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Protein

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Objectives

Compare the available methods to determine protein and amino acid utilization.

Contrast normal, starvation, and injury protein metabolism.

Identify the “conditionally essential” amino acids, and explain in what conditions they become essential.

Identify the most clinically useful method for assessing protein status.

Test Your Knowledge Questions

1. Which of the following statements is true relating to hydrochloric acid (HCl) and protein digestion?

A. HCl aids in the conversion of pepsin to pepsinogen.

B. HCl denatures protein structures to make them more susceptible to enzymatic action.
C. HCl is secreted by the parietal cells within the duodenum in response to dietary proteins.
D. HCl's release is stimulated by the hormone insulin.

2. During protein metabolism, branched-chain amino acids (BCAAs):
A. Are extracted primarily by the liver after a protein-containing meal.
B. Are released by the skeletal muscle at a higher rate than other amino acids.
C. Serve as the primary fuel sources for the enterocytes.
D. Produce oxidative wastes during metabolism within the skeletal muscle, which are removed by alanine and glutamine.

3. Proteins perform all the following physiological functions except:
A. Provide a major source of energy.
B. Maintain acid-base balance.
C. Contribute to immune defense.
D. Serve as a mode of transport for substances.

4. The rate of protein turnover in catabolic, critically ill patients:
A. Does not change.
B. Decreases.
C. Increases.
D. Is not affected by nutrition support.

Test Your Knowledge Answers

1) The correct answer is B. Denaturing protein structures, making them more susceptible to enzymatic action, is a primary role of HCl. HCl plays several roles in protein digestion, including conversion of the proenzyme pepsinogen to its active form pepsin. HCl is secreted by the parietal cells within the stomach, not the duodenum. HCl secretion is stimulated by gastrin, not insulin.
2) The correct answer is D. Nitrogen end products produced during BCAA oxidation within the skeletal muscle are removed by the nitrogen carriers alanine and glutamine. BCAAs are extracted primarily by the skeletal muscle, with minimal extraction by the liver. BCAAs are primarily oxidized in the skeletal muscle (not the enterocytes) and are released from the muscle at a lower rate than other amino acids.
3) The correct answer is A. Carbohydrates and fats are the major energy source in the human diet. Protein is not preferentially used as a source of energy in health. Protein is used as the body's

primary buffer to maintain acid-base balance. All cells of the immune system (ie, white blood cells, macrophages, and so on) are made up of proteins. Proteins are the primary carriers for substances such as minerals, vitamins, and hormones.

4) The correct answer is C. Protein turnover rates increase dramatically in critical illness. Nutrition support will improve protein synthesis somewhat, but it has little effect on protein degradation.

Introduction

Proteins are an essential component of all living organisms and are important components of body mass and structure. The word protein is derived from the Greek protos ("first"); as the name suggests, protein is clearly thought of as the most important macronutrient in the body. A thorough knowledge of how proteins are used by the healthy body and the changes in protein metabolism that occur with injury and disease is essential in nutrition support practice.

Amino Acids and Peptides

Amino Acids

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. The "nutritional value" of dietary protein is closely related to the in vivo biochemical processes of the metabolism or use of individual amino acids released from the dietary proteins after digestion. The 20 amino acids found within proteins convey a vast array of chemical versatility. The content and sequence of the amino acids of a specific protein are determined by the sequence of the bases in the gene that encodes that protein through complex transcription, translation, and posttranslational modifications. The chemical properties of a protein's amino acids and their spatial configurations in a protein determine their biological activity. Proteins not only catalyze most of the reactions in living cells; they control virtually all cellular processes.1 Understanding amino acid structure and properties is key to understanding protein structure and properties.

Dietary proteins are important sources of amino acids. Some amino acids are considered nutritionally essential (indispensable) because they cannot be physiologically synthesized in the human body. Our bodies cannot manufacture them, so they must be obtained exogenously. There are also nonessential (dispensable) amino acids, which are synthesized from available carbon and nitrogen precursors. In addition, there are a few amino acids that can be manufactured by the body under normal circumstances but become "conditionally essential" under certain clinical conditions, meaning the demand exceeds what can be produced. For example, tyrosine is a nonessential amino acid that is normally synthesized from the essential amino acid phenylalanine. However, tyrosine becomes conditionally essential if inadequate phenylalanine is provided, or if, under certain clinical conditions, the tyrosine converted from phenylalanine cannot meet the body's requirements. Other examples of conditionally essential amino acids include cysteine, glutamine, histidine, and taurine.

The classification of an amino acid as essential or nonessential is based on whether the amino acid can be synthesized in the body under physiological conditions. Humans can produce 11 of the 20 amino acids that are the building blocks of proteins. The others must be supplied exogenously. Muscle protein is catabolized when the exogenous essential amino acid requirement for even a single amino acid is not met. Unlike fat and starch, excessive amino acids are not stored for later use. Therefore, amino acids must be consumed from exogenous sources or the body must accelerate the breakdown of its own proteins.

The nutritionally essential amino acids are phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine, and histidine; the latter is essential primarily in infants and children. Foods of animal origin, such as meat, poultry, fish, eggs, and dairy products, are the richest sources of the essential amino acids. Plant sources of protein are often deficient in one or more of the essential amino acids. It was once believed that all the essential amino acids had to be "balanced" at each meal for vegetarians to obtain adequate amounts of protein. For example, it was thought that grains and beans had to be consumed at the same meal. However, more recent research has indicated that consuming a proper mixture of amino acids is important, but it is not necessary to consume all types at the same meal.2

The distinctions between essential and nonessential amino acids are not always clearly delineated in all physiological and pathophysiological conditions. The labels "indispensable" and "dispensable" were originally defined not only in dietary terms but also in relation to the role of amino acids in supporting protein deposition and growth. Reeds3 has examined these labels as they apply to amino acids from 3 different perspectives. The first perspective comes from a traditional nutrition-focused view that some amino acids are absolute dietary necessities if normal growth is to be maintained. The second perspective comes from a metabolism-focused view that there may be significant limitations on the synthesis of some amino acids, thereby rendering potential limitations to growth. In addition, a consideration of in vivo amino acid metabolism leads to the definition of a third class of amino acids, termed conditionally essential, whose synthesis can be carried out by mammals but is limited by a variety of factors. These factors include the dietary supply of the appropriate precursors and the maturity and health of the individual. Finally, from a functional perspective, all amino acids, including nonessential and conditionally essential amino acids, are essential to optimize physiological function.3

Peptides

Peptides are composed of amino acids linked together chemically by peptide bonds. The link between one amino acid residue and the next is an amide bond and is sometimes referred to as a peptide bond. The peptide bond always involves a single covalent link between the α-carboxy (oxygen-bearing carbon) of one amino acid and the amino nitrogen of a second amino acid. In the formation of a peptide bond from 2 amino acids, a molecule of water is eliminated. An amide bond is somewhat shorter than a typical carbon-nitrogen single bond and has a partial double-bond character because the participating carbon atom is double-bonded to an oxygen atom and the nitrogen has a lone pair of electrons available for bonding. Dipeptides are 2 amino acids bonded together, whereas 3 amino acids bonded together by

peptide bonds are called tripeptides. Polypeptides consist of 10 or more amino acids bonded together by peptide bonds and an intermediate string between 4 and 10 amino acids is an oligopeptide.

Peptides differ from proteins, which are also long chains of amino acids, by virtue of their size. Traditionally, peptide chains that are short enough to be made synthetically from the constituent amino acids are called peptides, rather than proteins. In essence, a peptide is a small protein.4

The gastrointestinal (GI) tract produces a variety of chemical transmitters (GI peptides) that are involved in GI motility, secretion, absorption, growth, and development.5 Dietary protein, for example, is digested in the GI tract to free amino acids and peptides by the action of gastric acid and enzymatic hydrolysis. Multiple amino acid transport systems exist for absorption of the amino acids (see Chapter 2). The cells that produce GI peptides are dispersed throughout the GI tract, and regulatory peptides are found in the esophagus, stomach, small and large bowel, and the pancreas. Although GI peptides are typically thought of as hormones, not all peptides act via the traditional endocrine pathway by which they are secreted into the bloodstream and act at a distant site. Many of the peptides in the GI tract are also found in the enteric nervous system and the central nervous system. GI peptides can act on the same cells from which they are released, on neighboring cells via classic endocrine mechanisms, and on cells following their release from nerves.6 Table 6-1 lists the secretory sites, targets, and functions of the nutritionally important GI peptides.

TABLE 6-1 Gastrointestinal Peptides and Their Functions

Peptide	Secretory Sites	Main Target Sites	Functions
Gastrin	G cell (pyloric antrum, duodenum, and pancreas)	Parietal cell	Acid secretion, gastric contraction
Secretin	S cell (duodenum)	Pancreas	Water and bicarbonate secretion
Cholecystokinin	I cell (duodenum and jejunum)	Pancreas, gallbladder	Pancreatic enzyme secretion, gallbladder contraction
Gastric inhibitory peptide	K cell (duodenum and jejunum)	Pancreas	Insulin secretion
Glucagon-like peptide-1	L cell (small intestine)	Pancreas	Insulin secretion
Glucagon-like peptide-2	L cell (small intestine)	Small intestine	Intestinal growth (crypt cell)
Peptide YY	L cell (small intestine)	Brain stem	Slowing the gastric emptying, reducing appetite
Somatostatin	Delta cell (pyloric antrum), hypothalamus	GI tract, pituitary gland	Suppressing the release of GI hormones

GI, gastrointestinal.

Functions and Structure of Proteins

Functions of Proteins

Proteins are the most abundant organic molecules in cells and are fundamental to cell structure and function. All proteins are constructed from the same basic set of 20 amino acids, covalently linked in characteristic sequences. Because each of these amino acids has a distinctive side chain that lends it

chemical individuality, this group of 20 building-block molecules may be regarded as the “alphabet” of protein structure. All proteins contain carbon, hydrogen, oxygen, and nitrogen. Some contain sulfur, and others contain phosphorus, iron, zinc, and copper.

The roles of protein are versatile. Table 6-2 lists some of the many important roles proteins play in the body.7 Newer research reveals increasingly complex roles for protein and amino acids in regulation of body composition and bone health, GI function and bacterial flora, glucose homeostasis, cell signaling, and satiety.8

TABLE 6-2 Functions of Proteins and Amino Acids

- Growth, maintenance, and movement: Proteins form integral parts of most body structures, such as skin, tendons, membranes, muscles, organs, and bones. As such, they support the growth and repair of body tissues.
- Enzymes: Proteins facilitate chemical reactions.
- Hormones: Some, but not all, hormones are made of protein. They function as messengers and signals and regulate body processes.
- Immunity: Antibodies, cytokines, and chemokines are proteins involved in immunological processes. Proteins regulate gene transcription and translation through mTOR signaling pathway.
- Fluid and electrolyte balance: Proteins help maintain fluid volume and the composition of body fluids.
- Acid-base balance: Proteins help maintain the acid-base balance of body fluids by acting as buffers.
- Transportation and storage: Proteins transport substances, such as lipids, vitamins, minerals, and oxygen, throughout the body. Some proteins, such as ferritin, store a specific micronutrient, such as iron.

mTOR, mammalian target of rapamycin.

Structure of Proteins

To understand protein function, one must understand protein structure. Two classes of strong bonds (peptide and disulfide) and 3 classes of weak bonds (hydrogen, hydrophobic, and electrostatic or salt) stabilize most protein structures. Protein structure is broken down into the following 4 levels:

- Primary structure refers to the “linear” sequence of amino acids in the polypeptide chain(s) and the location of disulfide bonds, if these are present. In contrast, the higher orders of protein structure (secondary, tertiary, and quaternary) involve mainly noncovalent interactions.
- Secondary structure refers to “local” ordered structure brought about via hydrogen bonding mainly within the peptide backbone. The most common secondary structure elements in proteins are the alpha (α) helix and the beta-13 sheet (sometimes called “13-pleated sheet”).

- Tertiary structure refers to the “global” folding of a single polypeptide chain. Hydrogen bonding involving groups from both the peptide backbone and the side chains is important in stabilizing tertiary structure. Disulfide bonds between cysteine residues stabilize the tertiary structure of some proteins.
- Quaternary structure refers to the stable association of multiple polypeptide chains resulting in an active unit united by forces other than covalent bonds (not peptide or disulfide bonds). The forces that stabilize these aggregates are hydrogen bonds and electrostatic (or salt) bonds formed between residues on the surfaces of the polypeptide chains. The protein structure is formed through the processes of translation and posttranslational modification. The configuration of a protein determines the physical property and the function of the protein. Taking enzyme protein as an example, the configuration of an enzyme protein determines the catalytic ability of the individual enzyme.

Transport Proteins

Transport proteins in plasma bind and carry specific molecules or ions from one organ to another. Some transport proteins are not attached to membranes; instead, they move via bodily fluids, carrying nutrients and other molecules. Some examples of proteins acting as transport proteins include hemoglobin carrying oxygen from the lungs to the cells, lipoproteins transporting lipids around the body, and special proteins that carry vitamins (eg, retinol-binding protein), minerals (eg, albumin), or hormones (eg, prealbumin). Other kinds of transport proteins are present in cell membranes and are adapted to bind and transport glucose, amino acids, and other nutrients across the membranes into cells. Iron transport provides a good illustration of these proteins’ specificity and precision. Dietary iron is mainly absorbed in the duodenum and proximal jejunum, where it may be stored bound to ferritin or transported into circulation. As iron is transported by an export protein from the cell into circulation, it is attached to the carrier protein transferrin, which is necessary to transport the iron to other tissues such as bone marrow or other storage sites where it is stored as ferritin or hemosiderin. During periods of physiological need, iron is incorporated into proteins such as those in the red blood cells and muscles that assist in oxygen transport and use. Since plasma proteins play such important roles in directly transporting substrates (such as minerals) as well as metabolic mediators (such as various hormones and growth factors), the dynamic status of plasma proteins is closely related to the delivery of the protein-bound substrates and the functions of these metabolic mediators.

Protein Signaling

Amino acids and proteins also have important functions as signaling molecules modulating the process of protein synthesis. The target of rapamycin is a protein kinase initially discovered in yeast, and it is the major signaling pathway regulating messenger ribonucleic acid translation. It is involved in the control of cell growth and proliferation and is activated by hormones and growth factors as well as amino acids, specifically leucine, and cellular energy status. Research on the mammalian target of rapamycin–signaling pathway may help identify the mechanisms involved in the longevity associated with energy restriction observed in certain species. Research on this topic may also have implications for the

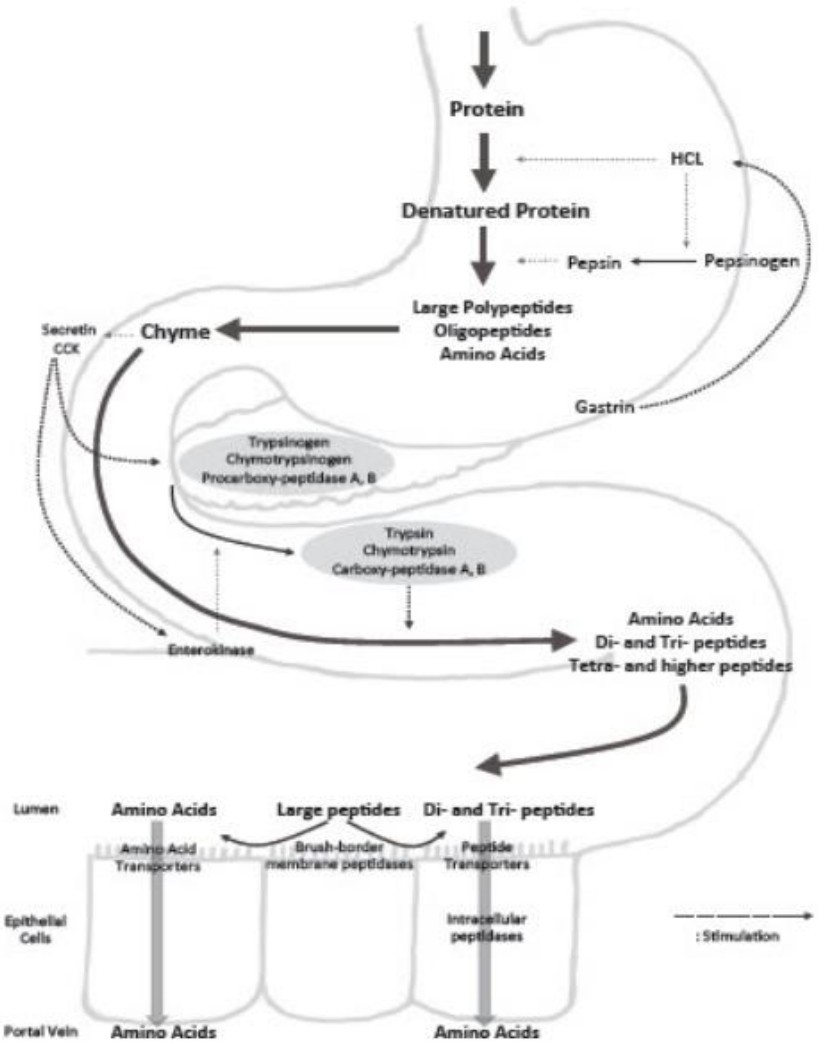
regulation of cancer cell growth, as well as our understanding of the role of muscle protein turnover in critical illness.

Digestion and Absorption

Protein and its amino acid components enter the GI tract via both endogenous and exogenous routes. Endogenous sources include desquamated mucosal cells, digestive enzymes, and other glycoproteins such as mucus. Exogenous sources (ie, dietary protein) in the form of meat, poultry, fish, milk products, grains, legumes, and vegetables provide both energy and amino acids essential to the body in roles, as previously described.8

As exogenous proteins travel through the GI tract, they are digested into peptides and free amino acids through a series of hydrolytic reactions and feedback mechanisms (Figure 6-1). Minimal protein digestion occurs within the mouth or esophagus. However, once proteins reach the stomach, HCl is released by the parietal cells of the stomach. HCl release may be stimulated by the hormone gastrin, the neurotransmitter acetylcholine from vagal nerve stimulation, the neuropeptide gastrin-releasing peptide, or the amine histamine.8 HCl denatures protein structures to make them more susceptible to enzymatic action and converts pepsinogen, an inactive proenzyme or zymogen released by the stomach's chief cells, to its active form, pepsin (see Chapter 1). Pepsin in turn may activate other pepsinogen molecules or hydrolyze specific peptide bonds into the end products of large polypeptides, oligopeptides, and free amino acids.9

FIGURE 6-1 Schemata of Digestion and Absorption of Proteins



CCK, cholecystokinin; HCL, hydrochloric acid.

This mixture, known as acid chyme, next passes into the duodenum and small intestine where most protein digestion takes place. The end products within the acid chyme stimulate the secretion of the hormones secretin and cholecystokinin (CCK), which in turn travel through the bloodstream to the acinar cells of the pancreas to stimulate the release of alkaline pancreatic juices. Secretin and CCK also stimulate the release of digestive proenzymes trypsinogen, procarboxypeptidases, chymotrypsinogen, and proelastase (Table 6-3). While deactivating pepsin, the higher pH of the duodenum is now optimal for the activity of the pancreatic enzymes. By releasing proteolytic enzymes initially in their inactive form, the enzyme-forming cells are protected from self-digestion.

TABLE 6-3 Selected Enzymes Responsible for the Digestion of Protein

Zymogen	Enzyme or Activator	Enzyme	Site of Activity
Pepsinogen	HCl or pepsin	Pepsin	Stomach
Trypsinogen	Enteropeptidase trypsin	Trypsin	Intestine
Chymotrypsinogen	Trypsin	Chymotrypsin	Intestine
Procarboxy-peptidases	Trypsin	Carboxypeptidase A, B Aminopeptidases	Intestine

HCl, hydrochloric acid.

Enterokinase (also known as enteropeptidase) is an enzyme secreted from the brush border of the small intestine, also in response to secretin and CCK. Enterokinase serves to activate trypsinogen into trypsin, which in turn converts many of the other proenzymes into their respective enzymatic forms for ongoing protein hydrolysis.9 At this point, endogenous protein sources from sloughing mucosal cells or other secretory proteins are also recycled through the digestive process to form the general pool of amino acids. The presence of protein within the gut signals further enzyme secretions. When most of the protein has been digested, the presence of unbound trypsin acts as a feedback mechanism to turn off further secretion of trypsinogen by the pancreas.10 At this point, the byproducts of pancreatic protease digestion, free amino acids and peptides of 2 to 6 amino acid components, now enter the final phases of digestion. The brush border of the small intestine produces a variety of peptidases, which allow peptide digestion to continue through the distal small intestine and ileum.8,11,12

The end products of protein digestion (eg, free amino acids, dipeptides, and tripeptides) are now ready for absorption within the small intestine. The absorption of intact proteins may occur through leaks in cell junctions to a lesser extent, which accounts for the immune response associated with food allergies.10,13 However, most amino acids are absorbed in the proximal small intestine, leaving less than 1% of dietary protein to be excreted in the feces.8,10 Through both sodium-dependent and sodium-independent active transport systems, amino acids pass from the lumen of the small intestine into the enterocyte. The various amino acid carrier systems have an affinity for specific amino acids.14 The rate of amino acid absorption may vary depending on the chemical properties of the amino acid. Essential (indispensable) amino acids are absorbed faster than nonessential (dispensable) amino acids. Methionine and the BCAAs leucine, isoleucine, and valine are the most rapidly absorbed.15 Competition can occur between amino acids for transport by a carrier system.

The active transport of dipeptides and tripeptides across the brush border membrane of the enterocyte also involves a competition for transporters but uses different carrier systems than those of the free amino acids. In fact, peptide absorption occurs more rapidly than an equivalent mixture of free amino acid.16,17 It is estimated that 67% of dietary protein is absorbed as dipeptides and tripeptides, with the remaining 33% absorbed as free amino acids10,18,19 Once inside the enterocyte, these peptides are converted to free amino acids by peptide hydrolases. The final phase of amino acid absorption occurs

across the basolateral membrane of the enterocyte by diffusion or active transport into the portal circulation (Figure 6-1).

Hydrolyzed Enteral Formulas

The use of peptide-based enteral formulas for patients with impaired GI function and malabsorption remains controversial. The GI tract has a large reserve capacity for digestive and absorptive processes with a multitude of intestinal folds, villi, and microvilli increasing its surface area. Malabsorption of some nutrients may not be manifested until 90% of organ function is impaired, such as in exocrine pancreatic disease.20,21 Peptide length may also play a role in effectiveness of GI absorption, with dipeptides and tripeptides being most readily absorbed. A meta-analysis of elemental (free amino acids) versus polymeric (intact protein) formulas did not show an advantage with the former for patients with Crohn's disease.22,23 Free amino acid formulas also have a higher osmolality, are less palatable, and cost more, further limiting their application in the clinical setting.8

Protein Synthesis, Turnover, and Breakdown

Protein Synthesis

The human body contains an estimated 10,000 to 50,000 different kinds of proteins. Each protein has a specific function, and that function is determined during protein synthesis. Protein synthesis is a fundamental process for all living organisms. The liver is the major site of amino acid metabolism. The sequence of amino acids in each protein determines its configuration, which supports a specific function. If a genetic error alters the amino acid sequence of a protein, or if a mistake is made in copying the sequence, an altered protein will result, sometimes with dramatic consequences. Because proteins are composed of amino acids, the information necessary for their production resides in the cellular nucleus as deoxyribonucleic acid (DNA). The instructions for protein synthesis are transmitted by genetic information received at conception. There are 5 major stages in protein synthesis, each requiring multiple components. A brief outline of the different stages follows:

Stage 1: Activation of amino acids: To inform a cell of the sequence of amino acids for a needed protein, a stretch of DNA serves as a template for making a strand of RNA that carries a code, which lists in order the amino acids that will be needed to make a given protein. Each messenger RNA strand copies exactly the instructions for making a protein that the cell needs.

Stage 2: Initiation of the polypeptide chain: The messenger RNA leaves the nucleus through the nuclear membrane. DNA remains inside the nucleus. The messenger RNA, bearing the code for the polypeptide to be made, attaches itself to the protein-making machinery of the cell in the cytosol, the ribosomes.

Stage 3: Elongation: Another form of RNA, transfer RNA, collects amino acids from the cell fluid. Each transfer RNA carries its amino acids to the messenger RNA, which dictates the sequence in which the amino acids will be attached to form the protein strands. Thus, the messenger RNA ensures that the amino acids are arranged in the correct sequence.

Stage 4: Termination and release: As the amino acids are lined up in the right sequence and the ribosome moves along the messenger, an enzyme bonds one amino acid after another to the growing protein strand. The transfer RNA is freed to return for more amino acids. When all the amino acids have been attached, the completed protein is released.

Stage 5: Folding and processing: Finally, the messenger RNA is degraded. To achieve its native biologically active form, the polypeptide must undergo folding into its proper 3-dimensional conformation. Before or after folding, the new polypeptide may undergo processing by enzymatic action to remove initiating amino acids; to introduce phosphate, methyl, carboxyl, or other groups into certain amino acid residues; or to attach oligosaccharides or prosthetic groups. Between 40 and 100 amino acids can be rapidly added to a growing protein strand in a cell. The completed protein strand is released (stage 4), the messenger is degraded, and the transfer RNA is freed to return for another load of amino acids.

Measurement of Protein Turnover and Breakdown

Protein turnover is the measurement of protein synthesis and breakdown rates. Protein turnover constantly occurs in the body with all proteins participating at varying rates. The amino acids liberated during protein turnover contribute to the free amino acid pool. There is constant flux of amino acids between muscle and organs, and the turnover rate of individual proteins varies depending on the function of the protein. For example, peptides and hormones have relatively high rates of synthesis and degradation in contrast to structural or plasma proteins. In a normal 24-hour period, dietary intake matches nitrogen output; thus, protein breakdown matches synthesis. This process is altered by disease and certain physiological states as well as changes in nutrient intake.25 Typically, daily protein intake provides approximately 25% of total nitrogen turnover in the body, with other sources coming from muscle, the viscera, and plasma proteins.

Body protein turnover in both healthy individuals and critically ill patients may be determined by several different methods (Table 6-4). They range from simple noninvasive measurements that can easily be performed in a clinical setting, such as nitrogen balance (NB), to expensive and more complicated tests, such as tracer methodology, arteriovenous differences across tissue beds, and the use of molecular biology techniques.10 All methods have limitations.

TABLE 6-4 Measurement of Protein Metabolism

Method	Characteristics
Nitrogen balance	• Measure of nitrogen input minus nitrogen output. • Can be used in clinical setting. • Decreased accuracy in certain clinical conditions (renal dysfunction, high-output fistula, and ostomy).

Arteriovenous amino acid differences across organs	• Invasive method. • A tracer amino acid should be infused. • Samples of blood are obtained from both the arterial and venous catheter. • The measurements are made across muscle beds.
Tracer methodology	• Constant infusion method used in human studies. • No requirement for a steady state. • Ideal for measuring acute changes in patients. • Important research tool.
Urinary creatinine excreted over 24 hours	• Indirect measurement of muscle mass. • Has high variation.
Urinary 3-methyl histidine excretion	• Can be used as a measure of muscle protein degradation. • Compromised accuracy with organ dysfunction.

Nitrogen Balance

NB is calculated in clinical settings to help assess protein requirements and adequacy. This technique is used to determine minimum levels of human protein and amino acid requirements. Determining NB using urinary urea is an approximation based on certain assumptions, such as urea accounting for about 80% of the total urinary nitrogen losses. The urinary urea nitrogen concentration is affected by stress and increased urinary excretion of non–urea nitrogen. NB determined with total urinary nitrogen (Kjeldahl method) is consistently more reliable than the urine urea nitrogen measurement but is typically not available for routine clinical measurements. Stool and skin losses of nitrogen are usually estimated to be about 2 g/d, unless there is excessive diarrhea or ostomy/fistula losses. In such cases, the effluent should also be collected and measured for total nitrogen, if possible. There are also negligible losses that are not accounted for clinically, such as menstrual losses and nasal secretion.

The NB equation used clinically is as follows:

NB = Nitrogen Intake – Nitrogen Output

where nitrogen intake is the total nitrogen intake, including enteral tube feeding and/or oral intake and parenteral nutrition (PN), and nitrogen output is the sum of nitrogen losses from urine, stool, skin, and body fluids:26

Nitrogen Output (g/d) = [Urinary Urea Nitrogen (mg/100 mL) × Urinary Volume (L/d)/100] + 20% of Urinary Urea Losses + 2 g

In clinical settings, a 24-hour urine collection is necessary for accurate urea nitrogen measurements. Adjustments need to be made in certain patients. Many other factors affect the accuracy of NB measurement. In addition to renal dysfunction, errors in estimating intake, or incomplete collections of urine, stool, fistula, or ostomy losses may affect balance results. NB, which may also be affected by changes in the muscle glutamine pool, has been reported to be significantly decreased in critical illness.27

Because NB measures the net difference between nitrogen intake and output, it is a useful value to measure the gross utilization of administered protein; however, it does not provide information about how the administrated protein is used within the body (eg, for protein synthesis, formation of nonessential amino acids, oxidation, or gluconeogenesis). NB is a poor indicator for characterizing interorgan amino acid flux and changes in protein turnover. More sensitive tools such as isotopically labeled tracers can be used to determine substrate balance across an organ and amino acid circulation. Practice Scenario 6-1 discusses the use of NB in a critical care setting.

Practice Scenario 6-1

Question: What are the best ways to evaluate protein adequacy after traumatic injury?

Scenario: A 36-year-old man was admitted to the trauma center after a collision with a tree while riding a motorcycle. He sustained major abdominal injuries, including a ruptured spleen, severe liver contusions, and small bowel lacerations, as well as 3 fractured ribs, a fractured pelvis, and a right femur fracture. He required multiple blood transfusions (10 units) during the first 24 hours in the hospital. After resuscitation and stabilization, he was taken to the operating room for intra-abdominal exploration, splenectomy, and repair of small bowel lacerations, which required 2 small bowel resections. Also noted was a large right retroperitoneal hematoma, which appeared stable. He also underwent an open reduction internal fixation of his femur fracture and stabilization of his pelvic fracture. His previous medical history was notable for alcohol abuse. His pertinent objective data include the following:

Height: 73 inches

Weight: 95 kg

Ideal body weight: 84 kg

Estimated energy needs (25 to 30 kcal/kg): 2375 to 2850 kcal/d

Estimated protein needs (1.5 g/kg): 142 g/d

He was initially started on nasogastric feedings on postoperative Day 3 with a standard high-calorie, high-protein tube feeding, which was discontinued after 24 hours because of very high gastric residuals

(>250 mL). Parenteral nutrition (PN) was started as a total nutrient admixture to meet the patient's needs until a nasojejunal tube was placed.

Intervention: On Day 3 of PN, the nutrition support team was consulted because the patient was experiencing worsening azotemia, with his blood urea nitrogen (BUN) rising to 63 mg/dL and a creatinine level of 1.9 mg/dL. His urine output and creatinine clearance were within normal limits. His liver function tests were also elevated, with the transaminases 5 times normal, total bilirubin 2.9 mg/dL, and direct bilirubin 1.8 mg/dL.

The primary team requested an evaluation of the protein dose provided by the feeding regimen for potential overfeeding.

Answer: A clinically useful and practical tool for assessing protein requirements in hospitalized patients is nitrogen balance (NB). When PN or tube feeding is the sole source of nutrition intake, accurate determination of nitrogen intake is easier. Collection of output is usually where errors in calculating NB occur. Gastrointestinal (GI) losses need to be accounted for, but they are often difficult to collect and analyze in a clinical setting. If there is significant fluctuation in daily plasma BUN levels (>10 mg/dL), the change in plasma urea nitrogen generation over the 24-hour collection period should also be included in the output calculations.

Rationale: Although clinical guidelines help define protein requirements in critical illness, it is often necessary to adjust protein goals depending on the clinical condition of the patient (eg, worsening renal function, where protein may need to be decreased) or results of NB data used in clinical monitoring.26

The NB equation was determined as follows: A 24-hour urine collection was started on Day 4 of PN. The patient's BUN was stable, and there were no additional excess GI losses via drains, tubes, and so on. The PN solution provided 2450 kcal and 130 g protein over the same 24-hour period as the urine collection. The patient was on nothing by mouth status.

The PN provided 20.8 g nitrogen; urine output was 2870 mL; and urine urea nitrogen was 16 g. Therefore, the NB was calculated as follows:

20.8 g Nitrogen In – [16 g Urea Nitrogen + (20% for Other Urine Nitrogen, or 3.2 g) + (Estimated Other Nitrogen from Skin and GI Losses, or 2 g)] = –0.4 g, or Nitrogen Equilibrium

This NB equation suggests that the feeding regimen is adequate.

Arteriovenous Amino Acid Differences Across Organs

A more invasive tool for measuring amino acid flux across tissues and organs is arteriovenous differences. Catheters placed in an artery and vein are used to measure the amino acids in blood delivered to the tissue and the amino acids from the blood leaving the tissue. Most commonly, these measurements are made across muscle beds, such as the leg or arm. By combined measurements of

arteriovenous differences of both amino acid concentrations and the isotopically labeled amino acid tracer in and out of the organs, this method can provide quantitative information on the rates of protein synthesis and degradation, namely protein turnover in the organ or tissue. The tracer amino acid is usually given by primed continuous infusion, and replicate samples of blood are obtained from both the arterial and venous catheters at steady-state conditions. Typically, net fluxes of 13C-, 15N-, and 2H-labeled amino acids (and sometime their metabolites) across tissues are used to investigate changes in protein turnover. Muscle biopsies have also been used along with arteriovenous difference measurements to obtain information on the dynamics of intramuscular pool of amino acids. This method allows assessment of intracellular amino acid recycling and transmembrane transport.28

Tracer Methodology to Measure Protein Turnover

Nonradioactive stable isotope tracer amino acids to measure whole body protein turnover were first used in the 1950s, but the accuracy was limited by because the technology lacked the necessary sensitivity to detect the stable isotope enrichment of 15N-glycine in biological samples. It was not until 1969 that a simpler tracer model using 15N-glycine was developed by Picou and Taylor-Roberts. The tracer was originally administered orally with the metabolite being measured in the urine. Current investigations in human subjects are commonly using the method of primed constant intravenous (IV) or intragastric infusion of amino acid tracers to achieve a steady state, in which the isotope abundance in the metabolic pool reaches a plateau level. The labeled tracers are identical to the endogenous metabolites in the body except for the substituted atom that is labeled by a heavy isotope. The major assumption of the mathematical model is that the amino acid tracer is well mixed with the metabolic pool of the free amino acids within the body; then the ratio of the labeled/natural amino acids in the metabolic pool is used to calculate the metabolic rates of this amino acid along the major pathways of its metabolism, including the rates of protein synthesis and breakdown.

Another approach of measuring tissue protein metabolism is the tracer incorporation method. The advancement of mass spectrometry technology has enabled the enrichment of free and protein-bound amino acids to be measured in very small samples of plasma or tissue. The protein synthesis rate of a special tissue is calculated based on the incorporation rate of the labeled amino acid into the proteins. A limitation of the tracer incorporation method is the time required to reach a steady state in isotope infusions; additionally, it is not useful in those critically ill patients who cannot be in a pathophysiological steady state during the period of isotope infusion. An alternative approach to measure protein synthesis rates in isolated tissues and cell cultures is the flooding-dose technique. This technique minimizes the intra- and extracellular amino acid enrichment differences. It does not require a long period of stable isotope infusion, and the measurement can be completed in less than 30 minutes. This technique is ideal for measuring acute changes of protein synthesis rate in specific tissues of interest in patients.29 More recent methodology is being developed using pulse tracer injections, coupled with muscle biopsies, to measure fractional protein breakdown as well as synthetic rates of muscle proteins. Tracer methodology is an important research tool for gaining further insight into protein and amino acid flux in health and disease in living subjects in vivo.

Other Measures of Protein Breakdown

Other methods have been used to specifically evaluate muscle protein breakdown, such as 3-methyl histidine excretion and creatinine excretion to measure creatinine height index. The amount of urinary creatinine excreted over 24 hours is an indirect measure of muscle mass; however, significant day-to-day variation in creatinine excretion occurs in both healthy individuals and hospitalized patients with acute illness and renal dysfunction.25 Three-methyl histidine is an amino acid residue formed only by the myofibrillar proteins actin and myosin. It is not reused for protein synthesis; therefore, its excretion rate from the urine can be used as a measure of myofibrillar protein degradation. However, its accuracy is affected in patients with compromised renal function; also, muscle myofibrillar contents in the diet interfere the measurements. In addition, there are no available objective standards for comparison.25 However, urinary 3-methyl histidine can serve as one of the parameters, combined with NB and/or protein turnover measurements, to assess the protein metabolism status of critically ill patients.

Role of Proteins in Organs and Body Systems

Gastrointestinal Tract

Because the GI tract is a metabolically active organ, it plays an important role in amino acid homeostasis. The GI tract actively regulates amino acid flow from the diet by extracting those amino acids it needs for maintenance and function. Reed and Burrin30 estimate that a minimum of 25% of protein intake is used within the splanchnic bed, largely by the GI tract. In the presence of a low-protein diet, GI tract growth is preserved over growth of other peripheral tissues or muscle.30,31 A large proportion of the amino acids used by the GI tract, especially the small intestine, are involved in secretory protein synthesis. Within the intestinal cell, amino acids may also be used for synthesis of nucleic acids, the antioxidant glutathione, apoproteins as components of lipoproteins, or other nitrogen-containing compounds.30 In addition, amino acids provide an important energy source to the GI tract. Dietary glutamate and aspartate account for a significant proportion of the energy source for the small intestine.30 Glutamine released by the lung and skeletal muscle is used as a primary source of energy for the enterocyte, and it may have trophic effects on the mucosal cells of the GI tract. Glutamine is also used by the immune system—which is abundant within the GI tract as gut-associated lymphoid tissue—as a precursor to nucleotide synthesis, especially for the quick-turnover proteins.32

Liver

The liver is a key organ for protein metabolism because of its high capacity for uptake and metabolism of amino acids. It also plays an essential role in regulating the flow of dietary amino acids and other nitrogenous compounds derived from tissue degradation into the systemic circulation.33 Approximately 57% of the amino acids extracted by the liver are either oxidized or used to synthesize plasma proteins.34 Some of these essential plasma proteins made by the liver play important roles such as maintenance of oncotic pressure in the blood (albumin), transport functions (albumin, prealbumin, transferrin, lipoproteins, some globulins), clotting functions (fibrinogen, prothrombin), and enzymatic reactions (alanine aminotransferase, aspartate aminotransferase). Amino acids are transported across the basolateral membrane of the enterocyte to the portal vein circulation (Figure 6-1). Carrier systems

also exist for amino acid transport into the hepatocyte as the only site for oxidation of the essential (indispensable) amino acids, excluding the BCAAs.8,10

Another important function of the liver is the metabolism of toxic nitrogenous wastes that have accumulated from exogenous protein intake or from protein breakdown during catabolic states such as trauma or sepsis.35 The accumulation and elimination of nitrogenous wastes such as ammonia from protein degradation is accomplished by hepatic ureagenesis. Urea is subsequently excreted in the urine by the kidneys. Carbon skeletons from the degradation of the gluconeogenic amino acids (alanine, glycine, cysteine, serine, threonine, asparagine, arginine, aspartic acid, histidine, glutamic acid, glutamine, isoleucine, methionine, proline, valine, and phenylalanine) can be metabolized by the liver to glucose. Gluconeogenesis, another major homeostatic role of the liver, provides glucose when glucose supply is limited in the fasting state. Also, in critically ill patients, hepatic glucose production is increased, which can contribute to hyperglycemia in these patients.

Musculoskeletal System

BCAAs are minimally extracted by the liver; instead, they mostly enter the systemic circulation for metabolism largely by muscles. Musculoskeletal uptake of amino acids following a protein-containing meal leads to a net protein synthesis within muscle.8 The fed state supplies amino acids from dietary protein as a source of energy and helps replenish the amino acid stores. Muscle mass is maintained in a relative steady state with a fraction of the muscle protein broken down and resynthesized daily (protein turnover).34 Most amino acids are released from the muscle in proportion to their concentrations within the muscle.36 The release of BCAA is more conservative than that of other amino acids. However, alanine and glutamine release from muscle is higher than their concentrations within muscle. Alanine and glutamine have a key role in muscle BCAA metabolism by participating in the process to remove the nitrogen wastes that are produced during BCAA oxidation.10,36

The glucose-alanine cycle is another means for the musculoskeletal system to eliminate nitrogen waste while replenishing its energy supply. Glycolysis is the process of glucose oxidation within the muscle that produces alanine via the action of alanine transaminase or alanine aminotransferase. Alanine is then transported through the bloodstream to the liver where its carbon atoms are used in gluconeogenesis to produce glucose, which is delivered back to the muscle. The amino group from alanine degradation within the liver is converted to urea and eventually excreted by the kidneys.37

Leucine is the only amino acid that undergoes a complete oxidation pathway via the formation of acetyl coenzyme A in muscle, although some of its intermediates, such as alpha-ketoisocaproate, after transamination may leave muscle and be further oxidized in other tissues or organs. In the fasting state, leucine levels rise both in the bloodstream and within the muscle. In the muscle, it is oxidized to acetyl coenzyme A, an energy source for that muscle. This process simultaneously spares pyruvate oxidation in the tricarboxylic acid cycle. Pyruvate could be reduced to lactate in the absence of oxygen (anaerobic respiration) by the action of lactate dehydrogenase and released by the muscle. The carbon skeleton of pyruvate is also transferred to liver in the form of either lactate or alanine (by transamination) as the

precursor for gluconeogenesis. Therefore, leucine oxidation by the skeletal muscle may have some effect in sparing some essential amino acids as gluconeogenic precursors.8

Kidney

The kidney plays an important role in the interorgan exchange and synthesis of a variety of amino acids.38 The kidney, preferentially, extracts specific amino acids, such as glycine, alanine, glutamine, glutamate, phenylalanine, and aspartate. The kidney is also a major site for arginine, histidine, and serine production,8 and it is involved in the conversions of phenylalanine to tyrosine, and glycine to serine.38 Aside from the liver, the kidney is the only organ with the necessary enzymes for conversion of the gluconeogenic amino acids to glucose during the process of gluconeogenesis.

Kidneys also remove nitrogenous waste from the body. Enzymes within the kidney catalyze the removal of ammonia from glutamate and glutamine, promoting the excretion of nitrogenous wastes in the urine. Urea, the hepatic metabolite of ammonia, is excreted by the kidneys, and, under normal circumstances, 80% of the body's nitrogenous wastes are excreted as urinary urea nitrogen.8

Specialized parenteral glutamine-dipeptides are thought to require the kidney as a metabolic mediator. The kidney uses some of the glutamine, whereas the remainder is released into the bloodstream.38 The kidney is also the site of conversion of enterally supplemented glutamine into arginine. Glutamine is first metabolized by the gut, and its nitrogen contributes to the formation of the nonproteinogenic amino acid citrulline, which is subsequently converted by the kidneys to arginine (see "Conditionally Essential Amino Acids in Clinical Practice" section, later in this chapter).35,38

Brain and Central Nervous System

Essential amino acids are actively transported into the neurons and are present in higher concentrations in neurons than within the plasma. These amino acids perform a variety of important functions as neurotransmitters, evoking signals between neurons and other cells. Tryptophan acts as a precursor to the sleep-regulating hormone melatonin and the excitatory neurotransmitter serotonin.8

Many nonessential amino acids are required for neurotransmitter synthesis and themselves act as neurotransmitters. Tyrosine is used to synthesize the catecholamines (dopamine, norepinephrine, and epinephrine). Catecholamine levels rise within the blood during stress, causing an increase in heart rate, blood pressure, and blood glucose. Both glycine and taurine act as inhibitory neurotransmitters. In a vitamin B6–dependent reaction, glutamate is converted to gamma-aminobutyric acid, which acts as an inhibitory neurotransmitter within the central nervous system. Glutamate may also act as an excitatory neurotransmitter or combine with ammonia through the action of glutamine synthetase to form glutamine. Glutamine then diffuses freely into the blood or cerebrospinal fluid, helping to rid the brain of ammonia.8

Neuropeptides also act as potent neurotransmitters, primarily in the hypothalamus, and consist of more than 100 different peptides. These complex molecules are more potent than other neurotransmitters, thus requiring only small amounts to produce profound effects on hormone release, endocrine function,

and modulation of mood and behavior. Neuropeptides are responsible for mediating sensory and emotional responses including hunger, thirst, sex drive, pleasure, and pain. The enkephalins and endorphins are similar to opiates, affecting pain sensation, body temperature, and blood pressure regulation. CCK is one of the most abundant neuropeptides in the central nervous system (see Chapter 1). Although the function of CCK within the brain is not fully understood, it may play a role in the control of food intake or in the pathogenesis of certain types of anxiety and/or schizophrenia. Vasopressin also performs a variety of functions within the body, including specific hormonal actions in the brain with respect to memory formation, blood pressure and temperature regulation, and pair bonding.8,39

Other Organs and Body Systems

In addition, amino acids are precursors for at least 2 important compounds that widely exist in almost all tissues and organs and exert important regulatory roles in metabolism—namely, (1) glutathione formed from glycine, cysteine, and glutamate via the gamma glutamyl cycle, and (2) nitric oxide formed from arginine via nitric oxide synthase. Glutathione exists in all tissues and plays important roles against oxidative damage to the tissues. Nitric oxide plays multiple roles in modulating hemodynamics, immune function, and neurotransmission. These functions are important in disease conditions, especially in critical illness. The availability of their precursor amino acids in nutrition support is a factor related to the quantity and function of these important mediators.

Food and Protein

Adaptation to Fasting and Starvation

Protein is the primary substrate for gluconeogenesis during starvation metabolism. The fasting state occurs as blood glucose levels return to a baseline level before the start of the next meal with a decrease in insulin levels and rise in glucagon levels.40 Tissues such as the brain and red blood cells require a constant supply of glucose, even when there is no exogenous supply (as during fasting). The breakdown of hepatic glycogen stores (glycogenolysis) for glucose production begins within 2 to 3 hours of fasting, and stores could be depleted in approximately 24 hours.10

Gluconeogenesis from amino acids and other noncarbohydrate sources, such as glycerol and lactic acid, occurs in both the liver and the kidney and begins within 4 to 6 hours of the last meal.41 After approximately 2 days of starvation, the brain switches its fuel source from glucose to ketone bodies; however, blood cells and the adrenal medulla continue to rely on glucose as the primary source of energy substrate. This adaptation to starvation involves the conversion in the liver of free fatty acids to ketone bodies. As the metabolically active lean mass is diminished with starvation, metabolic processes occurring within these tissues are also reduced, accounting for a reduction in resting energy expenditure. Within approximately 1 week, adaptation to starvation with a ketone-based fuel system minimizes gluconeogenesis and further protein breakdown.42,43

Determining Protein Quality

Protein quality is evaluated by calculating the protein digestibility-corrected amino acid score (PDCAAS). PDCAAS was proposed in 1991 by the Food and Agriculture Organization/World Health Organization (FAO/WHO) and has been adopted by the Food and Nutrition Board of the Health and Medicine Division (formerly Institute of Medicine) of the National Academies of Science.44 The PDCAAS indicates the overall quality of a protein because it represents the relative adequacy of its most limiting amino acid. The PDCAAS is estimated as the concentration of the most limiting, digestibility-corrected amino acid in a test protein, as follows:

PDCAAS = Concentration of That Amino Acid in the 1991 FAO/WHO Amino Acid Scoring Reference Pattern

For example, if a protein has a PDCAAS of 40 and the lowest amino acid content is for lysine, then 40% of that protein can be used for protein synthesis. The highest PDCAAS is 100, which would mean that 100% of the amino acids of that protein after digestion can be used for protein synthesis, or that the protein contains all essential amino acids in adequate proportions. Given that typical diets contain many sources of dietary protein, foods could function as “complementary proteins” if they are higher in the amino acid that is limiting in other protein.45

An important benefit to using the PDCAAS is that this approach uses human amino acid requirements whereas animal bioassays were used in the past.35 An additional step is added to this method: the lowest amino acid ratio is then multiplied by its true protein digestibility. This ensures that the appropriate levels of essential amino acids are provided in the diet.

Determining Protein Content in Food

Historically, the protein content of foods was determined by multiplying total nitrogen, as measured by the Kjeldahl method, by a conversion factor. This approach is based on 2 assumptions: first, that carbohydrates and fats do not contain nitrogen, and, second, that almost all nitrogen in the diet is present as amino acids in proteins. Based on early estimates, the average nitrogen content of proteins was estimated to be 16%, which led to the use of the following calculation to convert nitrogen to protein:46

Protein = Nitrogen × 6.25

where the conversion factor 6.25 was calculated by dividing 1 by 16%.

However, estimating nitrogen content of dietary protein with the use of a single factor may not accurately reflect true nitrogen content. Not all nitrogen in foods is protein-based. For example, nitrogen may be part of amino acids, nucleotides, creatine, and choline, where it is referred to as nonprotein nitrogen. Only a small percentage of nonprotein nitrogen is available to synthesize nonessential amino acids. Moreover, the nitrogen content of specific amino acids varies with the molecular weight of the amino acid and the number of nitrogen atoms it contains (1 to 4, depending on

the amino acid in question). Based on this information, we know that the nitrogen content of proteins varies from 13% to 19%, and nitrogen conversion factors range from 5.26 to 7.69.

Other factors that have been used to determine dietary protein content include “Jones” factors, which are nitrogen conversion factors for specific foods.47 For example, Jones factors for animal protein such as meat, milk, and eggs are between 6.25 and 6.38, whereas those for vegetable proteins are in the range of 5.7 to 6.25. The difference between the conventional 6.25 conversion factor and the Jones factor is only about 1% for mixed diets. The protein content for enteral and parenteral formulations is also determined by amino acid analysis. The protein content of the enteral or parenteral formula can be calculated from its amino acid concentrations. It is important to realize, however, that the total amount of protein provided from an IV amino acid mixture is about 17% less than what would be assumed. When a peptide bond is made, a molecule of water is released; thus, the weight of a peptide bond is 18 mass units less than its molecular weight.48 In essence, to provide 1.0 to 1.5 g of protein per kg of body weight via PN, one needs to administer 1.2 to 1.8 g of a mixed amino acid solution.48

Protein in Clinical Practice

Optimizing Nitrogen-Energy Relationships

Loss of significant body protein is associated with disability and death. This process is accelerated in critical illness due to increased protein catabolism and prolonged bed rest and can be further hastened by an inadequate nutrient intake. However, providing energy from nonprotein sources in amounts to meet energy demands has important effects on NB. Calloway and Spector reported nitrogen sparing in early experiments with healthy active men when energy was supplied without protein.49 Maximum protein sparing occurred at approximately 700 nonprotein kcal. Additional increases in calories energy intake did not improve NB. However, addition of protein in dietary intake further improved nitrogen sparing. The researchers concluded that, with fixed but adequate protein intake, the energy level is a determinant factor in nitrogen equilibrium, and, with fixed and adequate energy intake, protein intake determines nitrogen equilibrium.

The Recommended Dietary Allowance for protein is set at 0.8 g/kg/d for healthy adults, based on NB studies performed in men and women who were receiving their full energy requirements. Adding 25%, or 2 standard deviations, to the balance data meets the needs of 97.5% of a normally distributed population.44

Critical illness is associated with increased protein turnover, protein catabolism, and negative NB, usually associated with hypermetabolism. The increased metabolic rate and accelerated net protein loss demand greater energy and protein intake. In fact, protein requirements double in critical illness, to approximately 15% to 20% of total energy; this increment could be much higher in very severe injury such as a large-area burn. For example, the estimated protein requirement for a patient with multiple traumas who requires 2600 kcal/d for maintenance would be at least 98 to 120 g/d. With regard to critically ill patients, there is controversy regarding whether full energy provision in the first week of intensive care is beneficial.50 Many of the permissive underfeeding studies have some methodological limitations. However, outcomes seem to be similar when comparing full energy feeds with underfeeding

in the first week of intensive care, keeping in mind that it is unusual to provide full energy needs in the first week if relying on enteral feedings alone.

Clearly, the goals for a patient who is at nutrition risk or malnourished should be to meet full energy and protein needs as early as possible.51 Current practice dictates that energy goals should meet energy demands—which, ideally, are determined by indirect calorimetry, or, alternatively, are calculated with energy equations—and protein intake should be at least 1.5 g/kg/d. However, recommendations in trauma and burns suggest protein requirements as high as 2.5 g/kg/d.52,53 NB is difficult to attain in critically ill patients and may not be a realistic goal. Exogenous protein will improve nitrogen retention by increasing synthesis, but it has little effect on protein catabolic rates.

Protein Requirements in Critical Illness

Nutrient requirements of critically ill patients are remarkably different than in normal individuals because of profound alterations in carbohydrate, lipid, and protein metabolism. The primary goal of nutrition therapy in critical illness is to preserve lean body mass and support metabolic functions. Lean-tissue loss inevitably occurs because of the rapid rates of protein breakdown, mobilization of amino acids from the periphery to the liver for gluconeogenesis, and production of acute-phase proteins, to support the immune system in host defense, to the wound to promote healing, and to the kidney for acid-base balance. Immobilization after critical illness and, in some cases, starvation and prolonged bed rest also contribute to lean-tissue wasting. A generalized hypoaminoacidemia occurs in critical illness due to the increased uptake of amino acids to meet the needs of the rapidly turning over central proteins (liver, kidney, immune cells). Therefore, providing adequate exogenous amino acids could potentially improve clinical outcomes by increasing protein synthesis to ameliorate the inflammatory response and by mitigating the loss of muscle protein observed in the first week of critical illness and beyond when critical illness is prolonged.52,54–57

After injury, sepsis, or critical illness, protein turnover rates, synthesis, breakdown, and oxidation of protein are increased. The degree of increase is determined by the severity of the illness and metabolic response. The negative NB seen after minor elective surgery is due primarily to a decrease in protein synthesis. However, with severe sepsis and injury, both synthesis and catabolism are elevated and feeding further increases turnover. An older (1987) protein turnover study by Shaw and associates suggested that feeding protein in patients with sepsis at 1.1 g/kg/d decreases net protein-loss catabolism. When protein intake was increased to 1.6 g/kg/d, catabolism was decreased further; however, catabolism increased again when protein intake was greater than 1.6 g/kg/d.58 Studies such as the one by Shaw et al58 from 30-plus years ago could be criticized for their low numbers of patient enrollment and for possibly overfeeding critically ill patients; however, numerous other studies using different methods and clinical observations mostly serve as a basis for the current protein recommendations of 1.2 to 1.5 g/kg/d. Several recently published comprehensive reviews of protein requirements in critical illness suggest that most critically ill patients may require much more than 1.5 g/kg/d,52,54,55 and this recommendation is corroborated by recently published research suggesting the same.59–61 Further support can be lent from the report that the oxidation rates of the most essential amino acids measured by their 13C-labeled tracers were about 50% higher in critically ill patients than in

the healthy subjects.61 Therefore, considering the protein requirement for healthy individuals is 0.8 to 1.0 g/kg/d, the protein requirement of critically ill patients could be at the level of 1.5g/kg/d. Using data from the 2013 International Nutrition Survey, Nicolo and colleagues evaluated the energy and protein intakes of 2828 patients who were in the intensive care unit (ICU) for 4 days or longer.61 Patients who received at least 80% of their protein requirements had shorter times to discharge alive (TDA) and reduced mortality. Receiving more than 80% of energy requirements did not have any effect on TDA or survival.

The risks of feeding too much protein include prerenal azotemia, with the excess protein increasing the burden to the kidneys to work to excrete excess urea nitrogen. Excess protein feeding over the long term can result in kidney stones and increased risk for osteoporosis. Nevertheless, adequately fed, underweight patients may require protein up to 25% more than their normal weight counterparts, or more than 1.9 g/kg/d.62,63

To determine the adequate protein supply to support a specific patient, the clinician must take into consideration the nutrition status of that patient on admission and the underlying disease. Clinical studies of underweight critically ill patients have not been performed; however, as weight decreases, there is a loss of both fat and muscle and the more rapidly turning over or metabolically active central protein compartments (liver, heart, lungs, brain) make up a greater proportion of the total body weight; therefore, metabolic rate and protein requirements may be higher per kilogram of body weight in an underweight versus normal or overweight individual.64–67

Conditionally Essential Amino Acids in Clinical Practice

Some amino acids may exert pharmacological action during critical illness when administered at higher doses than normally consumed in food or standard nutrition support. For example, glutamine metabolism during critical illness has been extensively investigated during the past 20 years. Glutamine is the most abundant amino acid in the body, accounting for more than 50% of the intracellular free amino acid pool in muscle. It is the vital fuel for rapidly dividing cells such as fibroblasts, reticuloendothelial cells, malignant cells, and gut epithelial cells. Glutamine carries 2 nitrogen moieties per molecule, and, along with alanine, it serves as the body's primary nitrogen shuttle between muscle and visceral organs. In some clinical conditions, such as exercise, trauma, and sepsis, the body's glutamine requirement exceeds the mammalian's ability to synthesize glutamine; this imbalance leads to a fall in plasma and intracellular glutamine levels. This fall in glutamine concentration is associated with atrophy of intestinal mucosa, impairment of immune function, decreased protein synthesis, and even "second-hit" sepsis due to the impaired gut barrier function against bacterial translocation. Low plasma and muscle concentrations of glutamine in critical illness are correlated with increased mortality, leading to the hypothesis that replenishment of glutamine levels has a therapeutic value in certain clinical conditions. However, the REDOX trial, which included 40 ICUs and randomly assigned 1223 ventilated ICU patients to receive glutamine (IV and enteral), antioxidants, both glutamine and antioxidants, or placebo, suggested a trend toward an increased 28-day mortality and significant hospital and 6-month mortality in those patients that received glutamine.68 However, the amount of glutamine (approximately 60 g) administered in this trial was much higher than that administered in

previous trials. Some smaller recent trials suggest glutamine supplementation may be beneficial in selected burn and surgical patient populations, but larger randomized controlled trials need to be performed before glutamine supplementation can be routinely recommended.69–71

Arginine and cysteine, the former having an important role in immunomodulation, may also have pharmacological properties. Arginine has demonstrated importance in immune function and wound healing. It is essential for young mammals, but adults can synthesize it de novo. Plasma arginine levels are usually dependent on dietary intake because the rate of endogenous synthesis cannot increase in response to an inadequate supply. Arginine is a urea-cycle intermediate. It is deaminated to citrulline and used to synthesize polyamines and nitric oxide, which affects respiratory, cardiovascular, renal, and immunological function. Arginine and cysteine also enhance lymphocyte function and may prove useful in treating inflammatory diseases and AIDS.62 Arginine is a key component of some immune-enhancing enteral nutrition formulas.72 It also plays a role in promoting wound healing, partially because of its relationship to the metabolic pathway of arginine-ornithine-proline. See Chapters 23 and 24 for more on this topic.

Additional proteins with pharmacological potential include carnitine and choline. Carnitine is a trimethylated amino acid similar in structure to choline, which is required as a cofactor for transformation of free long-chain fatty acids into acylcarnitines and their transport into the mitochondria. Although a primary deficiency in carnitine is rare, it has been documented in preterm infants and in chronic renal failure. Carnitine metabolism is discussed in greater detail in Chapter 5.

Choline is also an essential nutrient necessary for proper membrane structure, which is not part of PN formulations. It can be manufactured in the liver from methionine, but synthesis may be impaired in patients on long-term PN or in premature infants. IV methionine is metabolized differently than orally or enterally consumed methionine, which may be partially responsible for the choline deficiency that develops with PN.73 Small amounts of choline are available in the form of lecithin present in lipid injectable emulsions, but, despite this, low choline levels have been documented in patients receiving PN.74 There is some evidence that choline deficiency is partially responsible for the hepatic abnormalities prevalent in patients on long-term PN, specifically hepatic steatosis. There are no available IV forms of choline. Repletion, if necessary, needs to be via the oral or enteral route.

Finally, creatine and beta-hydroxy beta-methylbutyrate (HMB) have been marketed as ergogenic aids in the sports medicine literature. They have both been extensively studied and have consistently been shown to increase lean body mass and improve strength when combined with resistance training.75 Creatine has also been studied in diseases associated with muscle wasting (amyotrophic lateral sclerosis and muscular dystrophy).76 HMB is a metabolite of leucine, but its mechanism of action remains elusive. It has been used as a supplement with glutamine in wasting associated with AIDS.77

The ingestion of 3 g of HMB per day in the elderly promotes an increase in strength and lean body mass when combined with resistance exercise.78,79 Its role in the treatment of the sarcopenia of aging is currently under investigation.