 14 Through 18 Years

Since data were not available to determine the maintenance protein requirement in children older than 14 years of age (Table 10-8), and since the maintenance nitrogen requirement of children (110 mg/kg/d) is similar to that for adults (105 mg/kg/d as shown in Table 10-12), the EAR for adolescents 14 through 18 years of age is based on the adult estimates of maintenance requirements from nitrogen balance studies (Rand et al., 2003), plus an additional amount to cover the needs for growth for this age as determined by the factorial method.

Protein EAR Summary, Ages 14 Through 18 Years

The maintenance requirement of adults of 105 mg nitrogen/kg/d or 656 mg protein/kg/d is added to the product of 2.13 (the reciprocal of the slope [0.47], which is the estimate of the efficiency of protein utilization in adults) (Rand et al., 2003), times the mean protein deposition as adjusted for efficiency of protein utilization (0.43), and calculated for boys or girls 14 through 18 years of age using the polynomial equations given in Table 10-9 to estimate protein deposition.

EAR for Boys

14–18 years	**0.73 g/kg/d of protein**

EAR for Girls

14–18 years	**0.71 g/kg/d of protein**

Protein RDA Summary, Ages 14 Through 18 Years

The RDA for protein for adolescents is set by determining the CV for maintenance and protein deposition. Since the CV of the maintenance requirement could not be calculated from the data shown in Table 10-8, and because of the similarity in maintenance requirements in children (Table 10-8; 110 mg N/kg/d) and adults (105 mg N/kg/d as estimated by

[3]Due to a calculation error in the prepublication copy, the value is changed from 1.10 g/kg/d to 1.05 g/kg/d.

Rand et al., [2003]), the CV in adults (12 percent) was also utilized to determine the variation in maintenance requirements for children and adolescents (see section "Protein RDA Summary, Ages 19–50 Years"). A CV of 43 percent for protein deposition was determined in the study of Butte and coworkers (2000), and this varied little with age and gender. Therefore, this value was used as the CV for growth for all ages.

The RDA is set for older adolescents as indicated at the 97.5th percentile, estimated as follows:

$$\text{RDA} = \text{EAR} + 2\ (\sqrt{\ [0.12 \times 0.656\ \text{g protein/kg/d}]^2 + [0.43 \times 2.13 \times \text{Y g protein/kg/d}]^2}),$$

where Y = 0.010 g for girls and 0.025 g for boys. Numbers are rounded to nearest 0.05 g.

Using the reference values for body weight shown in Table 1-1, the RDA for protein for girls 14–18 years of age would be 46 g/d, and for boys, 52 g/d.

RDA for Boys	
14–18 years	**0. 85 g/kg/d of protein or 52 g/d of protein**
RDA for Girls	
14–18 years	**0.85 g/kg/d of protein or 46 g/d of protein**

Adults Ages 19 Through 50 Years

Evidence Considered in Estimating the Average Requirement

In adults, protein requirement estimates have depended on one of two main approaches, namely, the factorial method and nitrogen balance response to different levels of intake of defined quality protein intakes. While the nitrogen balance method for estimation of protein requirements has serious shortcomings (see "Nitrogen Balance Method"), this method remains the primary approach for determining the protein requirement in adults, in large part because there is no validated or accepted alternative.

Nitrogen Balance Studies

Over the last 40 years, a number of analyses of available data on adult nitrogen balance studies have been utilized to estimate adult protein requirements; some reports are listed in Table 10-10. A growing body of data has accumulated that allows a more refined approach to such estimates, as improved techniques for measuring nitrogen output and controlling for

TABLE 10-10 Estimates of Adult Protein Requirements Using Nitrogen Balance Data

Reference	Estimates of Adult Protein Requirements for High Quality Protein (includes estimate of variation in requirements) (g/kg/d)		Estimation of Variation in Requirements (%)
	Men	Women	
FAO/WHO, 1965	0.71	0.71	10
FAO/WHO, 1973	0.60	0.60	15
FAO/WHO/UNU, 1985	0.75	0.75	12.5
Rand et al., 2003	0.80	0.80	12

external variables that impact nitrogen utilization have been implemented, and there has been a move toward standardization of study protocols.

The most recent in-depth analysis conducted at the request of the International Dietary Energy Consultative Group in 1996 and then more recently by the Food and Agriculture Organization, World Health Organization, and the United Nations University (FAO/WHO/UNU) included 19 studies conducted across the globe that measured and published nitrogen balance responses for 235 individuals given at least three levels of nitrogen intake for periods of 10 to 14 days (to be included in the analysis, it was required that individual data be available for at least three levels of intake adapted to by consuming the diet for least 10 days, with urinary and fecal nitrogen collection in the final 5 days of the diet period) (Rand et al., 2003). This was considered important so that estimates of individual requirements could be interpolated. In addition, 9 studies of individuals fed a single level of nitrogen intake or that only provided group data for multiple levels of intake (n = 174 individuals) were used to assess the fit of the analyses conducted (Rand et al., 2003). The studies used were classified on the basis of age of the adults (young: 19 through 52 years of age; old: 53 years of age and older); protein source (animal [animal sources provided > 90 percent of the total protein], vegetable [vegetable sources provided > 90 percent of the total protein], or mixed), as well as gender and climatic origin (temperate or tropical area), and corrected for skin and miscellaneous losses when not included in the nitrogen balance data (Rand et al., 2003). (See Appendix M for data on studies used.)

Analyses have also been made estimating endogenous protein loss in healthy adults when consuming protein-free diets adequate in all other respects. Estimates of endogenous loss from some of the various analyses of protein requirements are included in Table 10-11.

Methods Used to Estimate Individual Requirements

Earlier estimates of adult protein requirements (FAO/WHO, 1965) utilized information from endogenous nitrogen losses as the basis for determining protein requirements, assuming maximal utilization at levels near endogenous losses. However, as discussed in earlier sections, the efficiency of utilization of dietary protein declines as nitrogen equilibrium is reached. More recent approaches have averaged nitrogen balance data obtained from various studies where healthy individuals were given high-quality protein sources so that total nitrogen is considered the limiting dietary component rather than a specific indispensable amino acid (FAO/WHO/UNU, 1985).

With additional data it is possible to estimate requirements using regression analysis. Linear regression of nitrogen balance on nitrogen intake was utilized to estimate the nitrogen intake that would produce zero nitrogen balance in the most recent carefully done analysis available (Rand et al., 2003). In adults, it is generally presumed that the protein requirement is achieved when an individual is in zero nitrogen balance. To some extent, this assumption poses problems that may lead to underestimates of the true protein requirement (see "Nitrogen Balance Method").

Although the authors (Rand et al., 2003) acknowledge that it is known that the nitrogen response curve is nonlinear (because at high intakes the efficiency of nitrogen retention decreases), linear interpolation was utilized because the primary data utilized for the regression were gathered at intake levels close to those that were expected to produce zero balance. In this range there is no indication, either visually or statistically, for the utilization of an interpolation scheme other than linear (Rand et al., 2003). It was also recognized that while the use of more complex models would improve the standard error of fit, these models did not statistically improve the fits, in large part because of the small number of data points (3 to 6) for each individual (Rand et al., 2003).

Estimation of the Median Requirement

Utilizing the recent analysis of nitrogen balance data (Rand et al., 2003), the individual requirement estimates were found to be both significantly skewed and kurtotic, being characterized by more than expected very large or very small requirements (see Figure 10-5) (Rand et al., 2003).

TABLE 10-11 Estimates of Endogenous Loss of Nitrogen

Reference	Estimates of Urinary Losses (mg N/kg/d)		Estimates of Fecal Losses (mg N/kg/d)	Integumental Obligatory and Miscellaneous Losses (mg N/kg/d)	Total Endogenous Loss of Nitrogen (mg N/kg/d)
	Men	Women			
FAO/WHO, 1965	46	46	46	46	~ 100
FAO/WHO, 1973				5	
FAO/WHO/UNU, 1985 (n = 11 studies)	34	27	34	27	Men = 54 Women = 47 All = 52
Rand et al., 2003 (n = 14 studies)				5 temperate 11 tropical	Men = 50 Women = 35 All = 47

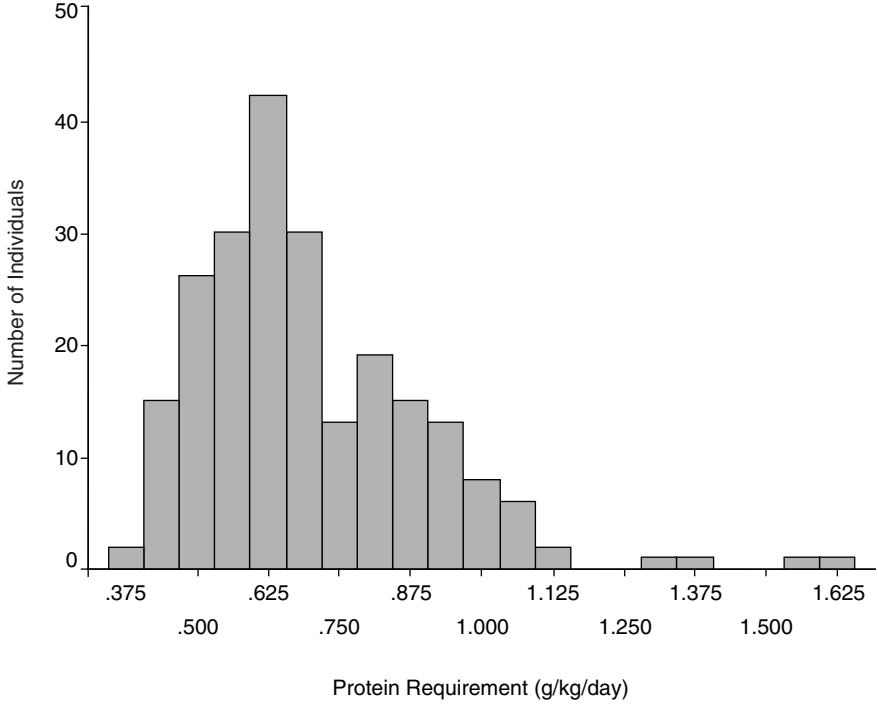


FIGURE 10-5 Distribution of the estimated protein requirements for 225 individuals (Rand et al., 2003) in a trimmed data set showing the skewness of protein requirement.
SOURCE: William Rand, personal communication, 2002.

The median requirement, potentially of use as the EAR, was calculated in two different ways: first as the median of the entire sample of 235 individuals in the primary data set (weighting all individuals the same), and second as the median of the medians of each distinct substudy (weighting each substudy equally) (Rand et al., 2003). In either case, data from all individuals were included in the analysis.

The results of these analyses are included in Table 10-12. Because of the non-normality of the individual data, nonparametric tests were used (Mann-Whitney and Kruskal-Wallis) to compare requirements between the age, gender, diet, and climate subgroups (Table 10-13). Where nonsignificant differences were found, Analysis of Variance was used for power calculations to roughly estimate the differences that could have been found with the data and variability. Separate analyses were conducted for all the

TABLE 10-12 Analyses of Linear Regression Analysis of Nitrogen Requirements in Adults

Data Sets Used	Linear Regression—Median Nitrogen Requirement (mg/kg/d)			Linear Regression—Slope	Intercept at 0 Intake (mg N/kg/d)
	Men	Women	All		
19 Estimation studies (n = 235; men = 181, women = 54)	109	91	105	0.47	–48
95% confidence intervals			(101, 110)	(0.44, 0.50)	(–51, –45)
32 substudies (n = 32 group medians; men = 24, women = 8)	102	102	102	0.49	–47
All individual data (n = 1,593)			103		
Linear regression model (grouped data)			122		
Quadratic regression model (grouped data)			114		
Asymptotic growth exponential (grouped data)			116		
Asymptote			42		
Linear bi-phase			108		
Breakpoint			126		
Asymptote			–7.2		

SOURCE: Rand et al. (2003).

individual requirements and for substudies, both excluding and including secondary estimation data (Rand et al., 2003).

Statistical Analysis of Nitrogen Balance Data to Determine the Protein Requirement

Data Analysis. The relationship between nitrogen balances, corrected for integumental and miscellaneous losses, and nitrogen intake from Rand and coworkers (2003) is shown in Figure 10-6. This figure includes individual data from the linear regression of nitrogen balance in adults examined (Rand et al., 2003). The authors noted that positive nitrogen balance was found in some individuals at nitrogen intakes as low as 60 mg/kg/d, and in other individuals negative balance was noted at nitrogen intakes as high as 200 mg/kg/d. This suggests that at least some of these individuals were not at constant nitrogen balance equilibrium.

In addition, while the nitrogen balance response to increasing nitrogen intake is theoretically expected to be nonlinear, the primary individual data points near the equilibrium balance point demonstrate a linear relationship, which appears to become nonlinear at high intakes. This can be attributed to different study designs in the test data included in Figure 10-6. The data points from only the estimation studies show a linear response over the relatively narrow range of intakes studied, while data points from the test studies also show a response that is not different from linear, although more variable and with a lower slope. Much variability is noted in the response data because the studies differ in methodology, individuals differ from each other, and an individual's response differs from day to day.

Table 10-12, a summary of the nitrogen requirement for all the data points included in the analysis by Rand and coworkers (2003), shows a nitrogen requirement of 105 mg/kg/d or 0.66 g protein/kg/d (105 mg N/kg/d × 6.25), with an approximately 95 percent confidence interval of 101 to 110 mg/kg/d (0.63 to 0.69 g protein/kg/d). When only the individual data points in the primary estimation studies are considered, the nitrogen requirement is 102 mg/kg/d (0.64 g protein/kg/d), and when all of the estimation study data points are considered, the nitrogen requirement is 103 mg/kg/d (0.64 g/kg/d). The median slope of the nitrogen balance response regression was 0.47 mg N/kg/d for all the data points, with a 95 percent confidence interval of 0.44 to 0.50 mg N/kg/d, which is in close agreement with the median slope of the primary estimation studies of 0.49 mg N/kg/d and all estimation studies of 0.47 mg N/kg/d.

Factor Analysis. Since all the data meeting the criteria for the meta-analysis were combined in the regression analysis by the authors (Rand et

TABLE 10-13 Factor Analysis: Estimation of Nitrogen Requirement with Medians and Mann-Whitney or Kruskal-Wallis Testing

Data	Factor	Number of Points	Median Slope	Median Intercept (mg/kg/d)	Median Nitrogen Requirement[a] (mg/kg/d)
Primary estimation studies	All	32	0.49	–47.1	102
Climate	Temperate	22	0.45	–43.0	101
	Tropical	10	0.52	–54.8	111
	P-value		0.10	0.020	0.27
Age	Young	30	0.50	–48.9	102
	Old	2	0.31	–36.7	111
	P-value		0.12	0.23	0.97
Gender	Male	24	0.50	–48.9	102
	Female	8	0.46	–42.0	102
	P-value		0.45	0.27	0.62
Diet	Animal	9	0.50	–48.1	101
	Vegetable	11	0.50	–45.9	104
	Mixed	12	0.48	–49.7	102
	P-value		0.88	0.83	0.72
Other estimation studies	All	45	0.47	–46.0	103
Climate	Temperate	33	0.42	–41.8	101
	Tropical	12	0.51	–54.5	111
	P-value		0.026	0.002	0.29

Age	Young	43	0.48	–47.0	103
	Old	2	0.31	–36.7	111
	P-value		0.18	0.27	0.98
Gender	Male	36	0.48	–47.6	103
	Female	9	0.44	–39.2	103
	P-value		0.47	0.40	0.94
Diet	Animal	17	0.46	–40.6	101
	Vegetable	13	0.50	–45.0	108
	Mixed	15	0.47	–50.5	105
	P-value		0.85	0.40	0.64
All data	All	235	0.47	–48.1	105
Climate	Temperate	154	0.45	–45.3	103
	Tropical	81	0.50	–51.9	113
	P-value		0.20	0.011	0.047
Age	Young	221	0.48	–49.4	104
	Old	14	0.31	–36.7	131
	P-value		0.003	0.025	0.401
Gender	Male	181	0.46	–49.4	109
	Female	54	0.47	–43.1	91
	P-value		0.47	0.20	< 0.001
Diet	Animal	64	0.46	–48.8	104
	Vegetable	77	0.47	–49.4	107
	Mixed	94	0.48	–46.6	104
	P-value		0.62	0.81	0.62

[a] Protein requirement = 6.25 × nitrogen requirement.

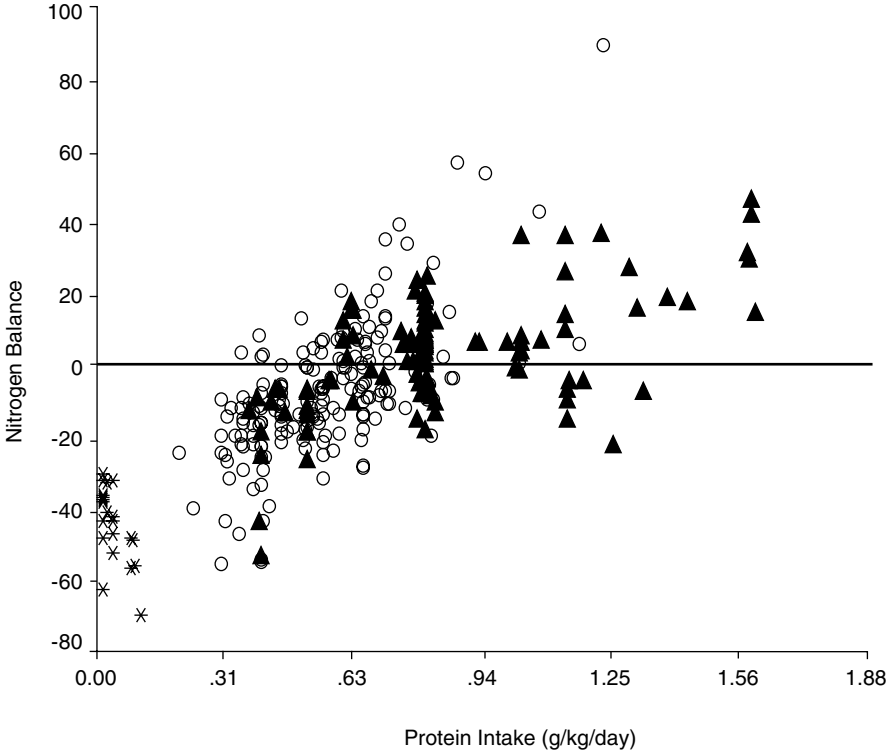


FIGURE 10-6 Relationship between individual nitrogen balances, corrected for integumental and nitrogen losses and nitrogen intake in random selection of data. □ = primary data; ▲ = test data; * = obligatory losses data.
SOURCE: Rand et al. (2003).

al., 2003), a separate analysis was conducted to evaluate the extent to which the four factors thought to have the most influence on protein requirements—climate, age, gender, and dietary protein source—were analyzed. As shown in Table 10-13, expected climate in the country of the study had a significant effect ($p < 0.47$), with differences of the magnitude of about +10 mg N/kg/d (0.06 g protein/kg/d) in tropical climates. The effect of age, as shown in Table 10-13, was a nonsignificant difference of 27 mg N/d (0.17 g protein/d) in the nitrogen requirement between young (19 to 52 years of age) and older (53 years of age and older) individuals per kg of body weight. Although the young individuals had a lower nitrogen requirement than the older individuals, the requirement of young individuals was more variable and more positively skewed than that for the older individuals.

In addition, men had a statistically significant higher median nitrogen requirement by about 18 mg N/kg/d (0.11 g protein/kg/d) than did the women studied, although this difference disappears when medians of the primary estimation studies are compared. Ninety-five percent confidence intervals for these estimates are 104 and 114 mg N/kg/d (0.65 and 0.71 g protein/kg/d) for men and 85 and 104 mg N/kg/d (0.53 and 0.65 g protein/kg/d) for women. Finally, the source of protein (90 percent animal, 90 percent vegetable, or mixed) did not significantly affect the median nitrogen requirement, slope, or intercept. It should be noted that almost all of the studies included as 90 percent vegetable were based on complementary proteins. For further discussion on this aspect of the data analysis and for information on vegetarian diets see later sections on "Protein Quality" and on "Vegetarians."

All of the various estimates of protein requirements (Table 10-10) are confounded by variations in energy intake relative to energy balance and expenditure. It has been estimated that an error of about 10 percent in energy intake as estimated from a diet history or a prediction equation (FAO/WHO/UNU, 1985) would cause the nitrogen balance estimate to be affected by about ± 6 mg/kg/d (Pellett and Young, 1992).

Other Approaches to Determine the Protein Requirement Based on the Recent Meta-Analysis

In addition to the linear statistical approach to determine protein requirements described in detail above, the authors considered three other statistical approaches to the nitrogen balance analysis (Rand et al., 2003). All data from the studies in the meta-analysis were fitted to the following models: linear, quadratic, asymptotic exponential growth and linear biphase (see Table 10-12).

Since the above analyses used all of the available data points without linking the individuals or restricting the range of intakes, the authors made the decision to use nitrogen equilibrium as the criterion and individual linear regressions, using only those individuals in the primary data set to determine the protein requirement (Rand et al., 2003). However, due to the shortcomings of the nitrogen balance method noted earlier, it is recommended that the use of nitrogen balance should no longer be regarded as the "gold standard" for the assessment of the adequacy of protein intake and that alternative means should be sought.

Protein EAR Summary, Ages 19 Through 50 Years

Using the recent meta-analysis of the nitrogen balance studies (Rand et al., 2003), the best estimate of the nitrogen EAR in the healthy adult popu-

lation is determined to be 105 mg N/kg/d, the median requirement for all data (Table 10-13), or 0.66 g/kg/d of protein (105 mg N/kg/d × 6.25). The criterion of adequacy used for the protein EAR is based on the lowest continuing intake of dietary protein that is sufficient to achieve body nitrogen equilibrium (zero balance). While the data as analyzed in the meta analysis (Rand et al., 2003) do not provide any basis for assuming different requirements for climate, age, or source of protein in the diet, it must be recognized that such a lack of statistically significant differences in this data may well be artifacts of the method and the variability in both its determination and in the individuals measured.

Although the data indicate that women have a lower nitrogen requirement than men per kilogram of body weight, this was only statistically significant when all studies were included, but not when the analysis was restricted to the primary data sets. This difference may be due to differences in body composition between men and women, with women and men having on average 28 and 15 percent fat mass, respectively. When controlled for lean body mass, no gender differences in the protein requirements were found. However, in view of the uncertain significance of the difference between the genders, the same protein EAR on a body weight basis for both men and women is chosen. Based on the reference body weights of 70 kg and 57 kg for men and women, respectively, from Table 1-1, the EAR for protein is 47 g/d for men and 38 g/d for women.

EAR for Men	
19–30 years	**0.66 g/kg/d of protein**
31–50 years	**0.66 g/kg/d of protein**
EAR for Women	
19–30 years	**0.66 g/kg/d of protein**
31–50 years	**0.66 g/kg/d of protein**

Protein RDA Summary, Ages 19 Through 50 Years

The RDA for protein is set using the nitrogen balance database and methodology detailed by Rand and colleagues (2003) who demonstrated that the natural logarithm of requirement (in mg nitrogen/kg/day) has a normal distribution with a mean of 4.65 and a standard deviation of 0.12. The 97.5th percentile of the log requirement is then calculated as 4.89 (the mean plus 1.96 times the standard deviation) and the RDA is the exponentiation (exp) of this value, 132 mg nitrogen/kg/d, or equivalently, 0.80 g protein/kg/d (rounding to the nearest 0.1 g). It should be noted that protein requirement having a log normal distribution permits the

estimation the protein intake adequate for any percentile (P) of a healthy population from the equivalent formulae:

$$\exp[0.12 \times z(P) + 4.65] \text{ for nitrogen (mg/kg/d), or}$$
$$\exp[0.12 \times z(P) - 0.425] \text{ for protein (g/kg/d)}$$

In these equations,

(1) "4.65" and "–0.425" are, respectively, the means of the log requirement distributions in mg nitrogen/kg/d and g protein/kg/d [the EAR in mg nitrogen/kg/d = exp(4.65) = 105, while the EAR in g protein/kg/d = exp(–0.425) = 0.65];

(2) "0.12" is the standard deviation of the log of requirement (note that one feature of log normal distributions is that their standard deviation does not change when the units are changed); and

(3) "z(P)" is the value of the standardized normal distribution associated with P.

For example, the intake that is estimated to be adequate for 80 percent of a healthy population is exp [0.12 × z(0.8) – 0.425] = exp [0.12 × 0.84 – 0.425] = exp[–0.324] = 0.72 g protein/kg/d.

Based on the reference body weights of 70 kg for men and 57 kg for women from Table 1-1, the RDA for protein is 56 g/d for men and 46 g/d for women ages 19 through 50 years.

Because the distribution of individual requirements for protein is log normal, and thus skewed, the calculated standard deviation and coefficient of variation of requirement itself does not have the usual intuitive meaning (that the mean plus two standard deviations exceeds all but about 2.5 percent of the population's requirement). However, because this skewing is not extreme, an approximate standard deviation can be calculated as half the distance from the 16th to the 84th percentile of the protein requirement distribution as estimated from the log normal distribution of requirements. This gives a value of 12.5 mg nitrogen/kg/d (CV = 12 percent) which can be used to estimate the RDA's of other age groups and for individual amino acids where fewer data from the following formula: RDA = EAR + 2 CV, or RDA = 1.24 × EAR.

RDA for Men

19–30 years	**0.80 g/kg/d or 56 g/d of protein**
31–50 years	**0.80 g/kg/d or 56 g/d of protein**

RDA for Women

19–30 years	**0.80 g/kg/d or 46 g/d of protein**
31–50 years	**0.80 g/kg/d or 46 g/d of protein**

Adults Ages 51 Years and Older

Evidence Considered in Estimating the Average Requirement

In the meta-analysis described in Table 10-12 and used as the basis to determine adult protein requirements (Rand et al., 2003), there were six studies to assess the protein requirement of individuals aged 51 years and older. These have been analyzed and evaluated in various publications (Campbell and Evans, 1996; Campbell et al., 1994; Millward and Roberts, 1996; Millward et al., 1997). Table 10-14 shows the value for the EAR derived by the original authors, plus the values obtained in the reassessments of the original data by Campbell's group in 1994 and Millward's group in 1997. The variability among the derived values, and the changes due to reassessment, are the result of the many inadequacies in the original data, which are described below.

Only the study of Cheng and coworkers (1978) involved a direct comparison of old with young adults; however, the authors made no assessment of the miscellaneous nitrogen losses and were not able to show any clear difference in the requirement of older and younger adults. In inter-

TABLE 10-14 Nitrogen Balance Studies in Older Individuals

Reference	Study Population	Protein Intake Levels (g/kg/d)
Cheng et al., 1978	7 men, 60–73 y	0.40, 0.80, 1.60
Uauy et al., 1978	7 men, 7 women	0.57, 0.70, 0.85 0.52, 0.65, 0.80
Zanni et al., 1979	6 men, 63–77 y	0.38, 0.44
Gersovitz et al., 1982	7 men, 70–82 y 8 women, 71–99 y	0.80 0.80
Campbell et al., 1994	8 men, 56–68 y 4 women, 66–80 y	0.80, 1.62 0.80, 1.62
Castaneda et al., 1995b	12 women, 66–79 y	0.45, 0.92

[a] Estimates of average requirements derived from studies on elderly adults by the original authors and in subsequent reanalyses by Millward et al. (1997) and Campbell et al. (1994).

preting the data of Cheng's group, it was suggested that the energy intake of the older Chilean men was too high, 40 kcal/kg/d, as it was the same as that given to the younger men, who would be expected to have higher energy expenditures (Campbell et al., 1994).

Dietary energy excess is believed to give rise to erroneously low estimates of protein requirements (Garza et al., 1976, 1977a). However, the energy requirements of the elderly have been shown to be higher than previously believed (Roberts, 1996). Moreover, the urinary creatinine to body weight ratio reported by Cheng and coworkers (1978) was the same in the old (0.023 g/kg/d) as in the young (0.022 g/kg/d) men, suggesting that the two groups were of similar body composition. This is in contrast to studies in the United States where lower creatinine to body weight ratios were observed in the older adults (0.014 to 0.018 g/kg/d) (Campbell et al., 1994; Uauy et al., 1978; Zanni et al., 1979).

The study of nitrogen balance by Zanni and coworkers (1979) suggested that the average amount of protein intake required to maintain nitrogen balance in older adults was very low (0.46 g/kg/d). This study was performed under almost the same conditions as those used with younger adults in an earlier study from the same laboratory (Calloway and

	Average Requirement (g protein/kg/d)[a] as calculated by:		
Energy Intake (kcal/kg/d)[b]	Authors	Campbell et al. (1994)	Millward et al. (1997)
40 (constant)	0.77	0.93	> 0.4
32	0.70–0.85	0.81	≤ 0.57
28 (varied)	0.83	0.81	Uncertain
31 (varied)	0.46	0.65	Uncertain
32	> 0.8	—	< 0.8
29 (varied)			
32	1.0	1.0	< 0.8
29 (varied)			
32 (varied)	0.78–0.82	—	< 0.92

[b] Energy intake was either held constant for duration of nitrogen balance period, or varied to maintain body weight; varied levels are average intakes of group as reported by authors.

Margen, 1971) and demonstrated that the amount of protein needed by older adults (0.46 g/kg/d) was quite similar per kg of body weight compared to the younger adults (0.42 g/kg/d). Since the two different diets studied were relatively low in protein (0.38 and 0.44 g/kg/d), Millward and coworkers (1997) suggested that these low protein intakes led to an underestimate of the requirement. Moreover, since the adults were on a protein-free diet for 17 days preceding the two low-protein diets (each fed in random order for 15 days), this could have resulted in significant protein depletion, probably leading to a further underestimate of requirement. On the other hand, the study of Uauy and coworkers (1978) employed energy intakes (30 kcal/kg/d) that may have been too low, suggesting that their estimate of requirement (~0.8 g/kg/d) might have been an overestimate (Millward et al., 1997). It can be seen from Table 10-14 that the reanalysis by Campbell and coworkers (1994) led to overall higher estimates of the requirements of older adults than the original authors, whereas the reanalysis by Millward's group (1997) led to lower estimates.

In studies designed to evaluate the adequacy of diets containing 0.8 g/kg/d of protein (the 1973 FAO/WHO recommendation for a safe level of intake of egg or milk protein in adults [FAO/WHO, 1973]), nitrogen balance was measured in adults given single levels of protein for various periods. Gersovitz and coworkers (1982) showed that almost 50 percent of older men and women were in negative nitrogen balance at this level after 30 days. Similar results were obtained by Campbell and coworkers (1994) in individuals given 0.8 g/kg/d of protein for 11 days, whereas Castaneda and coworkers (1995a) found that the majority of older women were in positive nitrogen balance after 3 and 9 weeks on a diet containing 0.92 g/kg/d of protein.

On the basis of these data and reanalysis of the original data from the studies discussed above, it was suggested that the estimated requirement should be increased (Campbell and Evans, 1996), although Millward and coworkers (1997) were not in agreement with this conclusion. More recent data have shown that elderly adults given 0.8 g/kg/d of protein were in nitrogen balance after 2 weeks, and in positive balance after 8 and 14 weeks (Campbell et al., 2001). However, the thigh muscle area was significantly reduced after 14 weeks compared with 2 weeks, although there were no changes in any other measured indices of body protein composition.

In order to address these problems of interpretation of the relevant literature, the meta-analysis evaluated the data from the studies on elderly adults compared with those from the studies used to evaluate the requirement in younger individuals (Rand et al., 2003). All the data from studies of nitrogen balance in the older adults were included in the regression procedure employed to determine the protein requirement of adults 19 to 50 years of age, and no significant effect of age in terms of the amount of protein required per kilogram of body weight was detected (Table 10-13).

Protein EAR Summary, Ages 51 Years and Older

In summary, no significant effect of age on protein requirement in older adults was detected using the linear regression model by Rand and coworkers (2003) when evaluated in terms of amount needed per kg of body weight, recognizing that lean body mass as a percent of body weight and the protein content of the body both decrease with age. Therefore, for older adults, no additional protein allowance based on body weight beyond that of younger adults is warranted.

EAR for Men

51–70 years	**0.66 g/kg/d of protein**
> 70 years	**0.66 g/kg/d of protein**

EAR for Women

51–70 years	**0.66 g/kg/d of protein**
> 70 years	**0.66 g/kg/d of protein**

Protein RDA Summary, Ages 51 Years and Older

As with younger adults, because the distribution of individual requirements for protein is not a normal distribution and is skewed, its calculated standard deviation and coefficient of variation do not have the usual intuitive meaning (the mean plus two standard deviations exceeding all but about 2.5 percent of the population's requirement). However, an approximate standard deviation can be calculated as half of the distance from the 16th to the 84th percentiles of the protein requirement distribution as estimated from the log normal distribution of requirements. This gives, for comparative purposes, an approximate standard deviation of 12.5 mg N/kg/d (CV = 12 percent). It can thus be assumed as with younger adults percent that the RDA = EAR + 2 CV for protein and individual amino acids, or RDA = 1.24 × EAR. The calculated RDA is rounded to the nearest 0.05.

RDA for Men

51–70 years	**0.80 g/kg/d or 56 g/d of protein**
> 70 years	**0.80 g/kg/d or 56 g/d of protein**

RDA for Women

51–70 years	**0.80 g/kg/d or 46 g/d of protein**
> 70 years	**0.80 g/kg/d or 46 g/d of protein**

Pregnancy

Physiological Adaptations to Protein Metabolism During Pregnancy

Whole body protein turnover, measured by leucine kinetics, is increased in pregnant women at weeks 24 and 35 compared with pregnant women at 13 weeks or with nonpregnant women (Thompson and Halliday, 1992). Similar observations of increased whole body protein turnover during pregnancy have been made using ^{15}N lysine as a tracer (Kalhan and Devapatla, 1999). A significant reduction in urea synthesis has been shown to occur in the first trimester and is sustained throughout pregnancy (Kalhan et al., 1998). There is general agreement that the amount of nitrogen accreted due to a pregnancy involving 12.5 kg of maternal weight gain (which includes a term infant weighing 3.3 kg) is 148 g (equivalent to 925 g protein if using a conversion factor of 6.25) (Hytten and Leitch, 1971; King, 1975). This amount of protein accumulation is predicted by a summation of the protein components of the fetus (440 g), uterus (166 g), expanded maternal blood volume (81 g), placenta (100 g), extracellular fluid (135 g), and amniotic fluid (3 g) (IOM, 1990). There is also evidence from both nitrogen balance studies and whole body potassium counting that there are additional maternal protein-containing tissues that accumulate during pregnancy and are presumed to be in skeletal muscle (Kalhan, 2000; King, 1975; King et al., 1973).

Evidence Considered in Estimating the Average Requirement

Nitrogen and Potassium Balance. King and coworkers (1973) studied 10 adolescent women aged 15 to 19 years during the last trimester of pregnancy. Since all but one of the individuals were more than 4 years beyond menarche, the authors excluded consideration of maternal height growth. Nitrogen retention was linearly related to protein intake when five different nitrogen levels (9.3 to 20.0 g of N/d [58 to 125 g of protein/d]) were fed for 12-day periods, and the slope of the relationship was 0.3 ($r = 0.68$, $p < 0.001$). The average nitrogen retention (corrected for skin and miscellaneous nitrogen losses) was 2.4 g/d.

Nitrogen balance studies in pregnant women that account for skin and miscellaneous losses have shown that nitrogen retention during all periods of pregnancy is double the theoretical factorial gain (Calloway, 1974; King, 1975; King et al., 1973) and as noted previously (see "Nitrogen Balance Methods"), at high nitrogen intakes erroneous positive nitrogen balances have frequently been obtained.

The rate of protein accretion has also been calculated indirectly from the increase in whole body potassium. The average potassium deposition, measured by total body ^{40}potassium counts, was 3.41 mmol/d in the adolescent girls (King et al., 1973), but a review of the literature suggests that this value may be too high. The results of measurements of total body potassium during pregnancy from the study of King's group (1973) and five other reports are shown in Table 10-15, and yield a weighted mean value of 2.48 mmol/d from 120 individuals, most of whom were in their third trimester of pregnancy. To calculate nitrogen deposition, King and coworkers (1973) used the potassium/nitrogen ratio of 2.15 mmol of potassium/g of nitrogen as determined by carcass analysis of 21 whole human infants (Fee and Weil, 1963; Hamilton and Moriarty, 1929; Iob and Swanson, 1934; Widdowson and Dickerson, 1964). Using this ratio, the accumulation of 2.48 mmol/d of potassium is equivalent to a nitrogen deposition of 1.16 g/d (2.48 mmol of potassium/2.15 mmol of potassium/g of nitrogen/d) or 7.2 g protein/d using the factor of 6.25 g of protein/g of nitrogen. This estimates the average amount of protein deposited dur-

TABLE 10-15 Total Body Potassium Content During Pregnancy

Reference	*n*	Total Body Potassium (mmol/d)	*n* × Total Body Potassium (mmol/d)	Average Protein Deposition (g/d)[a]
MacGillivray and Buchanan, 1958	6	3.22	19.3	9.4
King et al., 1973	10	3.41	34.1	9.9
Emerson et al., 1975	5	3.43	17.2	10.0
Pipe et al., 1979	27	1.78	48.1	5.2
Forbes, 1987	50	2.64	132	7.7
Forsum et al., 1988	22	2.13	48.9	6.2
Total	120		297.9	
Mean		2.48[b]		7.2

[a] Protein deposition = Total potassium accumulated (mmol/d) ÷ 2.15 (mmol potassium/g nitrogen) × 6.25.
[b] Mean total body potassium calculated as the sum of column 3 divided by the total number of cases (298 ÷ 120) = 2.48).

ing the third trimester of pregnancy at a total of approximately 670 g of protein.

Calculation of the amount of dietary protein needed for a deposition of 7.2 g of protein/d during the third trimester of pregnancy requires a value for the efficiency of utilization of dietary protein. This was reported as being about 30 percent in a group of adolescent women in the third trimester of pregnancy (King et al., 1973). Closer review of the data indicates that for those six adolescents who demonstrated a positive efficiency at multiple levels of protein intake, the mean of the slope of the positive nitrogen balances was 0.43 ± 0.21 (median = 0.44). Compared with the slope for maintenance of adults of 0.47, which was calculated from a much larger data set (see "Adults Ages 19 Through 50 Years"), it is possible that the paucity of the data for both infants and during pregnancy has obscured the true rate of efficiency of deposition. While other physiological changes occurring in pregnancy appear to enhance nutrient utilization during periods of increased need (e.g., calcium absorption), it would be surprising to find that efficiency of protein utilization during pregnancy is diminished over that of other life stages. However, to ensure adequate intakes, 0.43 was chosen to use based on the six women studied. As calculated in Table 10-16, the average protein deposition was converted to the amount of intake needed to provide this level: 7.2 ÷ 0.43 = 16.7 g of protein/d for accretion during the third trimester.

The protein needed to maintain the new tissue accreted during pregnancy must also be added. The increase of body weight during a full-term pregnancy averages approximately 16 kg, which is the median weight gain of 4,218 women who had good pregnancy outcomes (Carmichael et al., 1997). Weight gain during pregnancy is made up of both additional fat and new lean tissue (including fetus, amniotic fluid, increased plasma volume, etc.), which has been estimated at 91 percent water (van Raaij et al., 1988), compared with the expected 73 percent of water in general nonpregnant lean tissue. The incremental weight gain at the 50th percentile for normal weight individuals with good pregnancy outcomes at the end of the first trimester is 2.2 kg; for the second trimester, 7.3 kg; and for the third trimester, 6.5 kg, which totals 16 kg (Carmichael et al., 1997).

The amount of protein to support additional tissue is calculated in Table 10-16 using a factor of 0.66 g/kg of body weight, the EAR for protein for adults. While it is recognized that pregnancy lean tissue contains a greater amount of water, correction for assumed differences in body composition have not been made given the lack of actual data delineating protein maintenance needs in pregnant women. This results in an average total additional need for protein during the last two trimesters of pregnancy of about 21 g/d over prepregnancy requirements.

TABLE 10-16 Derivation of Protein Requirements During Pregnancy

Trimester	Average Additional Body Weight Gained by the End of Trimester (kg)[a]	Total Weight Gain by End of Trimester	[A] Additional Protein to Maintain Increased Body Weight[b] (g/d)	Average Protein Deposition (additional lean tissue)[c] (g/d)	[B] Protein Deposition Corrected for Conversion Efficiency[d] (g/d)	[A + B] Average Total Additional Protein Required (g/d)[e]	RDA (g/d)[f]
1	Δ2.2	2.2	+1.4	~		~	
2	Δ7.3	9.5	+6.3	3.6	8.4	+14.7	
3	Δ6.5	16.0	+10.6	7.2	16.7	+27.3	
Average over 2nd and 3rd trimesters				5.4	12.6	+21.0	+ 25

[a] Carmichael et al. (1997); average body weight gain by end of trimester; divided by 2 to get approximate increase mid-trimester.
[b] End of trimester increase in body weight × 0.66 g/kg/d, the Estimated Average Requirement (EAR) for maintenance of protein in adults.
[c] From Table 10-15 where protein deposition = total potassium accumulated (mmol/d) ÷ 2.15 (mmol potassium/g nitrogen) × 6.25; and assumption that nitrogen accretion during second trimester is ~ 50% that of third trimester.
[d] Protein deposition ÷ 0.43, slope of regression line of protein intake versus nitrogen balance (recalculated from King et al., 1973).
[e] Average required additional amount needed during pregnancy.
[f] RDA is based on EAR + assumed variation in requirements; amount needed above nonpregnant needs.

Outcome of Food Supplementation Trials. Burke and coworkers (1943) conducted an observational study of 216 mothers giving birth to single infants in Boston and found a significant correlation between average daily protein intake and birth length and birth weight. They concluded that for practical purposes, a protein intake less than 75 g/d was associated with an infant who would be short and light in weight. Studies from the Montreal Diet Dispensary have also shown a relationship between maternal protein-energy intake and birth weight (Higgins, 1976). This study involved 1,736 low-income pregnant women, 20 years of age or more, whose average maternal protein and energy intakes at various stages of pregnancy were 68 g and 2,249 kcal/d during pregnancy, and were increased to an average of 101 g of protein and 2,778 kcal/d by supplementing the mothers with whole milk and eggs during a subsequent pregnancy. Birth weights were significantly higher for siblings with supplemented mothers compared with their older siblings born to the same mothers when they did not receive the supplementary milk and eggs. These data support the value increased intake of foods high in protein and energy during pregnancy and the additional requirements outlined above.

Adolescent Pregnancy. It is well established that both the mother's pre-pregnant weight and weight gain during pregnancy are correlated with the birth weight of the infant (Higgins, 1976; IOM, 1990; Wynn and Wynn, 1979). The problem of adolescent pregnancy is that the mother may still be completing her growth (Frisancho et al., 1983; Hediger et al., 1990; Scholl et al., 1990, 1994). In those pregnancies in which the mother's growth is not yet completed, it appears that there is competition between maternal and fetal growth needs (Hediger et al., 1990; Scholl et al., 1990, 1994).

The Montreal Diet Dispensary studied the effect of supplementing 1,203 low-income pregnant adolescents with whole milk and eggs and compared them with 1,203 pregnant adolescents who did not receive the additional milk and eggs in their diets (Dubois et al., 1997). The adolescents in the intervention group increased their protein intake from 73 g/d to approximately 125 g/d in addition to significantly increasing their energy intake. Participation in the intervention resulted in significantly increased mean birth weights and reduced the rate of low birth weights by 39 percent ($p < 0.001$) in adolescent girls, which again is attributed to the increased consumption of foods rich in protein and energy.

Protein EAR Summary, Pregnancy

Based upon the nitrogen balance study of King and coworkers (1973) and the estimated average protein deposition during pregnancy based on

potassium retention in six studies (Table 10-15), the average requirement for additional protein needed for adult pregnant women at the end of the trimester during pregnancy in adult women is calculated and given in Table 10-16. It is composed of two components: the amount needed to maintain the new pregnant tissue and the amount needed for initial deposition. The amount of protein deposition is corrected for the efficiency of protein deposition (using the estimate from the slope of 0.43 from the King and coworkers study [1973], recalculated as described above). Since little weight gain occurs during the first trimester, it is assumed that roughly one-third of the total increase in protein deposition during the 40 weeks of pregnancy (~ 925 g) occurs during the second trimester, with two-thirds occurring during the third trimester.

As described above, by the end of the third trimester, ~17 g/d is needed to allow for adequate protein deposition; it can be assumed that roughly half that amount is needed for growth during the second trimester, or 8 g/d (Table 10-16). Given the small amount of protein accretion expected to occur during the first trimester (as demonstrated by Thompson and Halliday [1992] in protein turnover studies during each trimester), the need for additional protein is rather low at this stage. Thus no additional increase in protein requirements is estimated for the first trimester. Averaging the overall protein needs over the last two trimesters of pregnancy, the EAR is set at 21 g/d above protein needs at the prepregnancy weight. Since this figure includes the protein needs for the additional tissue deposited, when calculating the amount needed per kilogram of body weight to use with pregnant women, only the amount needed for protein deposition is considered. Thus the increased amount on a body-weight basis is +12.6 g of protein/d ÷ 57 kg (reference woman) = +0.22 g of protein/kg/d. This is added to the factor for nonpregnant women of 0.66 g of protein/kg/d, and results in an EAR of 0.88 g of protein/kg/d.

Pregnant individuals who were studied ranged from 15 to 19 years of age (King et al., 1973); however, they were considered mature and physiologically similar to adults, as all but one of the ten young women was 4 to 7 years post-menarche. For adolescents, the additional need for protein during the second and third trimesters is assumed to be the same as for adult women.

EAR for Pregnancy

All age groups	**0.88 g/kg/d of protein or +21 g/d of additional protein**

Protein RDA Summary, Pregnancy

The protein RDA for pregnancy is in addition to the RDA for the nonpregnant woman, which is based on an estimated CV of about 12 percent (see "Protein RDA Summary, Adults 19 Years and Older"). Data for the variability of protein deposition in the fetus and mother was not available. The RDA is thus equal to the EAR + 24 percent. Thus the 1.24 multiplied by the EAR of +21 g protein/d = 26 g; rounded to the nearest 5 g/d, the RDA = +25 g/d.

Again, in considering the amount needed per kilogram of body weight, only that due to protein deposition is considered. The increase in the RDA is thus +12.6 g/d × 1.24 ÷ 57 kg (reference woman) = +0.27 g protein/kg/d. This is added to the factor for the RDA for non-pregnant women of 0.8 g protein/kg/d = 1.1 g protein/kg/d.

RDA for Pregnancy	
All age groups	**1.1 g/kg/d of protein or +25 g/d of additional protein**

Special Considerations

It is well recognized that multiparous pregnancies are associated with a marked increase in low birth weight and perinatal mortality (Hays and Smeltzer, 1986). Thus, it is logical to assume that a woman supporting the growth of twins has higher protein needs than a woman having a singleton birth. In a study in which the mothers of twins received nutritional intervention (target supplementation was an additional 50 g of protein/d and 1,000 kcal/d) from the 20th week, pregnancy outcome was improved, with a decrease in the low birth weight rate by 25 percent and the very low birth weight rate by 50 percent (Dubois et al., 1991). Although this study did not measure the dietary protein or energy intake of the women bearing twins, they gained 2 kg more than the controls. No study could be found that investigated dietary protein intervention in twin pregnancy. On the basis of these data, it seems prudent to provide women carrying twins with protein intakes of an additional 50 g/d beginning in the second trimester, along with sufficient energy to utilize the protein as efficiently as possible.

Lactation

Evidence Considered in Estimating the Average Requirement

The literature on the relationship between nutritional status and lactation performance is not extensive and suggests that most lactating women, even those who are undernourished with chronically low body mass index,

establish adequate lactation (Prentice et al., 1994). While it appears that the concentration of protein in human milk is not influenced by diet or body composition even in undernourished mothers (Lönnerdal 1986), protein intakes of 1 g/kg of body weight/d promoted the conservation of skeletal muscle in order to maintain good milk production in lactating mothers (Motil et al., 1996). Lactating women with these protein intakes appear to adapt by down-regulating protein metabolism (Motil et al., 1996).

The factorial approach is utilized for determining the protein requirement during lactation. In this approach, it is assumed that the process of lactation does not alter the maintenance protein requirement of the nonlactating woman and that the protein and amino acid requirements are increased in proportion to milk production. It is important to emphasize that human milk is characterized by a relatively high concentration of nonprotein nitrogenous substances, which contribute approximately 20 to 27 percent of total milk nitrogen (Butte et al., 1984a, 1984b; Dewey et al., 1996). The quantitatively important component of this fraction of milk is urea. Whether this merely reflects a diversion of urea loss from urine (plus some colonic fermentation) to milk is not known, but in the calculations it is assumed that part of the increased nitrogen needs of the lactating woman will of necessity be derived from her dietary protein. The factor of 6.25, the figure that is utilized to convert nitrogen to protein, was used to convert nonprotein nitrogenous substances to protein.

The additional protein requirement for lactation therefore is defined as the output of total protein and nonprotein nitrogen in milk. Data on the output of protein in human milk are summarized in Table 10-17. This table shows the factorial estimate of the increase in protein requirement associated with lactation and assumes that the incremental efficiency of nitrogen utilization of 0.47 in adults (Table 10-12) and in adolescents (data on the efficiency of nitrogen utilization are not available in this age group) is the same as that noted for the restoration of nitrogen equilibrium in nonlactating women and adolescents. It is assumed that the cost of making protein for maintenance requirements is the same as that for growth and lactation. Whether this assumption is valid is not known.

Protein EAR Summary, Lactation

To estimate the increase in the EAR for lactation, the average protein equivalent of human milk nitrogen output during the first six months of lactation was divided by the average incremental efficiency of dietary protein utilization (0.47 for lactating mothers 19 years of age and older [Table 10-12] and 0.47 for mothers 14 through 18 years of age because data were not available in this age group). The values shown in Table 10-17

TABLE 10-17 Factorial Estimate of the Increment in Protein Requirement Associated with Lactation

Stage (mo)	Protein Content of Human Milk (g protein/d)	Nonprotein Nitrogen Content of Human Milk[a] (g protein/d)	Total Human Milk Nitrogen Output (g protein/d)	Increase in Protein Need[b] (g/d)	
				14–18 y	> 18 y
1	8.93 ± 1.97[a]	2.05 ± 0.4	11.0 ± 2.4	23.4	23.4
2	8.26 ± 1.08[c]	2.02 ± 1.6	10.3 ± 2.7	21.9	21.9
3	8.24 ± 1.54[d]	1.71 ± 1.1	10.0 ± 2.6	21.3	21.3
4–6	7.29 ± 1.27[c]	1.28 ± 0.5	8.6 ± 1.8	18.3	18.3
Mean	8.18	1.76		21.2	21.2

[a] Butte et al. (1984b); Lemons et al. (1982).
[b] The increase in the Estimated Average Requirement (EAR) for protein was calculated by dividing the average protein equivalent of milk nitrogen output by the average incremental efficiency of dietary protein utilization (0.47 for lactating mothers 19 years of age and older [Table 10-12] as well as for lactating mothers 14–18 years of age because data are not available for this age group).
[c] Butte et al. (1984b).
[d] Butte et al. (1984b); Dewey and Lönnerdal (1983); Heinig et al. (1993); Motil et al. (1998); Nommsen et al. (1991).

for the various months of lactation were then averaged to set the amount by which the EAR for nonlactating girls or women should be increased. The result was +21.2 g/d. When the absolute increase was converted to weight-specific intakes by using the reference weights of adolescent girls 14 to 18 years (54 kg) and adult women 19 to 50 years (57 kg) from Chapter 1 (Table 1-1), the numbers were quite close, so the highest value (that for the 14- to 18-year-old category) is provided as the overall recommendation. Adding the average requirement for additional protein needed is calculated as +21.2 ÷ 54 kg (reference weight) = +0.39 g of protein/kg/d. This is added to the recommendation for nonpregnant women of 0.66 g of protein/kg/d to obtain 1.05 g of protein/kg/d.

EAR for Lactation	
All age groups	**1.05 g/kg/d of protein or +21 g/d of additional protein**

Protein RDA Summary, Lactation

The RDA for protein for lactation is set by assuming a CV of 12 percent used for total protein in nonlactating women (see "Protein RDA Summary, Ages 19 Years through 50 Years"). Again, given the closeness of the values, one value is recommended for all age groups. The recommendations are rounded to the nearest +5 g/d and +0.05 g/kg/d of additional protein.

The RDA is thus equal to the EAR plus 24 percent. So, 1.24 multiplied by the EAR of + 21 g of protein/d = +26 g; rounded to the nearest 5 g/d, the RDA = +25 g/d.

Again, in considering the amount needed per kg of body weight, the increase in the RDA is calculated as the EAR of +21 g/d × 1.24 ÷ 54 kg (reference weight) = +0.48 g of protein/kg/d. This is added to the factor for the RDA for nonpregnant women of 0.8 g of protein/kg/d = 1.3 g of protein/kg/d (rounded to nearest 0.1g).

RDA for Lactation	
All age groups	**1.3 g/kg/d of protein or +25 g/d of additional protein**[4]

[4]Due to a calculation error in the prepublication copy, the value is changed from 1.1 g/kg/d to 1.3 g/kg/d.

Special Considerations

Physical Activity

Although there have been few studies of the requirement for protein by individuals undertaking high levels of physical exercise, it is commonly believed by athletes that a higher than normal protein intake is required to maintain optimum physical performance (Lemon, 1996). Whether or not this is true has significance not only for athletes, but also for those with muscle wasting who wish to preserve muscle mass by training, such as elderly or immobile adults, or those suffering from muscle-wasting diseases. The available literature includes studies of both resistance (bodybuilding) and endurance training.

Endurance Training. Endurance training does not result in muscle building, which would increase muscle protein deposition, but it is well recognized that endurance exercise is accompanied by an increase in the oxidation of branched chain amino acids (Lemon et al., 1982, 1985; Rennie et al., 1981; Wagenmakers, 1998; White and Brooks, 1981), which has been suggested to imply an increased need for dietary protein (Lemon, 1996). However, these were acute studies performed around the time of the exercise itself, and did not take into account the remaining part of the day. An examination of leucine oxidation over a 24-hour period, including exercise during each of the fed and fasting periods, showed that the increase in oxidation, although statistically significant, was small in relation to the total daily amount of oxidation (4 to 7 percent) (El-Khoury et al., 1997). Moreover, the increase in leucine oxidation was proportionally similar with diets containing 1 or 2.5 g/kg/d fed over 7 days prior to the measurement of oxidation during exercise on day 7 (Forslund et al., 1998). Neither leucine nor nitrogen balance was significantly negative, suggesting that the exercise did not compromise body protein homeostasis at either level of protein intake. Although no control group without exercise was studied, the results were similar to those reported previously from individuals at an intake of 1 g/kg/d of protein undergoing the same experimental procedures without exercise (El-Khoury et al., 1994b). Similarly, a study designed to determine the protein requirement of endurance-trained men led to an average requirement estimate in young and older men of 0.94 g/kg/d (Meredith et al., 1989). This value is higher than that derived from the meta-analysis of data from nonexercising individuals (see "Protein EAR Summary, Ages 19 Through 50 Years"). However, as no controls without exercise were included in the study, it is not possible to conclude that the exercise led to a higher protein requirement.

Resistance Training. The effects of resistance training on nitrogen balance have been investigated in older adults (8 men and 4 women, aged 56 to 80 years) at one of two levels of protein intake, 0.8 or 1.6 g/kg/d (Campbell et al., 1995). Before training began, the mean corrected nitrogen balance was not significantly different from zero in the three men and three women receiving the lower protein intake, and was positive in the five men and one woman receiving the higher intake, suggesting a requirement about 0.8 g/kg/d. However, after 12 weeks of resistance training, nitrogen balance became more positive by a similar amount at the two intakes, which the authors suggested was the result of an increased efficiency of protein retention that was more pronounced in those on the lower protein diet as a percent of protein intake. In particular, the improvement in nitrogen balance was independent of the protein intake. However, various aspects of body composition, as well as mid-thigh composition and areas of Type I and II muscle fibers, did not change with resistance training, making the increase in nitrogen retention difficult to interpret.

A similar study was performed by Lemon and coworkers (1992), which compared protein intakes of 1.35 and 2.62 g/kg/d during the first 12 weeks of resistance training in young male strength athletes. Linear interpolation of the nitrogen balances (–3.4 and +8.9 g/d) suggested a protein requirement of 1.4 to 1.5 g/kg/d. However, this estimate of requirement cannot be taken as realistic, because the positive nitrogen balance of 8.9 g/d would correspond to an increase of lean tissue of about 300 g/d. Measurements of body composition showed no changes in lean body mass, creatinine excretion, or biceps muscle nitrogen content in either dietary group. In addition, although there were increases in some measurements of strength, there was no effect attributable to diet. Therefore, the available data do not support the conclusion that the protein requirement for resistance training individuals is greater than that of nonexercising subjects.

Summary. In view of the lack of compelling evidence to the contrary, no additional dietary protein is suggested for healthy adults undertaking resistance or endurance exercise.

Vegetarians

In North America, plant proteins (e.g., those found in cereals, pulses, nuts, starchy roots, vegetables, fruits) account for only about 65 percent of the available food protein per capita (FAO/Agrostat, 1991). Individuals who restrict their diet to plant foods may be at risk of not getting adequate amounts of certain indispensable amino acids because the concentration of lysine, sulfur amino acids, and threonine are sometimes lower in plant food proteins than in animal food proteins (FAO/WHO/UNU, 1985).

However, vegetarian diets that include complementary mixtures of plant proteins can provide the same quality of protein (see "Protein Quality") as that from animal proteins (Young and Pellett, 1994). Plant proteins are generally less digestible than animal proteins; however, digestibility can be altered through processing and preparation. Therefore, consuming a varied diet ensures an adequate intake of protein for vegetarians.

Adult vegetarians consume less protein in their diet than non-vegetarians (Alexander et al., 1994; Ball and Bartlett, 1999; Barr and Broughton, 2000; Haddad et al., 1999; Janelle and Barr, 1995). However, only one of these studies indicated that total protein intakes of 10 of the 25 vegan women were potentially inadequate (Haddad et al., 1999). As was shown in Table 10-13, the nitrogen requirement for adults based on high-quality plant food proteins as determined by regression analysis was not significantly different than the requirement based on animal protein or protein from a mixed diet. In conclusion, available evidence does not support recommending a separate protein requirement for vegetarians who consume complementary mixtures of plant proteins.

FINDINGS BY LIFE STAGE AND GENDER GROUP FOR INDISPENSABLE AMINO ACIDS

The original technique used to determine amino acid requirements in individuals studied with graded levels of intake of the test amino acid was nitrogen balance (see "Nitrogen Balance Method"). Several new methods have been developed and applied in the last few decades. However, nitrogen balance could not be applied to histidine since individuals take 56 days or more to go into negative nitrogen balance on a low histidine or histidine-free diet (Cho et al., 1984), and there haven't been any useful studies on isoleucine, using either nitrogen balance or any of the newer methods.

The amino acid requirements thus developed are used as the basis for recommended protein scoring patterns discussed in a subsequent section.

Infants Ages 0 Through 6 Months

Method Used to Estimate the Adequate Intake

Human milk is recognized as the optimal source of nutrients for infants throughout at least the first year of life and is recommended as the sole nutritional source for infants during the first 4 to 6 months of life (IOM, 1991). Further, there are no reports of healthy full-term infants exclusively and freely fed human milk who manifest any sign of amino acid or protein deficiency (Heinig et al., 1993). Therefore, determination of

the adequate intake (AI) for amino acids for infants is based on data from infants fed human milk as the principal source of nutrients during the periods 0 through 6 months of age. The AI is set for ages 0 through 6 months at the mean value of each indispensable amino acid calculated from studies in which the intake of human milk was measured by test weighing volume, and the average concentration of the amino acid in human milk was determined using average values from several reported studies.

Four recent studies on the indispensable amino acid composition of human milk and their mean are shown in Table 10-18. The indispensable amino acid intake on a mg/L basis was calculated from the mean of the amino acid composition of mixed human milk proteins expressed as mg amino acid/g protein (Table 10-18) times the average protein content of human milk of 11.9 g/L from mothers whose infants were 0 through 6 months as assessed by Butte and coworkers (1984a), Dewey and Lönnerdal (1983), Dewey and coworkers (1984), and Nommsen and coworkers (1991) and is in the range of protein content reported in other studies in Table 10-7.

Indispensable Amino Acids AI Summary, Ages 0 Through 6 Months

The AI for infants 0 through 6 months of age is based on the average volume of milk intake of 0.78 L/d (Allen et al., 1991; Heinig et al., 1993), and the mean indispensable amino acid content of human milk (Table 10-18). Multiplying the mean concentration of histidine (274 mg/L), for example, by the average intake of human milk at 0 through 6 months, the AI would be 274 g/L × 0.78 L/d = 214 mg/d. This process was repeated for all the indispensable amino acids. As with the AI for protein, the AIs (which remain essentially the same from 2 weeks to 6 months of age) were converted to weight specific intakes by using the reference weight of 6 kg from Table 1-1.

AI for Infants	
0–6 months	**214 mg/d or 36 mg/kg/d of histidine**
	529 mg/d or 88 mg/kg/d of isoleucine
	938 mg/d or 156 mg/kg/d of leucine
	640 mg/d or 107 mg/kg/d of lysine
	353 mg/d or 59 mg/kg/d of methionine + cysteine
	807 mg/d or 135 mg/kg/d of phenylalanine + tyrosine
	436 mg/d or 73 mg/kg/d of threonine
	167 mg/d or 28 mg/kg/d of tryptophan
	519 mg/d or 87 mg/kg/d of valine

TABLE 10-18 Amino Acid Content of Human Milk

Amino Acid	Heine et al. (1991) (mg/g protein)	Davis et al. (1994)[a] (mg/g protein)	Villalpando et al. (1998)[a] (mg/g protein)
n	Not given	6	70
Stage of lactation	Not given	> 10 d pp[d]	4 or 6 mo pp
Histidine	23	23 ± 2	21± 2
Isoleucine	58	53 ± 3	64 ± 11
Leucine	101	104 ± 1	105 ± 11
Lysine	62	71 ± 6	79 ± 9
Methionine	18	16 ± 1	14 ± 3
Cysteine	17	20 ± 3	26 ± 3
Phenylalanine	44	37 ± 1	43 ± 15
Tyrosine	47	46 ± 2	58 ± 9
Threonine	46	44 ± 1	47 ± 5
Tryptophan	18	ND[e]	17 ± 2
Valine	60	51 ± 2	63 ± 8

[a] Mean ± Standard Deviation.
[b] Mean ± Standard Error.

Children Ages 7 Months Through 18 Years

Evidence Considered in Estimated the Average Requirement

Nitrogen Balance. The only data derived directly from experiments to determine the indispensable amino acids requirements of children have been obtained by studying nitrogen balance. Pineda and coworkers (1981) conducted nitrogen balance studies in 42 Guatemalan children ranging in age from 21 to 27 months. The children were considered to be in nitrogen balance if all children retained nitrogen in the amount of at least 16 mg/kg/d, the nitrogen requirement for growth in children in this age range derived by FAO/WHO (1973) when given a diet lacking one indispensable amino acid with all other indispensable amino acids at levels considered to be adequate. Their mean amino acid estimates were reported to be: lysine, 66 mg/kg/d; threonine, 37 to 53 mg/kg/d; tryptophan, 13 mg/kg/d; methionine + cysteine, 28 mg/kg/d; isoleucine, 32 mg/kg/d; and valine, 39 mg/kg/d. Unfortunately, with the exception of lysine, no estimates of variance were published.

Darragh and Moughan (1998)[b] (mg/g protein)	Mean (mg/g protein)	Mean[c] (mg/L)
20 10–14 wk pp		
24 ± 2	23	274
52 ± 4	57	678
94 ± 5	101	1,202
64 ± 4	69	821
14 ± 1	16	190
27 ± 3	22	262
38 ± 9	40	476
38 ± 2	47	559
50 ± 9	47	559
ND[e]	18	214
51 ± 5	56	666

[c] Mean (mg/g total protein) × 11.9 g protein/L of human milk.
[d] pp = Postpartum.
[e] ND = Not determined.

For older children, the only data are those published by Nakagawa and coworkers in the 1960s (1961a, 1961b, 1962, 1963, 1964) on Japanese boys 10 to 12 years of age. Although these data seem to be accurate as there was uniformly negative nitrogen balance when the test amino acid was at zero, the maximum rate of nitrogen retention found when the amino acids were given in adequate quantities was 33 ± 14 mg/kg/d. This is approximately 5-fold higher than that predicted for 11-year-old boys, 7.7 mg/kg/d, as calculated from estimates of the protein deposition for boys this age (48 mg of protein/kg/d ÷ 6.25 mg of protein/mg of nitrogen, from Table 10-9). Thus, it is likely that the values generated in this series of studies are overestimates of the actual requirement. Similar problems of interpreting nitrogen balance studies are apparent in the data for infants aged 0 to 6 months from a number of detailed studies in which infants were given multiple levels of amino acids (Pratt et al., 1955; Snyderman et al., 1955, 1959a, 1959b, 1961a, 1961b, 1963, 1964a, 1964b;). With these studies also, the measured nitrogen balance was higher than what would be expected from the growth rates observed or estimated.

An attempt was made to reanalyze the data from these studies in order to obtain estimates of the mean requirement and its interindividual vari-

ance. Nonlinear regression analysis was used to fit the data for nitrogen balance versus amino acid intake to various curves, such as exponential, sigmoid, and bilinear crossover, in order to detect an approach to an asymptote or a breakpoint that could be equated with a requirement. However, these attempts did not lead to interpretable results, which proved to be too sensitive to the specific criteria employed to define the point on the curve that would identify a requirement.

In view of the reservations expressed above, the data from nitrogen balance studies in children were not utilized. Instead, the factorial approach was employed for children from 7 months through 18 years of age.

Factorial Estimate. In view of the doubts about the accuracy of the values generated by the empirical data, the factorial approach using data for growth (and its amino acid composition) and maintenance was utilized to determine requirements. In this model, the growth component was estimated from estimates of the rate of protein deposition at different ages (Table 10-9), the amino acid composition of whole body protein (Table 10-19), and incremental efficiency of protein utilization as derived from the studies in Table 10-8.

The obligatory need for protein deposition (growth) was calculated as the product of the rate of protein deposition (Table 10-9) and the amino acid composition of whole body protein (Table 10-19). This was then converted to a dietary requirement for protein deposition by dividing the need by the incremental efficiency of dietary protein utilization, which is estimated by the average slope of the regression analyses evaluating the protein requirement from studies done in children 7 months through

TABLE 10-19 Indispensable Amino Acid Composition of Whole Body Protein

Amino Acid	mg/g Protein ± 1 Standard Deviation (from interspecies comparison)
Histidine	27 ± 2
Isoleucine	35 ± 3
Leucine	75 ± 2
Lysine	73 ± 3
Methionine + cysteine	35 ± 1
Phenylalanine + tyrosine	73 ± 4
Threonine	42 ± 3
Tryptophan	12 (no extensive data)
Valine	49 ± 4

SOURCE: Davis et al. (1994).

13 years (0.58) from Table 10-8 and in children 14 through 18 years (0.47) from Table 10-12.

It is also necessary to determine a maintenance amino acid requirement since by 7 months of age, the dietary requirement necessary to maintain the body in nitrogen equilibrium accounts for more than 50 percent of the total indispensable amino acid requirement. This was determined in three ways.

First, estimates of the amino acid requirements needed for maintenance were calculated based on estimates of the obligatory nitrogen loss, which is the total rate of loss of nitrogen by all routes (urine, feces, and miscellaneous) in children receiving a protein-free or very low protein intake. Assuming that each individual amino acid contributed to this loss in proportion to its content in body protein, and that this represents the minimal rate of loss for this amino acid, the amount of this amino acid that must be given to replace the loss and achieve nitrogen balance is taken as the maintenance requirement when corrected for the efficiency of nitrogen utilization. Thus, the lysine requirement for maintenance for children 7 months through 13 years of age is calculated by multiplying the obligatory nitrogen loss of 57.4 mg/kg/d (mean intercept from Table 10-8), which is equivalent to 359 mg of protein/kg/d (57.4 × 6.25), by the estimate of the proportion of lysine in body protein of 0.073 (Table 10-19), to yield a value for lysine of 26.2 mg/kg/d (i.e., 359 mg/kg/d × 0.073). Then this is divided by the slope of the regression line of protein intake versus nitrogen balance, which represents the efficiency protein utilization of 0.58 (Table 10-8) for children to yield a value of 45 mg/kg/d (i.e., 26.2 mg/kg/d divided by 0.58) for the lysine maintenance requirement. The calculated values for each indispensable amino acid are shown in Table 10-20.

A second method for estimating maintenance requirements is to assume that at nitrogen equilibrium, the relative requirement of each indispensable amino acid is in proportion to its contribution to body protein. Thus, the maintenance protein requirement of 688 mg/kg/d (110 mg of N/kg/d for children through age 13 in Table 10-8 × 6.25) can be converted into requirements for individual amino acids by multiplying the maintenance protein requirement by the proportional contribution of the amino acid to body protein (Table 10-19). This method is mathematically equivalent to the method described above, but because the values for obligatory loss and maintenance protein requirement were taken from the regression of protein intake against nitrogen balance, for statistical reasons they give slightly different results, and both are given in the Table 10-20.

Since it was noted that the maintenance nitrogen requirement of 110 mg/kg/d (Table 10-8) does not vary with age in children, and the value in children is very similar to that found for adults of 105 mg/kg/d

TABLE 10-20 Factorial Estimates of Maintenance Amino Acid Requirements for Children in Comparison to Adults

	Children		Adults		
Amino Acid	Based on Obligatory Nitrogen Loss (mg/kg/d)[a]	Based on Maintenance Protein Requirement (mg/kg/d)[b]	Based on Maintenance Protein Requirement (mg/kg/d)[c]	Direct Measurement of EAR (mg/kg/d)[d]	Ratio of Maintenance Amino Acid Requirements to EAR
Histidine	17	19	18	ND[e]	—
Isoleucine	22	24	23	ND[e]	—
Leucine	46	52	49	34	1.4
Lysine	45	50	48	31	1.5
Methionine + cysteine	22	24	23	15	1.5
Phenylalanine + tyrosine	45	50	48	27	1.7
Threonine	26	29	28	16	1.7
Tryptophan	7	8	8	4	2.0
Valine	30	34	32	19	1.7

[a] Determined by multiplying the estimated obligatory nitrogen (N) loss in children, 57.4 mg N kg/d × 6.25 (Table 10-8) by the estimates for the amino acid content of whole body protein (Table 10-19) and then dividing by the efficiency of protein utilization, 0.58 for children (slope in Table 10-8) and 0.47 for adults (slope in Table 10-12).

[b] Determined by multiplying the total protein maintenance needs in children aged 7 months through 13 years, 110 mg N/kg/d × 6.25 (Table 10-8) by the amino acid content of whole body protein (Table 10-19).

[c] Determined by multiplying the total protein maintenance needs in adults, 105 mg N/kg/d × 6.25 (Table 10-12) by the amino acid content of whole body protein (Table 10-19).

[d] EAR = Estimated Average Requirement.

[e] ND = Not determined. There have been no direct measurements of isoleucine or histidine requirements in adults.

(Table 10-12), the values for maintenance amino acid requirements were taken to be independent of age in subsequent calculations. However, for adults, who are by definition at maintenance, direct measurements of the estimated average requirement (EAR) for each amino acid have been determined (see "Adults Ages 19 Years and Older"), and are shown in Table 10-20 for comparison with the factorially derived estimates. For all amino acids for adults, the EAR as derived from direct measurements is lower than the factorial approach by a factor of 1.3 to 2.0, depending on the amino acid. This difference is predictable because of the imperfections in the factorial approach. It is likely that the obligatory loss of one amino acid is higher than that for other amino acids in relation to their content in body protein. If this loss cannot be reduced further under basal conditions, then this amino acid will determine the obligatory loss for all other amino acids, which can no longer be used for anabolic processes. In theory, this "limiting" amino acid should be identified as having the lowest ratio between the requirement estimates from maintenance and by direct measurement, which is isoleucine in this report (Table 10-20). However, this is the amino acid with no direct measurements of requirement, as the adult EAR was estimated from its content in egg protein in relation to the other branched chain amino acids.

The important conclusion from the above discussion is that the calculation of the maintenance requirement in adults from the obligatory nitrogen loss gives values in adults that are in general higher than the measured values, and therefore appear to overestimate true maintenance. Moreover, as the maintenance protein requirement is estimated to be the same per kilogram of body weight in adults and children, it is reasonable to conclude that the amino acid values for maintenance needs derived from the obligatory nitrogen loss are likely to be overestimates in children as well as in adults. Therefore, in the factorial calculations to estimate total requirements for indispensable amino acid needs in children, the maintenance requirements for the individual amino acids are those derived on a weight basis from direct measurements or the EAR in adults (Table 10-20).

Indispensable Amino Acid EAR and RDA Summary, Ages 7 Months Through 18 Years

To calculate a factorial estimate of the EAR for individual indispensable amino acids, the amino acid needs for growth or protein deposition are first calculated as the product of the average rate of protein deposition (Table 10-9) and the average amino acid composition of body protein (Table 10-19). Thus, for a 9- through 12-month-old infant depositing on average 242 mg of protein/kg/d (Table 10-9, average of 232 mg/kg/d for girls and 252 mg/kg/d for boys), the obligatory need for lysine (amino

acid deposition) is 242 × 0.073 (Table 10-19) = 17.7 mg/kg/d. This is then divided by the partial efficiency of protein deposition (0.58 as shown in Table 10-8 for children aged 7 months through 13 years and 0.47 for children aged 14 through 18 years [see "Adolescents, Ages 14 Through 18 Years"]) to yield a value of 30 mg/kg/d for protein deposition. (The same result would be achieved by multiplying the amino acid deposition figure by 1.72 [reciprocal of 0.58] or 2.13 [reciprocal of 0.47] as indicated in Table 10-21.) This value is then added to the estimated maintenance requirement, which is the same as the EAR in adults on a body weight basis (31 mg/kg/d in Tables 10-20 and 10-21). This gives an EAR for the 9- through 12-month-old infant of 62 mg of lysine/kg/d. In the same way, the EARs for each of the indispensable amino acids at different age groups were calculated and the results are shown in Table 10-21.

The RDA for the indispensable amino acids for children is set by determining the coefficients of variation for maintenance and for protein deposition. Since the maintenance requirement in adults was utilized, the estimate of the coefficient of variation in adults (12 percent) (see "Protein RDA Summary, Ages 19 Through 50 Years") was also utilized to determine the RDA for maintenance requirements for children. A coefficient of variation of 43 percent for protein deposition was determined in the study of Butte and coworkers (2000), and this varied little with age and gender. Therefore, this value was used for variation in growth for all ages. Since the RDA is defined as equal to the EAR plus twice the CV to cover the needs of 97 to 98 percent of the individuals in the group, the protein RDA is equal to the EAR + 2 × square root $[(0.12 \times \text{Maintenance})^2 + (0.43 \times 1.72$ for children 7 mo–13 y or 2.13 for children 14–18 y × Protein Deposition$)^2]$. The RDAs for each indispensable amino acid for each age group are shown in Table 10-21.

Adults Ages 19 Years and Older

Evidence Considered in Estimating the Average Requirement

Several different indicators have been used to determine indispensable amino acid requirements, which include nitrogen balance (N-balance), plasma amino acid concentrations, direct amino acid oxidation (DAAO), 24-hour amino acid balance (AAB), and indicator amino acid oxidation (IAAO). An explanation of each of these indicators is found in the section, "Selection of Indicators for Estimating the Requirement for Individual Amino Acids." In general, the latter three methods, which depend on amino acid kinetic measurements, give higher values for amino acid requirements than do the (classical) nitrogen balance studies.

Resolution of a Controversy. All of the above five methods are based on measuring a change in the particular endpoint in response to graded levels of the test amino acid. A key observation regarding nitrogen balance as an endpoint is that there is a curvilinear relationship between nitrogen balance and test amino acid intake, so that nitrogen retention (nitrogen balance) becomes less efficient as zero balance is approached (Figure 10-7) (Rand and Young, 1999). Furthermore, the earlier work did not include miscellaneous losses in their nitrogen balances. Finally, most studies did not attempt to consider the effect of between-individual variance.

Only two studies were found in which several individuals were studied at four or more different levels of intake of the test amino acid (Jones et al., 1956; Reynolds et al., 1958). Rand and Young (1999) reanalyzed the lysine data of Jones et al. (1956) using regression techniques and found that curvilinear models best fit the data (Figure 10-7). They also examined the effect of adding either 5 or 8 mg/kg/d of miscellaneous nitrogen losses. Whereas Jones and coworkers (1956) had concluded, based on their data, that the lysine requirement was 8 mg/kg/d, the reanalysis by Rand and Young (1999) came to the conclusion that the lysine requirement was in the range of 17 to 36 mg/kg/d, and that the data strongly support a requirement of about 30 mg/kg/d. As shown in Table 10-22, this requirement approximates values derived from DAAO (Meredith et al., 1986), is similar to values derived from 24-hour amino acid balances (Kurpad et al., 2001a, 2002b), and is comparable to values derived from two IAAO studies (Kriengsinyos et al., 2002; Zello et al., 1993).

It is important to note that in growing animals, nitrogen balance and IAAO give comparable values (Zello et al., 1995) as do DAAO and IAAO. All three approaches are based on different assumptions. The reanalysis of the Jones et al. (1956) data by Rand and Young (1999) using nonlinear regression and including miscellaneous losses, has closed the apparent gap between nitrogen balance and the amino acid oxidation techniques.

Twenty-four Hour Amino Acid Balance. As shown in Table 10-22, 24-hour amino acid balance studies have been completed for four amino acids: leucine (El-Khoury et al., 1994a; Kurpad et al., 2001b), lysine (Kurpad et al., 2001a, 2002a), phenylalanine + tyrosine (Basile-Filho et al., 1998), and most recently threonine (Borgonha et al., 2002; Kurpad et al., 2002b). Of the studies, lysine (Kurpad et al., 2001a, 2002a) and threonine (Borgonha et al., 2002; Kurpad et al., 2002b) employed the 24-hour indicator balance method. Furthermore, the initial 24-hour balance study for leucine (El-Khoury et al., 1994b) also included measurement of urea production as further support for the leucine requirement estimate obtained from DAAO (Meguid et al., 1986a). Similarly, the 24-hour lysine balance data lend

TABLE 10-21 Calculations of Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for Amino Acids for Children Ages 7 Months Through 18 Years

Age and Gender/Amino Acid	Maintenance[a] (mg/kg/d)	Amino Acid Deposition[b] (mg/kg/d)	Total = EAR[c] (mg/kg/d)	RDA[d] (mg/kg/d)
7–12 mo, Boys, Girls				
Histidine	11	7	22	32
Isoleucine	15	9	30	43
Leucine	34	18	65	93
Lysine	31	18	62	89
Methionine + cysteine	15	9	30	43
Phenylalanine + tyrosine	27	18	58	84
Threonine	16	10	34	49
Tryptophan	4	3	9	13
Valine	19	12	39	58
1–3 y, Boys, Girls				
Histidine	11	3	16	21
Isoleucine	15	4	22	28
Leucine	34	8	48	63
Lysine	31	8	45	58
Methionine + cysteine	15	4	22	28
Phenylalanine + tyrosine	27	8	41	54
Threonine	16	5	24	32
Tryptophan	4	1	6	8
Valine	19	5	28	37

4–8 y, Boys, Girls				
Histidine	11	1	13	16
Isoleucine	15	2	18	22
Leucine	34	4	40	49
Lysine	31	3	37	46
Methionine + cysteine	15	2	18	22
Phenylalanine + tyrosine	27	3	33	41
Threonine	16	2	19	24
Tryptophan	4	1	5	6
Valine	19	2	23	28
9–13 y, Boys				
Histidine	11	1	13	17
Isoleucine	15	2	18	22
Leucine	34	4	40	49
Lysine	31	4	37	46
Methionine + cysteine	15	2	18	22
Phenylalanine + tyrosine	27	4	33	41
Threonine	16	2	19	24
Tryptophan	4	1	5	6
Valine	19	42	23	28

continued

TABLE 10-21 Continued

Age and Gender/Amino Acid	Maintenance[a] (mg/kg/d)	Amino Acid Deposition[b] (mg/kg/d)	Total = EAR[c] (mg/kg/d)	RDA[d] (mg/kg/d)
9–13 y, Girls				
Histidine	11	1	12	15
Isoleucine	15	1	17	21
Leucine	34	2	38	47
Lysine	31	2	35	43
Methionine + cysteine	15	1	17	21
Phenylalanine + tyrosine	27	2	31	38
Threonine	16	1	18	22
Tryptophan	4	< 0.5	5	6
Valine	19	2	22	27
14–18 y, Boys				
Histidine	11	1	12	15
Isoleucine	15	1	17	21
Leucine	34	2	38	47
Lysine	31	2	35	43
Methionine + cysteine	15	1	17	21
Phenylalanine + tyrosine	27	2	31	38
Threonine	16	1	18	22
Tryptophan	4	< 0.5	5	6
Valine	19	1	22	27

14–18 y, Girls				
Histidine	11	< 0.5	12	14
Isoleucine	15	< 0.5	16	19
Leucine	34	1	35	44
Lysine	31	1	32	40
Methionine + cysteine	15	< 0.5	16	19
Phenylalanine + tyrosine	27	1	28	35
Threonine	16	< 0.5	17	21
Tryptophan	4	< 0.5	4	5
Valine	19	1	20	24

[a] Derived from the adult EAR for specified amino acids (from Table 10-20).
[b] Derived using the following equation: Amino acid deposition = mean protein deposition (from Table 10-9) × amino acid composition of whole body protein (from Table 10-19).
[c] EAR for ages 7 mo–13 y = maintenance + amino acid deposition × 1.72. EAR for ages 14–18 y = maintenance + amino acid deposition x 2.13.
[d] RDA for ages 7 mo–13 y = EAR + $2 \times \sqrt{[(0.12 \times \text{maintenance})^2 + (0.43 \times 1.72 \times \text{mean protein deposition})^2]}$. RDA for ages 14–18y = EAR + $2 \times \sqrt{[(0.12 \times \text{maintenance})^2 + (0.43 \times 2.13 \times \text{mean protein deposition})^2]}$.

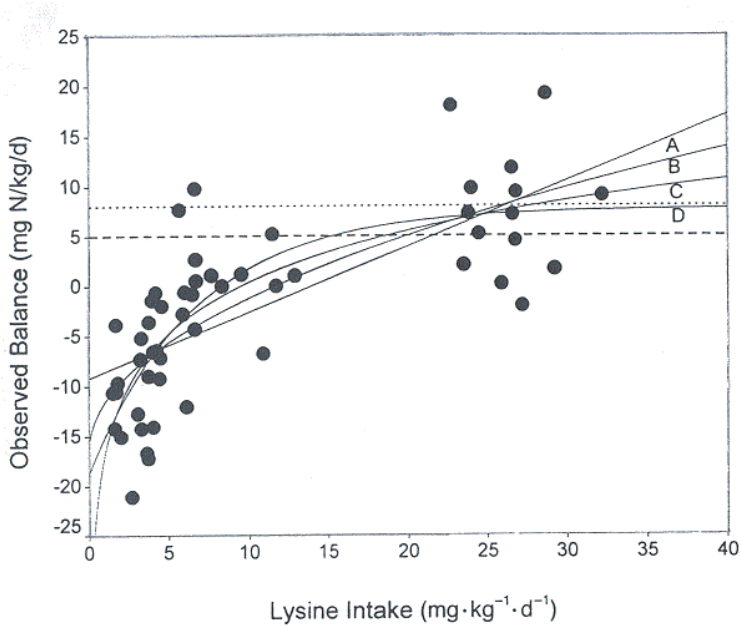


FIGURE 10-7 Relationship between nitrogen balance and test amino acid intake using four different one-fit regression equations: linear (A), square root (B), log (C), and exponential asymptotic (D), superimposed on the original data. Reprinted, with permission, from Rand and Young (1999). Copyright 1999 by the American Society for Nutritional Sciences.

support to the lysine DAAO estimate (Kurpad et al., 2001a; Meredith et al., 1986).

The 24-hour balance model is regarded as being the best from a theoretical point of view, especially when performed with the indicator approach. However, from a practical point of view, the 24-hour amino acid balance studies are very labor intensive with the result that only three or four levels of intake of the test amino acid have been studied for each of leucine, lysine, phenylalanine + tyrosine, and threonine.

Direct Amino Acid Oxidation. The DAAO method has been utilized to investigate six or seven amino acid levels, so it was possible to reanalyze these data using two-phase linear crossover regression analysis and define a breakpoint (which is regarded as the EAR). DAAO can only be used if the carboxyl group of the test amino acid is released to the body bicarbonate pool when the amino acid is committed to degradation. As shown in

TABLE 10-22 Indispensable Amino Acid Studies in Adults

Reference	Amino Acid	Method Used[a] (Number of Levels/ Number of Data Points)	Estimated Average Requirement (mg/kg/d)
Meguid et al., 1986a	Leucine	DAAO reanalyzed (8/52)	24.5
El-Khoury et al., 1994a	Leucine	24-h AAB (3/10)	38.3
Kurpad et al., 2001b	Leucine	24-h AAB (4/40)	40
Meredith et al., 1986	Lysine	DAAO reanalyzed (8/28)	26.6
Zello et al., 1993	Lysine	IAAO (7/42)	36.9
Rand and Young, 1999	Lysine	N-Balance reanalyzed (8/53)	30
Kurpad et al., 2001a	Lysine	24-h IAAB (4/32)	29
Kriengsinyos et al., 2002	Lysine	IAAO (5/60)	35
Kurpad et al., 2002a	Lysine	24-h IAAB (4/36)	29
Reynolds et al., 1958	Methionine + cysteine	N-balance reanalyzed (6/42)	20
Young et al., 1991	Methionine + cysteine	Methionine balance (1/5)	13
Di Buono et al., 2001	Methionine + cysteine	IAAO (6/36)	12.6
Zello et al., 1990	Phenylalanine	DAAO (7/41)	9.1
Roberts et al., 2001	Tyrosine	IAAO (7/42)	6.0
	Phenylalanine + tyrosine		15.1
Basile-Filho et al., 1998	Phenylalanine + tyrosine	24-h AAB	39.0
Zhao et al., 1986	Threonine	DAAO reanalyzed (7/33)	13.5
Wilson et al., 2000	Threonine	IAAO (7/36)	19.0
Borgonha et al., 2002	Threonine	24-h IAAB (3/15)	15.0
Kurpad et al., 2002b	Threonine	24-h IAAB (6/48)	15.0
Lazaris-Brunner et al., 1998	Tryptophan	IAAO (8/36)	4.0
Meguid et al., 1986b	Valine	DAAO reanalyzed (7/37)	19.2

[a] AAB = amino acid balance, DAAO = direct amino acid oxidation, IAAB = indicator amino acid balance and oxidation, IAAO = indicator amino acid oxidation.

Table 10-22, DAAO studies of indispensable amino acid requirements are limited to leucine (Meguid et al., 1986a), lysine (Meredith et al., 1986), phenylalanine (Zello et al., 1990), and valine (Meguid et al., 1986b). DAAO was also utilized to determine the threonine requirement (Zhao et al., 1986). However there are theoretical concerns for this amino acid,

since there are two pathways of degradation for threonine; the second pathway, threonine dehydrogenase (TDG), ends in the label being retained in glycine. In practical terms this may not be a serious error since others have shown that the TDG pathway is a minor pathway in adults (Darling et al., 2000).

Indicator Amino Acid Oxidation. IAAO has the advantage that the requirement of any amino acid can be determined, since either phenylalanine (in the presence of an excess of tyrosine to ensure that there is no label retention in the body tyrosine pools) or lysine can and have been used as indicator amino acids in humans and in animals (Bross et al., 2000; Brunton et al., 1998; Zello et al., 1995). A further strength of the IAAO studies is that each adult was fed at six or seven levels of the test amino acid, which has made it possible to define requirements for individuals by two-phase, linear cross-over regression analysis (Brunton et al., 1998; Zello et al., 1995). As shown in Table 10-22, IAAO estimates have been reported for lysine (Kriengsinyos et al., 2002; Zello et al., 1993), methionine + cysteine (Di Buono et al., 2001), tyrosine (Roberts et al., 2001), threonine (Wilson et al., 2000), and tryptophan (Lazaris-Brunner et al., 1998).

As shown in Table 10-22, currently there are amino acid oxidation estimates in which two-phase linear crossover regression analysis has been performed for leucine (DAAO), lysine (both DAAO and IAAO), methionine + cysteine (IAAO), phenylalanine (DAAO), tyrosine (IAAO), threonine (both DAAO and IAAO), tryptophan (IAAO), and valine (DAAO).

Other Indicators. Nonlinear regression was used on two sets of nitrogen balance data as shown by Rand and Young (1999). The first was for lysine in which the original data were in women, each of whom were studied at two to five levels (Jones et al., 1956). This data set was reanalyzed using nonlinear regression, including the addition of 5 or 8 mg of nitrogen/kg/d as miscellaneous losses (Rand and Young, 1999), and these reanalyzed data are included in Table 10-22. Using a similar approach, the data of Reynolds and coworkers (1958) for methionine + cysteine were reanalyzed, and these data are included in Table 10-22. The result is consistent with the data of Zezulka and Calloway (1976a, 1976b), who studied the effect on nitrogen balance of three levels of methionine added to soy protein at a constant and adequate level of total nitrogen. Since there are no direct estimates of the isoleucine requirement, it is estimated from the leucine and valine estimates. The isoleucine requirement was therefore calculated by multiplying the isoleucine requirement calculated from the protein requirement (Table 10-20) by 1.55, the average of the ratios for leucine and valine. Similarly, the requirement for histidine, for which there have also been no direct determinations, is calculated from the protein require-

ment (Table 10-20) multiplied by 1.7, the average ratio for all amino acids in Table 10-19.

Indispensable Amino Acid EAR Summary, Ages 19 Years and Older

An EAR was derived for each of the indispensable amino acids from the data in Table 10-22. Where more than one EAR was given for an amino acid in Table 10-22, the values were averaged and rounded to the nearest whole number. This approach is weakest with the phenylalanine + tyrosine requirements where there is a large range—from 15.1 to 39 mg/kg/d giving an average value of 27 mg/kg/d. Lysine is the indispensable amino acid with the most estimates (six in all), with the EAR varying from 26.6 to 36.9 mg/kg/d for an average value of 31 mg/kg/d. Given the very few studies available, separate requirements could not be determined for women versus men, or for young and older adults.

EAR for Adults

19 years and older	**11 mg/kg/d of histidine**
	15 mg/kg/d of isoleucine
	34 mg/kg/d of leucine
	31 mg/kg/d of lysine
	15 mg/kg/d of methionine + cysteine
	27 mg/kg/d of phenylalanine + tyrosine
	16 mg/kg/d of threonine
	4 mg/kg/d of tryptophan
	19 mg/kg/d of valine

Indispensable Amino Acid RDA Summary, Ages 19 Years and Older

With protein (see "Protein RDA Summary, Ages 19 Through 50 Years"), because the distribution of individual requirements for protein is not a normal distribution and is skewed, its calculated standard deviation and coefficient of variation do not have the usual intuitive meaning (the mean plus two standard deviations exceeding all but about 2.5 percent of the population's requirement). However, an approximate standard deviation was calculated as half of the distance from the 16th to the 84th percentile of the protein requirement distribution as estimated from the log normal distribution of requirements. This gives, for comparative purposes, an approximate standard deviation of 12.5 mg N/kg/d (a CV = 12 percent). Given the paucity of data, it is assumed that for amino acids a similar deviation should be used; thus the RDA = EAR + 2 CV for amino acids as well as for protein, or RDA = 1.24 × EAR. The calculated RDA is rounded to the nearest whole number.

RDA for Adults	
19 years and older	**14 mg/kg/d of histidine**
	19 mg/kg/d of isoleucine
	42 mg/kg/d of leucine
	38 mg/kg/d of lysine
	19 mg/kg/d of methionine + cysteine
	33 mg/kg/d of phenylalanine + tyrosine
	20 mg/kg/d of threonine
	5 mg/kg/d of tryptophan
	4 mg/kg/d of valine

Pregnancy

Method Used to Estimate the Average Requirement

There are essentially no data with regard to amino acid requirements during pregnancy, so it is generally assumed that indispensable amino acid needs increase in proportion to the increased protein needs during pregnancy. Since the pregnancy EAR for total protein is 0.88 g/kg/d for women, the amino acid EARs for nonpregnant women were multiplied by 1.33 and rounded to the nearest whole number.

Amino Acid EAR and RDA Summary, Pregnancy

EAR for Pregnancy	
For all ages	**15 mg/kg/d of histidine**
	20 mg/kg/d of isoleucine
	45 mg/kg/d of leucine
	41 mg/kg/d of lysine
	20 mg/kg/d of methionine + cysteine
	36 mg/kg/d of phenylalanine + tyrosine
	21 mg/kg/d of threonine
	5 mg/kg/d of tryptophan
	25 mg/kg/d of valine

The RDA for amino acids for pregnancy is set by increasing the EAR by the variation in protein derived for adults ages 19 years and older ($1.24 \times$ EAR) and rounded to nearest whole number.

RDA for Pregnancy

For all ages	**18 mg/kg/d of histidine**
	25 mg/kg/d of isoleucine
	56 mg/kg/d of leucine
	51 mg/kg/d of lysine
	25 mg/kg/d of methionine + cysteine
	44 mg/kg/d of phenylalanine + tyrosine
	26 mg/kg/d of threonine
	7 mg/kg/d of tryptophan
	31 mg/kg/d of valine

Lactation

Method Used to Estimate the Average Requirement

There are essentially no data with regard to amino acid requirements during lactation, so it is generally assumed that indispensable amino acid needs will increase over the nonlactating needs by the amount of amino acids found in human milk (Table 10-18).

To estimate the EAR for amino acids for lactation, the average amounts of amino acids in human milk during the first 6 months of lactation expressed as mg/kg/d based on the reference weight of the adult woman in Table 1-1 (see "AI for Infants 0–6 Months"), are added to the EAR for amino acids for the nonlactating woman, expressed as mg/kg/d (see sections "Amino Acids EAR and RDA Summary, Ages 7 Months Through 18 Years" and "Amino Acids EAR and RDA Summary, Ages 19 Years and Older"). The calculated EARs in mg/kg body weight/d are rounded to the nearest whole number.

Amino Acid EAR and RDA Summary, Lactation

EAR for Lactation

For all ages	**15 mg/kg/d of histidine**
	24 mg/kg/d of isoleucine
	50 mg/kg/d of leucine
	42 mg/kg/d of lysine
	21 mg/kg/d of methionine + cysteine
	41 mg/kg/d of phenylalanine + tyrosine
	24 mg/kg/d of threonine
	7 mg/kg/d of tryptophan
	28 mg/kg/d of valine

The RDA for amino acids for lactation is set by assuming the same (CV) as that for total protein for lactation, 12 percent. The RDA is defined as the EAR plus twice the assumed CV to cover the needs of 97 to 98 percent of the individuals in the group. Therefore, for amino acids the RDA is 124 percent of the EAR for adolescents and adults. The calculated RDA in mg/kg of body weight/d is rounded.

RDA for Lactation	
For all ages	**19 mg/kg/d of histidine**
	30 mg/kg/d of isoleucine
	62 mg/kg/d of leucine
	52 mg/kg/d of lysine
	26 mg/kg/d of methionine + cysteine
	51 mg/kg/d of phenylalanine + tyrosine
	30 mg/kg/d of threonine
	9 mg/kg/d of tryptophan
	35 mg/kg/d of valine

INTAKE OF TOTAL PROTEIN AND AMINO ACIDS

Protein Quality

Different sources of protein vary widely in their chemical composition as well as in their nutritional value. The quality of a source of protein (or more specifically the source of nitrogen, since dietary protein is generally measured analytically in terms of nitrogen) is an expression of its ability to provide the nitrogen and amino acid requirements for growth, maintenance, and repair. In practice, protein quality is principally determined by two factors: digestibility and the amino acid composition of the protein in question. In food as opposed to relatively pure protein, the contribution of all of the indispensable amino acids to the total nitrogen content of the food has to be considered in assessing the overall protein quality of the diet.

Digestibility

Nitrogen is excreted in the feces in amounts that usually vary between 10 and 25 percent of the nitrogen intake. As mentioned earlier, only a part of this is derived directly from dietary nitrogen that was not absorbed; the other parts result from protein and other secretions into the gastrointestinal tract during the process of digestion and from nitrogen con-

tained in fecal bacteria. The unabsorbed part represents mainly proteins that, by reason of their physical characteristics or chemical composition, are resistant to breakdown by the proteolytic digestive enzymes. There is probably a variable contribution of nitrogen contained in other nonabsorbable components, such as amino sugars and other nitrogen-containing materials found in cell walls.

On the other hand, the secretions consist of specific proteins, such as mucins, which represent a loss that is of nutritional importance. These secretions appear to be rich in threonine and cysteine (Roberton et al., 1991), and thus contribute to the requirement for both amino acids. However, both the nonabsorbed and secreted components that make up nitrogen loss are difficult to quantify with any confidence, except in terms of total nitrogen, because of the overwhelming modifying effect of the intestinal microflora. Thus, digestibility (as estimated by nitrogen excretion) is usually determined by measuring the fecal nitrogen (N_{FP}) in individuals consuming the specific nitrogen source and subtracting the fecal nitrogen values obtained when a protein-free diet is given (N_{F0}). This value is then subtracted from the total nitrogen intake (N_I) and expressed as a proportion of the nitrogen intake.

$$\text{True Digestibility} = D_F = (N_I - N_{F\Delta}) / N_I,$$

where $N_{FD} = N_{FP} - N_{F0}$.

Fecal nitrogen from a protein-free diet is a measure of the amount of nitrogen from intestinal secretions, on the assumption (probably incorrect) that this component does not vary with different diets (de Lange et al., 1989). The values thus calculated are called "true" digestibility and represent the proportion of the dietary nitrogen that is absorbed. This portion can generally be assumed to be available to the host for meeting the needs for maintenance and growth.

It must be noted that a number of recent studies with isotopically labeled proteins suggest that true digestibility exceeds 90 percent for many common foods such as milk, cereals, and soy and other legumes (Darragh and Hodgkinson, 2000, de Vrese et al., 2000; Mariotti et al., 1999, Gausserès et al., 1997; Gaudichon et al., 1999). It should also be noted that, at present, calculation of the availability (or digestibility) of amino acids from food protein sources is based on the digestibility of total nitrogen as contrasted to that for the individual amino acid. However, there can be quite large differences between the digestibility coefficients for total nitrogen and the individual amino acid. These and other related aspects of protein quality have been reviewed elsewhere (Darragh and Hodgkinson, 2000; Schaafsma, 2000).

The digestive and intestinal phase of dietary protein utilization is currently an active area of research, but it is still, from a practical standpoint, not possible to make major improvements over the estimates of true protein digestibility made some years ago by the Food and Agriculture Organization and the World Health Organization (FAO/WHO, 1991). Therefore, the determination of true digestibility of proteins, diets, and amino acids in this report are based on the approaches and values proposed by FAO/WHO in 1991.

Nitrogen Versus Amino Acids

Absorbed nitrogen is mainly in the form of amino acids, but a proportion is in other compounds such as nucleic acids, creatine, amino sugars, ammonia, and urea. The quantitative extent to which these contribute to nitrogen retention and homeostasis is not known. Creatine can probably be utilized (Metges et al., 1999b), but in general it is unknown to what extent these different compounds can have a sparing effect on the utilization of the amino acids for which they are precursors. However, the major requirement for total nitrogen or protein is for the specific indispensable amino acids (and/or conditionally indispensable amino acids) and an additional source of α-amino nitrogen. At appropriate intakes these maintain protein homeostasis and adequate synthesis of those physiologically important compounds for which amino acids are the obligatory precursors (Table 10-5).

It is conventional to use a value of 6.25 to express the weight ratio of protein to the nitrogen content in foods, which assumes that nitrogen is, on average, 16 percent by weight of mixed protein. However, this factor is in fact quite variable among different proteins. For example, when protein intake is calculated by summing the weight of amino acids as analyzed in a food (less the water of hydrolysis), the protein/nitrogen ratio is 5.38 for egg, 5.62 for whole milk, 4.86 for cooked ham, 5.70 for whole-meal wheat bread, and 6.07 for soymilk. Thus when converting the amount of nitrogen present in a specific foodstuff to total protein, this factor becomes important to use.

These differences in the protein-to-nitrogen ratio of food proteins are not of specific importance in reference to the development of the recommendations for protein requirements given herein. This is because these recommendations have been based initially on nitrogen balance determinations, which in turn were based on analytical measurements of nitrogen intake (from different test proteins or mixtures of proteins). The nitrogen intake values were then converted to protein intakes using the conventional 6.25 factor, irrespective of the protein source used in the various experiments.

However, the protein-to-nitrogen conversion factor does matter in considering the quality of food protein sources when the protein-specific nitrogen conversion factor has been used to convert the chemically determined nitrogen content of the protein to a protein value. In this case, protein intakes and the relation between the amino acid concentrations in the protein should all be referred back to a nitrogen base. For this reason, amino acid requirement patterns delineated below are given in reference to both conventional protein (nitrogen × 6.25) and to a nitrogen basis.

Amino Acids Content of Proteins

The second and generally more important factor that influences the nutritional value of a protein source is the relative content and metabolic availability of the individual indispensable amino acids. If the content of a single indispensable amino acid in the diet is less than the individual's requirement, then it will limit the utilization of other amino acids and thus prevent normal rates of protein synthesis even when the total nitrogen intake level is adequate. Thus, the "limiting amino acid" will determine the nutritional value of the total nitrogen or protein in the diet. This has been illustrated in experiments comparing the relative ability of different protein sources to maintain nitrogen balance. For example, studies have shown, depending on its source and preparation, that more soy protein might be needed to maintain nitrogen balance when compared to egg-white protein, and that the difference may be eliminated by the addition of methionine to the soy diet. This indicates that sulfur amino acids can be limiting in soy (Zezulka and Calloway, 1976a, 1976b). Similarly, the limiting amino acid in wheat protein is lysine (Young et al., 1975a).

The concept of the limiting amino acid has led to the practice of amino acid (or chemical) scoring, whereby the indispensable amino acid composition of the specific protein source is compared with that of a reference amino acid composition profile. Earlier the amino acid composition of a good quality protein such as egg, which is regarded as being well balanced in amino acid content in relation to human needs (FAO/WHO, 1973), was used as a reference or benchmark. Table 10-23 shows the composition of various food protein sources expressed as mg of amino acid per g of protein (nitrogen × 6.25). The composition of amino acids of egg and milk proteins is similar with the exception of the sulfur amino acids methionine and cysteine. However, wheat and beans have lower proportions of indispensable amino acids, especially of lysine and sulfur amino acids, respectively.

The nutritional implications of these differences in the amino acid content of different proteins or mixtures of proteins can be evaluated by

TABLE 10-23 Amino Acid Composition of Major Food Protein Sources (mg/g protein)[a]

Amino Acid	Whole Wheat Flour	Navy Beans	Milk	Eggs
Histidine	22	28	28	24
Isoleucine	40	42	60	63
Leucine	63	76	98	88
Lysine	26	72	79	70
Methionine + cysteine	35	19	34	56
Phenylalanine + tyrosine	81	77	96	98
Threonine	27	39	45	49
Tryptophan	11	10	14	16
Valine	43	46	67	72

[a] Values for protein composition (N × 6.25) are from Young and Pellett (1990).

comparing the amino acid composition of the protein source with a suitable reference amino acid pattern.

Amino Acid Scoring and Protein Quality

In recent years, the amino acid requirement values for humans have been used to develop reference amino acid patterns for purposes of evaluating the quality of food proteins or their capacity to efficiently meet both the nitrogen and indispensable amino acid requirements of the individual. Based on the estimated average requirements for the individual indispensable amino acids presented earlier (Tables 10-20 and 10-21) and for total protein (nitrogen × 6.25) (Tables 10-9 and 10-13), it is possible to establish an amino acid requirement (or scoring) pattern for preschool children and for adults. These are given in Table 10-24 together with the amino acid requirement pattern used for breast-fed infants. It should be noted that this latter pattern is that for human milk and so it is derived quite differently compared to that for the other age groups.

There are three important points that need to be highlighted about the proposed amino acid scoring patterns. First, there are relatively small differences between the amino acid requirement and thus scoring patterns for children and adults, therefore use amino acid requirement pattern for 1 to 3 years of age is recommended as the reference pattern for purposes of assessment and planning of the protein component of diets.

Second, the requirement pattern proposed here for adults is fundamentally different from a number of previously recommended requirement patterns (Table 10-25). The pattern for adults (FAO/WHO/UNU,

TABLE 10-24 Proposed Amino Acid Scoring Patterns for Infants, Preschool Children, and Adults Based on Estimated Requirements for Protein and Indispensable Amino Acids

	Infants[a]	Preschool Children (1–3 y)		Adults (18+ y)	
Amino Acid	(mg/g protein)	(mg/g protein)[b]	(mg/g N)[c]	(mg/g protein)[b]	(mg/g N)[c]
Histidine	23	18	114	17	104
Isoleucine	57	25	156	23	142
Leucine	101	55	341	52	322
Lysine	69	51	320	47	294
Methionine + cysteine	38	25	156	23	142
Phenylalanine + tyrosine	87	47	291	41	256
Threonine	47	27	170	24	152
Tryptophan	18	7	43	6	38
Valine	56	32	199	29	180

[a] Pattern based on amino acid composition of human milk (from Table 10-18).
[b] Pattern derived from (EAR for amino acid ÷ EAR for protein); for 1–3 y group, where EAR for protein = 0.88 g/kg/d; for adults, EAR for protein = 0.66 g/kg/d. EAR is Estimated Average Requirement.
[c] Calculated as (mg/g protein) × 6.25.

TABLE 10-25 FNB/IOM Scoring Pattern Compared to Other Proposed Patterns (mg/g protein)

Amino Acid	MIT Pattern[a]	Millward Pattern[b]	FAO/WHO/ UNU Pattern[c]	Recommended FNB/IOM Pattern[d]
Histidine	—	—	—	18
Isoleucine	35	30	13	25
Leucine	65	44	19	55
Lysine	50	31	16	51
Methionine + cysteine	25	27	17	25
Phenylalanine + tyrosine	65	33	19	47
Threonine	25	26	9	27
Tryptophan	10	6	5	7
Valine	35	23	13	32

[a] Young and Pellett (1990).
[b] Millward et al. (1990).
[c] FAO/WHO/UNU (1985).
[d] Based on 1- to 3-year-old Estimated Average Requirements for protein and indispensable amino acids.

1985) has uniformly lower proportions of all the indispensable amino acids, as these requirement values were determined from studies of nitrogen balance, which are now considered to be not as reliable as values derived from metabolic amino acid data (see previous discussion). The other requirement patterns shown in Table 10-25 for adults were published in two recent reviews (Millward, 1999; Young and Borgonha, 2000). The pattern suggested by Millward (1999) is based on a reanalysis of nitrogen balance data that yields values that are generally lower than either the FNB/IOM reference pattern, based on the EARs estimated above, or the MIT pattern (Young and Borgonha, 2000). The MIT pattern includes much of the oxidation and carbon balance data contained in the EAR estimates given in this report, but the reference pattern recommended here is derived from a larger body of data than that used by Young and Borgonha. Thus, the reference amino acid scoring patterns shown in Table 10-24 are designed for use in the evaluation of dietary protein quality.

Third, in generating these amino acid scoring patterns, the EARs for the amino acids and for total protein were used. However, two important statistical considerations need to be raised here: first, the extent to which there is a correlation between nitrogen (protein) and the requirement for a specific indispensable amino acid; second, the impact of the variance for both protein and amino acid requirements on the derived amino acid reference pattern. The extent to which the requirements for specific indispensable amino acids and total protein are correlated is not known. In this report it is assumed that the variance in requirement for each indispensable amino acid is the same as that for the adult protein requirement.

This analysis illustrates one of the uncertainties faced in establishing a reference or scoring pattern and judging the nutritional value of a protein source for an individual. However, on the basis of different experimental studies in groups of subjects, experience shows that a reasonable approximation of the mean value for the relative quality of a protein source or mixture of proteins can be obtained by use of the amino acid scoring pattern proposed in Table 10-26 and a standard amino acid scoring approach, examples of which are given in the following section.

Calculation of Amino Acid Scores for Different Food Proteins

The method for evaluating the relative nutritional quality of different protein sources that is used in this report is based on calculating the protein digestibility corrected amino acid score (PDCAAS) as proposed by FAO/WHO (1991). It is calculated as follows:

TABLE 10-26 Summary FNB/IOM 2002 Amino Acid Scoring Pattern for Use in Children ≥ 1 Year of Age and in All Other Older Age Groups

Amino Acid	mg/g protein[a]	mg/g N
Histidine	18	114
Isoleucine	25	156
Leucine	55	341
Lysine	51	320
Methionine + cysteine	25	156
Phenylalanine + tyrosine	47	291
Threonine	27	170
Tryptophan	7	43
Valine	32	199

[a] Protein = nitrogen × 6.25.

$$\text{PDCAAS (\%)} = \frac{\text{[mg of limiting amino acid in 1-g test protein]}}{\text{mg of same amino acid in 1-g reference protein}} \times \text{[true digestibility } (D_F)\text{(\%)]}$$

As mentioned earlier, in comparing the amino acid reference (or scoring) patterns (Table 10-24) for 1- through 3-year-old children and the adult age groups, it would be hard to justify proposing separate amino acid scoring patterns for these populations for practical purposes. Therefore, for calculation of the amino acid score, corrected for digestibility (PDCAAS, %), it is recommended that one scoring pattern be used to cover all ages from 1 year and above, as shown in Table 10-26.

A number of examples of the PDCAAS for different food proteins or diets based on three major protein sources are given in Table 10-27. As shown, wheat (lysine limiting) and chickpea proteins (sulfur amino acid limiting) have a PDCAAS of 44 and 87 percent, respectively. For a diet based on a mixture of wheat, chickpea, and skim milk proteins the PDCAAS is 110 percent, which is truncated to a value of 100, since the relative efficiency of utilization of the limiting amino acid cannot be greater than that of the amino acid scoring pattern at nitrogen intakes sufficient to meet nitrogen needs. Finally, it should be noted that PDCAAS scores have only been calculated here based on four indispensable amino acids (lysine, sulfur amino acids, threonine, and tryptophan). These are

TABLE 10-27 Calculation of PDCAAS[a] for Selected Individual Food Proteins and for a Mixture of These Proteins, Based on the FNB/IOM 2002 Amino Acid Scoring Pattern

Protein	Amino Acid Content (mg/g protein)[b]				Protein Digestibility	PDCAAS (%)
	Lys	SAA	Thr	Trp		
Wheat	25	35	30	11	0.85	42 (Lysine)[c]
Chickpea	70	25	42	13	0.80	80 (SAA)
Milk powder	80	30	37	12	0.95	100 (114—SAA)[d]
Mixture (% of protein)						
Wheat (19)						
Chickpea (32)						
Milk powder (49)						
For combination[e]	64	29	37	12	0.88	100 (102—SAA)

[a] Data for proteins taken from Table 10 of FAO/WHO (1991) using the procedure described therein to determine PDCAAS (Protein Digestibility Corrected Amino Acid Score).
[b] Lys = lysine; SAA = sulfur amino acids; Thr = threonine; Trp = Tryptophan.
[c] Lysine or sulfur amino acid = limiting amino acid.
[d] Where relevant, the nontruncated value for the PDCAAS is given prior to truncation to a value of 100.
[e] Weighted values based on the proportion of the total protein in the mixture that is contributed by each protein source.

the most likely limiting amino acids in common food protein sources and so have been considered here for illustrative purposes.

There have been discussions on ways to improve the PDCAAS procedure and further developments in this context are needed. Until better methods are developed, the foregoing procedure is recommended, using the digestibility values proposed by FAO/WHO (1991).

Comments on Protein Quality for Adults

While the importance of considering protein quality in relation to the protein nutrition of the young has been firmly established and accepted over the years, the significance of protein quality (other than digestibility) of protein sources in adults has been controversial or less clear. The amino acid scoring pattern given in Table 10-24 for adults is not markedly different from that for the preschool age group, implying that protein quality should also be an important consideration in adult protein nutrition.

In the published meta-analysis of nitrogen balance studies by Rand and coworkers (2003), there were no significant differences in the intakes of dietary nitrogen required to meet nitrogen equilibrium between those studies that supplied dietary protein predominantly from animal, vegetable, or mixed protein sources. It is important to realize however, that this aggregate analysis does not suggest that dietary protein quality is of no importance in adult protein nutrition. The examined and aggregated studies included an analysis of those that were designed to compare good quality soy protein (Istfan et al., 1983; Young et al., 1984) as well as one that involved comparison of whole-wheat proteins (Young et al., 1975a) with animal proteins sources using parallel experimental diet groups. The results of these studies showed clearly that the quality of well-processed soy proteins was equivalent to animal protein in the adults evaluated (which would be predicted from the amino acid reference pattern in Table 10-26), while wheat proteins were used with significantly lower efficiency than the animal protein (beef) (again this would be predicted from the procedure above). Similar studies compared rice and egg proteins (Inoue et al., 1973), wheat gluten and egg proteins (Yanez et al., 1982), and lupin and egg proteins (Egana et al., 1992), all demonstrating the higher quality of the animal protein reference sources.

Thus, the aggregate analyses of all available studies analyzed by Rand and coworkers (2003) obscured these results and illustrate the conservative nature of their meta-analysis of the primary nitrogen balance. Moreover, this discussion and presentation of data in Table 10-27 underscores the fact that while lysine is likely to be the most limiting of the indispensable amino acids in diets based predominantly on cereal proteins, the risk of a lysine inadequacy is essentially removed by inclusion of relatively modest amounts of animal or other vegetable proteins, such as those from legumes and oilseeds, or through lysine fortification of cereal flour.

Food Sources

Protein from animal sources such as meat, poultry, fish, eggs, milk, cheese, and yogurt provide all nine indispensable amino acids, and for this reason are referred to as “complete proteins.” Proteins from plants, legumes, grains, nuts, seeds, and vegetables tend to be deficient in one or more of the indispensable amino acids and are called “incomplete proteins.” Three ounces of lean meat or poultry contain about 25 g of protein, and 3 ounces of fish or 1 cup of soybeans supplies about 20 g of protein. The protein content of 1 cup of yogurt is approximately 8 g, 1 cup of milk is 8 g, and 1 egg or 1 ounce of cheese contains about 6 g. One cup of legumes has approximately 15 g of protein. Cereals, grains, nuts, and vegetables contain about 2 g of protein per serving.

Dietary Intake

Data from nationally representative U.S. and Canadian surveys are available to estimate protein intakes (Appendix Tables E-16 and F-5). In the United States, the median dietary intake of protein by adult men during 1994–1996 and 1998 ranged from 71 to 101 g/d for various age groups (Appendix Table E-16). For women, the median intake ranged from 55 to 62 g/d. For both men and women, protein provided approximately 15 percent of total calories (Appendix Table E-17). Similarly, in Canada, protein provided approximately 15 percent of total calories for adults (Appendix Table F-5).

The amino acids intakes for the U.S. population are found in Appendix Tables D-2 through D-19. The median dietary intake of lysine by adult men during 1988–1994 ranged from 4.65 to 7.50 g/d for various ages (Appendix Table D-11), and by adult women from 3.59 to 4.56 g/d. The median dietary intake of threonine by adult men during 1988–1994 ranged from 2.74 to 4.21 g/d for various ages (Appendix Table D-16) and by adult women from 2.10 to 2.59 g/d. The median dietary intake of tryptophan by adult men and women during 1988–1994 ranged from 0.84 to 1.26 g/d and from 0.65 to 0.78 g/d, respectively (Appendix Table D-17).

TOLERABLE UPPER INTAKE LEVELS FOR PROTEIN

Humans consume a wide range of intakes of protein. As intake is increased, the concentrations of free amino acids and urea in the blood increase postprandially. The nitrogenous substances in the urine also increase, especially urea. These changes are part of the normal regulation of the amino acids and nitrogen and represent no hazards per se, at least within the range of intakes normally consumed by apparently healthy individuals. Nonetheless, a number of adverse effects have been reported, especially at the very high intakes that might be achieved with supplement use, but also at more modest levels.

In addition, some naturally occurring proteins are allergenic to certain sensitive individuals; for example, the glycoprotein fractions of foods have been implicated in allergic responses. However, relatively few protein foods cause most allergic reactions: milk, eggs, peanuts, and soy in children; and fish, shellfish, peanuts, and tree nuts in adults.

Hazard Identification

Adverse Effects

There is little scientific literature on the effects of consuming very high protein diets, but it has been suggested from evidence of the dietary

practices of hunter-gatherer populations, both present day and historical, that humans avoid diets that contain too much protein (Cordain et al., 2000; Speth, 1989). Even when meat is the dominant food, diets of a wide range of populations do not usually contain more than about 40 percent of energy as protein (Speth, 1989). Indeed, Eskimos, when eating only meat, maintain a protein intake below 50 percent of energy by eating fat; protein intake estimated from data collected in 1855 was estimated to be about 44 percent (Krogh and Krogh, 1913).

There have been case reports of high levels of intake. Two arctic explorers, Stefansson and Andersen, ate only meat for a whole year while living in New York City (Lieb, 1929; McClellan and Du Bois, 1930; McClellan et al., 1930, 1931). For most of the period, the diet contained 15 to 25 percent of energy as protein, with fat (75 to 85 percent) and carbohydrate (1 to 2 percent) providing the rest, and no ill effects were observed (McClellan and Du Bois, 1930). However, consumption of greater portions of lean meat (45 percent of calories from protein) by one of the two explorers led rapidly to the development of weakness, nausea, and diarrhea, which was resolved when the dietary protein content was reduced to 20 to 25 percent of calories (McClellan and Du Bois, 1930).

If continued, a diet too high in protein results in death after several weeks, a condition known as "rabbit starvation" by early American explorers, as rabbit meat contains very little fat (Speth and Spielmann, 1983; Stefansson, 1944a). Similar symptoms of eating only lean meat were described by Lewis and Clark (McGilvery, 1983). Conversely, an all-meat diet with a protein content between 20 and 35 percent has been reported in explorers, trappers, and hunters during the winters in northern America surviving exclusively on pemmican for extended periods with no adverse effects (McGilvery, 1983; Speth, 1989; Stefansson, 1944b). Pemmican is a concentrated food made by taking lean dried meat that has been pounded finely and then blending it with melted fat. It contains about 20 to 35 percent protein; the remainder is fat (Stefansson, 1944b).

Nitrogen Balance Studies. Nitrogen balance studies at protein intakes of 212 to 300 g/d consistently have shown positive nitrogen balance (Fisher et al. 1967; Oddoye and Margen, 1979; Tarnopolsky et al., 1988), although this is usually attributed to the cumulative errors of the nitrogen balance procedure (Garlick et al., 1999; Hegsted, 1978; Oddoye and Margen, 1979). In particular, no negative nitrogen balances were reported, suggesting that the high protein intake had no detrimental effect on protein homeostasis.

Maximum Urea Synthesis. Rudman and coworkers (1973) studied the effect of meals containing graded levels of protein on the rate of urea production by human liver in vivo. With increasing protein content of the

meals, a maximum rate of urea synthesis of 65 mg of urea nitrogen/hour/kg body weight$^{0.75}$ was observed. At higher intakes, the rate was not increased further, but the maximum rate continued longer. In a 70-kg sedentary person, this maximum rate corresponds to about 250 g/d of protein, or about 40 percent of energy. The correspondence of this maximum to the apparent upper level of protein intake (45 percent of energy) described in the earlier section related to the experiences reported by explorers has therefore been suggested as cause and effect (Cordain et al., 2000). However, this interpretation should be made with caution, as there was no period of adaptation to the meal in the study of Rudman's group (1973). It is probable that when high protein diets are given, the capacities to oxidize amino acids and synthesize urea are increased, as has been demonstrated in animals (Das and Waterlow, 1974). However, this does not appear to have been investigated in humans.

Chronic Disease. High protein intakes have also been implicated in chronic diseases such as osteoporosis, renal stones, renal insufficiency, cancer, coronary artery disease, and obesity (see "High Protein Diets" in Chapter 11). However, the current state of the literature does not permit any recommendation of the upper level for protein to be made on the basis of chronic disease risk.

Dose–Response Assessment

The data on the potential for high protein diets to produce gastrointestinal effects, changes in nitrogen balance, maximum urea synthesis, or chronic disease are often conflicting and do not provide dose–response information or clear indications of a lowest-observed-adverse-effect level (LOAEL) or no-observed-adverse-effect level (NOAEL) for these endpoints. Thus, there are insufficient data to establish a Tolerable Upper Intake Level (UL) for total protein. Because of the current widespread use of protein supplements, more research is needed to assess the safety of high protein intakes from supplements; until such information is available, caution is warranted.

The potential implications of high dietary protein for bone and kidney stone metabolism are not sufficiently clear at present to make recommendations for the general population to restrict their protein intake. However, in those who have idiopathic hypercalciuria, the occurrence of kidney stones is much increased, and although there is no evidence to indicate reducing protein intake will decrease the risk of developing kidney stones, these individuals should not be encouraged to consume more protein than the Recommended Dietary Allowance (RDA).

Intake Assessment

Based on distribution data from the 1994–1996, 1998 Continuing Survey of Food Intakes by Individuals (CSFII), the highest mean intake of protein from diet for any gender and life stage group was estimated to be 104 g/d (Appendix Table E-16) for men aged 19 through 30 years of age. For the 70-kg reference man (Table 1-1), this would equate to 1.5 g/kg/d. This life stage group also had the highest reported protein intake at the 99th percentile of intake at 190 g/d, or 2.7 g/kg/d, for the reference 70 kg-man.

Risk Characterization

The risk of adverse effects resulting from excess intakes of protein from foods appears to be very low at the highest intake noted above. Based on distribution data from the 1994–1996, 1998 CSFII (Appendix Table E-17), these 19-30-year-old men would be consuming a mean of 15.2 percent of their energy from protein, and at the 99th percentile, 21.5 percent. Women over the age of 50 had the highest reported percentage of total energy from protein at the 99th percentile of 23.7 percent. Although a UL for protein could not be established, this does not mean that there is not a potential for adverse effects resulting from high protein intakes from food or supplements. Because the data on adverse effects resulting from high protein intakes are limited, caution may be warranted.

TOLERABLE UPPER INTAKE LEVELS FOR INDIVIDUAL AMINO ACIDS

In establishing tolerable upper intake levels (ULs) for amino acids several general points, common to all the amino acids, were noted.

- There is no evidence that amino acids derived from usual or even high intakes of protein from foodstuffs present any risk. Therefore, attention was focused on intakes of amino acids from dietary supplements and when utilized as food ingredients, such as monosodium glutamate in food or aspartic acid and phenylalanine in aspartame.
- This review was confined to those amino acids that are found in dietary protein and only the L-forms of amino acids were considered.
- Recognizing that the ULs are for chronic intake and in keeping with the UL model, only limited emphasis was placed on the results of acute and short-term toxicity studies, while longer-term studies were considered most appropriate for establishing ULs.

- More emphasis was placed on observations of adverse effects in humans than on effects observed in animals. Pharmacokinetic studies were sought to bridge potential differences between animals and humans.
- It was noted that blood concentrations could be considerably higher when amino acids were consumed as supplements as opposed to a component of protein in food, and this was considered in establishing ULs.
- Many animal studies of amino acid toxicity were conducted with diets deficient in protein. Less emphasis was placed on these studies than those with adequate protein diets because of concern over the creation of amino acid imbalances.
- For some well-studied amino acids, there were no adverse effects reported at the highest dose tested in long-term studies. In such cases it was not possible to establish a Lowest-Observed-Adverse-Effect Level (LOAEL) or a No-Observed-Adverse-Effect Level (NOAEL) that was supported by toxicity data. Under these circumstances, it was not possible to establish a UL in keeping with the criteria and procedures required by the UL model.

Alanine

L-Alanine is a dispensable amino acid with glycogenic properties. Studies of food intake, growth, and hematological changes resulting from the oral ingestion of L-alanine in animals and humans reveal little data to suggest a LOAEL or a NOAEL (LSRO, 1992). Based on intake distribution data from the 1988–1994 (NHANES III) mean daily intake for all life stage and gender groups of alanine from food and supplements is approximately 3.6 g/d (Appendix Table D-2). Men 51 through 70 years of age had the highest reported intake at the 99th percentile of 8.5 g/d.

Hazard Identification

Adverse Effects in Animals. In animals, L-alanine exhibits neural inhibitory actions as well as hypothermogenicity (Glyn and Lipton, 1981). There are no adequate data to characterize dose–response relationships for L-alanine in animals.

Adverse Effects in Humans. Oral administration of a single L-alanine dose, up to 50 g/d, increased plasma insulin levels (Genuth, 1973; Genuth and Castro, 1974; Rose et al., 1977). However, there are no chronic studies that can be utilized to establish a UL for supplemental L-alanine in humans.

Dose–Response Assessment

The very limited data on adverse effects of L-alanine intake from dietary supplements (Genuth, 1973; Genuth and Castro, 1974) were considered insufficient for a dose–response assessment and derivation of a UL for L-alanine.

Arginine

L-Arginine is incorporated into tissue proteins, and is required for the synthesis of other amino acids, polyamines, and creatine, as well as for the detoxification of ammonia via the urea cycle (Rodwell, 1990). It is a dispensable glycogenic amino acid, synthesized in adequate amounts from the urea cycle intermediate ornithine. Ornithine, in turn, can be synthesized from proline and possibly from glutamate (Brunton et al., 1999). However, in children with congenital defects of argininosuccinic acid synthetase or argininosuccinase, both urea cycle enzymes, arginine is an indispensable amino acid with daily supplementation required (Brusilow and Horwich, 1989). Based on intake distribution data from the 1988–1994 NHANES III, mean daily intakes for all life stage and gender groups of arginine from food and supplements is approximately 4.2 g/d (Appendix Table D-3). Men 51 through 70 years of age had the highest reported intake at the 99th percentile of 10.1 g/d.

Hazard Identification

Adverse Effects in Animals. Feeding low-protein diets supplemented with 4, 5, or 7.5 percent arginine resulted in depressed body weight gains in rats (Harper et al., 1966; Sauberlich, 1961). However, the growth suppression by excess arginine was lessened when the protein content of the diet was increased and when the quality of protein was improved (Harper et al., 1970).

Oral doses of L-arginine of 0.1, 0.5, and 1.0 g/kg of body weight were given to rats 1 hour before behavioral trials for a period of 5 or 7 days. Avoidance behavior was increased in CDR rats (a strain with poor learning capacity) at the highest dose only. Conditioned avoidance was not affected in Wistar rats, but increased locomotion was reported (Drago et al., 1984).

Studies on the effects of orally administered arginine on the immune system have provided conflicting results. Barbul and coworkers (1980) reported significant increases in thymus weights, thymic lymphocyte content, and in vitro activity of thymic lymphocytes after supplementing the diet of male mice with 0.5, 1, 2, and 3 percent arginine hydrochloride (one-half in the diet and one-half in drinking water) for 6 days. No dose–

response was found, with the maximum stimulation noted at 0.5 percent supplementation of the normal chow diet containing 1.8 percent arginine. Reynolds and coworkers (1990) reported significantly increased thymus weight, spleen cell mitogenesis, and inducible natural killer cell activity in mice after oral arginine (drinking water) doses of 60, 120, or 240 mg/kg of body weight/d. No dose–response was reported with maximum stimulation noted at 60 mg/kg of body weight/d. In young or aged rats, ingestion of diets supplemented with 3 percent L-arginine for 15 days did not result in increased thymus weights and little effect was reported on lymphocyte proliferation or interleukin-2 production as compared to controls (Ronnenberg et al., 1991).

Adverse Effects in Humans. Feeding 30 g of L-arginine hydrochloride/d for 7 days to 21 healthy human volunteers resulted in no changes in liver function, blood urea nitrogen (BUN), creatinine, or blood glucose (Barbul et al., 1981). The nausea and diarrhea reported by two and three adults, respectively, were ameliorated by altering the amount given at any time without decreasing the total daily intake. However, administration of 5 or 10 g of arginine as arginine aspartate for 80 days produced such dose-related reversible effects as increased weight, gastrointestinal disturbances, and somnolence (De Aloysio et al., 1982).

Thirty-six healthy volunteers were divided into 3 equal groups of 12 and orally administered 30 g of arginine hydrochloride (24.8 g of free arginine), 30 g of arginine aspartate (17 g free arginine), or a placebo daily for 14 days (Barbul et al., 1990). Dietary consumption of arginine was not controlled. Supplementation with arginine hydrochloride resulted in the development of mild hyperchloremic acidosis. Side effects of bloating, mild anorexia, and diarrhea were reported by one in the group receiving placebo, three in the group receiving arginine aspartate, and six in the group receiving arginine hydrochloride (Barbul et al., 1990). In another study of 30 elderly adults receiving 17 g of free arginine/d as arginine aspartate for 14 days, no adverse effects were observed (Hurson et al., 1995).

Park and coworkers (1992) administered orally 30 g of arginine free base/d to 10 patients with breast cancer during the three days immediately prior to surgery. A second group of ten cancer patients did not receive arginine supplementation prior to surgery and served as controls. The daily median rate of tumor protein synthesis in arginine-supplemented patients was slightly more than double that found in controls (25.6 percent/d, range 9 to 37 percent/d; 10 percent/d, range 5.5 to 15.8 percent/d, respectively). In addition, in patients receiving arginine supplementation there was a marked stimulation in the expression of the activation antigen Ki67 as measured histologically (~40 percent tumor cells staining with Ki67

compared to ~9 percent in controls). These data indicate that large oral doses of arginine may stimulate tumor growth in humans.

Studies in experimental animals have indicated a suppression of tumor growth after oral administration of arginine (Barbul, 1986; Reynolds et al., 1988; Tachibana, et al., 1985). Paradoxically, there are also published studies showing that arginine can stimulate tumor growth in animal models. Yeatman and coworkers (1991) showed that an arginine-enriched diet stimulated the growth of a murine colon tumor, whereas an arginine-depleted diet inhibited the tumor growth. Arginine was also shown to stimulate tumors in total parenteral nutrition-fed rats, while substitution of ornithine for arginine abolished the effect (Grossie et al., 1992). Moreover, Levy and coworkers (1954) showed that subcutaneous injections of arginine either inhibited or stimulated the tumor, depending on its size at the start of treatment. The mechanism of these effects is unknown, but might in part involve the immune system. Reynolds and coworkers (1988) observed an inhibition of tumor growth with tumors of high immunogenicity, but stimulation when a tumor of low immunogenicity was used, suggesting that inhibition might only occur when tumors can be recognized and killed by the immune system.

Batshaw and coworkers (1984) treated 17 hyperammonemic infants with 175 to 350 mg L-arginine/kg of body weight/d for 6 to 8 weeks. No adverse effects were reported. Plasma arginine concentrations were approximately twice those in the controls but less than one-third of the minimal concentration postulated to result in neurological effects in hyperargininemia. A follow-up at 18 months of age showed similar IQ scores in all groups. It should be mentioned that Brusilow and coworkers (1984) have used arginine supplements of 210 to 840 mg/kg of body weight/d for 5 years in the treatment of children with inborn errors of urea synthesis. No evidence of intellectual deterioration or visual effects was reported in these patients. In addition, there are several reports regarding patients treated intravenously with arginine hydrochloride for metabolic alkalosis or as a provocative test for growth hormone, where life-threatening hyperkalemia (Bushinsky and Gennari, 1978; Massara et al., 1981) or fatal hyponatremia (Gerard and Luisiri, 1997) were observed. These are acute toxicity reports and thus are not useful to evaluate chronic intakes.

Dose–Response Assessment

Studies of oral administration of supplemental arginine in humans (in excess of normal dietary intakes of approximately 5.4 g/100 g of mixed dietary proteins) were not designed to systematically study the toxicity of chronic oral exposure to this amino acid. They are generally of short dura-

tion, do not present dose–response data, and involve small numbers of individuals. Although data from these studies do not support the development of an LOAEL and thus a UL, they do give some indication of the effects from oral arginine intakes of up to 30 g/d. Oral intakes of arginine aspartate providing 5 and 10 g/d of free arginine for 80 days resulted in dose-related weight increases, digestive disturbances, and sleepiness (De Aloysio et al., 1982). Daily intakes of 20 to 30 g of arginine hydrochloride for 7 to 14 days resulted in gastrointestinal disturbances (Barbul et al., 1981, 1990). Such effects were considered mild and responded to lowering the oral dose at various times during the day without affecting the total daily intake.

Although the data appear to indicate minimal effects from arginine supplementation at intakes up to 24.8 g/d of free arginine base, the unconfirmed finding that 30 g/d of arginine for 3 days resulted in a stimulation of tumor growth in breast cancer patients (Park et al., 1992) indicates that dietary supplementation with arginine is not advisable other than in at-risk children with congenital defects of argininosuccinic acid synthetase or argininosuccinase. Therefore, since neither a NOAEL nor LOAEL can be identified for intake of L-arginine from dietary supplements in healthy individuals, a UL could not be determined.

Asparagine

L-Asparagine is a dispensable amino acid, the amide of the dicarboxylic amino acid aspartic acid that is either deaminated during food processing or converted into aspartate by the mucosal cells. Daily human intakes of L-asparagine from dietary protein are about 7.4 g/100 g of dietary protein (LSRO, 1992).

Hazard Identification

There are no data available regarding the toxicity of L-asparagine as a single amino acid supplement, which are relevant for setting an UL.

Dose–Response Assessment

There are no data to characterize a dose–response assessment for supplemental asparagine. However, asparagine is rapidly converted to aspartic acid in the gastrointestinal tract, and the potential adverse health effects from asparagine intake should be considered when developing the UL for aspartic acid.

Aspartic Acid

L-Aspartic acid is a dispensable dicarboxylic amino acid that can be produced by the transamination of oxaloacetic acid arising from glucose breakdown. In the presence of α-ketoglutarate, aspartate is converted to oxaloacetate and glutamate. Based on distribution data from the 1988–1994 NHANES III, mean daily intakes for all life stage and gender groups of aspartic acid from food and supplements are 6.5 g/d (Appendix Table D-4). Men 31 through 50 years of age had the highest intake at the 99th percentile of 15.4 g/d.

Hazard Identification

Adverse Effects in Animals. Neonatal mice (24-hours postpartum) received four subcutaneous injections of L-aspartic acid at 2 g/kg of body weight and were followed for 7 months (Schainker and Olney, 1974). When compared to controls, there was an increase in hypothalmic lesions, obesity, skeletal stunting, and reduced reproductive organ size. Neither blood nor brain concentrations of aspartic acid were measured. Using a similar protocol, Pizzi and coworkers (1978) replicated these findings in mice given gradually increasing doses of monosodium L-aspartic acid (2.2 to 4.4 g/kg of body weight) by subcutaneous injection on days 2 to 11 of life. Animals were followed for 150 days for growth and reproductive behavior and sacrificed between 200 and 300 days of age. Females had reduced litter sizes and fewer pregnancies, and males had reduced fertility. At 190 and 195 days of age, behavioral tests were carried out on the male mice and significant reductions in activity and exploratory behavior were observed in treated animals.

Finkelstein and coworkers (1988) have proposed that some of the adverse effects reported may be the result of insufficient carbohydrate in the diet of mice receiving large acute doses of aspartic acid. When neonatal mice were orally administered 750 mg aspartate/kg of body weight, the characteristic hypothalmic lesions were observed. However, when mice were treated simultaneously by gavage with aspartate and 1 g of Polycose®/kg of body weight, no lesions were found. At a dose of 1 g of aspartate/kg of body weight administered with carbohydrate, there was a reduction of more than 60 percent in the lesions observed compared to the animals treated with aspartate only. Prior injection of insulin (at pharmacological doses) 4 hours before aspartate treatment (750 mg/kg of body weight) reduced, but did not eliminate, the numbers of animals with lesions from 12/12 to 6/10 and decreased the maximum number of necrotic neurons per brain section. This paper reported a threshold dose for a single oral

administration of aspartate producing neurotoxicity in infant mice at 650 mg/kg of body weight (Finkelstein et al., 1988).

Finkelstein and coworkers (1983) also conducted an oral exposure study with L-aspartic acid in slightly older infant mice (8 days old). Aspartic acid was administered by oral gavage at a single dose of 0, 250, 500, 650, 750, or 1,000 mg/kg of body weight. Brain regions were assessed at 5 hours after exposure. No hypothalamic neuronal necrosis was observed in animals treated with a single dose of aspartic acid up to and including 500 mg/kg of body weight. Increasing numbers of animals with hypothalamic lesions and severity of lesions (as assessed by numbers of necrotic neurons per brain section) were observed with increasing doses. In contrast, Reynolds and coworkers (1980) gave infant monkeys a single dose of 2 g/kg of body weight of aspartame by gastric tube and found no hypothalamic damage.

None of the above studies on the effects of aspartic acid on hypothalamic structure and function include data on food consumption of the treated animals and the observations of adverse effects have been made in rodents only. The only study in nonhuman primates found no change in the hypothalamus of infant monkeys given an acute dose of aspartame (Reynolds et al., 1980).

Adverse Effects in Humans. Carlson and coworkers (1989) measured the effects of a 10-g bolus dose of L-aspartic acid on pituitary hormone secretion in healthy male and female adults. Aspartic acid had no consistent effect on any hormone measured.

The potassium magnesium salt of aspartic acid (KMA) has been used as a supplement in exercise regimens (Ahlborg et al., 1968; de Haan et al., 1985; Maughan and Sadler, 1983; Sen Gupta and Srivastava, 1973). Acute oral doses in these studies ranged from approximately 75 to 130 mg of KMA/kg of body weight. While no adverse effects were reported, it was not clear from the reports what adverse effects were examined, and plasma aspartic acid concentrations were not reported.

Since the artificial sweetener aspartame contains about 40 percent aspartic acid, studies on the effects of oral administration of this dipeptide provide useful information on the safety of aspartic acid. Twelve normal adults were orally given 34 mg/kg of body weight of aspartame and the equimolar amount of aspartic acid (13 mg/kg of body weight) in a crossover design (Stegink et al., 1977). No increase in plasma or erythrocyte aspartate was found during the 24 hours after dosing. Plasma phenylalanine levels doubled over fasting concentrations 45 to 60 minutes after dosing with aspartame but returned to baseline after 4 hours. Plasma concentrations of other amino acids remained unchanged.

Frey (1976) studied the effects of the oral administration of aspartame to 126 children and adolescents (30 to 77 mg of aspartame/kg body weight/d,

equal to 12 to 30 mg of aspartate/kg body weight/d) for 13 weeks in a double-blind study. Each child received a physical examination and special eye examinations before and after the study. In addition, tests for liver and renal function, hematological status, and plasma levels of phenylalanine and tyrosine were conducted. The results of all tests were within normal limits. Using a similar study design and a dose of 36 mg aspartame/kg body weight/d (14 mg aspartate/kg/d) given orally to young adults (mean age 19.3 years), Knopp and coworkers (1976) reported no meaningful effects on plasma triglycerides and cholesterol nor on tests measuring hematological parameters, and liver and renal function.

Dose–Response Assessment

All human studies on the effects of aspartic acid involve acute exposures (Ahlborg et al., 1968; Carlson et al., 1989; de Haan et al., 1985; Maughan and Sadler, 1983; Sen Gupta and Srivastava, 1973). There are some subchronic studies on the oral administration of aspartame to humans (Frey, 1976; Stegink et al., 1977); however, in both studies no dose–response data are available. Although some studies in experimental animals were designed to obtain dose–response data, the effects measured were usually found in all doses studied. Therefore, even if the protocol had used dosing regimens appropriate for the development of a UL, no NOAEL was identified.

The most serious endpoint identified in animal studies was the development of neuronal necrosis in the hypothalamus of newborn rodents after dosing with aspartic acid a few days postpartum. This is a property of dicarboxylic amino acids, since glutamic acid dosing in this animal model results in similar necrotic effects (Stegink, 1976; Stegink et al., 1974). There is still some uncertainty over the relevance to humans of the newborn rodent model for assessing the neuronal necrosis potential of aspartic acid. Neuronal necrosis in the hypothalamus was not found in newborn nonhuman primates with levels of plasma dicarboxylic amino acids 10 times those found in newborn mice with neuronal necrosis (Stegink, 1976; Stegink et al., 1974). In addition, human studies where high doses of aspartic acid or aspartame were given failed to find a significant increase in the plasma level of aspartic acid.

In view of the ongoing scientific debate regarding the sensitivity of newborn animals to the consumption of supplemental dicarboxylic amino acids, it is concluded that aspartic acid dietary supplements are not advisable for infants and pregnant women. Although the scientific data are not sufficient to develop a UL for aspartic acid, it should be noted that dietary supplement doses of up to 8 g/d (approximately 120 mg/kg body weight/d) have not resulted in any documented adverse effects.

Branched-Chain Amino Acids (Leucine, Isoleucine, Valine)

The branched-chain amino acids (BCAA)—leucine, isoleucine, and valine—differ from most other indispensable amino acids in that the enzymes initially responsible for their catabolism are found primarily in extrahepatic tissues. Each undergoes reversible transamination, catalyzed by a branched-chain aminotransferase (BCAT), and yields α-ketoisocaproate (KIC, from leucine), α-keto-β-methylvalerate (KMV, from isoleucine), and α-ketoisovalerate (KIV, from valine). Each of these ketoacids then undergoes an irreversible, oxidative decarboxylation, catalyzed by a branched-chain ketoacid dehydrogenase (BCKAD). The latter is a multienzyme system located in mitochondrial membranes (Danner et al., 1979). The products of these oxidation reactions undergo further transformations to yield acetyl CoA, propionyl CoA, acetoacetate, and succinyl CoA; the BCAA are thus keto- and glucogenic.

Based on distribution data from the 1988–1994 NHANES III, mean daily intakes for all life stage and gender groups of leucine (Appendix Table D-10), isoleucine (Appendix Table D-9) and valine (Appendix Table D-19) from food and supplements are 6.1, 3.6, and 4.0 g/d, respectively. Men 51 through 70 years of age had the highest intakes at the 99th percentile for leucine at 14.1 g/d, isoleucine at 8.2 g/d, and valine at 9.1 g/d.

Hazard Identification

Blood and tissue concentrations of BCAA are altered by several disease and abnormal physiological states, including diabetes mellitus, liver dysfunction, starvation, protein–calorie malnutrition, alcoholism, and obesity. These and other conditions sometimes produce drastic alterations in plasma pools of BCAA (Amen and Yoshimura, 1981). Markedly elevated concentrations of BCAA and branched-chain α-keto acids are associated with maple-syrup urine disease; the latter is caused by an inborn error of metabolism in which BCKAD is low or absent (Hutson and Harper, 1981). BCAA imbalances appear not to cause these various diseases and physiological abnormalities, but rather result from them. Numerous investigations of interrelationships of BCAA in patients having one or more of these conditions have been undertaken; their usefulness for assessing risks to healthy populations is in most cases questionable (LSRO, 1992).

One other set of interactions, in this case concerning BCAA and other amino acids, may be of significance in assessing human risks associated with supplementation. Thus, it has been well established that the BCAA compete with other large neutral amino acids (LNAA, particularly tryptophan and tyrosine) for membrane transport (Anderson and Johnston, 1983). Although the BCAA do not act as direct precursors for neurotransmitters,

they can affect transport of certain LNAA across the blood–brain barrier, and thereby influence central nervous system concentrations of certain neurotransmitters. Fernstrom and coworkers (1973) demonstrated, for example, that brain tryptophan levels in rats decrease as the ratio of plasma tryptophan to other LNAA, including the BCAA, declines.

Influences on and Consequences of Metabolism. BCAA-transaminase (BCAAT) exists in at least three different subtypes, and its tissue and cellular distribution varies across species. Differences between rats and humans in this regard raise the possibility that, to the extent that adverse biological effects of the BCAA are dependent upon metabolism, rodent data may not be completely predictive of human responses (Harper et al., 1984). BCKA-dehydrogenase (BCKAD) appears to display similar interspecies variability. It should be noted, however, that in most of the animal studies reported below, it is not entirely clear that these various enzyme activities are critical determinants of the effects seen. Thus, while the animal data must be interpreted with caution, there is no well-established basis for disregarding them entirely.

Among the BCAA, leucine appears to exhibit the highest degree of metabolic activity, although this conclusion may arise at least partially because it has been the subject of more study than isoleucine and valine. Leucine may affect muscle protein turnover (Elia and Livesey, 1983) and stimulate insulin release and tissue sensitivity (Frexes-Steed et al., 1990) as well as somatostatin, glycogen, and zinc release (Danner and Elsas, 1989). It is unclear whether any of these effects have adverse health consequences.

Adverse Effects in Humans. BCAA-enriched protein or amino acid mixtures and, in some cases, BCAA alone, have been used in the treatment of a variety of metabolic disorders. These amino acids have received considerable attention in efforts to reduce brain uptake of aromatic amino acids and to raise low circulating levels of BCAA in patients with chronic liver disease and encephalopathy (LSRO, 1992; Marchesini et al., 1990; Skeie et al., 1990). They have also been used in parenteral nutrition of patients with sepsis and other abnormalities. Although no adverse effects have been reported in these studies, it is not clear that such effects have been carefully monitored (Skeie et al., 1990). Additionally, the data from these studies, because they involved patients with significant and sometimes unusual disease states, are not directly relevant to the problem of assessing risks to normal, healthy humans.

Most studies of the effects of BCAA supplementation involving healthy individuals have been directed at their potential for improving physical or mental performance. It has been hypothesized that BCAA supplementation may reduce muscle catabolism associated with exercise, fasting, or

metabolic stress (Hood and Terjung, 1990), and may reduce fatigue associated with increased central nervous system concentrations of serotonin (Newsholme et al., 1991). The first hypothesis is based on the fact that, in humans, the highest concentrations of BCAAT, the catalyst for BCAA oxidation, are found in muscle; the second hypotheses relates to the fact that high circulatory levels of BCAA interfere with the transport of tryptophan, a serotonin precursor, across the blood–brain barrier. Of the individual BCAA, leucine has received the most study, because of its relatively greater rate of oxidation and because it is associated with the rapid release of glucogenic precursors from muscle.

There have been several reports of clinical trials in which groups of healthy humans, in most cases trained athletes, were given high doses of leucine by intravenous infusion (Abumrad et al., 1982; Elia and Livesey, 1983; Eriksson et al., 1983; Hagenfeldt et al., 1980; Tarnopolsky et al., 1991). Most of the studies involved a single dose of the amino acid. These trials measured physical and mental performance, the impact on blood levels of other amino acids, and in one case, of insulin and glucose output. Although some evidence of reduced muscle catabolism and clear evidence of an impact on blood concentrations of other amino acids (most especially, declines in the other BCAA and several other neutral amino acids) can be found in these reports, none of these provides evidence of an adverse effect of leucine. In fact, in one study glutamine output from forearm muscle was significantly increased (Abumrad et al., 1982). It should be noted, however, that possible side effects in all studies were those that might have been recognized subjectively. No potential functional changes were investigated in any of these studies. Thus, although this collection of studies provides no evidence of adverse effects of high doses of leucine, they are of highly limited value in assessing health risks.

Maple Syrup Urine Disease (MSUD). The most common disorder associated with genetic anomalies in BCAA metabolism is Maple Syrup Urine Disease (MSUD), a condition brought about by lack of adequate function in BCKAD. The disorder, which can be diagnosed in the neonatal period, is characterized by very high plasma levels of BCAA, especially leucine. There are six other forms of the condition that have onsets later in life; these different forms are associated with different abnormal subtypes of BCKAD. MSUD is associated with mental retardation and even early death and is treated by dietary control of BCAA. Other less common metabolic disorders are associated with genetic anomalies in specific enzymes involved in BCAA metabolism (LSRO, 1992; Sweetman, 1989). Information on these disorders provides no data helpful to assessing risks in normal populations; the affected populations require medical management involving severe restriction of BCAA consumption.

Adverse Effects in Animals. There have been a relatively large number of studies in rats of high levels of BCAA administration and, in some cases, of individual BCAA (particularly leucine). The largest share of these investigations followed the observation that the BCAA compete with other LNAA (tryptophan, tyrosine) for the blood–brain carrier system (Ashley and Anderson, 1975; Fernstrom et al., 1973). Of particular interest has been the effect of BCAA-induced changes in LNAA/BCAA ratios, and the effects of LNAA and neurotransmitter brain concentrations on food intake and body weight.

Peters and Harper (1987) demonstrated that protein intake was, however, not affected by BCAA-induced changes in neurotransmitter concentrations. In another study, BCAA dosing lowered plasma and brain concentrations of all indispensable amino acids, but there appeared to be no consistent association of these alterations with protein selection (Anderson et al., 1990). Indeed, given a choice, rats adjusted their dietary intakes in response to supplementation with BCAA.

It appears, however, that the creation of imbalances among the BCAA (e.g., by dosing with high levels of any one of them) may sometimes induce reductions in appetite and growth (Block, 1989; Harper et al., 1984). However, these imbalances, which lead to catabolism of muscle, occur only in rats on marginally adequate protein diets (Block, 1989). Thus, for example, Harper et al. (1984) demonstrated that high dietary levels of leucine suppressed the growth of rats fed a low protein diet, and that the growth suppression could be prevented by supplementation with isoleucine and valine. There have been a number of attempts to study BCAA antagonisms in various tissues, and it appears that muscle is the major contributor to the depletion of isoleucine and valine pools in animals consuming high leucine diets. It is not at all clear that induced BCAA imbalances (except possibly in the case of animals on marginally adequate protein diets) have any adverse effects on growth.

The consequences of reduced brain concentrations of neurotransmitters observed in these animal studies that may be associated with high level BCAA supplementation are not entirely clear, nor is their relevance to humans certain, given the known interspecies differences in the activities and tissue distribution of BCAAT and BCKAD.

Kawabe and coworkers (1996) reported on a subchronic feeding study in which L-isoleucine was administered to groups of 10 rats at dietary concentrations of 0, 1.25, 2.5, 5.0, or 8.0 percent for 13 weeks. The amino acid caused no changes in body weights, food consumption, or hematological parameters. At the highest dietary level, increased urine volumes and relative kidney weights and urine pH, together with some alterations in serum electrolytes, were clearly related to treatment. Minimal changes were observed at the 5.0 percent dietary level, although no histopathological

alterations were observed in any organs of either gender. No alterations of any type were observed at the 2.5 percent dietary level (corresponding to about 1,800 mg/kg/d).

There is evidence that isoleucine acts as a promoter of urinary bladder carcinogenesis in rats (Kakizoe et al., 1983; Nishio et al., 1986). Thus, Kakizoe and coworkers (1983) exposed 6-week-old rats to low doses of N-butyl-N (4-hydroxybutyl) nitrosamine (BHBN), a known initiator of cancer of the urinary bladder, and supplemented their diets with isoleucine or leucine. After 40 weeks, the incidence of papillomas was significantly elevated in rats receiving isoleucine plus BHBN over that observed in the group receiving BHBN alone. In a follow-up study of similar design, Nishio and coworkers (1986) extended the experimental period to 60 weeks and included diets supplemented with 2 or 4 percent isoleucine or leucine. In this case, both dose levels of both amino acids significantly increased bladder carcinoma incidence over groups receiving BHBN alone or groups receiving amino acids alone (see Table 10-28). It thus appears that both leucine and isoleucine are potent promoters of bladder neoplasms in rats at dietary levels of 2 percent and above; a no-effect level was not identified in either of the above studies. There is no evidence that either amino acid is carcinogenic in the absence of an initiating agent.

Developmental Studies. Persaud (1969) reported that leucine is a teratogen when it is administered by intraperitoneal injection in pregnant female rats at doses as low as 15 mg/kg of body weight. The author suggested that the effects, which were multiple and serious, may have resulted from amino acid imbalances that adversely affected protein synthesis dur-

TABLE 10-28 Incidences of Bladder Carcinomas in Rats After 60 Weeks

	Dietary Levels[a]		
Added Substance	0%	2%	4%
Isoleucine or leucine	0	0	0
BHBN	0		
BHBN and isoleucine		46	77
BHBN and leucine		52	74

[a] Dietary level refers to level of amino acid addition. N-butyl-N (4-hydroxybutyl) nitrosamine (BHNB) was administered at a dose below that known to induce bladder tumors. No papillomas or preneoplastic lesions were observed in the control groups or in the amino acid groups.

ing embryonic development. No attempt has been made to determine whether orally administered BCAA have any such effect.

Matsueda and Niiyama (1982) reported on the effects of dietary supplementation with the individual BCAA on maintenance and outcome of pregnancy in rats. Pregnant rats were fed a low protein (6 percent casein) diet supplemented with 5 percent leucine, isoleucine, or valine. Four control groups were administered the 6 percent casein diet; it was stated (without documentation) that the four control groups were given the 6 percent diets in amounts matching those of pair-fed BCAA groups.

Only 11 out of 20 possible pregnancies were maintained in rats administered leucine and isoleucine (2/10 for the leucine groups and 9/10 for the isoleucine groups). No consistent effects on food intake and maternal body weight gain were observed, except for an increase in both in valine-supplemented dams. All fetal weights in the BCAA groups were less than those in ad libitum controls, and fetal weights in the isoleucine and valine groups (but not the leucine groups) were less than those in pair-fed controls; this same pattern was observed when fetal brain weights were analyzed. In all three BCAA-fed groups, brain concentrations of BCAA, histidine, and arginine were greatly increased relative to ad libitum controls, but no such effects were seen for glutamate or phenylalanine.

This study suggests that BCAA when administered to pregnant rats at high doses (dietary levels of 5 percent, corresponding roughly to a daily dose of 2,000 mg/kg) may reduce fetal body weight and relative brain weights and cause sharp increases in brain concentrations of certain amino acids.

Thoemke and Huether (1984) bred rats for three generations on diets enriched with BCAA at 10 g/kg for each amino acid. They also concurrently studied the effects of tryptophan, tyrosine, and phenylalanine supplementation. Feeding of the supplemented diets commenced in both genders two weeks before mating, and continued through three generations (F1, F2, F3). BCAA caused, as expected, decreases in serum levels of tryptophan and tyrosine in F3 dams, and increases in serum glycine. There were, however, no such differences observed in dams of the F1 and F2 generations and no changes in BCAA levels were observed in any generation. In the F1 generation, diets supplemented with BCAA caused significant decreases in brain weights at days 5 and 10 postpartum, but weights were, in all cases, normal by day 20. In the F2 and F3 generations, however, pup brain weights were reduced at day 5 and did not recover by day 20. The concentrations of neurotransmitters were decreased in the brain in all three generations, with the most significant decrease seen for aspartate; no functional measurements were made to assess the possible effects of these declines in neurotransmitter concentrations.

It is thus clear that alterations in brain chemistry, most especially declines in neurotransmitter concentrations and reductions in brain weight, can be seen in offspring of rats fed supplemental BCAA at 30 g/kg diet (10 g/kg for each amino acid). Assuming that female rats weigh an average of 200 g during gestation and consume about 15 g food/d, then the 30 g/kg level of BCAA corresponds to about 450 mg/d, or a daily dose of 2,250 mg/kg (about 750 mg/kg for each amino acid). This study involved only a single level of supplementation, so a "no-effect" level was not identified.

Summary. There are no reports of adverse health effects associated with normal diets containing BCAA, nor have such effects been reported in healthy persons receiving single, infused supplemental BCAA doses as high as 9.75 g. The several studies in which such large supplemental doses were given are highly limited as a basis for reaching conclusions about safety because most involved only a single dose, and none involved an attempt to assess any functional changes. In some human studies, especially those involving high doses of leucine, metabolic alterations were observed, typically expressed as declines in blood levels of LNAA, including neurotransmitter precursors. In one study, insulin sensitivity was increased by BCAA supplementation.

The effects of BCAA on plasma and whole blood concentrations of amino acids have been convincingly and repeatedly observed under a variety of conditions in experimental animal studies. BCAA compete among themselves and with other LNAA, and these competitive interactions may affect growth and appetite (although significant only in animals on diets marginally adequate in protein). Changes in brain concentrations of neurotransmitters precursors (tryptophan and tyrosine) have also been demonstrated at various levels of supplementation.

Developmental studies in rats also reveal the effect of BCAA supplementation on fetal brain concentrations of neurotransmitters in successive generations of animals. Fetal brain weights are also reduced across generations. Decreases in viable pregnancies have been seen in rats administered supplemental leucine and isoleucine.

Leucine and isoleucine have both been shown to promote bladder carcinogenesis in a two-stage rat model. Neither has been demonstrated to be carcinogenically active in the absence of an initiating agent. A recent 13-week study in rats involving isoleucine provided no evidence that this amino acid could induce pre-neoplastic lesions in the urinary bladder, but did reveal that isoleucine could increase urine volume and pH and relative kidney weights at very high dietary levels. Such effects are generally species specific.

Dose–Response Assessment

There are no adequate dose–response data from human or animal studies upon which to base a UL for BCAA. Tumor promotion data from rat studies cannot be used reliably to assess human risk. It is not at all clear that such two-stage models, involving an initiating agent, are relevant to expected conditions of human exposure (Williams and Whysner, 1996).

Cysteine

L-Cysteine, a dispensable amino acid, is formed metabolically from L-methionine and L-serine. It is interconvertible to cystine, and for purposes of this report, L-cysteine and L-cystine are considered together. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of cysteine from food and supplements is 1.0 g/d (Appendix Table D-5). Men 51 through 70 years of age had the highest intakes at the 99th percentile of 2.2 g/d.

Hazard Identification

Acute Adverse Effects in Animals. L-Cysteine is mutagenic in bacteria (Glatt, 1989), but not in mammalian cells (Glatt, 1990). L-Cysteine has been identified as a neuro excitotoxin due to its interaction with N-methyl-D-aspartate (NDMA) receptors (Olney, 1994). Administration to perinatal mice or rats that have an immature blood–brain barrier produces neurotoxicity. Swiss Webster albino mice, 10 to 12 days old, were given a single oral dose of 3 g/kg of body weight of L-cysteine (Olney and Ho, 1970). At 5 hours after treatment, necrosis of hypothalamic neurons was found, as well as retinal lesions.

In male Wistar rats injected intraperitoneally with 1.0 mmol/kg of body weight of cysteine, blood levels of cysteine peaked at about 2 mM at 30 minutes (Calabrese et al., 1997). At 1 hour, exposure produced elevated brain levels of malondialdehyde in the substantia nigra. Subcutaneous injection of 4-day-old Sprague-Dawley rats with L-cysteine 0.5 g/kg of body weight produced no subsequent effect on neurotransmitter or neuropeptide systems in the striatum at 35 days of age (Sivam and Chermak, 1992).

In addition to the report of Olney and Ho (1970) on retinal lesions in mice, subcutaneous injection of 9- to 10-day-old Wistar rats with L-cysteine at 1.2 mg/g body weight produced permanent dystrophy of the inner layers of the retina (Karlsen and Pedersen, 1982).

Acute administration of L-cysteine to rats at a dose of 1.9 g/kg was reported to produce ultrastructural alterations of testicular Sertoli cells and spermatids (Bernacchi et al., 1993).

Acute Adverse Effects in Humans. Single oral doses of 5 and 10 g of L-cysteine have produced nausea and light-headedness in normal humans (Carlson et al., 1989). Reports of chronic administration of L-cysteine to humans were not found.

Dose–Response Assessment

The data on adverse effects of L-cysteine and L-cystine intake from supplements were not considered sufficient for a dose–response assessment and derivation of a UL.

Glutamic Acid, Including Its Sodium Salt

Dietary glutamate is almost totally extracted by the gut and is metabolized rapidly by transamination to α-ketoglutarate, and hence to other intermediary metabolites, notably alanine. The glutamate that escapes capture by the gut is largely taken up by the liver. Glutamate is also synthesized endogenously as a product of transamination of other amino acids during the catabolism of arginine, proline, and histidine, and by the action of glutaminase on glutamine. Its importance in metabolism is that it is a dispensable amino acid that plays a role in the shuttle of nitrogen from amino acid catabolism to urea synthesis through its transamination reamination reactions, and behaves as a neurotransmitter in the brain.

Based on distribution data from the 1988–1994 NHANES III, mean daily intakes for all life stage and gender groups of glutamic acid from food and supplements are approximately 15 g/d (Appendix Table D-6). Men 31 through 50 years of age had the highest intakes at the 99th percentile of 33.7 g/d.

Hazard Identification

Most of the body's free glutamate pool is concentrated in the tissues, especially brain (homogenate, 10 mmol/L; synaptic vesicles, 100 mmol/L) (Meldrum, 2000). By contrast, the concentration of glutamate in the blood is low, typically about 50 μmol/L in the fasting state (Stegink et al., 1982a, 1983a, 1983b). During absorption of a high-protein meal (1g protein/kg/d), there is about a twofold rise in the concentration of glutamic acid in the systemic plasma (Stegink et al., 1982a), returning to baseline 8 hours after the meal. Addition of monosodium glutamate (34 mg/kg) to the meal,

which increased the total glutamate intake by 16 percent, did not result in any further increase in glutamate concentration. However, a larger dose of glutamate, 150 mg/kg/d, which increased the total intake by 69 percent, resulted in a larger increase in glutamate level than the meal alone (by about 50 percent) (Stegink et al., 1983b). Both the peak level achieved and the time course of rise in glutamate level have been shown to be highly dependent on the way in which the glutamate is ingested. A single drink of glutamate (150 mg/kg) in water resulted in a large and rapid rise in the plasma level, peaking at about 12 times the basal level at 45 minutes, and falling quickly thereafter (Stegink et al., 1983a). By contrast, a meal consisting of a liquid formula substantially inhibited the rise in glutamate level (Stegink et al., 1983a).

Adverse Effects in Animals. The adverse effects of glutamic acid and its salts have been reviewed in great detail by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 1988) and the American Institute of Nutrition of the Federation of American Societies for Experimental Biology (FASEB) (Raiten et al., 1995). The acute toxicity has been evaluated in several animal species, with LD_{50} values for the oral route of administration ranging from 16,200 to 19,200 mg/kg of body weight in mice, 10,000 to 19,900 mg/kg of body weight in rats, and greater than 2,300 mg/kg of body weight in rabbits (JECFA, 1988), indicating a low level of acute toxicity. Subchronic studies in mice showed an increase in body fat and female sterility in animals that had been subcutaneously injected with glutamate (2.2 to 4.2 g/kg/d) from day 1 to day 10 of life (Olney, 1969). Mice given subcutaneous injections of glutamate (3 g/kg) at 2 days of age were also found to have higher body weights (Olney, 1969). In similar studies on rats given up to 2.0 g/kg/d of glutamate, no effects on body weight, growth, and the volume and weights of several organs were detected (Hara et al., 1962). Other studies showed no effects of glutamate on learning or recovery from electroconvulsive shock (Porter and Griffin, 1950; Stellar and McElroy, 1948).

Longer-term investigations of the effects of glutamate in animals have revealed few adverse effects. In two studies on mice given glutamate (1 or 4 percent of the diet) for 2 years, no increase in the incidence of malignant tumors was shown, and in other respects the animals were normal (Ebert, 1979b; JECFA, 1988). Similar negative results were reported from chronic studies (2 year) in rats given diets containing 0, 0.1, and 0.4 percent glutamate (JECFA, 1988) and in rats given diets containing 0, 1, 2, and 4 percent glutamate (Owen et al., 1978a). In addition, rats given diets with 0.1 or 0.4 percent glutamate showed no adverse effects on fertility and survival of the young (Ebert, 1979a). Moreover, no adverse effects on body weight gain, food consumption, behavior, electrocardiogram, ophthal-

mology, hematology, blood chemistry, organ weights, kidney function, or mortality were observed in dogs given diets with 0, 2.5, 5, or 10 percent glutamate (Owen et al., 1978b).

Adverse Effects in Humans. In humans there is a direct relationship between serum glutamate level and nausea and vomiting with concentrations above 1 mmol/L resulting in vomiting in 50 percent of the individuals (Levey et al., 1949). Glutamate has been used for treatment of a variety of medical conditions. For example, arginine glutamate has been given to treat ammonia intoxication, at a dose of 50 g every 8 hours, but no more than 25 g over 1 to 2 hours in order to avoid vomiting (Martindale, 1967). Chronic glutamate treatment of children with approximately 0.3 g/d of glutamic acid for 6 months (Zimmerman and Burgemeister, 1959) and adults with 45 g/d for 10 weeks (Himwich et al., 1954) showed no adverse effects.

Despite the generally low level of toxicity of glutamic acid demonstrated in the studies on animals and humans, there has remained concern over its continued use as a flavor-enhancing agent. This has been fueled by the discovery that high doses of glutamate can under certain circumstances be neurotoxic (Olney, 1969), and by the reported occurrence of distressing symptoms after the consumption of Asian foods, generally known as Chinese restaurant syndrome. As glutamate is an excitatory neurotransmitter, its potential for neurotoxicity has been studied extensively. In 1957 it was shown that injection of glutamate into suckling mice resulted in degeneration of the inner neural layers of the retina (Lucas and Newhouse, 1957). Later work showed that neuronal destruction also occurred in several regions of the brain in mice after glutamate was parenterally administered (Olney, 1969). Neurons are destroyed by excessive activation by glutamate of excitatory receptors located on the dendrosomal surfaces of neurons (Olney, 1989). The most sensitive areas of the brain are those that are relatively unprotected by the blood–brain barrier, notably the arcuate nucleus of the hypothalamus.

In a detailed analysis of the literature on the neurotoxic effects of glutamate in several species, JECFA (1988) concluded that parenteral administration of glutamate results in reproducible lesions in the central nervous system. However, lesions have never been observed in animals taking glutamate with food, although lesions were noted when the glutamate was given as a large dose by gavage. The neonatal mouse is the most sensitive, the sensitivity declining in weanlings through adults. Moreover, the sensitivity is lower in rats, hamsters, guinea pigs, and rabbits, and effects have rarely been detected in nonhuman primates. In addition, there have been a number of reports of behavioral abnormalities in mice and rats given large doses of glutamate in the early neonatal period (Berry et

al., 1974; Iwata et al., 1979; Nikoletseas, 1977; Olivo et al., 1986; Pinto-Scognamiglio et al., 1972; Poon and Cameron, 1978; Pradhan and Lynch, 1972). There are also reports of reproductive abnormalities in animals given glutamate as neonates (Lamperti and Blaha, 1976, 1980; Matsuzawa et al., 1979; Pizzi et al., 1977). However, a number of other studies have shown no effect on reproduction (Anantharaman, 1979; Prosky and O'Dell, 1972; Yonetani et al., 1979), and one study reported an enhancement of fertility (Semprini et al., 1971).

No signs of neurological damage have been reported in humans. For example, in adult males given a chemically defined diet in which glutamate was the only source of dispensable nitrogen for periods of 14 to 42 days, no changes in neurologic or hepatic function were detected (Bazzano et al., 1970). However, concern was raised by a report that a large dose of glutamate taken orally stimulated the secretion of prolactin and cortisol (Carlson et al., 1989). Earlier findings that rats injected with 1 g/kg of glutamate showed stimulation in the secretion of luteinizing hormone and testosterone (Olney et al., 1976) were interpreted as indicating that the high concentration of glutamate had penetrated the neuroendocrine parts of the hypothalamus. Similarly, it was shown that the same dose of glutamate stimulated release of prolactin and inhibited the release of growth hormone (Terry et al., 1981). The data of Carlson and coworkers (1989) might therefore be interpreted to imply that the elevated concentration of glutamate was penetrating the hypothalamus in humans, and that neuroendocrine disturbances might be a potential consequence. However, a more recent and more strictly controlled study, employing 12.7 g of monosodium glutamate (160 mg/kg of body weight), failed to show significant changes in prolactin and cortisol or of luteinizing hormone, follicle stimulating hormone, growth hormone, or thyroid stimulating hormone (Fernstrom et al., 1996).

Chinese Restaurant Syndrome. Despite the failure to show any neurological damage in humans resulting from glutamate ingestion, there are many reports of symptoms associated with Chinese Restaurant Syndrome, also called MSG (monosodium glutamate) Symptom Complex (Raiten et al., 1995) and Idiosyncratic Intolerance. These symptoms, which have frequently been reported anecdotally after eating Asian food, have been described as a burning sensation at the back of the neck, forearms, and chest; facial pressure or tightness; chest pain; headache; nausea; upper body tingling and weakness; palpitation; numbness in the back of the neck, arms, and back; and drowsiness. After initial reports of this complaint, the symptoms were attributed to the high concentration of MSG in Asian food (Ambos et al., 1968; Schaumburg and Byck, 1968).

Studies indicated that some of those who reported being susceptible were sensitive to less than 3 g, and that all but one of those studied suffered some symptom at sufficiently high doses (Schaumburg et al., 1969). Later work suggested that as many as 25 to 30 percent of the population might be susceptible (Kenney and Tidball, 1972; Reif-Lehrer, 1976). However, a more recent assessment, using a randomized double-blind crossover design in which the characteristic taste of MSG had been carefully disguised, failed to detect any greater incidence of adverse symptoms after consuming glutamate at a meal (1.5 or 3 g) compared with the placebo (Tarasoff and Kelly, 1993). In fact, a significant negative correlation was found between MSG dose and adverse symptoms. In another study, six adults who believed themselves to be sensitive to MSG were challenged with MSG (6 g) or placebo in a strongly flavored drink to mask the MSG in a double-blind study (Kenney, 1986). Four of the six did not react to either MSG or the placebo, whereas the other two reacted to both. Similarly, 24 individuals, 18 of whom believed themselves to be subject to flushing symptoms after eating Chinese food, were challenged with MSG (3 to 18.5 g), but no cases of flushing occurred (Wilkin, 1986).

Thus in 1988, JECFA concluded that properly conducted and controlled clinical trials had failed to establish a relationship between Chinese Restaurant Syndrome and the ingestion of MSG. Subsequently, the FASEB report (Raiten et al., 1995) concluded that there was no scientifically verifiable evidence of adverse effects in most individuals exposed to high levels of MSG.

FASEB (Raiten et al., 1995) also acknowledged that there was sufficient evidence for the existence of a small subgroup of healthy people that were sensitive to MSG, and that they showed symptoms when exposed to an oral dose of 3 g in the absence of food. A recent double-blind, placebo-controlled study on a self-selected group of individuals who believed themselves to be sensitive to MSG has shown that many have the specific symptoms under experimental conditions that they had previously identified as representing their sensitivity to MSG (Yang et al., 1997). They also identified a dose of 2.5 g as the threshold for the induction of symptoms. A more recent study of similar design confirmed these findings, and also reported that responses did not occur when MSG was given with food (Geha et al., 2000). It was also noted that neither persistent nor serious effects from MSG were observed.

Asthma. Triggering of asthma was another, and potentially more serious, symptom of the MSG Symptom Complex listed by FASEB (Raiten et al., 1995). A recent review by Stevenson (2000) analyzed six studies on asthmatic patients, and has pointed out a number of deficiencies. Two studies indicated that single-blind administration of MSG (1.5 to 2.5 g) was

associated with bronchospasm in 14 of 32 (Allen et al., 1987) and 2 of 30 asthmatics (Moneret-Vautrin, 1987). However, the subsequent four studies, employing double-blind approaches, showed no incidence of bronchospasm after MSG ingestion in a total of 45 asthmatic patients (Germano et al., 1991; Schwartzstein et al., 1987; Woessner et al., 1999; Woods et al., 1998). Clearly there is a need for further study in this area to clarify the inconsistencies, but overall they show no convincing evidence that MSG precipitates asthma attacks.

It has also been suggested that MSG exacerbates urticaria. In a single systematic study of patients with chronic idiopathic urticaria, involving single- and double-blind, placebo-controlled challenges, two patients had positive single-blind, but neither had positive double-blind challenges, suggesting that only a very small proportion of the patients, if any, were sensitive to MSG (Simon, 2000).

Dose–Response Assessment

Despite the large number of studies of glutamate toxicity in animals and humans, there appear to be very few adverse effects of L-glutamate consumption that have significance for humans. The possible involvement of glutamate in the MSG Symptom Complex is not yet established and is of little concern, as there is no evidence that it has any impact on overall health. Although there is no convincing evidence that MSG precipitates asthma attacks, this is an area that needs further study. There is continuing controversy about the potential neurotoxicity of glutamate, but data in this area are conflicting and not sufficient for a dose–response assessment. Thus, a UL for L-glutamate from supplements cannot be established at the present time.

Glutamine

L-Glutamine, a dispensable amino acid, taken orally, is metabolized primarily in the splanchnic tissues. After absorption it is extensively metabolized to citrulline, arginine, glutamate, and proline (Reeds and Burrin, 2001). Extensive metabolism also occurs in lymphocytes, kidney, and liver. However, glutamine is simultaneously being synthesized in many tissues, especially muscle, intestine, brain, and liver (LSRO, 1992). The endogenous rate of production by the adult whole body has been estimated to be 60 to 100 g/d (van Acker et al., 1999). The two enzymes primarily responsible for glutamine metabolism are glutaminase, which converts glutamine to glutamate and ammonia, and glutamine synthetase, which synthesizes glutamine from glutamate and ammonia. Because high concentrations of either glutamic acid or ammonia are known to be

neurotoxic, hyperammonemia and hyperglutamic-acidemia are important potential hazards of glutamine consumption.

Hazard Identification

Ziegler and coworkers (1990) performed several individual studies to examine glutamine safety under different circumstances. In the first study, six volunteers were given a single oral dose of glutamine at three different doses (0, 0.1, and 0.3 g/kg of body weight) and monitored for 4 hours. A second study in nine volunteers was performed to investigate the effects of intravenous infusion of glutamine at three doses (0, 0.0125, and 0.025 g/kg body weight/hour) for 4 hours. A third study in seven volunteers was designed to investigate the effects of glutamine-supplemented total parenteral nutrition (TPN) (0, 0.285, and 0.570 g/kg body weight/d) over 5 days. A pharmacokinetic study over 4 hours was also performed in three volunteers. After single oral doses, plasma glutamine concentrations rose in proportion to the dose given, by approximately twofold after 1 hour for the higher dose, and returned to basal within 4 hours. During infusions of glutamine in volunteers, with or without TPN, the plasma glutamine concentration remained elevated by about 30 percent, and no significant changes in plasma glutamate or ammonia were seen. In the two studies of glutamine-supplemented TPN, when serial assessments of mental status were made, there was no evidence of neurotoxicity. Overall, there were no indications of adverse effects at any dose when glutamine was given by either the oral or intravenous route.

Hornsby-Lewis and coworkers (1994) examined the effects of glutamine supplementation in seven patients for up to 4 weeks while receiving TPN plus glutamine at a single dose of 0.285 g/kg body weight/d. There was no significant increase in plasma glutamine concentration, and no other adverse effects were observed, but the authors noted their concern regarding elevations in liver enzymes.

In a randomized, double-blind, controlled study, normal TPN in 60 patients was compared with isonitrogenous TPN including alanyl-glutamine (0.5 g/kg body weight/d, equivalent to 0.34 g/kg/d of glutamine) in 60 patients for 6 days (Jiang et al., 1999). After 6 days the plasma glutamine was increased by 8 percent in the treated group compared with a decrease of 15 percent in the controls. No indications of adverse effects were apparent. Morlion and coworkers (1998) described the results of 28 elective surgery patients given TPN containing alanyl-glutamine (0.3 g/kg/d) or an isonitrogenous control. Plasma glutamine was modestly increased and nitrogen balances were improved compared with the control group. In addition, no adverse effects were observed.

Lacey and coworkers (1996) carried out a randomized, double-blind study of glutamine-supplemented parenteral nutrition (20 percent of amino acids) in 44 preterm neonates for 15 days. On the basis of plasma ammonia and glutamate levels and the absence of clinical signs of neurotoxicity, it was concluded that glutamine at this dose is safe in preterm infants. Also, Roig and coworkers (1996) reported no increases in the concentrations of glutamine, glutamate, and ammonia in very low birthweight infants given enteral supplements of glutamine (0.3 g/kg/d).

It is notable that despite the substantial number of published investigations in which glutamine has been administered to humans, very few, if any adverse effects have been reported. However, the published studies of toxicity have not fully taken account of a number of important factors, including the chronic consumption of glutamine. Glutamine is an important fuel utilized by most rapidly growing tumors (Kovacevic and Morris, 1972), which may deplete the body's ability to provide glutamine (Chen et al., 1991, 1993; Klimberg and McClellan, 1996). Moreover, tumor cells are dependent on a supply of glutamine for growth (Colquhoun and Newsholme, 1997), and the growth rates correlate with the activity of glutaminase (Knox et al., 1969; Linder-Horowitz et al., 1969). Therefore, although providing supplemental glutamine might restore the body glutamine pool, it is also important to examine the possibility that glutamine supplements may promote cancer. However, the evidence points to the contrary, and in vivo studies have not confirmed this suspicion (Klimberg and McClellan, 1996; Souba, 1993). Oral administration of glutamine did not enhance tumor growth in rats in vivo (Klimberg et al., 1990), and may even depress tumor growth (Fahr et al., 1994; Klimberg and McClellan, 1996).

Dose–Response Assessment

The only reported adverse effect of glutamine was an increase in liver enzymes in patients on TPN supplemented with glutamine (0.285 g/kg body weight/d, corresponding to about 20 g/d), which resolved after cessation of treatment (Hornsby-Lewis et al., 1994). However, in other studies, doses up to 0.57 g/kg/d have been given without any adverse effect being reported. Thus, the data on L-glutamine from supplements are conflicting and are not sufficient for a dose–response assessment and derivation of a UL.

Glycine

Glycine is a dispensable amino acid with glycogenic properties. It is the only amino acid that does not have an asymmetric carbon atom, and its metabolism is linked to that of L-serine. Based on distribution data

from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of glycine from food and supplements is 3.2 g/d (Appendix Table D-7). Men 19 through 30 years of age had the highest intakes at the 99th percentile of 7.8 g/d.

Hazard Identification

Adverse Effects in Animals. Growth depression in rats and chicks has been reported after feeding diets containing as much as l0 percent glycine (Harper et al., 1970). Nitrosated glycine can be genotoxic in vitro (Gaspar et al., 1996). It is not, however, mutagenic using a modified Ames test (Hoorn, 1989).

Adverse Effects in Humans. Surgical irrigation solutions of glycine containing 1.5 or 2.2 percent glycine reportedly cause some transient adverse effects (e.g., nausea, diarrhea, and visual disturbances) in patients after transurethral resection of the prostate (Creel et al., 1987; Hahn, 1988; Mizutani et al., 1990; Wang et al., 1989). In patients with schizophrenia, oral doses of approximately 60 g/d of glycine for several weeks failed to reveal adverse effects (Leiderman et al., 1996). There have been no chronic dose–response studies with L-glycine in healthy humans.

Dose–Response Assessment

The data on adverse effects of glycine intake from supplements were considered not sufficient for a dose–response assessment and derivation of a UL.

Histidine

Although histidine is generally regarded as an indispensable amino acid (FAO/WHO/UNU, 1985), removal of histidine from the diet, unlike the eight classical indispensable amino acids, does not induce negative nitrogen balance in the first 10 days (Rose et al., 1951). Further, men fed amino acid-based diets containing 10 g of nitrogen/d devoid of histidine remained in nitrogen balance for up to 2.4 months (Rose, 1957). There were similar reports in women (Reynolds et al., 1958) and children (Nakagawa et al., 1963). Conversely, it has been observed that nitrogen balance becomes gradually negative over a longer period of time and nitrogen balance rapidly became positive upon the reintroduction of histidine (Kopple and Swendseid, 1975).

Histidine is an important component of hemoglobin (8 percent), with the bulk being in the globin portion. The rate of erythropoiesis decreases

and hemoglobin falls in adults on a histidine-free diet that is reversed when histidine is restored (Kopple and Swendseid, 1975). In addition, the dipeptide carnosine, found in skeletal muscle, is a large store of histidine and serve as a source of histidine (Christman, 1971). Because of these large body pools of histidine it takes a prolonged period (more than 60 days) to deplete an adult of histidine. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of histidine from food and supplements is 2.2 g/d (Appendix Table D-8). Men 51 through 70 years of age had the highest intakes at the 99th percentile of 5.2 g/d.

Hazard Identification

Adverse Effects in Animals. Histidine given acutely by intraperitoneal injection or intravenously has been shown to result in changes in the concentration of brain amino acids (Oishi et al., 1989) and histamine (Schwartz et al., 1972). Young rats (4 to 5 weeks old) treated with an inhibitor of histidinase exhibited reduced locomotor activity after an intraperitoneal injection of histidine (250 mg/kg of body weight) (Dutra-Filho et al., 1989). Pilc and coworkers (1982) reported "bizarre behavior" in rats dosed intraperitoneally with histidine (400 to 800 mg/kg of body weight). These effects have not been examined in rats fed L-histidine and are of minimal use in deriving a UL for the chronic exposure of humans to oral L-histidine.

Feeding low-protein diets supplemented with L-histidine for 3 to 4 weeks resulted in significant body weight losses after only several days in rats. However, the effects became less as increasing levels of high-quality protein were added to the diet (Benevenga and Steele, 1984).

Short-term feeding studies (7 to 46 days) in rats have shown growth retardation, hepatomegaly, and hypercholesterolemia at L-histidine levels of approximately 2 to 4 g/kg body weight/d (Harvey et al., 1981; Hitomi-Ohmura et al., 1992; Ohmura et al., 1986; Solomon and Geison, 1978). Harvey and coworkers (1981) reported significantly reduced concentrations of copper and zinc in the plasma and reduced liver concentrations of copper after feeding diets containing 8 percent L-histidine (~4 g/kg body weight/d) for 46 days. Hypercholesterolemia was eliminated by the simultaneous feeding of an L-histidine- and copper-supplemented diet, supporting the hypothesis that the histidine-induced hypercholesterolemia was a result of changes in copper status. Feeding mice 1.3 g L-histidine/kg body weight/d for 21 days resulted in an increase in the absorption and utiliza-

tion of zinc with higher concentrations of zinc in liver, muscle, spleen, and pancreas (van Wouwe et al., 1989).

The long-term toxicity and carcinogenicity of L-histidine monohydrochloride (HMHC) was studied in 50 male and 50 female rats (Ikezaki et al., 1996). Male rats were fed diets containing 0.47 and 0.96 g/kg body weight/d of HMHC for 104 weeks; female rats were fed 0.56 and 1.1 g/kg body weight/d for the same period. No significant treatment-related increases in any tumors were reported when compared to matched controls. No neoplastic changes were reported in controls or treatment groups. In male rats fed 0.96 g of HMHC/kg body weight/d, increases in red blood cell counts, hemoglobin concentrations, and hematocrit were reported. No evidence of sperm granulomas were observed in male rats fed either 1.6 g of HMHC/kg body weight/d for 13 weeks or 0.97 g/kg body weight/d for 104 weeks (Ikezaki et al., 1994, 1996).

Adverse Effects in Humans. Pinals and coworkers (1977) treated 30 rheumatoid arthritis patients and 30 controls daily with capsules containing 4.5 g of L-histidine for 30 weeks in a double-blind trial followed by 19 patients receiving this dosage for 10 additional months in a period of open treatment. It is not clear which adverse effects were examined; however, the authors concluded that no adverse effects of the histidine therapy were noted. In a similar double-blind treatment design, Blumenkrantz and coworkers (1975) treated 42 patients (16 chronic uremic and 26 undergoing maintenance dialysis) with oral doses of 4 g/d of L-histidine for 17.5 weeks. No adverse effects were reported; however, it was not evident from the report which adverse effects were examined.

Studies on the effects of L-histidine on taste and smell acuity in humans have produced conflicting results. Henkin and coworkers (1975) reported decreased taste and smell acuity in six patients given 8 to 65 g of histidine/d for up to 24 days. In view of the increased urinary excretion of zinc and a decreased concentration of serum zinc, the authors postulated that the effects of histidine administration were due to a zinc-deficient state. In a study of eight healthy men given 4 g/d of histidine for 2 weeks, no effects on smell or taste acuity were reported (Schechter and Prakash, 1979). Similarly, Geliebter and coworkers (1981) failed to find any effect of L-histidine on taste and smell after oral dosing of L-histidine between 24 and 64 g/d for 4 weeks. Even at the lower dose (4 g/d), adverse effects such as headaches, weakness, drowsiness, and nausea were reported, while at the highest doses (24 and 64 g/d) anorexia, painful sensations in the eyes, and changes in visual acuity were reported in two females.

Zlotkin (1989) reported an approximate 70 percent increase in urinary zinc excretion in infants on TPN when the fluid contained 165 mg of

histidine/kg body weight/d compared to 95 mg/kg body weight/d in controls. Although the study examined parental administration, it provides further evidence that excess histidine intake in humans can lead to histidine/zinc interactions that might lead to a zinc-deficient state.

Dose–Response Assessment

In experimental animals, the only dose–response study on the chronic oral administration of L-histidine was that of Ikezaki and coworkers (1996). However, this study utilized only two doses, neither of which demonstrated any adverse effects. In addition, no data were reported on the possible effect of the doses on zinc or copper metabolism, an effect reported in both humans and experimental animals.

None of the studies in humans on the effects of L-histidine were designed for developing a UL—they were designed to study the efficacy of utilizing L-histidine as a therapeutic agent in certain disease states. They provide only minimal support for the evaluation of a UL for histidine for apparently healthy individuals. The chronic study on the effects of orally administered histidine in rodents was not considered appropriate for the development of a UL.

There is evidence in humans that doses of L-histidine between 4 and 4.5 g/d over the amounts found in the diet do not result in adverse effects. However, this evidence should be considered tentative given the few individuals studied and lack of dose–response information. There is evidence from studies in experimental animals and humans that intakes of high levels of histidine can alter copper and zinc metabolism. However, the lack of dose–response data precludes identifying the intake concentrations in humans required to elicit such responses.

In conclusion, the available scientific data are not adequate to derive a UL for the chronic oral intake of L-histidine from supplements.

Lysine

L-Lysine, a dibasic amino acid, is indispensable in humans. Lysine, as well as threonine, does not participate in transamination reactions. Carnitine is required for the transport of long-chain fatty acids and is synthesized from lysine and methionine in the liver and kidney (Mayes, 1990). Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of lysine from food and supplements is 5.3 g/d (Appendix Table D-11). Men 51 through 70 years of age had the highest intakes at the 99th percentile of 12.6 g/d.

Hazard Identification

Acute intake of high levels of lysine interferes with dietary protein metabolism and competes with the transport of arginine, suggesting that adverse effects from high levels of lysine are more likely to occur if protein intake or dietary arginine intake is low. Intravenous L-lysine (16.5 to 41.3 g/d in young men) has been shown to inhibit renal tubular protein reabsorption (Mogensen and Solling, 1977). L-Lysine shares an intestinal transport system with L-arginine (McCarthy et al., 1964; Rosenberg et al., 1966), and competes with L-arginine for reabsorption from renal tubules (Kamin and Handler, 1951; Webber et al., 1961). In addition, increased liver total lipids, triacylglycerol, and cholesterol concentrations were seen in rats fed 5 percent L-lysine and 15 percent casein for 2 weeks (Hevia et al., 1980a), an effect that can be reversed by feeding arginine (Hevia et al., 1980b).

Acute Adverse Effects in Animals. Administration of lysine to pregnant rats does not appear to result in gross morphological changes, but higher fetal mortality and decreases in maternal and fetal body and brain weights have been found (Cohlan and Stone, 1961; Funk et al., 1991, Matsueda and Niiyama, 1982).

Adverse Effects in Humans. Studies of lysine tolerance of human infants have not found adverse effects. In one study, six infants (4 to 11 months of age) were given 60 to 1,080 mg of lysine monohydrochloride per 8 ounces of milk in a series of seven incremental doses for 3 to 4 days at each dose. No behavioral effects were observed, nor was there anorexia, diarrhea, or other signs of gastrointestinal upset, and no evidence of cystinuria (Dubow et al., 1958). Similarly, no adverse effects were reported when 1- to 5-month-old infants were given up to 220 mg/kg body weight of lysine for 15 days (Snyderman et al., 1959b).

Higher plasma and urinary concentrations of carnitine were found in six healthy adult males given a single 5-g oral dose of lysine (Vijayasarathy et al., 1987). In another study of eight healthy males (15 to 20 years of age) given a single oral dose of 1.2 g of L-lysine hydrochloride, growth hormone release was not significantly stimulated and no side effects were reported (Isidori et al., 1981).

Several clinical trials of lysine intakes from 0.6 to 3.0 g/d for 3 to 6 months in people with herpes infections have, in general, not found or reported any adverse effects (DiGiovanna and Blank, 1984, 1985; Griffith et al., 1978, 1987; McCune et al., 1984; Milman et al., 1980; Simon et al., 1985; Thein and Hurt, 1984). The one adverse effect was an upset stomach in 3 of 27 patients given 3 g/d of L-lysine hydrochloride for 6 months and in 1 of the 25 controls (Griffith et al., 1987).

A limitation of these clinical studies is that they were done in humans with a disease. Also, the longest study was only 6 months. Finally, only a limited number of endpoints were investigated. McCune and coworkers (1984) reported no effects on plasma sodium, potassium, and chloride in 41 patients treated for 24 weeks with 1,248 mg/d of L-lysine monohydrochloride.

Dose–Response Assessment

As mentioned above, very few adverse effects of L-lysine have been observed in humans or animals after high, mostly acute, doses. Thus, the data on the adverse effects of L-lysine from supplements were considered not sufficient for a dose–response assessment and derivation of a UL for apparently healthy humans.

Methionine

L-Methionine is an indispensable amino acid with glycogenic properties. In animal studies, it has been described as one of the more toxic amino acids (Health and Welfare Canada, 1990). Humans, as well as other mammals, cannot fix inorganic sulfur into organic molecules and must rely on ingested sulfur amino acids, such as methionine, for the synthesis of protein and biologically active sulfur. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of methionine from food and supplements is 1.8 g/d (Appendix Table D-12). Men 51 through 70 years of age had the highest intakes at the 99th percentile of 4.1 g/d.

Hazard Identification

Adverse Effects in Animals. Dietary excesses of L-methionine (2.7 percent of the diet) for 6, 13, or 20 days have been associated with erythrocyte engorgement and accumulation of hemosiderine in rats (Benevenga et al., 1976), and there was a depression of growth and splenic damage. A single dietary dose (2.7 percent of the diet) of L-methionine decreased body growth and also reduced food intake in rats (Steele et al., 1979).

Dietary intakes of 2 to 4 percent of L-methionine caused slight changes in liver cells in rats (Stekol and Szaran, 1962) and slight decreases in liver iron content (Klavins et al., 1963). Darkened spleens caused by increases in iron deposition have been observed in weanling rats fed 1.8 percent methionine diets for 28 days (Celander and George, 1963).

Viau and Leathem (1973) fed pregnant rats 4 percent of their diet as methionine and reported subnormal fetal and placental weights. However, supplemental methionine prevented neural tube defects in rat embryos treated with teratogenic antivisceral yolk sac serum (Fawcett et al., 2000). In the mouse, the administration of methionine reduced experimentally induced spina bifida (Ehlers et al., 1994). Other studies in rodent and primate models support the beneficial effect of methionine supplementation in improving pregnancy outcomes (Chambers et al., 1995; Chatot et al., 1984; Coelho and Klein, 1990; Ferrari et al., 1994; Moephuli et al., 1997).

Adverse Effects in Humans. Single oral doses of about 0.6 g (adults) and 0.08 g (infants) led to increased plasma levels of L-methionine and L-alanine, and decreased plasma concentrations of leucine, isoleucine, valine, tyrosine, tryptophan, and phenylalanine (Stegink et al., 1980, 1982b). Neither report included mention of any adverse effects. Methionine supplements (5 g/d) for periods of weeks were reportedly innocuous in humans (Health and Welfare Canada, 1990). A single oral dose of 7 g has been associated with increased plasma concentrations of methionine and the presence of mixed sulfides (Brattstrom et al., 1984). Single oral doses of 7 g produced lethargy in six individuals and oral administration of 10.5 g of L-methionine to one produced nausea and vomiting (Perry et al., 1965). After an oral administration of 8 g/d of methionine (isomer not specified) for 4 days, serum folate concentrations were decreased in five otherwise healthy adults (Connor et al., 1978).

High doses of methionine (~100 mg/kg of body weight) led to elevated plasma methionine and homocysteine concentrations (Brattstrom et al., 1984, 1990; Clarke et al., 1991; Wilcken et al., 1983). Thus, it was concluded that elevated plasma homocysteine concentrations may be a risk factor for coronary disease (Clarke et al., 1991).

Infants more rapidly metabolized methionine than adults (Stegink et al., 1982b). In women whose average daily intake of methionine was above the lowest quartile of intake (greater than 1.34 g/d), a 30 to 40 percent reduction in neural tube defect-affected pregnancies was observed (Shaw et al., 1997). These reductions were observed for both anencephaly and spina bifida.

Dose–Response Assessment

There are no adequate data to characterize a dose–response relationship for L-methionine. Thus the data on the adverse effects of L-methionine from supplements were considered not sufficient for a dose–response assessment and derivation of a UL for apparently healthy humans.

Phenylalanine

L-Phenylalanine is an indispensable amino acid that has both glycogenic and ketogenic properties. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of phenylalanine from food and supplements is 3.4 g/d (Appendix Table D-13). Men 31 through 50 years of age had the highest intakes at the 99th percentile of 7.7 g/d. About 16 percent of the ingested L-phenylalanine is converted to tyrosine in humans (Clarke and Bier, 1982). Unlike most other amino acids, excessive ingestion of L-phenylalanine can be complicated by the coexistence of genetic disorders.

Hazard Identification

Adverse Effects in Animals. Because of major species differences in phenylalanine metabolism between humans and rodents (Clarke and Bier, 1982; Moldawer et al., 1983), studies in which high doses of L-phenylalanine were fed to rodents could not be utilized in developing a UL for L-phenylalanine. There is one study indicating that high concentrations of L-phenylalanine (3 g/kg body weight/d) fed to monkeys from a few days after birth until 2 or 3 years of age can produce irreversible brain damage (Waisman and Harlow, 1965). However, this study does not provide any dose–response data to utilize in determining a UL.

Adverse Effects in Humans. Data are not available on the effects of chronic ingestion of supplemental phenylalanine by apparently healthy adults. Adverse effects were not evident following acute single oral doses of L-phenylalanine as high as l0 g in 13 adult men (Ryan-Harshman et al., 1987).

Most of the literature on the consumption of large doses of L–phenylalanine consists of studies on the effects of large doses of the artificial sweetener aspartame, which is 50 percent by weight phenylalanine. In adults given oral doses of aspartame ranging from 4 to 200 mg/kg of body weight (2 to 100 mg/kg of body weight L-phenylalanine), dose-related increases in plasma phenylalanine were observed (Filer and Stegink, 1988). Ingestion of single doses up to 60 mg/kg of body weight aspartame (30 mg/kg of body weight L-phenylalanine) by normal weight adults had no effect on behavior or cognitive performance (Lieberman et al., 1988; Stokes et al., 1991).

Dose–Response Assessment

The data on the adverse effects of L-phenylalanine intake from supplements were not available for a dose–response assessment and derivation of a UL in apparently healthy humans.

Special Considerations

Phenylketonuria (PKU) is a genetic disorder that impairs phenylalanine hydroxylase (PAH) activity. Impaired PAH activity allows phenylalanine or its catabolic byproducts to accumulate above normal levels in the plasma during critical periods of brain development. Persistently elevated levels of L-phenylalanine in the plasma before and during infancy and childhood can result in irreversible brain damage, growth retardation, and dermatologic abnormalities if dietary phenylalanine is not restricted within 1 month of birth and continued at least through childhood and adolescence (Scriver et al., 1989). Restriction of phenylalanine intake throughout life in PKU patients is necessary to keep plasma phenylalanine levels low and to promote normal growth and brain development (Scriver et al., 1989). If PKU is detected early and treated effectively through strict metabolic control, infants can live a normal life-span (Hellekson, 2001). In the United States, approximately 1 of every 15,000 infants is born with PKU (Hellekson, 2001).

Maternal hyperphenylalaninemia due to deficient phenylalanine hydroxylation is a recognized human teratogen (Lenke and Levy, 1980). Because phenylalanine is actively transported across the placenta (Kudo and Boyd, 1990), a pregnant woman with PKU exposes her developing fetus to potentially harmful levels of phenylalanine. High maternal plasma phenylalanine levels are associated with high incidence of mental retardation, microcephaly, intrauterine growth delay, and congenital heart malformations in the fetus (Scriver et al., 1989). The fetal demand for phenylalanine for protein synthesis is exceeded by the placental supply of L-phenylalanine by only a small amount, suggesting that the safety margin of placental transfer may be small (Chien et al., 1993). Careful maintenance of plasma phenylalanine levels in the mother through dietary control, before conception and throughout her pregnancy, may prevent the teratogenic effects of phenylalanine.

Proline

L-Proline is a dispensable amino acid that can be formed from and converted to glutamic acid. It is incorporated into tissue proteins and can then be hydroxylated to form hydroxproline. Both proline and hydroxy-

proline are found in large quantities in collagen. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of proline from food and supplements is 5.2 g/d (Appendix Table D-14). Boys 14 through 18 years of age had the highest intakes at the 99th percentile of 12.0 g/d.

Hazard Identification

Adverse Effects in Animals. There are minimal data on the adverse effects of L-proline in either experimental animals or humans. Female Sprague Dawley rats given L-proline in drinking water for 1 month (mean dose 50 mg/kg body weight/d) did not exhibit any adverse effects (Kampel et al., 1990).

Genetically hyperprolinemic mice have 6 to 7 times the concentration of proline in the brain as control animals and 10 times the concentration of proline in plasma (Baxter et al., 1985). Hyperprolinemic hybrid mice took longer than control mice to make an initial avoidance response to foot shock in a T-maze and required more trials before learning of the avoidance response (Baxter et al., 1985). No other studies in experimental animals relevant to the evaluation of the toxicity of orally administered L-proline or hydroxyproline could be found.

Adverse Effects in Humans. The only study in humans on the effects of long-term oral administration of proline was a clinical study on the efficacy of proline (isomer not specified) to alter the progression of gyrate atrophy of the choriod and retina (Hayasaka et al., 1985). Four patients (aged 4 to 32 years) were treated with doses of proline between 2 and 10 g/d (mode = 3 g/d) for up to 5 years. No overt adverse effects were reported; however, it was uncertain from the paper which effects were studied.

Dose–Response Assessment

The data on adverse effects of L-proline intake from supplements were not available for a dose–response assessment and derivation of a UL in apparently healthy individuals.

Serine

Serine is a dispensable amino acid that is synthesized endogenously from D-3 phosphoglycerate or glycine. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of serine from food and supplements is 3.5 g/d (Appendix

Table D-15). Men 31 through 50 years of age had the highest intakes at the 99th percentile of 7.9 g/d.

Hazard Identification

Adverse Effects in Animals. There are limited data pertaining to the toxicity of supplemental serine. In rats given 100 mg/d of L-serine via stomach tube for 14 days, there was a decrease in food consumption but no other effects were noted (Artom et al., 1945). Other authors (Morehead et al., 1945; Wachstein, 1947) have reported that supplemental L-serine at levels as low as 10 mg/d resulted in decreased appetite, increased mortality, and renal necrosis in rats.

Adverse Effects in Humans. In four healthy adults given a single oral dose of 15 g of serine, no adverse effects were reported (Pepplinkhuizen et al., 1980). There are no studies in humans that would permit an evaluation of the possible adverse effects of repeated administration, thus the safety of repeated dose oral administration of supplemental serine cannot be assessed.

Dose–Response Assessment

The data on the adverse effects of L-serine intake from supplements were not available for a dose–response assessment and derivation of a UL in apparently healthy humans.

Threonine

L-Threonine is a large neutral amino acid that is indispensable. Similar to L-lysine, L-threonine does not take part in transamination reactions. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of threonine from food and supplements is 3.0 g/d (Appendix Table D-16). Men 51 through 70 years of age had the highest intakes at the 99th percentile of 7.1 g/d.

Hazard Identification

Adverse Effects in Animals. In rats fed 5 percent threonine added to a 10 percent casein diet, weight gain was reduced compared to controls fed casein alone but there were no changes in liver weight or hepatic DNA, RNA, or protein content (Muramatsu et al., 1971). The evidence indicates

that excess threonine is converted to carbohydrate, liver lipids, and carbon dioxide (Yamashita and Ashida, 1971). In weanling pigs, adding 0.5, 1, 2, or 4 percent L-threonine to a 20 percent crude protein diet did not change weight gain, food intake, and gain:feed ratios in comparison to the controls (Edmonds and Baker, 1987; Edmonds et al., 1987).

Adverse Effects in Humans. No data were found on apparently healthy humans given oral L-threonoine supplements. However, L-threonine has been used clinically with the aim of increasing glycine concentrations in the cerebral spinal fluid of patients with spasticity. When given in amounts of 4.5 to 6.0 g/d for 14 days, no adverse clinical effects were noted in such patients (Growdon et al., 1991). Threonine also has been studied in low birth weight infants. In a study of 163 low birth weight infants, threonine serum concentrations were directly related to the threonine concentrations of the formula (Rigo and Senterre, 1980). The authors suggested that threonine intakes should not exceed about 140 mg/kg body weight/d for premature infants.

Dose–Response Assessment

The data on the adverse effects of L-threonine intake from supplements were not available for a dose–response assessment and derivation of a UL in apparently healthy humans.

Tryptophan

L-Tryptophan, an indispensable amino acid, serves as a precursor for several small molecules of functional significance including the vitamin niacin, the neurotransmitter serotonin, the metabolite tryptamine, and the pineal hormone melatonin. Increases in tryptophan have been shown to increase synthesis of the neurotransmitters in brain, blood, and other body organs (Fregly et al., 1989; Leathwood and Fernstrom, 1990; Young, 1986). Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of tryptophan from food and supplements is 0.9 g/d (Appendix Table D-17). Men 51 through 70 years of age had the highest intakes at the 99th percentile of 2.1 g/d.

Hazard Identification

Adverse Effects in Animals. Several rodent studies have demonstrated that supplementation of low-protein diets with L-tryptophan (5 percent) reduces food intake and weight gain over a 4-day to 4-week period

(reviewed by Benevenga and Steele, 1984; Harper et al., 1970). Funk and coworkers (1991) found that rats given a 20 percent casein diet supplemented with 14.3 percent tryptophan for 4 weeks developed scaly tails and thinning hair. However, no adverse effects were seen when the diets contained 1.4 or 2.9 percent L-tryptophan. No cancers were observed over an 80-week period when rats were fed diets containing 2 percent added L-tryptophan (Birt et al., 1987). Addition of 2.5 or 5 percent L-tryptophan to diets of rats and mice for 2 years resulted in decreased body weights of male and female mice and male (but not female) rats (DHEW, 1978). In pigs, supplementation with 0.1 or 1 percent L-tryptophan for up to 40 days did not affect weight gain, but 2 or 4 percent decreased weight gain and 4 percent also decreased food intake (Chung et al., 1991).

Several developmental studies have shown that maternal weight gain is impaired and fetal weight is reduced when maternal rat diets are supplemented with 1.4 to 6 percent L-tryptophan (Funk et al., 1991; Matsueda and Niiyama, 1982). Decreased brain weights were observed when 1 percent L-tryptophan was added to diets of male and female rats beginning 2 weeks before mating (Thoemke and Huether, 1984). Over three successive generations, brain weights decreased with each generation.

Adverse Effects in Humans. Serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in human blood and brain cerebrospinal fluid (CSF) increase after tryptophan loading, which is similar to the effects of L-tryptophan in animals. For example, Young and Gauthier (1981) found elevations in blood and 5-HIAA and CSF serotonin after single doses of 3 or 6 g of L-tryptophan. However, Benedict and coworkers (1983) conducted a double-blind, placebo-controlled trial in six normal men fed 3 g/d of L-tryptophan in divided doses with meals for 3 days, and found a 113 percent elevation in plasma tryptophan, but no changes in platelet or plasma serotonin or in plasma catecholamines. They also found no changes in urinary catecholamines. Additionally, they found no changes in blood pressure, heart rate, plasma sodium levels or 24-hour sodium excretion in urine.

L-Tryptophan administration (2 g) as a single dose before a meal has been found to decrease subjective hunger ratings, food intake, and alertness in men (Hrboticky et al., 1985), but not women (Leiter et al., 1987). Hrboticky and coworkers (1985) also tested 15 humans only once with 0, 1, 2, and 3 g of L-tryptophan. Individuals receiving 2 and 3 g of L-tryptophan had decreased hunger and alertness and increased faintness and dizziness. Administration of 1 g of L-tryptophan with 10 g of carbohydrates before each meal (3 g L-tryptophan/d) for 3 months did not affect body weight of obese humans (Strain et al., 1985). Wurtman and coworkers (1981) found that daily doses of 2.4 g of L-tryptophan for 2 weeks did not produce a significant reduction in the consumption of carbohydrate snacks in the

majority of the 24 individuals. Ten healthy adults given 5 g of L-tryptophan in a double-blind, placebo-controlled study reported severe nausea and headache and increased drowsiness soon after ingestion (Greenwood et al., 1975).

Smith and Prockop (1962) reported sustained nystagmus and drowsiness in seven adults given 70 and 90 mg/kg of body weight of L-tryptophan orally in single doses, but found that these effects were absent at 30 or 50 mg/kg. However, Lieberman and coworkers (1985) reported decreased self-ratings of vigor and alertness and increased subjective fatigue in 20 men treated with a single oral dose of 50 mg/kg of tryptophan. Yuwiler and coworkers (1981) also reported that five individuals given 50 or 100 mg/kg/d of L-tryptophan as a single dose or 50 mg/kg/d for 14 days experienced prolonged lethargy and drowsiness within 30 minutes of ingestion under all loading conditions.

Newborns (2 to 3 days of age) given infant formula supplemented with L-tryptophan (about 20 mg) were found to enter active and then quiet sleep sooner than those newborns given unsupplemented formula (Yogman and Zeisel, 1983). In a later study, these same investigators found that low doses of L-tryptophan have sleep-inducing properties in full-term infants (Yogman and Zeisel, 1985).

Finally, retrospective studies covering the time prior to the 1989 eosinophilia-myalgia syndrome (EMS) outbreak—thought to be caused by L-tryptophan contaminated with 1,1-ethylidene-bis[tryptophan] (EBT)—indicate that use of L-tryptophan alone may increase risk of eosinophilic fasciitis. Blauvelt and Falanga (1991) examined the history of L-tryptophan use in 49 patients with cutaneous fibrosis. Eleven of 17 patients reported using L-tryptophan prior to onset of eosinophilic fasciitis, as did two of ten patients with localized scleroderma, but use of L-tryptophan was not reported in any of 22 patients with systemic sclerosis. Intakes of L-tryptophan were from 0.5 to 5 g/d for 1 month to 10 years before the onset of symptoms of eosinophilic fasciitis were noted. L-tryptophan use in individuals with localized scleroderma occurred for 3 or 10 months before onset of symptoms, and intake was 1.5 to 2 g/d. Hibbs and coworkers (1992) found that 9 of 45 patients with eosinophilic fasciitis used 0.5 to 2.5 g/d of L-tryptophan for 1 month to 10 years before symptom onset. It is unknown whether or not these results occurred because of impurities in the L-tryptophan supplements.

Dose–Response Assessment

Taken together, the above studies in humans indicate that relatively short-term (acute and subacute) use of L-tryptophan is associated with appetite suppression, nausea, and drowsiness. However, in the absence of

data on the relationship between chronic consumption of L-tryptophan and the potential for adverse effects, and because of continuing uncertainty of the possible role of L-tryptophan in the development of eosinophilic fasciitis, a UL was not established for L-tryptophan.

Tyrosine

L-Tyrosine is considered a conditionally indispensable amino acid because it can be synthesized from L-phenylalanine in the liver. L-Tyrosine is a precursor of several biologically active substances, including catecholamine neurotransmitters, hormones, and melanin skin pigments. Based on distribution data from the 1988–1994 NHANES III, the mean daily intake for all life stage and gender groups of tyrosine from food and supplements is 2.8 g/d (Appendix Table D-18). Men 31 through 50 years of age had the highest intakes at the 99th percentile of 6.4 g/d.

Hazard Identification

Adverse Effects in Animals. In the mouse with elevated tissue concentrations of tyrosine, decarboxylation to tyramine becomes increasingly important, reducing lethality (David et al., 1974). Evidence has been provided that hepatic biotransformation of tyrosine yields a toxic metabolite, possibly an epoxide (David, 1976).

In rodents, feeding studies document the toxicity of large supplements of L-tyrosine (Benevenga and Steele, 1984; Harper et al., 1970). Effects of tyrosine on weight-gain suppression are a function of the protein content of the diet. For example, feeding rats a low-protein diet, 6 or 9 percent casein, retarded weight gain over a 3-week period. This effect of an inadequate protein intake was exacerbated by the addition of 3 to 8 percent L-tyrosine in the diet (Ip and Harper, 1973). With higher protein intakes of 15 or 24 percent, the toxicity of L-tyrosine was reduced, although 8 percent L-tyrosine still resulted in mortality.

Gipson and coworkers (1975) reported corneal lesions in rats fed L-tyrosine. Subsequently, Rich and coworkers (1973) reported that young adult Simonson albino or Long-Evans pigmented rats fed diets containing 5 or 10 percent L-tyrosine for 15 days developed elevated serum tyrosine levels and experienced reduced weight gain. At 10 percent L-tyrosine in the diet, deaths occurred within 10 days. Corneal disease was the first sign of toxicity; keratopathy was evident by 1 day and progressed in severity. The change began as haziness of the cornea, followed by opacities, and vascularization. The corneal changes were accompanied by elevations of tyrosine concentration in the aqueous humor.

Thoemke and Huether (1984) fed 8-week-old rats a diet containing 2.64 percent L-tyrosine. Rats were fed the diet for 2 weeks prior to mating and continually for three generations. No details were reported on overall pregnancy outcomes or behavioral endpoints. Brain weight was measured in all three generations and no differences were seen except at days 15 and 20 postpartum in the F2 generation (92 and 95 percent of controls). Serum concentration of tyrosine of F3 generation rats was increased at postnatal day 5.

Adverse Effects in Humans. No adverse effects have been reported for L-tyrosine from food. Large single doses of L-tyrosine (500 mg/kg/d) or smaller daily doses (100 mg/kg/d) have not been associated with any adverse affects (Al-Damluji et al., 1988; Glaeser et al., 1979; Sole et al., 1985). Nevertheless, the occurrence of corneal and skin lesions in humans with the autosomal recessive genetic disease, tyrosinemia II, in which tyrosine blood levels can be elevated tenfold, suggests that high chronic intakes leading to high-sustained concentrations of tyrosine in plasma and tissues may have adverse effects.

Single oral doses of 100 or 150 mg/kg of L-tyrosine administered to humans lead to a two- to threefold increase in plasma tyrosine concentrations (Cuche et al., 1985; Glaeser et al., 1979) and in urinary excretion of catecholamines and their metabolites (Alonso et al., 1982). Similar amounts given over the day in three equal doses result in similar increments in plasma tyrosine (Benedict et al., 1983; Melamed et al., 1980) and an increase in urinary catecholamines (Agharanya et al., 1981) and their metabolites (Alonso et al., 1982). Tyrosine given at 7.5 g/d decreased both free and conjugated plasma norepinephrine concentrations (Benedict et al., 1983). An increase in the dopamine metabolite, homovanillic acid, has been found in cerebral spinal fluid after L-tyrosine loads (Growdon et al., 1982).

Loads of L-tyrosine of 100 to 150 mg/kg/d have not been found to have any adverse effects on physiological systems (Benedict et al., 1983; Glaeser et al., 1979; Neri et al., 1995). In 13 patients with mild hypertension and given 2.5 g of L-tyrosine for 2 weeks, blood tyrosine was doubled 2 hours after the supplement, but no differences were found in systolic, diastolic, or mean blood pressure, heart rate, or plasma nonepinephrine (Sole et al., 1985). No data on blood concentrations in humans predictive of corneal lesions are available.

Dose–Response Assessment

In the absence of dose response data to describe more fully the relationship of L-tyrosine loads to alteration in catecholamine synthesis, physiological function, and corneal lesions in humans, a UL for L-tyrosine cannot be set for apparently healthy humans.

Intake Assessment

Although no ULs could be set for any of the amino acids, highest median and 99th percentile intakes for the amino acids are found in Table 10-29. All amino acids had their highest median intake for any life stage and gender group in men aged 19 through 30 years. The highest intakes at the 99th percentile were also found in men, with those 51

TABLE 10-29 Highest Median and 99th Percentile of Usual Daily Intake of Amino Acids, United States, Third National Health and Nutrition Examination Survey, 1998–1994

Amino Acid	Highest Median Intake[a] (g/d)	Highest 99th Percentile of Intake (g/d)
Alanine	5.2	8.5[b]
Arginine	5.9	10.1[b]
Aspartic acid	9.2	15.4[c]
Cysteine	1.4	2.2[b]
Glutamic acid	21.1	33.7[c]
Glycine	4.6	7.8[a]
Histidine	3.1	5.2[b]
Isoleucine	4.9	8.2[b]
Leucine	8.5	14.1[b]
Lysine	7.5	12.6[b]
Methionine	2.5	4.1[b]
Phenylalanine	4.8	7.7[c]
Proline	7.2	12.0[d]
Serine	4.8	7.9[c]
Threonine	4.2	7.1[b]
Tryptophan	1.3	2.1[b]
Tyrosine	3.9	6.4[c]
Valine	5.5	9.1[b]

[a] Males, 19–30 y.
[b] Males, 51–70 y.
[c] Males, 31–50 y.
[d] Males, 14–18 y.
NOTE: Data are from Appendix Tables D-2 through D-19.

through 70 years of age consuming the highest intakes for the majority of the amino acids surveyed.

Risk Characterization

Since there is no evidence that amino acids derived from usual or even high intakes of protein from food present any risk, attention was focused on intakes of the L-form of the amino acid found in dietary protein and amino acid supplements. Even from well-studied amino acids, adequate dose–response data from human or animal studies on which to base a UL were not available, but this does not mean that there is no potential for adverse effects resulting from high intakes of amino acids from dietary supplements. Since data on the adverse effects of high levels of amino acids intakes from dietary supplements are limited, caution may be warranted.

RESEARCH RECOMMENDATIONS

- Research is needed on high-protein intakes (>145 mg N/kg/d) in relationship to positive nitrogen balance and requirement estimates, metabolic and possible toxic effects in children and adults, and pathways affected by these high intakes.
- More data are needed on indispensable amino acid requirements for infants, children, and adolescents, as they are very sparse.
- Few studies on additional needs for protein during pregnancy, including estimates of changes in efficiency of conversion of dietary protein for maintenance and tissue accretion, are available. Thus more studies conducted during the length of pregnancy are needed.
- New methods, other than nitrogen balance, need to be validated to determine protein requirements, particularly in regard to long-term health.
- The role of the gastrointestinal system in the metabolism of amino acids, the nature of the amino acid losses, and the extent of synthesis of indispensable amino acids need to be investigated.
- Research on adaptation mechanisms at various intakes of protein is needed.
- Currently protein data for the elderly are sparse and more data are needed. Available data for the very elderly, namely those from 80 to 100 years of age, consists of only two or three adults in their early 80s, and thus studies conducted with this age group need to be done.
- Since ULs could not be established for any of the amino acids (some of which are known to result in toxic effects at high doses) due to insuffi-

cient data on dose–response relationships, more data are needed on adverse effects of high intakes of amino acids.

REFERENCES

Abumrad NN, Robinson RP, Gooch BR, Lacy WW. 1982. The effect of leucine infusion on substrate flux across the human forearm. *J Surg Res* 32:453–463.

Agharanya JC, Alonso R, Wurtman RJ. 1981. Changes in catecholamine excretion after short-term tyrosine ingestion in normally fed human subjects. *Am J Clin Nutr* 34:82–87.

Ahlborg B, Ekelund LG, Nilsson CG. 1968. Effect of potassium-magnesium-aspartate on the capacity for prolonged exercise in man. *Acta Physiol Scand* 74:238–245.

Al-Damluji S, Ross G, Touzel R, Perrett D, White A, Besser GM. 1988. Modulation of the actions of tyrosine by α-2-adrenoceptor blockade. *Br J Pharmacol* 95:405–412.

Alexander D, Ball MJ, Mann J. 1994. Nutrient intake and haematological status of vegetarians and age-sex matched omnivores. *Eur J Clin Nutr* 48:538–546.

Allen DH, Delohery J, Baker G. 1987. Monosodium L-glutamate-induced asthma. *J Allergy Clin Immunol* 80:530–537.

Allen JC, Keller RP, Archer P, Neville MC. 1991. Studies in human lactation: Milk composition and daily secretion rates of macronutrients in the first year of lactation. *Am J Clin Nutr* 54:69–80.

Allison SP. 1992. The uses and limitations of nutritional support. *Clin Nutr* 11:319–330.

Allison SP. 1995. Cost-effectiveness of nutritional support in the elderly. *Proc Nutr Soc* 54:693–699.

Alonso R, Gibson CJ, Wurtman RJ, Agharanya JC, Prieto L. 1982. Elevation of urinary catecholamines and their metabolites following tyrosine administration in humans. *Biol Psychiatry* 17:781–790.

Ambos M, Leavitt NR, Marmorek L, Wolschina SB. 1968. Sin Cib-Syn: Accent on glutamate. *N Engl J Med* 279:105.

Amen RJ, Yoshimura NN. 1981. The pharmacology of branched-chain amino acids. *Nutr Pharmacol* 4:73–116.

Anantharaman K. 1979. In utero and dietary administration of monosodium L-glutamate to mice: Reproductive performance and development in a multigeneration study. In: Filer LJ, ed. *Glutamic Acid: Advances in Biochemistry and Physiology.* Pp. 231–253.

Anderson DM, Williams FH, Merkatz RB, Schulman PK, Kerr DS, Pittard WB. 1983. Length of gestation and nutritional composition of human milk. *Am J Clin Nutr* 37:810–814.

Anderson GH, Johnston JL. 1983. Nutrient control of brain neurotransmitter synthesis and function. *Can J Physiol Pharmacol* 61:271–281.

Anderson GH, Atkinson SA, Bryan MH. 1981. Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. *Am J Clin Nutr* 34:258–265.

Anderson SA, Tews JK, Harper AE. 1990. Dietary branched-chain amino acids and protein selection by rats. *J Nutr* 120:52–63.

ARS (Agricultural Research Service). 2001. *USDA Nutrient Database for Standard Reference, Release 14*. Online. U.S. Department of Agriculture. Available at http://www.nal.usda.gov/fnic/foodcomp/Data/SR14/sr14.html. Accessed July 3, 2002.

Artom C, Fishman WH, Morehead RP. 1945. The relative toxicity of l- and dl-serine in rats. *Proc Soc Exp Biol Med* 60:284–287.

Ashley DV, Anderson GH. 1975. Correlation between the plasma tryptophan to neutral amino acid ratio and protein intake in the self-selecting weanling rat. *J Nutr* 105:1412–1421.

Atkinson SA, Anderson GH, Bryan MH. 1980. Human milk: comparison of the nitrogen composition in milk from mothers of premature and full-term infants. *Am J Clin Nutr 33:811-814*.

Ball MJ, Bartlett MA. 1999. Dietary intake and iron status of Australian vegetarian women. *Am J Clin Nutr* 70:353–358.

Barbul A. 1986. Arginine: Biochemistry, physiology, and therapeutic implications. *J Parenter Enteral Nutr* 10:227–238.

Barbul A, Wasserkrug HL, Sisto DA, Seifter E, Rettura G, Levenson SM, Efron G. 1980. Thymic stimulatory actions of arginine. *J Parenter Enteral Nutr* 4:446–449.

Barbul A, Sisto DA, Wasserkrug HL, Efron G. 1981. Arginine stimulates lymphocyte immune response in healthy human beings. *Surgery* 90:244–251.

Barbul A, Lazarou SA, Efron DT, Wasserkrug HL, Efron G. 1990. Arginine enhances wound healing and lymphocyte immune responses in humans. *Surgery* 108:331–337.

Barr SI, Broughton TM. 2000. Relative weight, weight loss efforts and nutrient intakes among health-conscious vegetarian, past vegetarian and nonvegetarian women ages 18 to 50. *J Am Coll Nutr* 19:781–788.

Basile-Filho A, Beaumier L, El-Khoury AE, Yu Y, Kenneway M, Gleason RE, Young VR. 1998. Twenty-four-hour L-[1-^{13}C]tyrosine and L-[3,3-$^{2}H_2$]phenylalanine oral tracer studies at generous, intermediate, and low phenylalanine intakes to estimate aromatic amino acid requirements in adults. *Am J Clin Nutr* 67:640–659.

Batshaw ML, Wachtel RC, Thomas GH, Starrett A, Brusilow SW. 1984. Arginine-responsive asymptomatic hyperammonemia in the premature infant. *J Pediatr* 105:86–91.

Baxter CF, Baldwin RA, Davis JL, Flood JF. 1985. High proline levels in the brains of mice as related to specific learning deficits. *Pharmacol Biochem Behav* 22:1053–1059.

Bazzano G, D'Elia JA, Olson RE. 1970. Monosodium glutamate: Feeding of large amounts in man and gerbils. *Science* 169:1208–1209.

Benabe JE, Martinez-Maldonado M. 1998. The impact of malnutrition on kidney function. *Mineral Electrolyte Metab* 24:20–26.

Benedict CR, Anderson GH, Sole MJ. 1983. The influence of oral tyrosine and tryptophan feeding on plasma catecholamines in man. *Am J Clin Nutr* 38:429–435.

Benevenga NJ, Steele RD. 1984. Adverse effects of excessive consumption of amino acids. *Annu Rev Nutr* 4:157–181.

Benevenga NJ, Yeh M-H, Lalich JJ. 1976. Growth depression and tissue reaction to the consumption of excess dietary methionine and S-methyl-L-cysteine. *J Nutr* 106:1714–1720.

Bergner H, Schwandt H, Kruger U. 1990. Determination of a prececal N-absorption from natural feed by ^{15}N-labeled laboratory rats using the isotope diluation method. *Arch Tierernahr* 40:569–582.

Bernacchi AS, DeFerreyra EC, DeCastro CR, Castro JA. 1993. Ultrastructural alterations in testes from rats treated with cysteine. *Biomed Environ Sci* 6:172–178.

Berry HK, Butcher RE, Elliot LA, Brunner RL. 1974. The effect of monosodium glutamate on the early biochemical and behavioral development of the rat. *Dev Psychobiol* 7:165–173.

Birt DF, Julius AD, Hasegawa R, St. John M, Cohen S. 1987. Effect of L-tryptophan excess and vitamin B_6 deficiency on rat urinary bladder cancer promotion. *Cancer Res* 47:1244–1250.

Bistrian BR. 1990. Recent advances in parenteral and enteral nutrition: A personal perspective. *J Parenteral Enteral Nutr* 14:329–334.

Blauvelt A, Falanga V. 1991. Idiopathic and L-tryptophan-associated eosinophilic fasciitis before and after L-tryptophan contamination. *Arch Dermatol* 127:1159–1166.

Block KP. 1989. Interactions among leucine, isoleucine, and valine with special reference to the branched-chain amino acid antagonism. In: Friedman M, ed. *Absorption and Utilization of Amino Acids,* Vol. 1. Boca Raton, FL: CRC Press. Pp. 229–244.

Blumenkrantz MJ, Shapiro DJ, Swendseid ME, Kopple JD. 1975. Histidine supplementation for treatment of anaemia of uraemia. *Br Med J* 2:530–533.

Borgonha S, Regan MM, Oh SH, Condon M, Young VR. 2002. Threonine requirement of healthy adults, derived with a 24-h indicator amino acid balance technique. *Am J Clin Nutr* 75:698–704.

Brattstrom LE, Hardebo JE, Hultberg BL. 1984. Moderate homocysteinemia—A possible risk factor for arteriosclerotic cerebrovascular disease. *Stroke* 15:1012–1016.

Brattstrom L, Israelsson B, Norrving B, Bergqvist D, Thorne J, Hultberg B, Hamfelt A. 1990. Impaired homocysteine metabolism in early-onset cerebral and peripheral occlusive arterial disease. *Atherosclerosis* 81:51–60.

Bross R, Ball RO, Pencharz PB. 1998. Development of a minimally invasive protocol for the determination of phenylalanine and lysine kinetics in humans during the fed state. *J Nutr* 128:1913–1919.

Bross R, Ball RO, Clarke JTR, Pencharz PB. 2000. Tyrosine requirements in children with classical PKU determined by indicator amino acid oxidation. *Am J Physiol* 278:E195–E201.

Brunton JA, Ball RO, Pencharz PB. 1998. Determination of amino acid requirements by indicator amino acid oxidation: Applications in health and disease. *Curr Opin Clin Nutr Metab Care* 1:449–453.

Brunton JA, Bertolo RF, Pencharz PB, Ball RO. 1999. Proline ameliorates arginine deficiency during enteral but not parenteral feeding in neonatal piglets. *Am J Physiol* 277:E223–E231.

Brusilow SW, Horwich AL. 1989. Urea cycle enzymes. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic Basis of Inherited Disease, 6th ed.* New York: McGraw-Hill. Pp. 629–663.

Brusilow SW, Danney M, Waber LJ, Batshaw M, Burton B, Levitsky L, Roth K, McKeethren C, Ward J. 1984. Treatment of episodic hyperammonemia in children with inborn errors of urea synthesis. *N Engl J Med* 310:1630–1634.

Burke BS, Harding VV, Stuart HC. 1943. Nutrition studies during pregnancy. IV. Relation of protein content of mother's diet during pregnancy to birth length, birth weight, and condition of infant at birth. *J Pediatr* 23:506–515.

Bushinsky DA, Gennari FJ. 1978. Life-threatening hyperkalemia induced by arginine. *Ann Intern Med* 89:632–634.

Butte NF, Garza C, Johnson CA, O'Brian Smith E, Nichols BL. 1984a. Longitudinal changes in milk composition of mothers delivering preterm and term infants. *Early Hum Dev* 9:153–162.

Butte NF, Garza C, O'Brian Smith E, Nichols BL. 1984b. Human milk intake and growth in exclusively breast-fed infants. *J Pediatr* 104:187–195.

Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. 2000. Body composition during the first 2 years of life: An updated reference. *Pediatr Res* 47:578–585.

Calabrese V, Rausa N, Antico A, Mangiameli S, Rizza V. 1997. Cysteine-induced enhancement of lipid peroxidation in substantia nigra: Comparative effect with exogenous administration of reduced glutathione. *Drugs Exp Clin Res* 23:25–31.

Calloway DH. 1974. Nitrogen balance during pregnancy. In: Winnick M, ed. *Nutrition and Fetal Development*, Vol. 2. New York: John Wiley and Sons. Pp. 79–94.

Calloway DH, Margen S. 1971. Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. *J Nutr* 101:205–216.

Calloway DH, Odell AC, Margen S. 1971. Sweat and miscellaneous nitrogen losses in human balance studies. *J Nutr* 101:775–786.

Campbell WW, Evans WJ. 1996. Protein requirements of elderly people. *Eur J Clin Nutr* 50:S180–S185.

Campbell WW, Crim MC, Dallal GE, Young VR, Evans WJ. 1994. Increased protein requirements in elderly people: New data and retrospective reassessments. *Am J Clin Nutr* 60:501–509.

Campbell WW, Crim MC, Young VR, Joseph LJ, Evans WJ. 1995. Effects of resistance training and dietary protein intake on protein metabolism in older adults. *Am J Physiol* 268:E1143–E1153.

Campbell WW, Trappe TA, Wolfe RR, Evans WJ. 2001. The recommended dietary allowance for protein may not be adequate for older people to maintain skeletal muscle. *J Gerontol A Biol Sci Med Sci* 56:M373–M380.

Carlson HE, Miglietta JT, Roginsky MS, Stegink LD. 1989. Stimulation of pituitary hormone secretion by neurotransmitter amino acids in humans. *Metabolism* 38:1179–1182.

Carmichael S, Abrams B, Selvin S. 1997. The pattern of maternal weight gain in women with good pregnancy outcomes. *Am J Public Health* 87:1984–1988.

Castaneda C, Charnley JM, Evans WJ, Crim MC. 1995a. Elderly women accommodate to a low-protein diet with losses of body cell mass, muscle function, and immune response. *Am J Clin Nutr* 62:30–39.

Castaneda C, Dolnikowski GG, Dallal GE, Evans WJ, Crim MC. 1995b. Protein turnover and energy metabolism of elderly women fed a low-protein diet. *Am J Clin Nutr* 62:40–48.

Celander DR, George MJ. 1963. Dietary interrelationships of ethionine and methionine in the weanling rat. *Biochem J* 87:143–146.

Chambers BJ, Klein NW, Nosel PG, Khairallah LH, Romanow JS. 1995. Methionine overcomes neural tube defects in rat embryos cultured on sera from laminin-immunized monkeys. *J Nutr* 125:1587–1599.

Chatot CL, Klein NW, Clapper ML, Resor SR, Singer WD, Russman BS, Holmes GL, Mattson RH, Cramer JA. 1984. Human serum teratogenicity studied by rat embryo culture: Epilepsy, anticonvulsant drugs, and nutrition. *Epilepsia* 25:205–216.

Chen MK, Salloum RM, Austgen TR, Bland JB, Bland KI, Copeland EM, Souba WW. 1991. Tumor regulation of hepatic glutamine metabolism. *J Parenter Enteral Nutr* 15:159–164.

Chen MK, Espat NJ, Bland KI, Copeland EM, Souba WW. 1993. Influence of progressive tumor growth on glutamine metabolism in skeletal muscle and kidney. *Ann Surg* 217:655–667.

Cheng AH, Gomez A, Bergan JG, Lee TC, Monckeberg F, Chichester CO. 1978. Comparative nitrogen balance study between young and aged adults using three levels of protein intake from a combination wheat-soy-milk mixture. *Am J Clin Nutr* 31:12–22.

Chien PFW, Smith K, Watt PW, Scrimgeour CM, Taylor DJ, Rennie MJ. 1993. Protein turnover in the human fetus studied at term using stable isotope tracer amino acids. *Am J Physiol* 265:E31–E35.

Chipponi JX, Bleier JC, Santi MT, Rudman D. 1982. Deficiencies of essential and conditionally essential nutrients. *Am J Clin Nutr* 35:1112–1116.

Cho ES, Anderson HL, Wixom RL, Hanson KC, Krause GF. 1984. Long-term effects of low histidine intake on men. *J Nutr* 114:369–384.

Christman AA. 1971. Determination of anserine, carnosine, and other histidine compounds in muscle extractives. *Anal Biochem* 39:181–187.

Chung TK, Gelberg HB, Dorner JL, Baker DH. 1991. Safety of L-tryptophan for pigs. *J Anim Sci* 69:2955–2960.

Ciechanover A, DiGiuseppe JA, Bercovich B, Orian A, Richter JD, Schwartz AL, Brodeur GM. 1991. Degradation of nuclear oncoproteins by the ubiquitin system in vitro. *Proc Natl Acad Sci USA* 88:139–143.

Clarke JTR, Bier DM. 1982. The conversion of phenylalanine to tyrosine in man. Direct measurement by continuous intravenous tracer infusions of L-[ring-$^{2}H_{5}$] phenylalanine and L-[1-^{13}C] tyrosine in the postabsorptive state. *Metabolism* 31:999–1005.

Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I. 1991. Hyperhomocysteinemia: An independent risk factor for vascular disease. *N Engl J Med* 324:1149–1155.

Coelho CN, Klein NW. 1990. Methionine and neural tube closure in cultured rat embryos: Morphological and biochemical analyses. *Teratology* 42:437–451.

Cohlan SQ, Stone SM. 1961. Effects of dietary and intraperitoneal excess of L-lysine and L-leucine on rat pregnancy and offspring. *J Nutr* 74:93–95.

Colquhoun A, Newsholme EA. 1997. Aspects of glutamine metabolism in human tumour cells. *Biochem Mol Biol Int* 41:583–596.

Connor H, Newton DJ, Preston FE, Woods HF. 1978. Oral methionine loading as a cause of acute serum folate deficiency: Its relevance to parental nutrition. *Postgrad Med J* 54:318–320.

Cordain L, Miller JB, Eaton SB, Mann N, Holt SH, Speth JD. 2000. Plant-animal subsistence ratios and macronutrient energy estimations in worldwide hunter-gatherer diets. *Am J Clin Nutr* 71:682–692.

Corish CA, Kennedy NP. 2000. Protein-energy undernutrition in hospital in-patients. *Br J Nutr* 83:575–591.

Creel DJ, Wang JM, Wong KC. 1987. Transient blindness associated with transurethral resection of the prostate. *Arch Ophthalmol* 105:1537–1539.

Cuche JL, Prinseau J, Selz F, Ruget G, Tual JL, Reingeissen L, Devoisin M, Baglin A, Guedon J, Fritel D. 1985. Oral load of tyrosine or L-dopa and plasma levels of free and sulfoconjugated catecholamines in healthy men. *Hypertension* 7:81–89.

Cuervo AM, Dice JF. 1998. Lysosomes, a meeting point of proteins, chaperones, and proteases. *J Mol Med* 76:6–12.

Danner DJ, Elsas LF. 1989. Disorders of branched chain amino acid and keto acid metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic Basis of Inherited Disease, 6th ed.*, Vol. I. New York: McGraw-Hill. Pp. 671–692.

Danner DJ, Lemmon SK, Besharse JC, Elsas LJ. 1979. Purification and characterization of branched chain alpha-ketoacid dehydrogenase from bovine liver mitochondria. *J Biol Chem* 254:5522–5526.

Darling PB, Grunow J, Rafii M, Brookes S, Ball RO Pencharz PB. 2000. Threonine dehydrogenase is a minor degradative pathway of threonine catabolism in human adults. *Am J Physiol* 278:E877–E884.

Darragh AJ, Hodgkinson SM. 2000. Quantifying the digestibility of dietary protein. *J Nutr* 130:1850S–1856S.

Darragh AJ, Moughan PJ. 1998. The amino acid composition of human milk corrected for amino acid digestibility. *Br J Nutr* 80:25–34.

Das TK, Waterlow JC. 1974. The rate of adaptation of urea cycle enzymes, aminotransferases and glutamic dehydrogenase to changes in dietary protein intake. *Br J Nutr* 32:353–373.

David JC. 1976. Evidence for the possible formation of a toxic tyrosine metabolite by the liver microsomal drug metabolizing system. *Naunyn Schmiedebergs Arch Pharmacol* 292:79–86.

David JC, Dairman W, Udenfriend S. 1974. Decarboxylation to tyramine: A major route of tyrosine metabolism in mammals. *Proc Natl Acad Sci USA* 71:1771–1775.

Davis TA, Nguyen HV, Garcia-Bravo R, Fiorotto ML, Jackson EM, Lewis DS, Lee DR, Reeds PJ. 1994. Amino acid composition of human milk is not unique. *J Nutr* 124:1126–1132.

De Aloysio D, Mantuano R, Mauloni M, Nicoletti G. 1982. The clinical use of arginine aspartate in male infertility. *Acta Eur Fertil* 13:133–167.

de Blaauw I, Deutz NEP, Von Meyenfeldt MF. 1996. In vivo amino acid metabolism of gut and liver during short and prolonged starvation. *Am J Physiol* 270:G298–G306.

de Haan A, van Doorn JE, Westra HG. 1985. Effects of potassium + magnesium aspartate on muscle metabolism and force development during short intensive static exercise. *Int J Sports Med* 6:44–49.

Dekker J, Aelmans PH, Strous GJ. 1991. The oligomeric structure of rat and human gastric mucins. *Biochem J* 277:423–427.

de Lange CFM, Sauer WC, Mosenthin R, Souffrant WB. 1989. The effect of feeding different protein-free diets on the recovery and amino acid composition of endogenous protein collected from the distal ileum and feces in pigs. *J Anim Sci* 67:746–754.

de Vrese M, Frik R, Roos N, Hagemeister H. 2000. Protein-bound D-amino acids, and to a lesser extent lysinoalanine, decrease true ileal protein digestibility in minipigs as determined with ^{15}N-labeling. *J Nutr* 130:2026–2031.

Dewey KG, Lönnerdal B. 1983. Milk and nutrient intake of breast-fed infants from 1 to 6 months: Relation to growth and fatness. *J Pediatr Gastroenterol Nutr* 2:497–506.

Dewey KG, Finley DA, Lönnerdal B. 1984. Breast milk volume and composition during late lactation (7–20 months). *J Pediatr Gastroenterol Nutr* 3:713–720.

Dewey KG, Beaton G, Fjeld C, Lönnerdal B, Reeds P. 1996. Protein requirements of infants and children. *Eur J Clin Nutr* 50:S119–S150.

DHEW (U.S. Department of Health, Education and Welfare). 1978. *Bioassay of L-Tryptophan for Possible Carcinogenicity.* National Cancer Institute Technical Report Series No. 71. Washington, DC: U.S. Government Printing Office.

Di Buono M, Wykes LJ, Ball RO, Pencharz PB. 2001. Total sulfur amino acid requirement in young men determined by indicator amino acid oxidation with L-[1-^{13}C] phenylalanine. *Am J Clin Nutr* 74:756–760.

Diem K. 1962. *Documenta Geigy Scientific Tables, 6th ed.* Ardsley, NY: Geigy Pharmaceuticals. Pp.528.

DiGiovanna JJ, Blank H. 1984. Failure of lysine in frequently recurrent herpes simplex infection. Treatment and prophylaxis. *Arch Dermatol* 120:48–51.

DiGiovanna JJ, Blank H. 1985. Failure of lysine? *Arch Dermatol* 121:21.

Drago F, Continella G, Alloro MC, Auditore S, Pennisi G. 1984. Behavioral effects of arginine in male rats. *Pharmacol Res Commun* 16:899–907.

Dubois S, Dougherty C, Duquette M-P, Hanley JA, Moutquin J-M. 1991. Twin pregnancy: The impact of the Higgins Nutrition Intervention Program on maternal and neonatal outcomes. *Am J Clin Nutr* 53:1397–1403.

Dubois S, Coulombe C, Pencharz P, Pinsonneault O, Duquette M-P. 1997. Ability of the Higgins Nutrition Intervention Program to improve adolescent pregnancy outcome. *J Am Diet Assoc* 97:871–878.

Dubow E, Maher A, Gish D, Erk V. 1958. Lysine tolerance in infants. *J Pediatr* 52:30–37.

Duffy B, Gunn T, Collinge J, Pencharz PB. 1981. The effect of varying protein quality and energy intake on the nitrogen metabolism of parenterally fed very low birthweight (<1600 g) infants. *Pediatr Res* 15:1040–1044.

Dutra-Filho CS, Wannmacher CM, Pires RF, Gus G, Kalil AM, Wajner M. 1989. Reduced locomotor activity of rats made histidinemic by injection of histidine. *J Nutr* 119:1223–1227.

Ebert AG. 1979a. The dietary administration of L-monosodium glutamate, DL-monosodium glutamate, and L-glutamic acid to rats. *Toxicol Lett* 3:71–78.

Ebert AG. 1979b. The dietary administration of monosodium glutamate or glutamic acid to C-57 black mice for 2 years. *Toxicol Lett* 3:65–70.

Edmonds MS, Baker DH. 1987. Amino acid excesses for young pigs: Effects of excess methionine, tryptophan, threonine or leucine. *J Anim Sci* 64:1664–1671.

Edmonds MS, Gonyou HW, Baker DH. 1987. Effect of excess levels of methionine, tryptophan, arginine, lysine or threonine on growth and dietary choice in the pig. *J Anim Sci* 65:179–185.

Egana JI, Fuentes A, Uauy R. 1984. Protein needs of Chilean pre-school children fed milk and soy protein isolate diets. In: Rand WM, Uauy R, Scrimshaw NS, eds. *Protein-Energy Requirement Studies in Developing Countries: Results of International Research.* Tokyo, Japan: United Nations University Press. Pp. 249–257.

Egana JI, Uauy R, Cassorla X, Barrera G, Yanez E. 1992. Sweet lupin protein quality in young men. *J Nutr* 122:2341–2347.

Ehlers K, Drews E, Nau H. 1994. The amino acid methionine reduces the valproic acid-induced spina bifida rate in the mouse. *Teratology* 50:28A.

Elia M, Livesey G. 1983. Effects of ingested steak and infused leucine on forelimb metabolism in man and the fate of the carbon skeletons and amino groups of branched-chain amino acids. *Clin Sci* 64:517–526.

El-Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, Gleason RE, Chapman TE, Young VR. 1994a. The 24-h pattern and rate of leucine oxidation, with particular reference to tracer estimates of leucine requirements in healthy adults. *Am J Clin Nutr* 59:1012–1020.

El-Khoury AE, Fukagawa NK, Sanchez M, Tsay RH, Gleason RE, Chapman TE, Young VR. 1994b. Validation of the tracer-balance concept with reference to leucine: 24-h intravenous tracer studies with L-[1-^{13}C]leucine and [^{15}N-^{15}N]urea. *Am J Clin Nutr* 59:1000–1011.

El-Khoury AE, Forslund A, Olsson R, Branth S, Sjodin A, Andersson A, Atkinson A, Selvaraj A, Hambraeus L, Young VR. 1997. Moderate exercise at energy balance does not affect 24-h leucine oxidation or nitrogen retention in healthy men. *Am J Physiol* 273:E394–E407.

El-Khoury AE, Pereira PC, Borgonha S, Basile-Filho A, Beaumier L, Wang SY, Metges CC, Ajami AM, Young VR. 2000. Twenty-four-hour oral tracer studies with L-[1-^{13}C]lysine at a low (15 mg·kg^{-1}·d^{-1}) and intermediate (29 mg·kg^{-1}·d^{-1}) lysine intake in healthy adults. *Am J Clin Nutr* 72:122–130.

Ellis KJ, Shypailo RJ, Abrams SA, Wong WW. 2000. The reference child and adolescent models of body composition. A contemporary comparison. *Ann NY Acad Sci* 904:374–382.

Emerson K, Poindexter EL, Kothari M. 1975. Changes in total body composition during normal and diabetic pregnancy: Relation to oxygen consumption. *Obstet Gynecol* 45:505–511.

Eriksson LS, Hagenfeldt L, Felig P, Wahren J. 1983. Leucine uptake by splanchnic and leg tissues in man: Relative independence of insulin levels. *Clin Sci* 65:491–498.

Fahr MJ, Kornbluth J, Blossom S, Schaeffer R, Klimberg VS. 1994. Harry M. Vars Research Award. Glutamine enhances immunoregulation of tumor growth. *J Parenter Enteral Nutr* 18:471–476.

FAO (Food and Agriculture Organization). 2000. *The State of Food and Agriculture 2000.* Rome: FAO.

FAO/Agrostat. 1991. *Computerized information series No. 1. Food balance sheets.* Rome: FAO.

FAO/WHO (World Health Organization). 1965. *Protein Requirements.* Report of a Joint FAO/WHO Expert Group. Technical Report Series No. 37. Rome: FAO.

FAO/WHO. 1973. *Energy and Protein Requirements.* Report of a Joint FAO/WHO Ad Hoc Expert Committee. Technical Report Series No. 522. Geneva, Switzerland: WHO.

FAO/WHO. 1991. *Protein Quality Evaluation.* FAO Food and Nutrition Paper 51. Rome: FAO.

FAO/WHO/UNU (United Nations University). 1985. *Energy and Protein Requirements.* Report of a Joint FAO/WHO/UNU Expert Consultation. Technical Report Series No. 724. Geneva, Switzerland: WHO.

Fawcett LB, Pugarelli JE, Brent RL. 2000. Effects of supplemental methionine on antiserum-induced dysmorphology in rat embryos cultured in vitro. *Teratology* 61:332–341.

Fee BA, Weil WB. 1963. Body composition of infants of diabetic mothers by direct analysis. *Ann NY Acad Sci* 110:869.

Fernstrom JD, Larin F, Wurtman RJ. 1973. Correlations between brain tryptophan and plasma neutral amino acid levels following food consumption in rats. *Life Sci* 13:517–524.

Fernstrom JD, Cameron JL, Fernstrom MH, McConaha C, Weltzin TE, Kaye WH. 1996. Short-term neuroendocrine effects of a large oral dose of monosodium glutamate in fasting male subjects. *J Clin Endocrinol Metab* 81:184–191.

Ferrari DA, Gilles PA, Klein NW, Nadler D, Weeks BS, Lammi-Keefe CJ, Hillman RE, Carey SW, Ying Y-K, Maier D, Olsen P, Wemple DW, Greenstein R, Muechler EK, Miller RK, Mariona FG. 1994. Rat embryo development on human sera is related to numbers of previous spontaneous abortions and nutritional factors. *Am J Obstet Gynecol* 170:228–236.

Filer LJ, Stegink LD. 1988. Effect of aspartame on plasma phenylalanine concentration in humans. In: Wurtman RJ, Ritter-Walker E, eds. *Dietary Phenylalanine and Brain Function.* Boston: Birkhauser. Pp. 18–40.

Finkelstein MW, Daabees TT, Stegink LD, Applebaum AE. 1983. Correlation of aspartate dose, plasma dicarboxylic amino acid concentration, and neuronal necrosis in infant mice. *Toxicology* 29:109–119.

Finkelstein MW, Daabees TT, Stegink LD, Applebaum AE. 1988. Aspartate-induced neuronal necrosis in infant mice: Protective effect of carbohydrate and insulin. *J Toxicol Environ Health* 23:395–406.

Fisher H, Brush MK, Griminger P, Sostman ER. 1967. Nitrogen retention in adult man: A possible factor in protein requirements. *Am J Clin Nutr* 20:927–934.

Fomon S. 1991. Requirements and recommended dietary intakes of protein during infancy. *Pediatr Res* 30:391–395.

Forbes GB. 1987. *Human Body Composition: Growth, Aging, Nutrition, and Activity.* New York: Springer-Verlag.

Forslund AH, Hambraeus L, Olsson RM, El-Khoury AE, Yu Y-M, Young VR. 1998. The 24-h whole body leucine and urea kinetics at normal and high protein intakes with exercise in healthy adults. *Am J Physiol* 275:E310–E320.

Forsum E, Sadurskis A, Wager J. 1988. Resting metabolic rate and body composition of healthy Swedish women during pregnancy. *Am J Clin Nutr* 47:942–947.

Fregly MJ, Rowland NE, Sumners C. 1989. Effect of chronic dietary treatment with L-tryptophan on spontaneous salt appetite of rats. *Pharmacol Biochem Behav* 33:401–406.

Frexes-Steed M, Warner ML, Bulus N, Flakoll P, Abumrad NN. 1990. Role of insulin and branched-chain amino acids in regulating protein metabolism during fasting. *Am J Physiol* 258:E907–E917.

Frey GH. 1976. Use of aspartame by apparently healthy children and adolescents. *J Toxicol Environ Health* 2:401–415.

Frisancho AR, Matos J, Flegel P. 1983. Maternal nutritional status and adolescent pregnancy outcome. *Am J Clin Nutr* 38:739–746.

Fuller MF, Garlick PJ. 1994. Human amino acid requirements: Can the controversy be resolved? *Annu Rev Nutr* 14:217–241.

Fuller MF, Reeds PJ. 1998. Nitrogen cycling in the gut. *Annu Rev Nutr* 18:385–411.

Funk DN, Worthington-Roberts B, Fantel A. 1991. Impact of supplemental lysine or tryptophan on pregnancy course and outcome in rats. *Nutr Res* 11:501–512.

Furst P. 1989. Amino acid metabolism in uremia. *J Am Coll Nutr* 8:310–323.

Garlick PJ, Reeds PJ. 1993. Proteins. In: Garrow JS, James WPT, Ralph A, eds. *Human Nutrition and Dietetics.* Edinburgh: Churchill Livingstone. Pp. 56–76.

Garlick PJ, McNurlan MA, Patlak CS. 1999. Adaptation of protein metabolism in relation to limits to high dietary protein intake. *Eur J Clin Nutr* 53:S34–S43.

Garza C, Scrimshaw NS, Young VR. 1976. Human protein requirements: The effect of variations in energy intake within the maintenance range. *Am J Clin Nutr* 29:280–287.

Garza C, Scrimshaw NS, Young VR. 1977a. Human protein requirements: A long-term metabolic nitrogen balance study in young men to evaluate the 1973 FAO/WHO safe level of egg protein intake. *J Nutr* 107:335–352.

Garza C, Scrimshaw NS, Young VR. 1977b. Human protein requirements: Evaluation of the 1973 FAO/WHO safe level of protein intake for young men at high energy intakes. *Br J Nutr* 37:403–420.

Garza C, Scrimshaw NS, Young VR. 1978. Human protein requirements: Interrelationships between energy intake and nitrogen balance in young men consuming the 1973 FAO/WHO safe level of egg protein, with added nonessential amino acids. *J Nutr* 108:90–96.

Gaspar J, Laires A, Va S, Pereira S, Mariano A, Quina M, Rueff J. 1996. Mutagenic activity of glycine upon nitrosation in the presence of chloride and human gastric juice: A possible role in gastric carcinogenesis. *Teratog Carcinog Mutagen* 16:275–286.

Gattas V, Barrera GA, Riumallo JS, Uauy R. 1990. Protein-energy requirements of prepubertal school-age boys determined by using the nitrogen-balance response to a mixed-protein diet. *Am J Clin Nutr* 52:1037–1042.

Gattas V, Barrera GA, Riumallo JS, Uauy R. 1992. Protein-energy requirements of boys 12-14 y old determined by using the nitrogen-balance response to a mixed-protein diet. *Am J Clin Nutr* 56:499–503.

Gaudichon C, Mahe S, Benamouzig R, Luengo C, Fouillet H, Dare S, Van Oycke M, Ferriere F, Rautureau J, Tome D. 1999. Net postprandial utilization of [15N]-labeled milk protein nitrogen is influenced by diet composition in humans. *J Nutr* 129:890–895.

Gausserès N, Mahé S, Benamouzig R, Luengo C, Ferriere F, Rautureau J, Tomé D. 1997. [^{15}N]-Labeled pea flour protein nitrogen exhibits good ileal digestibility and postprandial retention in humans. *J Nutr* 127:1160–1165.

Geha RS, Beiser A, Ren C, Patterson R, Greenberger P, Grammer LC, Ditto AM, Harris KE, Shaughnessy MA, Yarnold PR, Corren J, Saxon A. 2000. Multicenter, double blind, placebo-controlled, multiple-challenge evaluation of reported reactions to monosodium glutamate. *J Allergy Clin Immunol* 106:973–980.

Geliebter AA, Hashim SA, Van Itallie TB. 1981. Oral L-histidine fails to reduce taste and smell acuity but induces anorexia and urinary zinc excretion. *Am J Clin Nutr* 34:119–120.

Genuth SM. 1973. Effects of oral alanine administration in fasting obese subjects. *Metabolism* 22:927–937.

Genuth SM, Castro J. 1974. Effect of oral alanine on blood beta-hydroxybutyrate and plasma glucose, insulin, free fatty acids, and growth hormone in normal and diabetic subjects. *Metabolism* 23:375–386.

Gerard JM, Luisiri A. 1997. A fatal overdose of arginine hydrochloride. *Clin Toxicol* 35:621–625.

Germano P, Cohen SG, Hahn B, Metcalfe DD. 1991. An evaluation of clinical reactions to monosodium glutamate (MSG) in asthmatics, using a blinded placebo-controlled challenge. *J Allergy Clin Immunol* 87:177.

Gersovitz M, Motil K, Munro HN, Scrimshaw NS, Young VR. 1982. Human protein requirements: Assessment of the adequacy of the current Recommended Dietary Allowance for dietary protein in elderly men and women. *Am J Clin Nutr* 35:6–14.

Gipson IK, Burns RP, Wolfe-Lande JD. 1975. Crystals in corneal epithelial lesions of tyrosine-fed rats. *Invest Ophthalmol* 14:937–941.

Glaeser BS, Melamed E, Growdon JH, Wurtman RJ. 1979. Evaluation of plasma tyrosine after a single oral dose of L-tyrosine. *Life Sci* 25:265–271.

Glatt H. 1989. Mutagenicity spectra in *Salmonella typhimurium* strains of glutathione, L-cysteine and active oxygen species. *Mutagenesis* 4:221–227.

Glatt H. 1990. Endogenous mutagens derived from amino acids. *Mutat Res* 238:235–243.

Glyn JR, Lipton JM. 1981. Effects of central administration of alanine on body temperature of the rabbit: Comparisons with the effects of serine, glycine and taurine. *Brain Res Bull* 6:467–472.

Goldberg AL, Rock KL. 1992. Proteolysis, proteasomes and antigen presentation. *Nature* 357:375–379.

Greenwood MH, Lader MH, Kantameneni BD, Curzon G. 1975. The acute effects of oral (–)-tryptophan in human subjects. *Br J Clin Pharmacol* 2:165–172.

Griffith RS, Norins AL, Kagan C. 1978. A multicentered study of lysine therapy in herpes simplex infection. *Dermatologica* 156:257–267.

Griffith RS, Walsh DE, Myrmel KH, Thompson RW, Behforooz A. 1987. Success of L-lysine therapy in frequently recurrent herpes simplex infection. Treatment and prophylaxis. *Dermatologica* 175:183–190.

Grossie VB, Nishioka K, Ajani JA, Ota DM. 1992. Substituting ornithine for arginine in total parenteral nutrition eliminates enhanced tumor growth. *J Surg Oncol* 50:161–167.

Growdon JH, Melamed E, Logue M, Hefti F, Wurtman RJ. 1982. Effects of oral L-tyrosine administration on CSF tyrosine and homovanillic acid levels in patients with Parkinson's disease. *Life Sci* 30:827–832.

Growdon JH, Nader TM, Schoenfeld J, Wurtman RJ. 1991. L-threonine in the treatment of spasticity. *Clin Neuropharmacol* 14:403–412.

Haddad EH, Berk LS, Kettering JD, Hubbard RW, Peters WR. 1999. Dietary intake and biochemical, hematologic, and immune status of vegans compared with nonvegetarians. *Am J Clin Nutr* 70:586S–593S.

Hagenfeldt L, Eriksson S, Wahren J. 1980. Influence of leucine on arterial concentrations and regional exchange of amino acids in healthy subjects. *Clin Sci* 59:173–181.

Hahn RG. 1988. Serum amino acid patterns and toxicity symptoms following the absorption of irrigant containing glycine in transurethral prostatic surgery. *Acta Anaesthesiol Scand* 32:493–501.

Hamilton B, Moriarty M. 1929. Comparison of growth in infancy. *Am J Dis Child* 37:1169.

Hansen RD, Raja C, Allen BJ. 2000. Total body protein in chronic diseases and in aging. *Ann NY Acad Sci* 904:345–352.

Hara S, Shibuya T, Nakakawaji K, Kyu M, Nakamura Y, Hoshikawa H, Takeuchi T, Iwao T, Ino H. 1962. Observations of pharmacological actions and toxicity of sodium glutamate, with comparisons between natural and synthetic products. *J Tokyo Med Coll* 20:69–100.

Harper AE. 1983. Dispensable and indispensable amino acid interrelationships. In: Blackburn GL, Grant JP, Young VR, eds. *Amino Acids. Metabolism and Medical Applications.* Boston: John Wright-PSG. Pp. 105–121.

Harper AE, Becker RV, Stucki WP. 1966. Some effects of excessive intakes of indispensable amino acids. *Proc Soc Exp Biol Med* 121:695–699.

Harper AE, Benevenga NJ, Wohlhueter RM. 1970. Effects of ingestion of disproportionate amounts of amino acids. *Physiol Rev* 50:428–558.

Harper AE, Miller RH, Block KP. 1984. Branched-chain amino acid metabolism. *Annu Rev Nutr* 4:409–454.

Harvey PW, Hunsaker HA, Allen KG. 1981. Dietary L-histidine-induced hypercholesterolemia and hypocupremia in the rat. *J Nutr* 111:639–647.

Hayasaka S, Saito T, Nakajima H, Takahashi O, Mizuno K, Tada K. 1985. Clinical trials of vitamin B_6 and proline supplementation for gyrate atrophy of the choroid and retina. *Br J Ophthalmol* 69:283–290.
Hays PM, Smeltzer JS. 1986. Multiple gestation. *Clin Obstet Gynecol* 29:264–285.
Health and Welfare Canada. 1990. *Report of the Expert Advisory Committee on Amino Acids*. Minister of Supply and Services Canada: Ottawa, Canada.
Hediger ML, Scholl TO, Ances IG, Belsky DH, Salmon RW. 1990. Rate and amount of weight gain during adolescent pregnancy: Associations with maternal weight-for-height and birth weight. *Am J Clin Nutr* 52:793–799.
Hegsted DM. 1963. Variation in requirements of nutrients: amino acids. *Fed Proc* 22:1420-1430.
Hegsted DM. 1976. Balance studies. *J Nutr* 106:307–311.
Hegsted DM. 1978. Assessment of nitrogen requirements. *Am J Clin Nutr* 31:1669–1677.
Heine WE, Klein PD, Reeds PJ. 1991. The importance of α-lactalbumin in infant nutrition. *J Nutr* 121:277–283.
Heinig MJ, Nommsen LA, Peerson JM, Lönnerdal B, Dewey KG. 1993. Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: The DARLING Study. *Am J Clin Nutr* 58:152–161.
Heird WC, Driscoll JM, Schullinger JN, Grebin B, Winters RW. 1972. Intravenous alimentation in pediatric patients. *J Pediatr* 80:351–372.
Hellekson KL. 2001. NIH consensus statement on phenylketonuria. *Am Fam Physician* 63:1430–1432.
Henkin RI, Patten BM, Re PK, Bronzert DA. 1975. A syndrome of acute zinc loss. Cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. *Arch Neurol* 32:745–751.
Hershko A, Ciechanover A. 1998. The ubiquiten system. *Ann Rev Biochem* 67:425–479.
Hevia P, Kari FW, Ulman EA, Visek WJ. 1980a. Serum and liver lipids in growing rats fed casein with L-lysine. *J Nutr* 110:1224–1230.
Hevia P, Ulman EA, Kari FW, Visek WJ. 1980b. Serum lipids of rats fed excess L-lysine and different carbohydrates. *J Nutr* 110:1231–1239.
Hibbs JR, Mittleman B, Hill P, Medsger TA. 1992. L-Tryptophan-associated eosinophilic fasciitis prior to the 1989 eosinophilia-myalgia syndrome outbreak. *Arthritis Rheum* 35:299–303.
Higgins AC. 1976. Nutritional status and the outcome of pregnancy. *J Can Diet Assoc* 37:17–35.
Hill GL. 1992. Body composition research: Implications for the practice of clinical nutrition. *J Parenteral Enteral Nutr* 16:197–218.
Himwich WA, Petersen IM, Graves JP. 1954. Ingested sodium glutamate and plasma levels of glutamic acid. *J Appl Physiol* 1:196–199.
Hitomi-Ohmura E, Amano N, Aoyama Y, Yoshida A. 1992. The effect of a histidine-excess diet on cholesterol synthesis and degradation in rats. *Lipids* 27:755–760.
Hood DA, Terjung RL. 1990. Amino acid metabolism during exercise and following endurance training. *Sports Med* 9:23–35.
Hoorn AJ. 1989. Dimethylglycine and chemically related amines tested for mutagenicity under potential nitrosation conditions. *Mutat Res* 222:343–350.
Hornsby-Lewis L, Shike M, Brown P, Klang M, Pearlstone D, Brennan MF. 1994. L-Glutamine supplementation in home total parenteral nutrition patients: Stability, safety, and effects on intestinal absorption. *J Parenteral Enteral Nutr* 18:268–273.

Howat PM, Korslund MK, Abernathy RP, Ritchy SJ. 1975. Sweat losses by and nitrogen balance of preadolescent girls consuming three levels of dietary protein. *Am J Clin Nutr* 28:879–882.

Hrboticky N, Leiter LA, Anderson GH. 1985. Effects of L-tryptophan on short term food intake in lean men. *Nutr Res* 5:595–607.

Huang P-C, Lin CP, Hsu JY. 1980. Protein requirements of normal infants at the age of 1 year: Maintenance nitrogen requirement and obligatory nitrogen losses. *J Nutr* 110:1727–1735.

Hurson M, Regan MC, Kirk SJ, Wasserkrug HL, Barbul A. 1995. Metabolic effects of arginine in a healthy elderly population. *J Parenteral Enteral Nutr* 19:227–230.

Hutson SM, Harper AE. 1981. Blood and tissue branched-chain amino and α-keto acid concentrations: Effect of diet, starvation, and disease. *Am J Clin Nutr* 34:173–183.

Hytten FE, Leitch I. 1971. *The Physiology of Human Pregnancy, 2nd ed.* Oxford: Blackwell.

Ikezaki S, Nishikawa A, Furukawa F, Imazawa T, Enami T, Mitsui M, Takahashi M. 1994. 13-Week subchronic toxicity study of L-histidine monohydrochloride in F344 rats. *Eisei Shikenjo Hokoku* 112:57–63.

Ikezaki S, Nishikawa A, Furukawa F, Enami T, Mitsui M, Tanakamaru Z, Kim HC, Lee IS, Imazawa T, Takahashi M. 1996. Long-term toxicity/carcinogenicity study of L-histidine monohydrochloride in F344 rats. *Food Chem Toxicol* 34:687–691.

Inoue G, Fujita Y, Niiyama Y. 1973. Studies on protein requirements of young men fed egg protein and rice protein with excess and maintenance energy intakes. *J Nutr* 103:1673–1687.

Intengan CL. 1984. Protein requirements of Filipino children 22–29 months old consuming local diets. In: Rand WM, Uauy R, Scrimshaw NS, eds. *Protein-Energy Requirement Studies in Developing Countries: Results of International Research.* Tokyo, Japan: United Nations University Press.

Intengan CL, Roxas BV, Loyola A, Carlos E. 1981. Protein requirements of Filipino children 20 to 29 months old consuming local diets. In: Torun B, Young VR, Rand WM, eds. *Protein-Energy Requirements of Developing Countries: Evaluation of New Data.* Tokyo, Japan: United Nations University Press. Pp. 172–181.

Inubushi T, Shikiji M, Endo K, Kakegawa H, Kishino Y, Katunuma N. 1996. Hormonal and dietary regulation of lysosomal cysteine proteinases in liver under gluconeogenesis conditions. *Biol Chem* 377:539–542.

Iob V, Swanson WW. 1934. Mineral growth of the human fetus. *Am J Dis Child* 47:302.

IOM (Institute of Medicine). 1990. *Nutrition During Pregnancy.* Washington, DC: National Academy Press.

IOM. 1991. *Nutrition During Lactation.* Washington, DC: National Academy Press.

Ip CC, Harper AE. 1973. Effects of dietary protein content and glucagon administration on tyrosine metabolism and tyrosine toxicity in the rat. *J Nutr* 103:1594–1607.

Isidori A, Lo Monaco A, Cappa M. 1981. A study of growth hormone release in man after oral administration of amino acids. *Curr Med Res Opin* 7:475–481.

Istfan N, Murray E, Janghorbani M, Young VR. 1983. An evaluation of the nutritional value of a soy protein concentrate in young adult men using the short-term N-balance method. *J Nutr* 113:2516–2523.

Iwata S, Ichimura M, Matsuzawa Y, Takasaki Y, Sasaoka M. 1979. Behavioural studies in rats treated with monosodium L-glutamate during the early stages of life. *Toxicol Lett* 4:345–357.

Jackson AA. 1989. Optimizing amino acid and protein supply and utilization in the newborn. *Proc Nutr Soc* 48:293–301.

Jackson AA. 1991. The glycine story. *Eur J Clin Nutr* 45:59–65.

Janas LM, Picciano MF, Hatch TF. 1985. Indices of protein metabolism in term infants fed human milk, whey-predominant formula, or cow's milk formula. *Pediatrics* 75:775–784.

Janas LM, Picciano MF, Hatch TF. 1987. Indices of protein metabolism in term infants fed either human milk or formulas with reduced protein concentration and various whey/casein ratios. *J Pediatrics* 10:838–848

Janelle KC, Barr SI. 1995. Nutrient intakes and eating behavior scores of vegetarian and nonvegetarian women. *J Am Diet Assoc* 95:180–186, 189.

Järvenpää AL, Räihä NCR, Rassin DK, Gaull GE. 1982a Milk protein quantity and quality in the term infant. I. Metabolic responses and effect on growth. *Pediatrics* 70:214–220.

Järvenpää AL, Räihä NCR, Rassin DK, Gaull GE. 1982b. Milk protein quantity and quality in the term infant. II. Effects on acidic and neutral amino acids. *Pediatrics* 70:221–230.

JECFA (Joint FAO/WHO Expert Committee on Food Additives). 1988. *Toxicological Evaluation of Certain Food Additives*. WHO Food Additive Series No. 22. Geneva: WHO/FAO.

Jefferson LS, Kimball S. 2001. Amino acid regulation of gene expression. *J Nutr* 131:2460S–2466S.

Jelliffe DB. 1966. The assessment of the nutritional status of the community. WHO Monograph Series No. 53. Geneva: WHO.

Jiang ZM, Cao JD, Zhu XG, Zhao WX, Yu JC, Ma EL, Wang XR, Zhu MW, Shu H, Liu YW. 1999. The impact of alanyl-glutamine on clinical safety, nitrogen balance, intestinal permeability, and clinical outcome in postoperative patients: A randomized, double-blind, controlled study in 120 patients. *J Parenter Enteral Nutr* 23:S62–S66.

Jones EM, Baumann CA, Reynolds MS. 1956. Nitrogen balances of women maintained on various levels of lysine. *J Nutr* 60:549–562.

Jungas RL, Halperin ML, Brosnan JT. 1992. Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiol Rev* 72:419–448.

Kakizoe T, Nishio Y, Honma Y, Niijima T, Sugimura T. 1983. L-Isoleucine and L-leucine are promoters of bladder cancer in rats. *Princess Takamatsu Symp* 14:373–380.

Kalhan SC. 2000. Protein metabolism in pregnancy. *Am J Clin Nutr* 71:1249S–1255S.

Kalhan SC, Devapatla S. 1999. Pregnancy, insulin resistance and nitrogen accretion. *Curr Opin Clin Nutr Metab Care* 2:359–363.

Kalhan SC, Rossi KQ, Gruca LL, Super DM, Savin SM. 1998. Relation between transamination of branched-chain amino acid and urea synthesis: Evidence from human pregnancy. *Am J Physiol* 275:E423–E431.

Kamin H, Handler P. 1951. Effect of infusion of single amino acids upon excretion of other amino acids. *Am J Physiol* 164:654–661.

Kampel D, Kupferschmidt R, Lubec G. 1990. Toxicity of D-proline. In: Lubec G, Rosenthal GA, eds. *Amino Acids: Chemistry, Biology, and Medicine*. ESCOM: Leiden, The Netherlands. Pp. 1164–1171.

Karlsen RL, Pedersen OO. 1982. A morphological study of the acute toxicity of L-cysteine on the retina of young rats. *Exp Eye Res* 34:65–69.
Katagiri M, Nakamura K. 2002. Animals are dependent on preformed α-amino nitrogen as an essential nutrient. *Life* 53:125–129.
Kawabe M, Takesada Y, Tamano S, Hagiwara A, Ito N, Shirai T. 1996. Subchronic toxicity study of L-isoleucine in F344 rats. *J Toxicol Environ Health* 47:499–508.
Kenney RA. 1986. The Chinese Restaurant Syndrome: An anecdote revisited. *Food Chem Toxicol* 24:351–354.
Kenney RA, Tidball CS. 1972. Human susceptibility to oral monosodium L-glutamate. *Am J Clin Nutr* 25:140–146.
Khatri IA, Forstner GG, Forstner F. 1998. Susceptibility of the cysteine-rich N-terminal and C-terminal ends of rat intestinal mucin Muc 2 to proteolytic cleavage. *Biochem J* 331:323–330.
Kim KI, McMillan I, Bayley HS. 1983. Determination of amino acid requirements of young pigs using an indicator amino acid. *Br J Nutr* 50:369–382.
King JC. 1975. Protein metabolism during pregnancy. *Clin Perinatol* 2:243–254.
King JC, Calloway DH, Margen S. 1973. Nitrogen retention, total body ^{40}K and weight gain in teenage pregnant girls. *J Nutr* 103:772–785.
Kirschner M. 1999. Intracellular proteolysis. *Trends Cell Biol* 9:M42–M45.
Klavins JV, Kinney TD, Kaufman N. 1963. Body iron levels and hematologic findings during excess methionine feeding. *J Nutr* 79:101–104.
Klein DG. 1990. Physiologic response to traumatic shock. *AACN Clin Issues Crit Care Nurs* 1:505–521.
Klimberg VS, McClellan J. 1996. Glutamine, cancer, and its therapy. *Am J Surg* 172:418–424.
Klimberg VS, Souba WW, Salloum RM, Plumley DA, Cohen FS, Dolson DJ, Bland KI, Copeland EM. 1990. Glutamine-enriched diets support muscle glutamine metabolism without stimulating tumor growth. *J Surg Res* 48:319–323.
Knopp RH, Brandt K, Arky RA. 1976. Effects of aspartame in young persons during weight reduction. *J Toxicol Environ Health* 2:417–428.
Knox WE, Horowitz ML, Friedell GH. 1969. The proportionality of glutaminase content to growth rate and morphology of rat neoplasms. *Cancer Res* 29:669–680.
Kopple JD. 1987. Uses and limitations of the balance technique. *J Parenter Enteral Nutr* 11:79S–85S.
Kopple JD, Swendseid ME. 1975. Evidence that histidine is an essential amino acid in normal and chronically uremic men. *J Clin Invest* 55:881–891.
Korslund MK, Leung EY, Meiners CR, Crews MG, Taper J, Abernathy RP, Ritchey SJ. 1976. The effects of sweat nitrogen losses in evaluating protein utilization by preadolescent children. *Am J Clin Nutr* 29:600–603.
Kovacevic Z, Morris HP. 1972. The role of glutamine in the oxidative metabolism of malignant cells. *Cancer Res* 32:326–333.
Kriengsinyos W, Wykes LJ, Ball RO, Pencharz PB. 2002. Oral and intravenous tracer protocols of the indicator amino acid oxidation method provide the same estimate of the lysine requirement in healthy men. *J Nutr* 132:2251–2257.
Krogh A, Krogh M. 1913. *A Study of the Diet and Metabolism of Eskimos.* Bianco Luno, Copenhagen.
Kudo Y, Boyd CA. 1990. Transport of amino acids by the human placenta: Predicted effects thereon of maternal hyperphenylalaninaemia. *J Inherit Metab Dis* 13:617–626.

Kurpad AV, Raj T, El-Khoury A, Beaumier L, Kuriyan R, Srivatsa A, Borgonha S, Selvaraj A, Regan MM, Young VR. 2001a. Lysine requirements of healthy adult Indian subjects, measured by an indicator amino acid balance technique. *Am J Clin Nutr* 73:900–907.

Kurpad AV, Raj T, El-Khoury A, Kuriyan R, Maruthy K, Borgonha S, Chandakudlu D, Regan MM, Young VR. 2001b. Daily requirement for and splanchnic uptake of leucine in healthy adult Indians. *Am J Clin Nutr* 74:747–755.

Kurpad AV, Regan MM, Raj T, El-Khoury A, Kuriyan R, Vaz M, Chandakudlu D, Venkataswamy VG, Borgonha S, Young VR. 2002a. Lysine requirements of healthy adult Indian subjects receiving long-term feeding, measured with a 24-h indicator amino acid oxidation and balance technique. *Am J Clin Nutr* 76:404–412.

Kurpad AV, Raj T, Regan MM, Vasudevan J, Caszo B, Nazareth D, Gnanou J, Young VR. 2002b. Threonine requirements of healthy Indian adults, measured by a 24-h indicator amino acid oxidation and balance technique. *Am J Clin Nutr* 76:789–797.

Labow BI, Souba WW. 2000. Glutamine. *World J Surg* 24:1503–1513.

Lacey JM, Crouch JB, Benfell K, Ringer SA, Wilmore CK, Maguire D, Wilmore DW. 1996. The effects of glutamine-supplemented parenteral nutrition in premature infants. *J Parenter Enteral Nutr* 20:74–80.

Laidlaw SA, Kopple JD. 1987. Newer concepts of the indispensable amino acids. *Am J Clin Nutr* 46:593–605.

Lammi-Keefe CJ, Ferris AM, Jensen RG. 1990. Changes in human milk at 0600, 1000, 1400, 1800, and 2200 h. *J Pediatr Gastroenterol Nutr* 11:83–88.

Lamperti A, Blaha G. 1976. The effects of neonatally-administered monosodium glutamate on the reproductive system of adult hamsters. *Biol Reprod* 14:362–369.

Lamperti A, Blaha G. 1980. Further observations on the effects of neonatally administered monosodium glutamate on the reproductive axis of hamsters. *Biol Reprod* 22:687–693.

Lazaris-Brunner G, Rafii M, Ball RO, Pencharz P. 1998. Tryptophan requirement in young adult women as determined by indicator amino acid oxidation with L-[^{13}C]-phenylalanine. *Am J Clin Nutr* 68:303–310.

Leathwood PD, Fernstrom JD. 1990. Effect of an oral tryptophan/carbohydrate load on tryptophan, large neutral amino acid, and serotonin and 5-hydroxyindoleacetic acid levels in monkey brain. *J Neural Transm Gen Sect* 79:25–34.

Leiderman E, Zylberman I, Zukin SR, Cooper TB, Javitt DC. 1996. Preliminary investigation of high-dose oral glycine on serum levels and negative symptoms in schizophrenia: An open-label trial. *Biol Psychiatry* 39:213–215.

Leiter LA, Hrboticky N, Anderson GH. 1987. Effects of L-tryptophan on food intake and selection in lean men and women. *Ann NY Acad Sci* 499:327–328.

Lemon PWR. 1996. Is increased dietary protein necessary or beneficial for individuals with a physically active lifestyle? *Nutr Rev* 54:S169–S175.

Lemon PW, Nagle FJ, Mullin JP, Benevenga NJ. 1982. In vivo leucine oxidation at rest and during two intensities of exercise. *J Appl Physiol* 53:947–954.

Lemon PW, Benevenga NJ, Mullin JP, Nagle FJ. 1985. Effect of daily exercise and food intake on leucine oxidation. *Biochem Med* 33:67–76.

Lemon PW, Tarnopolsky MA, MacDougall JD, Atkinson SA. 1992. Protein requirements and muscle mass/strength changes during intensive training in novice bodybuilders. *J Appl Physiol* 73(2):767-775.

Lemons JA, Moye L, Hall D, Simmons M. 1982. Differences in the composition of preterm and term human milk during early lactation. *Pediatr Res* 16:113–117.

Lenke RR, Levy HL. 1980. Maternal phenylketonuria and hyperphenylalaninemia. An international survey of the outcome of untreated and treated pregnancies. *N Engl J Med* 303:1202–1208.

Lentner C. 1981. *Geigy Scientific Tables, 8th ed.*, Vol. 1. *Units of Measurement, Body Fluids, Composition of the Body, Nutrition*. West Caldwell, NJ: Ciba-Geigy Corporation.

Leverton RM, Gram MR, Brodovsky E, Chaloupka M, Mitchell A, Johnson N. 1956a. The quantitative amino acid requirements of young women. II. Valine. *J Nutr* 58:83–93.

Leverton RM, Gram MR, Chaloupka M, Brodovsky E, Mitchell A. 1956b. The quantitative amino acid requirements of young women. I. Threonine. *J Nutr* 58:59–81.

Leverton RM, Johnson N, Ellison J, Geschwender D, Schmidt F. 1956c. The quantitative amino acid requirements of young women. IV. Phenylalanine, with and without tyrosine. *J Nutr* 58:341–353.

Leverton RM, Johnson N, Pazur J, Ellison J. 1956d. The quantitative amino acid requirements of young women. III. Tryptophan. *J Nutr* 58:219–229.

Levey S, Harroun JE, Smyth CJ. 1949. Serum glutamic acid levels and the occurrence of nausea and vomiting after the intravenous administration of amino acid mixtures. *J Lab Clin Med* 34:1238–1248.

Levy HM, Montanez G, Feaver ER, Murphy EA, Dunn MS. 1954. Effect of arginine on tumor growth in rats. *Cancer Res* 14:198–200.

Lieb CW. 1929. The effects on human beings of a twelve months' exclusive meat diet. *J Am Med Assoc* 93:20–22.

Lieberman HR, Corkin S, Spring BJ, Wurtman RJ, Growdon JH. 1985. The effects of dietary neurotransmitter precursors on human behavior. *Am J Clin Nutr* 42:366–370.

Lieberman HR, Caballero B, Emde GG, Bernstein JG. 1988. The effects of aspartame on human mood, performance, and plasma amino acid levels. In: Wurtman RJ, Ritter-Walker E, eds. *Dietary Phenylalanine and Brain Function*. Boston. Birkhauser. Pp. 198–200.

Linder-Horowitz M, Knox WE, Morris HP. 1969. Glutaminase activities and growth rates of rat hepatomas. *Cancer Res* 29:1195–1199.

Longenecker JB, Hause NL. 1959. Relationship between plasma amino acids and composition of ingested protein. *Arch Bioch Biophys* 84:46.

Longenecker JB, Hause NL. 1961. Relationship between plasma amino acids and composition of ingested protein. II. A shortened procedure to determine plasma amino acid (PAA) ratios. *Am J Clin Nutr* 9:356–362.

Lönnerdal B. 1986. Effects of maternal nutrition in human lactation. In: Hamosh M, Goldman AS, eds. *Human Lactation 2: Maternal and Environmental Factors*. New York: Plenum Press. Pp. 301–323.

Lönnerdal B, Chen CL. 1990. Effects of formula protein level and ration on infant growth, plasma amino acids and serum trace elements I: Cow's milk formula. *Acta Paediatr Scand* 79:257–265.

Lönnerdal B, Woodhouse LR, Glazier C. 1987. Compartmentalization and quantitation of protein in human milk. *J Nutr* 117:1385–1395.

LSRO (Life Sciences Research Office). 1992. *Safety of Amino Acids Used as Dietary Supplements*. Bethesda, MD: LSRO.

Lucas DR, Newhouse JP. 1957. The toxic effect of sodium L-glutamate on the inner layers of the retina. *AMA Arch Ophthalmol* 58:193–201.

Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. 1992. Breast milk and subsequent intelligence quotient in children born preterm. *Lancet* 339:261–264.

MacGillivray I, Buchanan TJ. 1958. Total exchangeable sodium and potassium in non-pregnant women and in normal and pre-eclamptic pregnancy. *Lancet* 2:1090–1093.

Manatt MW, Garcia PA. 1992. Nitrogen balance: Concepts and techniques. In: Nissen S, ed. *Modern Methods in Protein Nutrition and Metabolism.* San Diego: Academic Press. Pp. 9–63.

Marchesini G, Dioguardi FS, Bianchi GP, Zoli M, Bellati G, Roffi L, Martines D, Abbiati R. 1990. Long-term oral branched-chain amino acid treatment in chronic hepatic encephalopathy. A randomized double-blind casein-controlled trial. The Italian Multicenter Study Group. *J Hepatol* 11:92–101.

Mariotti F, Mahe S, Benamouzig R, Luengo C, Dare S, Gaudichon C, Tome D. 1999. Nutritional value of [^{15}N]-soy protein isolate assessed from ileal digestibility and postprandial protein utilization in humans. *J Nutr* 129:1992–1997.

Martindale LW. 1967. *Extra Pharmacopoeia, 25th ed.*. London: Pharmaceutical Press.

Massara F, Cagliero E, Bisbocci D, Passarino G, Carta Q, Molinatti GM. 1981. The risk of pronounced hyperkalaemia after arginine infusion in the diabetic subject. *Diabete Metab* 7:149–153.

Matsueda S, Niiyama Y. 1982. The effects of excess amino acids on maintenance of pregnancy and fetal growth in rats. *J Nutr Sci Vitaminol* 28:557–573.

Matsuzawa Y, Yonetani S, Takasaki Y, Iwata S, Sekine S. 1979. Studies on reproductive endocrine function in rats treated with monosodium L-glutamate early in life. *Toxicol Lett* 4:359–371.

Matthews DE. 1999. Proteins and amino acids. In: Shils ME, Olson JA, Shike M, Ross AC, eds. *Modern Nutrition in Health and Disease, 9th ed.* Baltimore: Williams and Wilkins. Pp. 11–48.

Maughan RJ, Sadler DJ. 1983. The effects of oral administration of salts of aspartic acid on the metabolic response to prolonged exhausting exercise in man. *Int J Sports Med* 4:119–123.

Mayes PA. 1990. Oxidation of fatty acids: Ketogenesis. In: Murray RK, Granner DK, Mayes PA, Rodwell VW, eds. *Harper's Biochemistry, 22nd ed.* Norwalk, CT: Appleton and Lange. Pp. 206–217.

McCarthy CF, Borland JL, Lynch HJ, Owen EE, Tyor MP. 1964. Defective uptake of basic amino acids and L-cystine by intestinal mucosa of patients with cystinuria. *J Clin Invest* 43:1518–1524.

McClellan WS, Du Bois EF. 1930. Clinical calorimetry XLV. Prolonged meat diets with a study of kidney function and ketosis. *J Biol Chem* 87:651–668.

McClellan WS, Rupp VR, Toscani V. 1930. Clinical calorimetry XLVI. Prolonged meat diets with a study of the metabolism of nitrogen, calcium and phosphorus. *J Biol Chem* 87:669–680.

McClellan WS, Spencer HJ, Falk EA 1931. Clinical calorimetry XLVII. Prolonged meat diets with a study of the respiratory metabolism. *J Biol Chem* 93:419–434.

McCune MA, Perry HO, Muller SA, O'Fallon WM. 1984. Treatment of recurrent herpes simplex infections with L-lysine monohydrochloride. *Cutis* 34:366–373.

McGilvery RW. 1983. *Biochemistry—A Functional Approach.* Philadelphia: WB Saunders. Pp. 791–793.

McNurlan MA, Garlick PJ. 1980. Contribution of rat liver and gastrointestinal tract to whole-body protein synthesis in the rat. *Biochem J* 186:381–383.

Meakins TS, Jackson AA. 1996. Salvage of exogenous urea nitrogen enhances nitrogen balance in normal men consuming marginally inadequate protein diets. *Clin Sci* 90:215–225.

Meguid MM, Matthews DE, Bier DM, Meredith CN, Soeldner JS, Young VR. 1986a. Leucine kinetics at graded leucine intakes in young men. *Am J Clin Nutr* 43:770–780.

Meguid MM, Matthews DE, Bier DM, Meredith CN, Young VR. 1986b. Valine kinetics at graded valine intakes in young men. *Am J Clin Nutr* 43:781–786.

Melamed E, Glaeser B, Growdon JH, Wurtman RJ. 1980. Plasma tyrosine in normal humans: Effects of oral tyrosine and protein-containing meals. *J Neural Trans* 47:299–306.

Meldrum BS. 2000. Glutamate as a neurotransmitter in the brain: Review of physiology and pathology. *J Nutr* 130:1007S–1015S.

Meredith CN, Wen ZM, Bier DM, Matthews DE, Young VR. 1986. Lysine kinetics at graded lysine intakes in young men. *Am J Clin Nutr* 43:787–794.

Meredith CN, Zackin MJ, Frontera WR, Evans WJ. 1989. Dietary protein requirements and body protein metabolism in endurance-trained men. *J Appl Physiol* 66:2850–2856.

Metges CC, El-Khoury AE, Henneman L, Petzke KJ, Grant I, Bedri S, Pereira PP, Ajami AM, Fuller MF, Young VR. 1999a. Availability of intestinal microbial lysine for whole body lysine homeostasis in human subjects. *Am J Physiol* 277:E597–E607.

Metges CC, Petzke KJ, El-Khoury AE, Henneman L, Grant I, Bedri S, Regan MM, Fuller MF, Young VR. 1999b. Incorporation of urea and ammonia nitrogen into ileal and fecal microbial proteins and plasma free amino acids in normal men and ileostomates. *Am J Clin Nutr* 70:1046–1058.

Millward DJ. 1998. Metabolic demands for amino acids and the human dietary requirement: Millward and Rivers (1988) revisited. *J Nutr* 128:2563S–2576S.

Millward DJ. 1999. The nutritional value of plant-based diets in relation to human amino acid and protein requirements. *Proc Nutr Soc* 58:249–260.

Millward DJ, Roberts SB. 1996. Protein requirements of older individuals. *Nutr Res Rev* 9:67–87.

Millward DJ, Price GM, Pacy PJ, Halliday D. 1990. Maintenance protein requirements: The need for conceptual re-evaluation. *Proc Nutr Soc* 49:473–487.

Millward DJ, Fereday A, Gibson N, Pacy PJ. 1997. Aging, protein requirements, and protein turnover. *Am J Clin Nutr* 66:774–786.

Milman N, Scheibel J, Jessen O. 1980. Lysine prophylaxis in recurrent herpes simplex labialis: A double-blind, controlled crossover study. *Acta Derm Venereol* 60:85–87.

Mizutani AR, Parker J, Katz J, Schmidt J. 1990. Visual disturbances, serum glycine levels and transurethral resection of the prostate. *J Urol* 144:697–699.

Moephuli SR, Klein NW, Baldwin MT, Krider HM. 1997. Effects of methionine on the cytoplasmic distribution of actin and tubulin during neural tube closure in rat embryos. *Proc Natl Acad Sci USA* 94:543–548.

Mogensen CE, Solling K. 1977. Studies on renal tubular protein reabsorption: Partial and near complete inhibition by certain amino acids. *Scand J Clin Lab Invest* 37:477–486.

Moldawer LL, Kawamura I, Bistrian BR, Blackburn GL. 1983. The contribution of phenylalanine to tyrosine in vivo: Studies in the post-absorptive and phenylalanine-loaded rat. *Biochem J* 210:811–817.

Moneret-Vautrin DA. 1987. Monosodium glutamate induced asthma: A study of the potential risk in 30 asthmatics and review of the literature. *Allerg Immunol (Paris)* 19:29–35.

Morehead RP, Fishman WH, Artom C. 1945. Renal injury in the rat following the administration of serine by stomach tube. *Am J Pathol* 21:803–815.

Morlion BJ, Stehle P, Wachtler P, Siedhoff HP, Koller M, Konig W, Furst P, Puchstein C. 1998. Total parenteral nutrition with glutamine dipeptide after major abdominal surgery: A randomised, double-blind, controlled study. *Ann Surg* 227:302–308.

Motil KJ, Opekun AR, Montandon CM, Berthold HK, Davis TA, Klein PD, Reeds PJ. 1994. Leucine oxidation changes rapidly after dietary protein intake is altered in adult women but lysine flux is unchanged as is lysine incorporation into VLDL-apolipoprotein B-100. *J Nutr* 124:41–51.

Motil KJ, Davis TA, Montandon CM, Wong WW, Klein PD, Reeds PJ. 1996. Whole-body protein turnover in the fed state is reduced in response to dietary protein restriction in lactating women. *Am J Clin Nutr* 64:32–39.

Motil KJ, Sheng H-P, Kertz BL, Montandon CM, Ellis KJ. 1998. Lean body mass of well-nourished women is preserved during lactation. *Am J Clin Nutr* 67:292–300.

Munro, HN. 1970. Free amino acid pools and their role in regulation. In: Munro HN, ed. *Mammalian Protein Metabolism*, Vol. IV. New York: Academic Press. Chap 34.

Muramatsu K, Odagiri H, Morishita S, Takeuchi H. 1971. Effect of excess levels of individual amino acids on growth of rats fed casein diets. *J Nutr* 101:1117–1125.

Nakagawa I, Takahashi T, Suzuki T. 1961a. Amino acid requirements of children: Isoleucine and leucine. *J Nutr* 73:186–190.

Nakagawa I, Takahashi T, Suzuki T. 1961b. Amino acid requirements of children: Minimal needs of lysine and methionine based on nitrogen balance method. *J Nutr* 74:401–407.

Nakagawa I, Takahashi T, Suzuki T, Kobayashi K. 1962. Amino acid requirements of children: Minimal needs of threonine, valine and phenylalanine based on nitrogen balance method. *J Nutr* 77:61–68.

Nakagawa I, Takahashi T, Suzuki T, Kobayashi K. 1963. Amino acid requirements of children: Minimal needs of tryptophan, arginine and histidine based on nitrogen balance method. *J Nutr* 80:305–310.

Nakagawa I, Takahashi T, Suzuki T, Kobayashi K. 1964. Amino acid requirements of children: Nitrogen balance at the minimal level of essential amino acids. *J Nutr* 83:115–118.

Neale RJ, Waterlow JC. 1974. The metabolism of ^{14}C-labelled essential amino acids given by intragastric or intravenous infusion to rats on normal and protein-free diets. *Br J Nutr* 32:11–25.

Neri DF, Wiegmann D, Stanny RR, Shappell SA, McCardie A, McKay DL. 1995. The effects of tyrosine on cognitive performance during extended wakefulness. *Aviat Space Environ Med* 66:313–319.

Neville MC, Keller RP, Seacat J, Casey CE, Allen JC, Archer P. 1984. Studies on human lactation. I. Within-feed and between-breast variation in selected components of human milk. *Am J Clin Nutr* 40:635–646.

Newsholme EA, Blomstrand E, Hassmen P, Ekblom B. 1991. Physical and mental fatigue: Do changes in plasma amino acids play a role? *Biochem Soc Trans* 19:358–362.

Nikoletseas MM. 1977. Obesity in exercising, hypophagic rats treated with monosodium glutamate. *Physiol Behav* 19:767–773.

Nishio Y, Kakizoe T, Ohtani M, Sato S, Sugimura T, Fukushima S. 1986. L-isoleucine and L-leucine: Tumor promoters of bladder cancer in rats. *Science* 231:843–845.

Nommsen LA, Lovelady CA, Heinig MJ, Lönnerdal B, Dewey KG. 1991. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: The DARLING Study. *Am J Clin Nutr* 53:457–465.

Oddoye EA, Margen S. 1979. Nitrogen balance studies in humans: Long-term effect of high nitrogen intake on nitrogen accretion. *J Nutr* 109:363–377.

Ohmura E, Aoyama Y, Yoshida A. 1986. Changes in lipids in liver and serum of rats fed a histidine-excess diet or cholesterol-supplemented diets. *Lipids* 21:748–753.

Oishi R, Furuno K, Gomita Y, Araki Y, Saeki K. 1989. Effect of acute treatment of mice with L-histidine on the brain levels of amino acids. *Jpn J Pharmacol* 49:143–146.

Olivo M, Kitahama K, Valatx JL, Jouvet M. 1986. Neonatal monosodium glutamate dosing alters the sleep-wake cycle of the mature rat. *Neurosci Lett* 67:186–190.

Olney JW. 1969. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* 164:719–721.

Olney JW. 1989. Glutamate, a neurotoxic transmitter. *J Child Neurol* 4:218–226.

Olney JW. 1994. Excitotoxins in foods. *Neuro Toxicol* 15:535–544.

Olney JW, Ho OL. 1970. Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature* 227:609–611.

Olney JW, Cicero TJ, Meyer ER, de Gubareff T. 1976. Acute glutamate-induced elevations in serum testosterone and luteinizing hormone. *Brain Res* 112:420–424.

Owen G, Cherry CP, Prentice DE, Worden AN. 1978a. The feeding of diets containing up to 4% monosodium glutamate to rats for 2 years. *Toxicol Lett* 1:221–226.

Owen G, Cherry CP, Prentice DE, Worden AN. 1978b. The feeding of diets containing up to 10% monosodium glutamate to beagle dogs for 2 years. *Toxicol Lett* 1:217–219.

Park KG, Heys SD, Blessing K, Kelly P, McNurlan MA, Eremin O, Garlick PJ. 1992. Stimulation of human breast cancers by dietary L-arginine. *Clin Sci* 82:413–417.

Patrick J, Pencharz PB, Belmonte M, Ste-Marie M, Boland MP, Issenman RM, Van Aerde JEE, Rousseau-Harsany E. 1994. Undernutrition in children with neurodevelopmental disability. *Can Med Assoc J* 151:753–759.

Pellett PL, Young VR. 1992. The effects of different levels of energy intake on protein metabolism and of different levels of protein intake on energy metabolism: A statistical evaluation from the published literature. In: Scrimshaw NS, Schürch B, eds. *Protein-Energy Interaction.* Lausanne, Switzerland: IDECG, Nestlé Foundation. Pp. 81–121.

Pencharz PB. 1985. Body composition and growth. In: Walker A, ed. *Nutrition in Pediatrics. Basic Science and Clinical Application.* Boston. Little, Brown. Pp. 77–85.

Pencharz PB, Azcue M. 1996. Use of bioelectrical impedance analysis (BIA) measurements in the clinical management of malnutrition. *Am J Clin Nutr* 64:S485–S488.

Pencharz BP, House JD, Wykes LJ, Ball RO. 1996. What are the essential amino acids for the preterm and term infant? In: Bindels JG, Goedhart A, Visser HKA, eds. *Recent Developments in Infant Nutrition. Nutricia Symposia Vol. 9.* Dordrecht, The Netherlands: Kluwer Academic Publishers. Pp. 278–296.

Pepplinkhuizen L, Bruinvels J, Blom W, Moleman P. 1980. Schizophrenia-like psychosis caused by a metabolic disorder. *Lancet* 1:454–456.

Perry TL, Hardwick DF, Dixon GH, Dolman CL, Hansen S. 1965. Hypermethioninemia: A metabolic disorder associated with cirrhosis, islet cell hyperplasia, and renal tubular degeneration. *Pediatrics* 36:236–250.

Persaud TV. 1969. The foetal toxicity of leucine in the rat. *West Indian Med J* 18:34–39.

Peters JC, Harper AE. 1987. Acute effects of dietary protein on food intake, tissue amino acids, and brain serotonin. *Am J Physiol* 252:R902–R914.

Picou D, Halliday D, Garrow JS. 1966. Total body protein, collagen and non-collagen protein in infantile protein malnutrition. *Clin Sci* 30:345–351.

Pilc A, Rogoz Z, Skuza G. 1982. Histidine-induced bizarre behaviour in rats: The possible involvement of central cholinergic system. *Neuropharmacology* 21:781–785.

Pinals RS, Harris ED, Burnett JB, Gerber DA. 1977. Treatment of rheumatoid arthritis with L-histidine: A randomized, placebo-controlled, double-blind trial. *J Rheumatol* 4:414–419.

Pineda O, Torun B, Viteri FE, Arroyave G. 1981. Protein quality in relation to estimates of essential amino acids requirements. In: Bodwell CE, Adkins JS, Hopkins DT, eds. *Protein Quality in Humans: Assessment and In Vitro Estimation.* Westport, CT: AVI Publishing. Pp. 29–42.

Pinto-Scognamiglio W, Amorico L, Gatti GL. 1972. Toxicity and tolerance to monosodium glutamate studied by a conditioned avoidance test. *Farmaco* 27:19–27.

Pipe NGJ, Smith T, Halliday D, Edmonds CJ, Williams C, Coltart TM. 1979. Changes in fat, fat-free mass and body water in human normal pregnancy. *Br J Obstet Gynaecol* 86:929–940.

Pizzi WJ, Barnhart JE, Fanslow DJ. 1977. Monosodium glutamate administration to the newborn reduces reproductive ability in female and male mice. *Science* 196:452–454.

Pizzi WJ, Tabor JM, Barnhart JE. 1978. Somatic, behavioral, and reproductive disturbances in mice following neonatal administration of sodium L-aspartate. *Pharmacol Biochem Behav* 9:481–485.

Pollitt E. 2000. Developmental sequel from early nutritional deficiencies: Conclusive and probability judgements. *J Nutr* 130:350S–353S.

Poon TK, Cameron DP. 1978. Measurement of oxygen consumption and locomotor activity in monosodium glutamate-induced obesity. *Am J Physiol* 234:E532–E534.

Porter PB, Griffin AC. 1950. Effects of glutamic acid on maze learning and recovery from electroconvulsive shocks in albino rats. *J Comp Physiol Psychol* 43:1–15.

Pradhan SN, Lynch JF. 1972. Behavioral changes in adult rats treated with monosodium glutamate in the neonatal stage. *Arch Int Pharmacodyn Ther* 197:301–304.

Pratt EL, Snyderman SE, Cheung MW, Norton P, Holt LE. 1955. The threonine requirement of the normal infant. *J Nutr* 56:231–251.

Prentice AM, Goldberg GR, Prentice A. 1994. Body mass index and lactation performance. *Eur J Clin Nutr* 48:S78–S86.

Prosky L, O'Dell RG. 1972. Biochemical changes of brain and liver in neonatal offspring of rats fed monosodium-L-glutamate. *Experientia* 28:260–263.

Raguso CA, Pereira P, Young VR. 1999. A tracer investigation of obligatory oxidative amino acids losses in healthy, young adults. *Am J Clin Nutr* 70:474–483.

Räihä N, Minoli I, Moro G. 1986a. Milk protein intake in the term infant I: Metabolic responses and effects on growth. *Acta Paediatr Scand* 75:881–886.

Räihä N, Minoli I, Moro G. 1986b. Milk protein intake in the term infant II: Effects on plasma amino acid concentrations. *Acta Paediatr Scand* 75:887–892.

Raiten DJ, Talbot JM, Fisher KD. 1995. *Analysis of Adverse Reactions to Monosodium Glutamate (MSG).* Bethesda, MD: Federation of American Societies for Experimental Biology.

Ramsey BW, Farrell P, Pencharz PB. 1992. Nutritional assessment and management in cystic fibrosis: a consensus report. *Am J Clin Nutr* 55:108–116.

Rand WM, Young VR. 1999. Statistical analysis of nitrogen balance data with reference to the lysine requirement in adults. *J Nutr* 129:1920–1926.
Rand WM, Young VR, Scrimshaw NS. 1976. Change of urinary nitrogen excretion in response to low-protein diets in adults. *Am J Clin Nutr* 29:639–644.
Rand WM, Scrimshaw NS, Young VR. 1981. Conventional ("long-term") nitrogen balance studies for protein quality evaluation in adults: Rationale and limitations. In: Bodwell CE, Adkins JS, Hopkins DT, eds. *Protein Quality in Humans: Assessment and In Vitro Estimation.* Westport, CT: AVI Publishing. Pp. 61–94.
Rand RM, Pellett PL, Young VR. 2003. Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. *Am J Clin Nutr* 77:109–127.
Reeds PJ, Burrin DG. 2001. Glutamine and the bowel. *J Nutr* 131:2505S–2508S.
Reeds PJ, Garlick PJ. 1984. Nutrition and protein turnover in man. *Adv Nutr Res* 6:93–138.
Reeds PJ, Field CR, Jahoor F. 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *J Nutr* 124:906–910.
Reif-Lehrer L. 1976. Possible significance of adverse reactions to glutamate in humans. *Fed Proc* 35:2205–2211.
Rennie MJ, Edwards RH, Krywawych S, Davies CT, Halliday D, Waterlow JC, Millward DJ. 1981. Effect of exercise on protein turnover in man. *Clin Sci (Lond)* 61:627–639.
Reynolds JV, Thom AK, Zhang SM, Ziegler MM, Naji A, Daly JM. 1988. Arginine, protein malnutrition, and cancer. *J Surg Res* 45:513–522.
Reynolds JV, Daly JM, Shou J, Sigal R, Ziegler MM, Naji A. 1990. Immunologic effects of arginine supplementation in tumor-bearing and non-tumor-bearing hosts. *Ann Surg* 211:202–210.
Reynolds JV, O'Farrelly C, Feighery C, Murchan P, Leonard N, Fulton G, O'Morain C, Keane FB, Tanner WA. 1996. Impaired gut barrier function in malnourished patients. *Br J Surg* 83:1288–1291.
Reynolds MS, Steel DL, Jones EM, Baumann CA. 1958. Nitrogen balances of women maintained on various levels of methionine and cystine. *J Nutr* 64:99–111.
Reynolds WA, Stegink LD, Filer LJ Jr, Renn E. 1980. Aspartame administration to the infant monkey: Hypothalamic morphology and plasma amino acid levels. *Anat Rec* 198:73–85.
Rich LF, Beard ME, Burns RP. 1973. Excess dietary tyrosine and corneal lesions. *Exp Eye Res* 17:87–97.
Rigo J, Senterre H. 1980. Optimal threonine intake for preterm infants fed on oral or parenteral nutrition. *J Parenteral Enteral Nutr* 4:15–17.
Roberton AM, Rabel B, Harding CA, Tasman-Jones C, Harris PJ, Lee SP. 1991. Use of the ileal conduit as a model for studying human small intestinal mucus glycoprotein secretion. *Am J Physiol* 261:G728–G734.
Roberts S. 1996. Energy requirements of older individuals. *Eur J Clin Nutr* 50:S112–S118.
Roberts S, Thorpe JM, Ball RO, Pencharz PB. 2001. Tyrosine requirement of healthy men receiving a fixed phenylalanine intake determined by using indicator amino acid oxidation. *Am J Clin Nutr* 73:276–282.
Rodwell VW. 1990. Conversion of amino acids to specialized products. In: Murray RK, Mayes PA, Granner DK, Rodwell VW, eds. *Harper's Biochemistry*, 22nd ed. Norwalk, CT: Appleton & Lange. Pp. 307–313.
Rogan WJ, Gladen BC. 1993. Breast-feeding and cognitive development. *Early Human Dev* 31:181–193.

Roig JC, Meetze WH, Auestad N, Jasionowski T, Veerman M, McMurray CA, Neu J. 1996. Enteral glutamine supplementation for the very low birthweight infant: Plasma amino acid concentrations. *J Nutr* 126:1115S–1120S.

Ronnenberg AG, Gross KL, Hartman WJ, Meydani SN, Prior RL. 1991. Dietary arginine supplementation does not enhance lymphocyte proliferation or interleukin-2 production in young and aged rats. *J Nutr* 121:1270–1278.

Rose DP, Leklem JE, Fardal L, Baron RB, Shrago E. 1977. Effect of oral alanine loads on the serum triglycerides of oral contraceptive users and normal subjects. *Am J Clin Nutr* 30:691–694.

Rose WC. 1957. The amino acid requirements of adult man. *Nutr Abs Rev* 27:631–647.

Rose WC, Haines WJ, Warner DT, Johnson JE. 1951. The amino acid requirements of man. II. The role of threonine and histidine. *J Biol Chem* 188:49–58.

Rose WC, Borman A, Coon MJ, Lambert GF. 1955a. The amino acid requirements of man. X. The lysine requirement. *J Biol Chem* 214:579–587.

Rose WC, Coon MJ, Lambert GF. 1955b. The amino acid requirements of man. VIII. The metabolic availability of the optical isomers of acetyltryptophan. *J Biol Chem* 212:201–205.

Rose WC, Coon MJ, Lockhart HB, Lambert GF. 1955c. The amino acid requirements of man. XI. The threonine and methionine requirements. *J Biol Chem* 215:101–110.

Rose WC, Eades CH, Coon MJ. 1955d. The amino acid requirements of man. XII. The leucine and isoleucine requirements. *J Biol Chem* 216:225–234.

Rose WC, Leach BE, Coon MJ, Lambert GF. 1955e. The amino acid requirements of man. IX. The phenylalanine requirement. *J Biol Chem* 213:913–922.

Rose WC, Wixom RL, Lockhart HB, Lambert GF. 1955f. The amino acid requirements of man. XV. The valine requirement; Summary and final observations. *J Biol Chem* 217:987–995.

Rosenberg LE, Downing S, Durant JL, Segal S. 1966. Cystinuria: Biochemical evidence for three genetically distinct diseases. *J Clin Invest* 45:365–371.

Rudman D, DiFulco TJ, Galambos JT, Smith RB, Salam AA, Warren WD. 1973. Maximal rates of excretion and synthesis of urea in normal and cirrhotic subjects. *J Clin Invest* 52:2241–2249.

Ryan-Harshman M, Leiter LA, Anderson GH. 1987. Phenylalanine and aspartame fail to alter feeding behavior, mood and arousal in men. *Physiol Behav* 39:247–253.

Said AK, Hegsted DM. 1970. Response of adult rats to low dietary levels of essential amino acids. *J Nutr* 100:1362–1375.

Sauberlich HE. 1961. Studies on the toxicity and antagonism of amino acids for weanling rats. *J Nutr* 75:61–72.

Schaafsma G. 2000. The protein digestibility-corrected amino acid score. *J Nutr* 130:1865S–1867S.

Schainker B, Olney JW. 1974. Glutamate-type hypothalamic-pituitary syndrome in mice treated with aspartate or cysteate in infancy. *J Neural Trans* 35:207–215.

Schaumburg HH, Byck R. 1968. Sin cib-syn: Accent on glutamate. *N Engl J Med* 279:105.

Schaumburg HH, Byck R, Gerstl R, Mashman JH. 1969. Monosodium L-glutamate: Its pharmacology and role in the Chinese restaurant syndrome. *Science* 163:826–828.

Schechter PJ, Prakash NJ. 1979. Failure of oral L-histidine to influence appetite or affect zinc metabolism in man: A double-blind study. *Am J Clin Nutr* 32:1011–1014.

Scholl TO, Hediger ML, Ances IG. 1990. Maternal growth during pregnancy and decreased infant birth weight. *Am J Clin Nutr* 51:790–793.
Scholl TO, Hediger ML, Schall JI, Khoo C-S, Fischer RL. 1994. Maternal growth during pregnancy and the competition for nutrients. *Am J Clin Nutr* 60:183–188.
Schwartz JC, Lampart C, Rose C. 1972. Histamine formation in rat brain in vivo: Effects of histidine loads. *J Neurochem* 19:801–810.
Schwartzstein RM, Kelleher M, Weinberger SE, Weiss JW, Drazen JM. 1987. Airways effects of monosodium glutamate in subjects with chronic stable asthma. *J Asthma* 24:167–172.
Scrimshaw NS, Hussein MA, Murray E, Rand WM, Young VR. 1972. Protein requirements of man: Variations in obligatory urinary and fecal nitrogen losses in young men. *J Nutr* 102:1595–1604.
Scriver CR, Kaufman S, Woo SL. 1989. The hyperphenylalaninemias. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic Basis of Inherited Disease*, 6th ed. New York: McGraw-Hill. Pp. 495–546.
Semprini ME, Frasca MA, Mariani A. 1971. Effects of monosodium glutamate (MSG) administration on rats during the intrauterine life and the neonatal period. *Quaderni delle Nutrizione* 31:85–100.
Sen Gupta J, Srivastava KK. 1973. Effect of potassium-magnesium aspartate on endurance work in man. *Ind J Exp Biol* 11:392–394.
Shaw GM, Velie EM, Schaffer DM. 1997. Is dietary intake of methionine associated with a reduction in risk for neural tube defect-affected pregnancies? *Teratology* 56:295–299.
Simon CA, Van Melle GD, Ramelet AA. 1985. Failure of lysine in frequently recurrent herpes simplex infection. *Arch Dermatol* 121:167–168.
Simon RA. 2000. Additive-induced urticaria: Experience with monosodium glutamate (MSG). *J Nutr* 130:1063S–1066S.
Sivam SP, Chermak T. 1992. Neonatal administration of L-cysteine does not produce long-term effects on neurotransmitter or neuropeptide systems in the rat striatum. *Res Comm Chem Pathol Pharm* 77:219–225.
Skeie B, Kvetan V, Gil KM, Rothkopf MM, Newsholme EA, Askanazi J. 1990. Branch-chain amino acids: Their metabolism and clinical utility. *Crit Care Med* 18:549–571.
Smith B, Prockop DJ. 1962. Central-nervous-system effects of ingestion of L-tryptophan by normal subjects. *N Engl J Med* 267:1338–1341.
Snyderman SE, Pratt EL, Cheung MW, Norton P, Holt LE, Hansen AE, Panos TC. 1955. The phenylalanine requirement of the normal infant. *J Nutr* 56: 253–263.
Snyderman SE, Holt LE, Smellie F, Boyer A, Westall RG. 1959a. The essential amino acid requirements of infants: Valine. *Am J Dis Child* 97:186–191.
Snyderman SE, Norton PM, Fowler DI, Holt LE. 1959b. The essential amino acid requirements of infants: Lysine. *Am J Dis Child* 97:175–185.
Snyderman SE, Boyer A, Phansalkar SV, Holt LE. 1961a. Essential amino acid requirements of infants. Tryptophan. *Am J Dis Child* 102:41–45.
Snyderman SE, Roitman EL, Boyer A, Holt LE. 1961b. Essential amino acid requirements of infants. Leucine. *Am J Dis Child* 102:35–40.
Snyderman SE, Boyer A, Roitman E, Holt LE, Prose PH. 1963. The histidine requirement of the infant. *Pediatrics* 31:786–801.
Snyderman SE, Boyer A, Norton PM, Roitman E, Holt LE. 1964a. The essential amino acid requirements of infants. IX. Isoleucine. *Am J Clin Nutr* 15:313–321.

Snyderman SE, Boyer A, Norton PM, Roitman E, Holt LE. 1964b. The essential amino acid requirements of infants. X. Methionine. *Am J Clin Nutr* 15:322–330.
Sole MJ, Benedict CR, Myers MG, Leenen FH, Anderson GH. 1985. Chronic dietary tyrosine supplements do not affect mild essential hypertension. *Hypertension* 7:593–596.
Solomon JK, Geison RL. 1978. Effect of excess dietary L-histidine on plasma cholesterol levels in weanling rats. *J Nutr* 108:936–943.
Souba WW. 1993. Glutamine and cancer. *Ann Surg* 218:715–728.
Speth JD. 1989. Early hominid hunting and scavenging: The role of meat as an energy source. *J Hum Evol* 18:329–343.
Speth JD, Spielmann KA. 1983. Energy source, protein metabolism, and hunter-gatherer subsistence strategies. *J Anthropol Archaeol* 2:1–31.
Stechmiller JK, Treloar D, Allen N. 1997. Gut dysfunction in critically ill patients: A review of the literature. *Am J Crit Care* 6:204–209.
Steele RD, Barber TA, Lalich J, Benevenga NJ. 1979. Effects of dietary 3-methylthiopropionate on metabolism, growth and hematopoiesis in the rat. *J Nutr* 109:1739–1751.
Stefansson V. 1944a. *Arctic Manual.* New York: Macmillan.
Stefansson V. 1944b. Pemmican. *Military Surg* 95:89–98.
Stegink LD. 1976. Absorption, utilization, and safety of aspartic acid. *J Toxicol Environ Health* 2:215–242.
Stegink LD, Shepherd JA, Brummel MC, Murray LM. 1974. Toxicity of protein hydrolysate solutions: Correlation of glutamate dose and neuronal necrosis to plasma amino acid levels in young mice. *Toxicology* 2:285–299.
Stegink LD, Filer LJ, Baker GL. 1977. Effect of aspartame and aspartate loading upon plasma and erythrocyte free amino acid levels in normal adult volunteers. *J Nutr* 107:1837–1845.
Stegink LD, Filer LJ, Baker GL. 1980. Plasma methionine levels in normal adult subjects after oral loading with L-methionine and N-acetyl-L-methionine. *J Nutr* 110:42–49.
Stegink LD, Filer LJ, Baker GL. 1982a. Plasma and erythrocyte amino acid levels in normal adult subjects fed a high protein meal with and without added monosodium glutamate. *J Nutr* 112:1953–1960.
Stegink LD, Filer LJ, Baker GL. 1982b. Plasma and urinary methionine levels in one-year-old infants after oral loading with L-methionine and N-acetyl-L-methionine. *J Nutr* 112:597–603.
Stegink LD, Filer LJ Jr, Baker GL. 1983a. Effect of carbohydrate on plasma and erythrocyte glutamate levels in humans ingesting large doses of monosodium L-glutamate in water. *Am J Clin Nutr* 37:961–968.
Stegink LD, Filer LJ Jr, Baker GL. 1983b. Plasma amino acid concentrations in normal adults fed meals with added monosodium L-glutamate and aspartame. *J Nutr* 113:1851–1860.
Stekol JA, Szaran J. 1962. Pathological effects of excessive methionine in the diet of growing rats. *J Nutr* 77:81–90.
Stellar E, McElroy WD. 1948. Does glutamic acid have any effect on learning? *Science* 108:281–283.
Stephenson LS, Lathan MC, Ottesen EA. 2000. Global malnutrition. *Parasitology* 121:S5–S22.
Stevenson DD. 2000. Monosodium glutamate and asthma. *J Nutr* 130:1067S–1073S.

Stokes AF, Belger A, Banich MT, Taylor H. 1991. Effects of acute aspartame and acute alcohol ingestion upon the cognitive performance of pilots. *Aviat Space Environ Med* 62:648–653.

Stoll B, Henry J, Reeds PJ, Yu H, Jahoor F, Burrin DG. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J Nutr* 128:606–614.

Strain GW, Strain JJ, Zumoff B. 1985. L-Tryptophan does not increase weight loss in carbohydrate-craving obese subjects. *Int J Obes* 9:375–380.

Sweetman L. 1989. Branched chain organic acidurias. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic Basis of Inherited Disease*, 6th ed. New York: McGraw-Hill. Pp. 791–819.

Swick RW, Benevenga NJ. 1977. Labile protein reserves and protein turnover. *J Dairy Sci* 60:505–515.

Tachibana K Mukai K, Hiraoka I, Moriguchi S, Takama S, Kishino Y. 1985. Evaluation of the effect of arginine-enriched amino acid solution on tumor growth. *J Parenter Enteral Nutr* 9:428–434.

Tarasoff L, Kelly MF. 1993. Monosodium L-glutamate: A double-blind study and review. *Food Chem Toxicol* 31:1019–1035.

Tarnopolsky MA, MacDougall JD, Atkinson SA. 1988. Influence of protein intake and training status on nitrogen balance and lean body mass. *J Appl Physiol* 64:187–193.

Tarnopolsky MA, Atkinson SA, MacDougall JD, Senor BB, Lemon PW, Schwarcz H. 1991. Whole body leucine metabolism during and after resistance exercise in fed humans. *Med Sci Sports Exer*c 23:326–333.

Taverner MR, Hume ID, Farrell DJ. 1981. Availability to pigs of amino acids in cereal grains. 1. Endogenous levels of amino acids in ileal digesta and faeces of pigs given cereal diets. *Br J Nutr* 46:149–158.

Terry LC, Epelbaum J, Martin JB. 1981. Monosodium glutamate: Acute and chronic effects on rhythmic growth hormone and prolactin secretion, and somatostatin in the undisturbed male rat. *Brain Res* 217:129–142.

Thein DJ, Hurt WC. 1984. Lysine as a prophylactic agent in the treatment of recurrent herpes simplex labialis. *Oral Surg* 58:659–666.

Thoemke F, Huether G. 1984. Breeding rats on amino acid imbalanced diets for three consecutive generations affects the concentrations of putative amino acid transmitters in the developing brain. *Int J Dev Neurosci* 2:567–574.

Thompson GN, Halliday D. 1992. Protein turnover in pregnancy. *Eur J Clin Nutr* 46:411–417.

Torun B, Viteri FE. 1981. Obligatory nitrogen losses and factorial calculations of protein requirements of pre-school children. In: Torun B, Young VR, Rand WM, eds. *Protein-Energy Requirements of Developing Countries: Evaluation of New Data*. Tokyo, Japan: United Nations University Press. Pp. 159–163.

Torun B, Cabrera Santiago M, Viteri FE. 1981. Protein requirements of pre-school children: Milk and soybean protein isolate. In: Torun B, Young VR, Rand WM, eds. *Protein-Energy Requirements of Developing Countries: Evaluation of New Data*. Tokyo, Japan: United Nations University Press. Pp. 182–190.

Uauy R, Scrimshaw NS, Young VR. 1978. Human protein requirements: Nitrogen balance response to graded levels of egg protein in elderly men and women. *Am J Clin Nutr* 31:779–785.

Uauy R, Yanez E, Ballester D, Barrera G, Guzman E, Saitua MT, Zacaris I. 1981. Obligatory urinary and faecal nitrogen losses in young Chilean men fed two levels of dietary energy intake. In: Torun B, Young VR, Rand WM, eds. *Protein-Energy Requirements of Developing Countries: Evaluation of New Data*. Tokyo, Japan: United Nations University Press.

van Acker BA, von Meyenfeldt MF, van der Hulst RR, Hulsewe KW, Wagenmakers AJ, Deutz NE, de Blaauw I, Dejong CH, van Kreel BK, Soeters PB. 1999. Glutamine: The pivot of our nitrogen economy? *J Parenter Enteral Nutr* 23:S45–S48.

van der Schoor SRD, van Goudoever JB, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Reeds PJ. 2001. The pattern of intestinal substrate oxidation is altered by protein restriction in pigs. *Gastroenterology* 121:1167–1175.

van Raaij JMA, Pee MEM, Vermaat-Miedema SH, Schonk CM, Hautvast JGAJ 1988. New equations for estimating body fat mass in pregnancy from body density or total body water. *Am J Clin Nutr* 48:24–29.

van Wouwe JP, Hoogenkamp S, Van den Hamer CJ. 1989. Histidine supplement and Zn status in Swiss random mice. *Biol Trace Elem Res* 22:35–43.

Viau AT, Leathem JH. 1973. Excess dietary methionine and pregnancy in the rat. *J Reprod Fertil* 33:109–111.

Vijayasarathy C, Khan-Siddiqui L, Murthy SN, Bamji MS. 1987. Rise in plasma trimethyllysine levels in humans after oral lysine load. *Am J Clin Nutr* 46:772–777.

Villalpando S, Butte NF, Flores-Huerta S, Thotathuchery M. 1998. Qualitative analysis of human milk produced by women consuming a maize-predominant diet typical of rural Mexico. *Ann Nutr Metab* 42:23–32.

Viteri FE, Martinez C. 1981. Integumental nitrogen losses of pre-school children with different levels and sources of dietary protein intake. In: Torun B, Young VR, Rand WM, eds. *Protein-Energy Requirements of Developing Countries: Evaluation of New Data*. Tokyo, Japan: United Nations University Press.

Wachstein M. 1947. Nephrotoxic action of dl-serine in the rat. II. The protective action of various amino acids and some other compounds. *Arch Pathol* 43:515–526.

Wagenmakers AJ. 1998. Muscle amino acid metabolism at rest and during exercise: Role in human physiology and metabolism. *Exerc Sport Sci Rev* 26:287–314.

Waisman HA, Harlow HF. 1965. Experimental phenylketonuria in infant monkeys: A high phenylalanine diet produces abnormalities simulating those of the hereditary disease. *Science* 147:685–695.

Wang JML, Creel DJ, Wong KC. 1989. Transurethral resection of the prostate, serum glycine levels, and ocular evoked potentials. *Anesthesiology* 70:36–41.

Waterlow JC. 1969. The assessment of protein nutrition and metabolism in the whole animal, with special reference to man. In: Munro HN ed. *Mammalian Protein Metabolism*, Vol III. New York: Academic Press. Pp. 347–348.

Waterlow JC. 1984. Protein turnover with special reference to man. *Quart J Exp Physiol* 69:409–438.

Waterlow JC, Garlick PJ, Millward DJ. 1978. *Protein Turnover in Mammalian Tissues and in the Whole Body*. Amsterdam: North-Holland Publishing.

Webber WA, Brown JL, Pitts RF. 1961. Interactions of amino acids in renal tubular transport. *Am J Physiol* 200:380–386.

White TP, Brooks GA. 1981. [U-^{14}C]glucose, -alanine, and -leucine oxidation in rates at rest and two intensities of running. *Am J Physiol* 240:E155–E165.

Widdowson EM, Dickerson JWT. 1964. Chemical composition of the body. In: Comar CL, Bronner F, eds. *Mineral Metabolism: An Advanced Treatise*, Vol 2. New York: Academic Press.

Wilcken DE, Reddy SG, Gupta VJ. 1983. Homocysteinemia, ischemic heart disease, and the carrier state for homocystinuria. *Metabolism* 32:363–370.

Wilkin JK. 1986. Does monosodium glutamate cause flushing (or merely "glutamania")? *J Am Acad Dermatol* 15:225–230.

Willard MD, Gilsdorf RB, Price RA. 1980. Protein-calorie malnutrition in a community hospital. *J Am Med Assoc* 243:1720–1722.

Williams GM, Whysner J. 1996. Epigenetic carcinogens: Evaluation and risk assessment. *Exp Toxicol Pathol* 48:189–195.

Wilson DC, Pencharz PB. 1997. Nutritional care of the chronically ill. In: Tsang RC, Zlotkin SH, Nichols BL, Hansen JW, eds. *Nutrition During Infancy: Birth to 2 Years.* Cincinnati: Digital Educational Publishing, Inc. Pp. 37–56.

Wilson D, Rafii M, Ball RO, Pencharz PB. 2000. Threonine requirement in young men determined by indicator amino acid oxidation with use of L-[1-^{13}C]-phenylalanine. *Am J Clin Nutr* 71:757–764.

Woessner KM, Simon RA, Stevenson DD. 1999. Monosodium glutamate sensitivity in asthma. *J Allergy Clin Immunol* 104:305–310.

Woods RK, Weiner JM, Thien F, Abramson M, Walters EH. 1998. The effects of monosodium glutamate in adults with asthma who perceive themselves to be monosodium glutamate-intolerant. *J Allergy Clin Immunol* 101:762–771.

Wurtman JJ, Wurtman RJ, Growdon JH, Henry P, Lipscomb A, Zeisel SH. 1981. Carbohydrate craving in obese people: Suppression by treatments affecting serotoninergic transmission. *Int J Eating Disord* 1:2–15.

Wynn M, Wynn A. 1979. *Prevention of Handicap and the Health of Women.* London: Routledge and Kegan Paul. Pp. 43–81.

Yamashita K, Ashida K. 1971. Effect of excessive levels of lysine and threonine on the metabolism of these amino acids in rats. *J Nutr* 101:1607–1614.

Yanez E, Uauy R, Ballester D, Barrera G, Chavez N, Guzman E, Saitua MT, Zacarias I. 1982. Capacity of the Chilean mixed diet to meet the protein and energy requirements of young adult males. *Br J Nutr* 47:1–10.

Yang WH, Drouin MA, Herbert M, Mao Y, Karsh J. 1997. The monosodium glutamate symptom complex: Assessment in a double-blind, placebo-controlled, randomized study. *J Allergy Clin Immunol* 99:757–762.

Yeatman TJ, Risley GL, Brunson ME. 1991. Depletion of dietary arginine inhibits growth of metastatic tumor. *Arch Surg* 126:1376–1382.

Yogman MW, Zeisel SH. 1983. Diet and sleep patterns in newborn infants. *N Engl J Med* 309:1147–1149.

Yogman MW, Zeisel SH. 1985. Nutrients, neurotransmitters and infant behavior. *Am J Clin Nutr* 42:352–360.

Yonetani S, Ishii H, Kirimura J. 1979. Effect of dietary administration of monosodium L-glutamate on growth and reproductive functions in mice. *Oyo Yakuri (Pharmacometrics)* 17:143–152.

Young SN. 1986. The clinical psychopharmacology of tryptophan. In: Wurtman RJ, Wurtman JJ, eds. *Nutrition and the Brain,* Vol. 7. New York: Raven Press. Pp. 49–88.

Young SN, Gauthier S. 1981. Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* 44:323–327.

Young VR. 1987. 1987 McCollum Award Lecture. Kinetics of human amino acid metabolism: Nutritional implications and some lessons. *Am J Clin Nutr* 46:709–725.

Young VR, Borgonha S. 2000. Nitrogen and amino acid requirements: The Massachusetts Institute of Technology Amino Acid Requirement Pattern. *J Nutr* 130:1841S–1849S.

Young VR, Pellett PL. 1990. Current concepts concerning indispensable amino acid needs in adults and their implications for international nutrition planning. *Food Nutr Bull* 12:289–300.

Young VR, Pellett PL. 1994. Plant proteins in relation to human protein and amino acid nutrition. *Am J Clin Nutr* 59:1203S–1212S.

Young VR, Hussein MA, Scrimshaw JS. 1968. Estimate of loss of labile body nitrogen during acute protein deprivation in young adults. *Nature.* 218:568–569.

Young VR, Tontisirin K, Ozalp I, Lakshmanan F, Scrimshaw NS. 1972. Plasma amino acid response curve and amino acid requirements in young men: Valine and lysine. *J Nutr* 102:1159–1169.

Young VR, Taylor YS, Rand WM, Scrimshaw NS. 1973. Protein requirements of man: Efficiency of egg protein utilization at maintenance and sub-maintenance levels in young men. *J Nutr* 103:1164–1174.

Young VR, Fajardo L, Murray E, Rand WM, Scrimshaw NS. 1975a. Protein requirements of man: Comparative nitrogen balance response within the submaintenance-to-maintenance range of intakes of wheat and beef proteins. *J Nutr* 105:534–542.

Young VR, Steffee WP, Pencharz PB, Winterer JC, Scrimshaw NS. 1975b. Total human body protein synthesis in relation to protein requirements at various ages. *Nature* 253:192–194.

Young VR, Puig M, Queiroz E, Scrimshaw NS, Rand WM. 1984. Evaluation of the protein quality of an isolated soy protein in young men: Relative nitrogen requirements and effect of methionine supplementation. *Am J Clin Nutr* 39:16–24.

Young VR, Gucalp C, Rand WM, Matthews DE, Bier DM. 1987. Leucine kinetics during three weeks at submaintenance-to-maintenance intakes of leucine in men: Adaptation and accommodation. *Hum Nutr Clin Nutr* 41:1–18.

Young VR, Bier DM, Pellett PL. 1989. A theoretical basis for increasing current estimates of the amino acid requirements in adult man, with experimental support. *Am J Clin Nutr* 50:80–92.

Young VR, Marchini JS, Cortiella J. 1990. Assessment of protein nutritional status. *J Nutr* 120:1496–1502.

Young VR, Wagner DA, Burini R, Storch KJ. 1991. Methionine kinetics and balance at the 1985 FAO/WHO/UNU intake requirement in adult men studied with L-[$^{2}H_{3}$-methyl-1-^{13}C]methionine as a tracer. *Am J Clin Nutr* 54:377–385.

Young VR, El-Khoury AE, Raguso CA, Forslund AH, Hambraeus L. 2000. Rates of urea production and hydrolysis and leucine oxidation change linearly over widely varying protein intakes in healthy adults. *J Nutr* 130:761–766.

Yuwiler A, Brammer GL, Morley JE, Raleigh MJ, Flannery JW, Geller E. 1981. Short-term and repetitive administration of oral tryptophan in normal men. Effects on blood tryptophan, serotonin, and kynurenine concentrations. *Arch Gen Psychiatry* 38:619–626.

Zanni E, Calloway DH, Zezulka AY. 1979. Protein requirements of elderly men. *J Nutr* 109:513–524.

Zello GA, Pencharz PB, Ball RO. 1990. Phenylalanine flux, oxidation and conversion to tyrosine in humans studied with L-[1-^{13}C]phenylalanine. *Am J Physiol* 259:E835–E843.

Zello GA, Pencharz PB, Ball RO. 1993. Dietary lysine requirement of young adult males determined by oxidation of L-[1-^{13}C]phenylalanine. *Am J Physiol* 264:E677–E685.

Zello GA, Wykes LJ, Ball RO, Pencharz PB. 1995. Recent advances in methods of assessing dietary amino acid requirements for adult humans. *J Nutr* 125:2907–2915.

Zezulka AY, Calloway DH. 1976a. Nitrogen retention in men fed isolated soybean protein supplemented with L-methionine, D-methionine, N-acetyl-L-methionine, or inorganic sulfate. *J Nutr* 106:1286–1291.

Zezulka AY, Calloway DH. 1976b. Nitrogen retention in men fed varying levels of amino acids from soy protein with or without added L-methionine. *J Nutr* 106:212–221.

Zhao X-H, Wen ZM, Meredith CN, Matthews DE, Bier DM, Young VR. 1986. Threonine kinetics at graded threonine intakes in young men. *Am J Clin Nutr* 43:795–802.

Ziegler TR, Benfell K, Smith RJ, Young LS, Brown E, Ferrari-Baliviera E, Lowe DK, Wilmore DW. 1990. Safety and metabolic effects of L-glutamine administration in humans. *J Parenter Enteral Nutr* 14:137S–146S.

Zimmerman FT, Burgemeister BB. 1959. A controlled experiment of glutamic acid therapy. *AMA Arch Neurol Psych* 81:639–648.

Zlotkin SH. 1989. Nutrient interactions with total parenteral nutrition: Effect of histidine and cysteine intake on urinary zinc excretion. *J Pediatr* 114:859–864.