Modification of Protein Metabolism Due to Disease

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Protein metabolism in human beings involves the breakdown of food protein into peptides and amino acids, and the distribution of and use of the amino acids as building blocks for protein molecules, as precursors for non-protein metabolites (e.g., neurotransmitters, purines and pyrimidines, creatinine, peptide hormones, etc.), or as a source of energy. Table 1 summarizes some of the functional properties of proteins. During infancy and childhood, protein metabolism is directed toward growth and maturation of tissues. At this stage in life, a dietary lack of protein or an injury or disease that interferes with protein metabolism can have the most dramatic effects on health and physical and mental development.

Protein turnover is highest in the premature infant and declines through childhood to adulthood. Because rates of whole body protein synthesis and breakdown are considerably greater than the intake of dietary protein estimated to meet the needs for growth or for the maintenance of nitrogen balance, there is extensive reutilization within the body of the amino acids liberated during the course of continual protein breakdown. This recycling of amino acids is subject to changes in response to various stimuli, including alterations in the level and adequacy of protein and energy intakes. Recently, it has been suggested that a relatively high rate of protein turnover is beneficial to the host and a useful indicator of the adequacy of amino acid intake (1). Support for this view stems from the principles of metabolic regulation described by Crabtree & Newsholme (2). These investigators argued that high fluxes of substrates through major pathways provide the organism with a precise and sensitive control mechanism for the provision of metabolic intermediates for various biosynthetic pathways. With respect to amino acid metabolism, Newsholme et al. (3) have shown that glutamine is both essential for and has a high flux in lymphocytes, macrophages, and other rapidly growing and dividing cells. The source of glutamine is either from dietary protein or via amino acid metabolism, leading to the possibility that high rates of muscle protein turnover facilitate the continued availability of glutamine for the support of lymphocyte metabolism and function. It is also proposed that high rates of muscle protein degradation in patients suffering from major trauma or
TABLE 1. Functional properties of proteins

<table>
<thead>
<tr>
<th>Function</th>
<th>Example</th>
<th>Clinical relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal/messenger proteins</td>
<td>Insulin</td>
<td>Primary anabolic hormone in the body; insulin stimulates amino acid uptake by cells, which supports cellular metabolism and nutrition</td>
</tr>
<tr>
<td>(hormones)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzymatic catalysis</td>
<td>Glutamine synthetase</td>
<td>A key enzyme in skeletal muscle and other tissues responsible for catalyzing the synthesis of glutamine, the most abundant amino acid in the body, and the amino acid most affected by surgical and other stress</td>
</tr>
<tr>
<td>Transport</td>
<td>Hemoglobin</td>
<td>Transports oxygen in erythrocytes</td>
</tr>
<tr>
<td>Storage</td>
<td>Myoglobin</td>
<td>Transports oxygen in muscle</td>
</tr>
<tr>
<td>Coordinated motion</td>
<td>Actin-myosin</td>
<td>Allows contraction of skeletal muscle</td>
</tr>
<tr>
<td>Mechanical support</td>
<td>Collagen</td>
<td>A fibrous protein made by fibroblasts; required for wound healing</td>
</tr>
<tr>
<td>Immune function</td>
<td>Antibodies</td>
<td>Specific proteins that recognize foreign antigens and bacteria and are essential in fighting infection; they help distinguish between self and non-self.</td>
</tr>
<tr>
<td>Generation and transmission of</td>
<td>Chemoreceptor proteins</td>
<td>Responsible for transmitting nerve impulses</td>
</tr>
<tr>
<td>nerve impulses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control of growth and differentiation</td>
<td>Epidermal growth factor (EGF)</td>
<td>Stimulates cell proliferation and gut mucosal growth; may promote wound healing in some patients</td>
</tr>
</tbody>
</table>

From Dudrick PS, Souba WW (24).

illness serve to assure an adequate supply of precursors and substrates to meet the increased metabolic demands of the immune system and tissue repair processes. Figure 1 shows that the most important quantitative aspect of amino acid metabolism is the flow of amino acids into and out of the tissue free amino acid pools (protein anabolism and catabolism); the flow into other pathways is less important. Changes in the flux of amino acids into and out of major tissue proteins may be expected to alter the ability of cells to use amino acids for other biosynthetic functions, especially when there is increased metabolic demand as a result of stressful stimuli.

PROTEIN METABOLISM DURING INFLAMMATION AND INFECTION

A prominent metabolic response in patients with fever and infection is a marked increase in the release of amino acids from peripheral tissues, associated with an enhanced uptake of these amino acids by the liver for gluconeogenesis and for use in the synthesis of acute-phase proteins (4). The response pattern for the accelerated proteolysis, increased nitrogen excretion, and prolonged negative nitrogen balance
occurring with sepsis is similar to that described for injury. A large proportion of the amino acid nitrogen is excreted in the form of urea, resulting in increased loss of nitrogen from the body. Acidosis frequently occurs in the septic patient, serving as a signal for increased glutamine uptake by the kidney. Glutamine liberates ammonia ions, which combine with hydrogen ions and are then excreted in the urine, thereby participating in the maintenance of acid-base balance. Glutamine originates from the breakdown of skeletal muscle protein; hence an increase in the renal uptake of glutamine acts as another stimulus for increased proteolysis (4). In addition, almost any kind of stress will induce a rise in plasma glucocorticoid levels. Increased cortisol concentrations result in protein breakdown with loss of both glutamine and alanine (5). Glucocorticoids have also been described as having effects on mediators of the stress-induced mechanisms described below (6).

The inflammatory response results from stimulation of macrophages and phagocytes by foreign antigens or tissue injury to produce cytokines, interleukin-1 (IL-1), and tumor necrosis factor (TNF). The physiologic responses initiated by IL-1 and/or TNF include fever, a redistribution of trace elements, skeletal muscle catabolism, and an increase in hepatic protein synthesis. Ramadori et al. (7) showed that IL-1
is involved in triggering the transcription and synthesis by the liver of acute-phase proteins, which play several roles in helping the organism to withstand the insult. For example, protease inhibitors such as $\alpha_2$-antitrypsin and $\alpha_2$-macroglobulin may accumulate at the site of injury and prevent additional damage caused by the release of proteolytic enzymes from phagocytic cells and already-damaged tissues (8). Fibrinogen may increase the tensile strength of a wound and stimulate fibroblast proliferation and growth (9). C-reactive proteins and albumin may act as essential nutrients to facilitate healing or aid in transporting trace metal cofactors (10). The redistribution of body zinc, an important component of metalloenzymes involved in carbohydrate, lipid, and protein metabolism, has also been shown to correlate with the increased production of acute-phase proteins by IL-1 (11). Other important functions of the acute-phase proteins include the modulation of the rate of synthesis of structural proteins, hormonal transport, inhibition of microbial invasion, neutralization of potentially toxic products, and local modulation of humoral effects.

Many metabolic responses to IL-1 and endotoxins, including granulocytosis, fever production, and the acute-phase protein response, have been shown to be less in the protein-depleted guinea pig model (12). Nonstressed protein-malnourished patients have been found to have a reduction in the IL-1 response (13) that returned to normal after adequate nutrition was provided (14). Bistrian et al. (15) recently reviewed work on cytokines, muscle proteolysis, and catabolic responses to infection and inflammation and concluded that nutritional intervention to restore the acute-phase response is very important. The role of other substrates such as fish oil in modulating the infection-injury response was also addressed; this is a new field to consider when planning nutritional therapies.

PROTEIN METABOLISM DURING SURGERY

It had been thought that infants responded qualitatively differently from adults to the insult of stress and trauma, but studies of postoperative neonatal metabolism suggest that the only differences are quantitative (16). Surgery is well known to increase protein catabolism (17). Patients often have diminished body protein content prior to surgery and as a result postoperative protein depletion may be both common and severe. As discussed earlier, protein depletion may be associated with impaired wound healing and increased susceptibility to infection (4). Several studies have shown that undernutrition and hypoproteinemia are associated with an overall increase in patient morbidity and mortality (18). There is little doubt that improved feeding techniques have decreased the morbidity and mortality in low birthweight neonates (19,20). The efficacy of nutritional support for infants with surgical gastrointestinal disorders has been documented (20,21). Early investigators suggested that the catabolic responses to operation were “obligatory” and nutritional intervention was directed at inhibiting peripheral proteolysis. More recent evidence suggests that accelerated amino acid flux from the periphery to the liver is beneficial to the organism and that nutritional support should be directed at supporting hepatic protein synthesis rather than blocking protein breakdown in muscle tissue (22,23). Attention
to the role of nutritional intervention in enhancing the individual's response to inflammation, infection, and surgical trauma is increasing and the role of specific amino acids in these responses is becoming clearer (24). Two of these amino acids—glutamine and arginine—will be described in greater detail.

**Glutamine**

Glutamine is an amino acid that has received considerable attention in the past decade because of a new awareness that its metabolism is affected by illness (23,25) and that it may be a conditionally essential amino acid (23,26). Glutamine is the most abundant amino acid in the body's free amino acid pool. Its concentration in skeletal muscle is 30 times the circulating concentration in blood. Glutamine functions as a vehicle for nitrogen transport, as a substrate for renal ammonia formation, hepatic urea formation, and gluconeogenesis, and as an essential precursor for nucleotide synthesis (23). Glutamine is also avidly consumed by replicating cells, such as gut mucosal cells (27), lymphocytes (3), and hepatocytes (25). The intestine uses glutamine as an oxidative fuel, sparing the use of glucose (27). In addition, alanine produced as a result of glutamine metabolism provides as much as 50% of the alanine removed by the liver during critical illness (28). In critically ill patients, glutamine concentrations in the body are not only quite high but are also very labile. A decline in glutamine levels correlates in general with the severity of the underlying insult and is reversed later in the course of recovery (26).

An important distinction between glutamine utilization resulting from the catabolic stress of surgery and that induced by sepsis or endotoxinemia is the site of enhanced glutamine uptake (23). Intestinal consumption of circulating glutamine is impaired during sepsis and in endotoxin-treated rats, while markedly increased in the liver. In contrast, the gut avidly consumes glutamine as a result of surgical stress. Nevertheless, recent reports suggest that glutamine-enriched diets are beneficial to patients in whom glutamine depletion is severe and/or when the intestinal mucosa is damaged by insults such as starvation, chemotherapy, radiation therapy, etc. (23,24,26). Glutamine supplementation has trophic effects on the intestinal mucosa (23) and reduces the translocation of bacteria across the gut in animals (29). Glutamine-supplemented parenteral nutrition has also been shown to improve nitrogen balance and reduce hepatic steatosis and pancreatic atrophy (23,30). Concern about the safety of excess glutamine intake has been raised because its metabolism can yield two ammonia molecules. However, glutamine has been given both orally and intravenously to healthy human volunteers without untoward effects (31) and preliminary data support its safety in infants (32). The implications for the very low birthweight infant are great and changes in nutritional recommendations may be forthcoming.

**Arginine**

Arginine is another amino acid that has received recent attention and is now considered to be conditionally indispensable. Recent studies have shown that arginine supplementation in amounts exceeding the estimated requirements may be of benefit to
stressed animals by enhancing mitogen-stimulated lymphocyte proliferation, decreasing stress-induced thymic involution, and improving wound healing (33). Arginine is also the immediate precursor of urea and nitric oxide (NO) (34–36). NO has been identified as an important mediator of cellular communication in several biological systems, including inflammatory cells (macrophages and neutrophils), brain tissue, and endothelial cells (34,35). The biological roles of NO depend on the specific tissues in question. These include the regulation of vascular tone, the stimulation of soluble guanylate cyclase in the central nervous system, the regulation of platelet aggregation, and the mediation of immune processes (e.g., cytotoxic cytostatic effect on certain microorganisms and tumor cells, modulation of lymphocyte behavior) (34–36). In other systems, data suggest that in vivo metabolic products of L-arginine, such as NO, may be directly or indirectly linked to immune complex–induced tissue injury (37). Certainly more work needs to be done to address the implications of the synthesis of NO in terms of potential novel treatments for different disease states.

HORMONAL REGULATION

Discussion of the alterations in protein metabolism due to disease must include a brief assessment of the role of hormones. Anand et al. (38) described the changes in stress-related hormones in preterm and term infants undergoing anesthesia and surgery. They suggested that minimizing the catabolic state characterized by glyco- genolysis, gluconeogenesis, lipolysis, and mobilization of gluconeogenic substrates by anesthetic or hormonal manipulation may be warranted. Recent work directed at augmenting body protein synthesis with recombinant human growth hormone has shown promotion of weight gain and whole body nitrogen retention in humans (39) and enhancement of muscle myosin mRNA and amino acid accumulation in humans (40). These encouraging results support the hypothesis that maintenance of anabolism through hormonal manipulation rather than limitation of catabolism has therapeutic potential. Along these lines, several investigators have reported improved growth and nitrogen retention (41), prevention of protein losses associated with pharmacologic doses of glucocorticosteroids (42), and increased lean body mass (43) as a result of growth hormone administration. More recently, Ziegler et al. (44) reported on a multicenter, randomized, double-blind study examining whether recombinant growth hormone improves the efficacy of total parenteral nutrition. Their study group included 15 patients requiring parenteral nutrition because of gastrointestinal or pancreatic disease, who were randomized to receive daily subcutaneous injections of saline or growth hormone for 14 days. Growth hormone administration was associated with a significant increase in nitrogen, potassium, and phosphorus balance, leading the authors to conclude that it may be a useful adjunct to parenteral nutrition in order to enhance nutrient retention and nutritional recovery. The implication for treatment of the very low birthweight infant remains to be clarified.

REFERENCES


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DISCUSSION

Dr. Guesry: You did not mention the role of tumor necrosis factor (TNF) and interleukin-2 in the genesis of the increased catabolic state during infection.

Dr. Fukagawa: There is evidence that both TNF and interleukin-1 are related to infection-induced catabolism and may indeed be mediators of it. There appears to be a difference in their effects. TNF tends to regulate protein turnover primarily in the periphery, redistributing amino acids to the liver for synthesis of acute-phase proteins. It appears to be the opposite with interleukin-1; namely, amino acids are mobilized from visceral stones and primarily catabolized. However, I think that the regulation of the release of cytokines and how they affect protein turnover is an area that needs further investigation.

Dr. Guesry: What tools can the clinician use to monitor catabolism? Can creatinine or 3-methylhistidine be used as indicators? If you have to inject C-13 leucine to monitor your babies it is going to be very difficult.

Dr. Fukagawa: This is a difficult issue. There is no really good index. However, following the plasma concentration of specific plasma proteins, for example, albumin (though it has a high turnover and is influenced by drug therapy), is quite a good index of protein nutrition per se. In the very low birthweight infant concentrations of the acute-phase proteins may be helpful, for example retinol-binding protein.

Dr. Priolisi: How much recycling of amino acids occurs during catabolic states?

Dr. Fukagawa: Recycling does occur but we believe that significant amounts of the amino
acids released from muscle during catabolism are reutilized for the synthesis of acute-phase proteins by the liver. Our techniques, however, have not up to now been precise enough to enable us to look in detail at turnover rates in specific organs and tissues.

**Dr. Micheli:** Could you tell us, at the level of the clinician, what are the factors that regulate protein breakdown?

**Dr. Fukagawa:** This is a difficult question for which there is no real answer as yet. However, I think that the major underlying regulator of protein breakdown is the need for precursors for other metabolic pathways. Through the 1970s we got into the way of thinking that increased nitrogen excretion was bad and that we should act to try to limit protein breakdown. Now our thinking is more that the body is responding to the need for more amino acids by increased protein turnover (and potentially increased nitrogen losses). The increased availability of free amino acids allows immediate access to precursors to fine-tune the synthesis of acute-phase proteins, neurotransmitters, and so on. Hence the answer is not to attempt to stop the increased nitrogen loss but to support the patient so that this increased availability of amino acids can be maintained.

**Dr. Jéquier:** You have said, and reiterated in the answer to Dr. Micheli's question, that efforts should be made to increase protein turnover in inflammation and infection rather than slowing it down. This implies that there must be some way of slowing down protein turnover. What is it?

**Dr. Fukagawa:** In fact numerous attempts to slow down protein turnover have been unsuccessful in the past 10 years. Maybe the body is trying to tell us that our attempts to normalize turnover are not in its best interests.

**Dr. Bucci:** About 10 years ago we began to give total parenteral nutrition to premature babies, using a preparation available in Italy at the time. It soon became apparent that our babies were developing metabolic alkalosis, whereas everywhere else in the world TPN is associated with acidosis! On looking more closely at the composition of the preparation we found that it contained large amounts of glutamic acid that were producing a considerable cation gap. We found huge quantities of glutamine in the blood so it was clear that glutamate was being converted to glutamine. We followed up these babies and found no evidence that the nervous system was affected by the high glutamine levels. It is clear that one way to get high blood glutamine is to give glutamate.

**Dr. Fukagawa:** This is a very important point. However, excessive free glutamate in the circulation could have adverse neurological effects because it acts as a neurotransmitter.

**Dr. Vidailhet:** Did you check for glutamic acid and the ratio of glutamine to glutamic acid during sepsis in your routine analyses?

**Dr. Fukagawa:** The problem with routine samples is that glutamine is hydrolyzed to glutamic acid so the measured values of glutamic acid do not reflect the values in the plasma when the samples were taken.

**Dr. Orzalesi:** As a clinician I was interested in the interrelationships between protein turnover, protein breakdown, and protein intake or nutritional adequacy. If the purpose of the increased turnover is to make single amino acids available as precursors for single functions, can we envisage a situation in which a disorder gives rise to the need for a particular amino acid, and where the absence of that amino acid could limit the speed of recovery? In such a case the correct approach would be to supply the particular amino acid rather than to increase the protein intake. An example would be an increased requirement for sulfur amino acids where mucus production has been increased, as in diarrhea.

**Dr. Fukagawa:** The problem is that it is very hard to know which amino acid or acids are
specifically needed, and even if one could have an informed guess, as in the example you gave, it is difficult to provide specific amino acids on their own in parenteral solutions, though they could be given orally as dipeptides.

Dr. Heird: I was interested in the relationship you described between glutamine and decreased hepatic steatosis. Is anything known about the mechanism? Can glutamine decrease the incidence of cholestasis, which is so common in small babies on parenteral nutrition?

Dr. Fukagawa: There is some evidence from the studies on parenteral glutamine that cholestasis is reduced, but this may only reflect the fact that the treated infants were able to come off TPN earlier. The reduction in hepatic steatosis has been related to increased glucose utilization and reduced availability of acetyl-COA for fatty acid synthesis.