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# Regulation of protein metabolism by glutamine: implications for nutrition and health

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## 1. ABSTRACT

Glutamine is the most abundant free alpha-amino acid in plasma and skeletal muscle. This nutrient plays an important role in regulating gene expression, protein turnover, anti-oxidative function, nutrient metabolism, immunity, and acid-base balance. Interestingly, intracellular and extracellular concentrations of glutamine exhibit marked reductions in response to infection, sepsis, severe burn, cancer, and other pathological factors. This raised an important question of whether glutamine may be a key mediator of muscle loss and negative nitrogen balance in critically ill and injured patients. Therefore, since the initial reports in late 1980s that glutamine could stimulate protein synthesis and inhibit proteolysis in rat skeletal muscle, there has been growing interest in the use of this functional amino acid to improve protein balance under various physiological and disease conditions. Although inconsistent results have appeared in the literature regarding a therapeutic role of glutamine in clinical medicine, a majority of studies indicate that supplementing appropriate doses of glutamine to enteral diets or parenteral solutions is beneficial for improving nitrogen balance in animals or humans with glutamine deficiency.

## 2. INTRODUCTION

L-Glutamine is a neutral amino acid which is present at high concentrations in plasma (0.5 to 1.0 mM), skeletal muscle (5 to 20 mM), milk (0.5 to 4 mM), and fetal allantoic fluid (1 to 25 mM) depending on species and developmental stages (1-5). This nutrient is synthesized from glutamate plus ammonia by glutamine synthetase in all animal species, with skeletal muscle being quantitatively the major site (6). It is now recognized that the intramuscular level of glutamine is regulated by both synthesis and degradation (7). Of particular note, glutamine is rapidly depleted in cells and tissues (e.g., blood and skeletal muscle) under a wide array of physiological (e.g., lactation and weaning) (4, 8) and pathological conditions (e.g., infection, sepsis, severe burn, and cancer) (9). These results indicate that glutamine synthesis is not sufficient to meet its optimal needs for lactating mothers, rapidly growing mammals, and catabolic subjects. Thus, glutamine is now classified as a conditionally essential amino acid for animals and humans (3,10). Besides its utilization for protein synthesis, glutamine is degraded by phosphate-activated glutaminase to form glutamate in all animal cells that contain mitochondria, with the small intestine, kidneys and

leukocytes being the major sites for its catabolism (11).

Rennie and co-workers made seminal observations in late 1980s regarding a novel role for glutamine in regulating muscle protein turnover. These authors reported that infusion of glutamine into rat skeletal muscle increased protein synthesis (12) and inhibited protein breakdown (13). Subsequently, Wu and Thompson (14) found that elevating extracellular concentrations of glutamine from 1 mM (physiological level in chick plasma) to 15 mM dose-dependently increased protein synthesis and decreased protein degradation in chicken skeletal muscle. Results of a recent *in vivo* study have firmly established that there is a positive relationship between intramuscular concentrations of glutamine and muscle protein synthesis in chickens (15). Besides skeletal muscle, glutamine also stimulates protein synthesis [often measured as fractional rate of synthesis (FRS) in %/day] and inhibits proteolysis in mucosal cells of the small intestine (16,17). The underlying mechanisms are largely unknown, but may involve the activation of the mammalian target of rapamycin (mTOR) signaling (18,19). In view of recent developments in glutamine biochemistry and nutrition, the objective of this article is to highlight nutritional and therapeutic role for this amino acid under a wide array of physiological and pathological conditions.

## 3. FUNCTIONS OF GLUTAMINE IN NUTRITION AND METABOLISM

Glutamine is a major energy substrate for rapidly dividing cells (including enterocytes and lymphocytes) and other cell types (e.g., macrophages and kidneys), providing ATP for intracellular protein turnover, nutrient transport through the plasma membrane, cell growth and migration, as well as the maintenance of cell integrity (9). Particularly, the formation of ammonia from glutamine is vital for the renal regulation of acid-base balance in animals. This amino acid is also a precursor for the synthesis of purine and pyrimidine nucleotides that are essential for the proliferation of cells, including intraepithelial lymphocytes, embryonic cells, and trophoblasts (16). Importantly, glutamine provides both nitrogen and carbon skeleton for the endogenous synthesis of arginine in most mammals (including humans, pigs, cattle, and sheep) via the intestinal-renal axis (11, 20). This synthetic pathway compensates for a deficiency of arginine (an essential amino acid for neonates) in milk during the suckling period and for the extensive catabolism of dietary arginine by the small intestine of postweaning animals (21). Furthermore, glutamine is required for the synthesis of N-acetylglucosamine-6-phosphate, a common substrate for the synthesis of glycoproteins that are particularly rich in intestinal mucosal cells (22). As a precursor of glutamate, glutamine plays a role in the synthesis of glutathione, the most abundant small-molecular-weight antioxidant in cells (23).

Glutamine increases expression of genes that beneficially regulate nutrient metabolism and cell survival (16, 24-27). These genes include ornithine decarboxylase, heat-shock proteins, anti-oxidative proteins, nitric oxide synthase, heme oxygenase. Notably, ornithine decarboxylase is a key enzyme for the synthesis of polyamines that function to stimulate DNA and protein synthesis; heat-shock proteins are crucial for protecting cells from death, and nitric oxide synthase converts arginine into nitric oxide, a signaling molecule that regulates virtually every cellular function (3). Moreover, glutamine enhances the activity of the mammalian target of rapamycin (mTOR), a protein kinase that regulates intracellular protein synthesis (16,18). Thus, increasing extracellular concentrations of glutamine stimulates protein synthesis and inhibits proteolysis in skeletal muscle of animals, including chickens (14). The discovery of the mTOR signaling pathway and its activation by glutamine is an exciting new development in amino acid research. Finally, glutamine stimulates the secretion of anabolic hormones (e.g., insulin and growth hormone) and inhibits the production of catabolic hormones (e.g., glucocorticoids), therefore favoring protein deposition and cell growth in animals (28,29).

The interconversion of glutamine and glutamate constitutes an intracellular, inter-cellular, or inter-organ glutamine-glutamate cycle in animals. Biochemically, glutamate can substitute glutamine for many functions (e.g., ATP production, arginine synthesis, and glutathione synthesis in epithelial cells of the small intestine). In addition, glutamate inhibits glutamine degradation by mitochondrial phosphate-dependent glutaminase in extrahepatic tissues and cells (6), therefore potentially sparing the use of glutamine as a fuel and increasing the availability of cellular glutamine. However, some key functions of glutamine (e.g., glucosamine synthesis, nucleotide synthesis, mTOR activation, and regulation of ornithine decarboxylase expression) cannot be served by glutamate (18). Additionally, although both glutamine and glutamate provided from the enteral diet are extensively catabolized by the small intestine, the gut takes up glutamine, but not glutamate, from the circulation (30-32). Thus, adequate provision of glutamine from the enteral diet is crucial for maintaining intestinal integrity and function (33-35).

## 4. EFFECTS OF GLUTAMINE SUPPLEMENTATION ON GLUTAMINE KINETICS AND PROTEIN METABOLISM IN HEALTHY HUMANS AND ANIMALS

Four considerations have led to glutamine supplementation to animals and humans. First, glutamine is a major amino acid in tissue proteins. Second, turnover rates of glutamine are high in all animals studied. Third, there are dynamic changes in glutamine concentrations among tissues and organs in response to physiological and pathological changes, which often result in a deficiency or reduced availability of glutamine in the body. Fourth, glutamine exhibits unique versatility in cellular metabolism and function, and cannot be replaced by any other amino acid.

Glutamine can be supplemented to animals or humans via oral or intravenous administration. While the

bioavailability of intravenously infused glutamine is 100%, the value is at most 30% for orally administered glutamine (3). This is because approximately 70% of glutamine in the enteral diet is degraded by the small intestine in first pass (36). Thus, whether oral administration of this amino acid can effectively increase its circulating levels depends on the dose and the time of blood sampling. Some studies have demonstrated that enteral or parenteral glutamine supplementation alter glutamine kinetics and protein metabolism in the whole-body and skeletal muscle of healthy humans and animals. For example, administration of exogenous free glutamine increased plasma glutamine concentrations in healthy human volunteers (37-39). However, when volunteers received enteral administration of protein-bound glutamine, plasma glutamine concentration did not reach the same levels as observed with free glutamine, despite similar intake of glutamine (39). Enteral provision of glutamine was associated with increased glutamine appearance rates (Ra) and oxidation, with no change in glutamine release from protein and even d decrease in glutamine synthesis in humans (37,40). However, enteral administration of glutamine to healthy subjects did not appear affect intramuscular glutamine concentration, but decrease intramuscular glutamine synthesis (41). In contrast, feeding a diet containing a glutamine-rich protein source could increase plasma and muscle glutamine concentrations in rats (42). Similarly, oral administration of glutamine (0.5 g/kg body weight per day) or dietary supplementation with 1% glutamine was effective in enhancing its circulating levels in young pigs (24,27). Thus, there are species differences in glutamine metabolism and responses to dietary supplementation.

Given its multiple functions, glutamine can modulate protein metabolism in the whole body, muscle, and gut of healthy humans and animals. For example, enteral administration of glutamine increased the nonoxidative leucine disposal (NOLD, an indicator of whole-body protein synthesis), whereas leucine Ra (an indicator of whole-body protein breakdown) did not change and leucine oxidation decreased (38,40). Enteral supplementation of glutamine had no effect on the rate of muscle protein synthesis in human volunteers (41), but stimulated the duodenal mucosal protein synthesis and decreased ubiquitin mRNA expression (17). Thus, glutamine may attenuate ubiquitin-dependent proteolysis, thereby improving protein balance in the gut (17). Moreover, glutamine enhanced protein synthesis in enterocytes from all levels of the villi in the rat jejunum, and a maximal effect was noted at a normal plasma concentration of glutamine (0.67 mmol/L) (43). Similarly, enteral glutamine provision increased intestinal protein synthesis in young pigs (44). These findings indicate that enetral glutamine may exert its anabolic effect on protein metabolism by increasing protein synthesis and inhibiting proteolysis in healthy humans and animals.

However, there are also reports that exogenous glutamine supplementation via intravenous or oral administration did not influence glutamine kinetics or protein metabolism in the whole-body, muscle and gut of healthy humans or unstressed animals. For example, oral glutamine intake did not affect whole-body protein synthesis in well-nourished humans (45) or animals (46). Similarly, glutamine provision neither stimulated the synthesis nor inhibited the breakdown of globular and myofibrillar proteins in skeletal muscles of healthy volunteers (47). Oral nutritional supplement [containing glutamine as the dipeptide L-alanyl-L-glutamine (Ala-Gln), carbohydrates, and antioxidants] improved gut protein metabolism in healthy humans by increasing protein synthesis and inhibiting cathepsin D–mediated proteolysis (48). Interestingly, provision of glutamine alone did not reproduce the effects of oral Ala-Gln supplement (48). Furthermore, in well-nourished, growing dogs, glutamine infusion doubled its concentration in plasma levels, but did not affect plasma leucine Ra or duodenal protein FSR, indicating that short-term iv infusion of glutamine does not stimulate duodenal protein synthesis (46). Additionally, glutamine did not influence intestinal protein synthesis when given intravenously to rats (49). Likewise, intravenous infusion of glutamine to piglets did not alter small intestinal protein or DNA content or the specific activities of lactase, sucrase, or maltase (50). These results should not be taken to indicate a lack of efficacy of glutamine on tissue or whole-body protein balance. Rather, the experimental conditions are such that it is highly possible that the dose or timing of glutamine supplementation was not optimal and that other factors (e.g., inadequate provision of both nutritionally essential and nonessential amino acids) might have compromised beneficial actions of glutamine on cells.

## 5. EFFECTS OF GLUTAMINE SUPPLEMENTATION ON GLUTAMINE KINETICS AND PROTEIN METABOLISM IN HUMANS AND ANIMALS UNDER STRESS AND DISEASED CONDITIONS

### 5.1. Surgical trauma

There has been a long history of studies regarding effects of glutamine on protein metabolism in patients with trauma because of a marked reduction in intramuscular and blood glutamine (Table 1). For example, in patients undergoing elective cholecystectomy (51-55), total hip replacement (56), elective abdominal surgery (57), or colorectal surgery (58), the intracellular concentrations of free glutamine, the total concentration of ribosomes and the relative proportion of polyribosome in skeletal muscle decreased markedly whereas the whole-body nitrogen balance was negative after operations. Therefore, the obligatory loss of nitrogen with concomitant reduction in skeletal muscle protein synthesis is accompanied by a decrease in muscle free glutamine, the extent of which is proportional to muscle protein catabolism. Other studies revealed that whole-body protein breakdown increased, but concentration of glutamine in plasma and muscle decreased in patient 2 d after elective gastrointestinal surgery (59). Moreover, studies with rats largely supported this conclusion and extended the work to liver and jejunum (60,61).

Previous research has been focused on effects of glutamine supplementation on protein metabolism in whole

**Table 1.** Effects of exogenous glutamine supplementation on whole-body protein metabolism in humans or rats with surgical trauma stress[1]

| Ref. | Species | Surgery type | Route of GLN supply | GLN source | Dose and duration of GLN supplementation | Whole body or tissue | [GLN] in tissue | PS in whole body or tissue | PB in whole body or tissue | Whole-body N balance |
|---|---|---|---|---|---|---|---|---|---|---|
| 57 | Human | Elective abdominal surgery | TPN | GLN | 0.29 g•kg $BW^{-1}$•$d^{-1}$, 3 d | Muscle | Maintained | NS | ND | ↑ |
| 58 | Human | Elective resection of carcinoma of colon or rectum | TPN | ALA-GLN | 54 mg peptide-N• kg $BW^{-1}$•$d^{-1}$•5 d | Muscle | Maintained | ND | ND | ↑ |
| 63 | Human | Elective cholecystectomy | TPN | ALA-GLN | 0.35 g•kg $BW^{-1}$ •24 $h^{-1}$ | Muscle | Maintained | Maintained | ND | ↑ |
| 54 | Human | Elective cholecystectomy | TPN | GLN or AKG | ND | Muscle | ↑ | ↑ | ND | ND |
| 52 | Human | Elective cholecystectomy | TPN | AKG | ND | Muscle | Maintained | Maintained | ND | ↑ |
| 56 | Human | Total hip replacement | Infusion | GLN or AKG | 0.28 g•kg $BW^{-1}$, 24 h | Muscle | Maintained | Maintained | ND | ND |
| 70 | Human | Trauma | Enteral | GLN | 0.35 g•kg $BW^{-1}$, 3 d | Whole body | ND | NS | NS | NS |
| 71 | Human | Elective abdominal surgery | TPN | GLY-GLN | 0.16 g GLN•kg $BW^{-1}$•24 $h^{-1}$, 5 d | Whole body | ND | ND | NS | NS |
| 69 | Human | Elective surgery for colon cancer | Infusion | GLN | 0.29 g•kg $BW^{-1}$, 5.5 h | Muscle | NS | NS | ND | ND |
| 65 | Human | Surgery | TPN | ARG and GLU | 129 mM ARG plus 83 mM GLU, 5 d | Whole body | ND | ND | ↓ | ↑ |
| 67 | Human | Abdominal surgery | TPN | GLN or GLN + GH | 0.28 g•kg $BW^{-1}$ •$d^{-1}$, 3 d | Muscle | Maintained | NS | ND | ↑ |
| 66 | Human | Surgery | Oral | GLN | 0.5 g•kg $BW^{-1}$• $d^{-1}$, 7 d | Whole body | ND | ↑ | ↓ | ND |
| 61 | Rat | Hepatectomy | Oral | GLN | 2 or 4 % GLN in diet, 7 d | Liver | ND | ↑ (for 2% GLN in diet) | ND | ND |
| 60 | Rat | Hepatectomy | TPN | GLN | 25% total N, 2 d | Liver | ND | ↑ | ND | ND |
| 72 | Rat | Femoral fracture | TPN | GLY-GLN | 2.2 g N• kg $BW^{-1}$• $d^{-1}$, 8 d | Jejunum | ND | NS | ND | ND |
| 68 | Rat | Small-bowel transplantation | TPN | GLN | ND | Whole body | ND | ↑ | ↓ | ↑ |

[1]Abbreviations: AKG, alpha-ketoglutarate; ALA-GLN, L-alanyl-L-glutamine; ARG, arginine; BW, body weight; GLN, glutamine; GLU, glutamate; GLY-GLN, glycyl-glutamine; N, nitrogen; ND, not determined; NS, no change; PD, protein degradation; PS, protein synthesis; TPN, total parental nutrition; ↑, increase; ↓, decrease.

body or tissue of human or animals undergoing surgical trauma stress (Table 1). Most of these studies demonstrate that the addition of free glutamine (54,56,57,62), Ala-Gln (54,63,64), alpha-ketoglutarate (AKG, the carbon skeleton of glutamine) (52,54,56), or ornithine-alpha-ketoglutarate (51,54) to total parenteral nutrition (TPN) counteracted the postoperative falls in intramuscular free glutamine concentrations and polyribosomes, and improved whole-body nitrogen balance in patients undergoing surgical trauma. Other studies also identified that TPN enriched with potential precursors of glutamine (e.g., arginine and glutamate) promoted a better nitrogen balance while limiting myofibrillar protein degradation in surgical patients. Arginine and glutamate are potentially substrates for the synthesis of glutamine via the formation of ornithine (65). Accordingly, Peng *et al.* (66) reported that, after 7 days of taking glutamine granules orally, plasma concentrations of glutamine, prealbumin and transferrin rose, which were associated with reduction of urine nitrogen excretion in severe burns and trauma patients. Also, positive correlations existed between the changes in muscle glutamine concentrations or muscle protein synthesis and postoperative nitrogen losses (54). Moreover, another study showed that in patients undergoing abdominal operation, TPN containing glutamine together with growth hormone (GH) prevented the decrease in the glutamine concentration in skeletal muscle and diminishing the loss of whole-body protein, compared with TPN containing glutamine alone. Thus, GH has an additive effect given together with glutamine on muscle amino acid metabolism (67). Likewise, in rats receiving hepatectomy, glutamine supplementation enhanced glutamine uptake by the liver and intestine, hepatic DNA and protein synthesis, the regeneration of the remnant liver, as well as protein synthesis in jejunum and colon, while improving mucosal integrity and reducing bacterial translocation (60). Similarly, a diet enriched with 2% glutamine increased liver growth, total protein content, and protein synthesis in the regenerating liver (61). Furthermore, glutamine-enriched TPN decreased the postoperative catabolism of protein, while promoting protein synthesis and positive nitrogen balance in rats after the small bowel transplantation, thereby minimizing the loss of body weight and ameliorating hypoalbuminemia (68). Collectively, these results indicate that glutamine or its precursors exert beneficial effects on patients and rats with surgical trauma.

In contrast, a few studies reported that exogenous glutamine supplementation had no effects on patients under any catabolic conditions tested. For example, after elective surgery for the treatment of colon cancer, a short-term postoperative infusion of glutamine-glucose did not affect glutamine concentration, the rate of protein synthesis, or the percentage of polyribosomes in human skeletal muscle (69). Similarly, glutamine-supplemented parenteral nutrition had no effect on nitrogen balance, protein turnover, or glucose metabolism (synthesis, oxidation and recycling) in trauma patients (70). Also, adding Ala-Gln to TPN had no beneficial effects on whole body protein metabolism in patients admitted for elective abdominal

surgery (71). Likewise, the supplementation of glycyl-glutamine to TPN did not influence either protein metabolism or the morphology of the jejunal mucosa in male Sprague-Dawley rats subjected to surgical stress via femoral fracture (72). In all of these studies, it is not clear whether the supplemental glutamine is sufficient to increase its concentrations in plasma.

**5.2. Sepsis**

Because of ethical concerns with human studies, animal models are often used to study effects of glutamine on protein turnover under septic conditions. Caecal ligation and puncture (CLP) in the rat is a clinically relevant model of severe sepsis and multiple-organ dysfunction (73). It produces bacteraemia with a slowly evolving septic insult, which is particularly suitable for metabolic studies. Furthermore, the clinical signs, physiological changes and metabolic responses are well described and are similar to those in human sepsis (73). Sepsis induced by CLP caused the marked changes in intramuscular glutamine metabolism, resulting in the decreases in glutamine concentrations in plasma and muscle as well as an increase in the rate of glutamine release from muscle (74). In rats rendered septic by CLP, plasma glutamine increased due to net whole-body proteolysis, muscle glutamine concentrations fell (75). However, there was no relationship between tissue glutamine levels and protein synthesis rates in either muscle or liver when tissue samples were obtained only at one time point (75). Other authors reported that after both CLP and sham operation, protein FSR in gastrocnemius muscle was reduced compared with unoperated animals, and 24 h after surgery, the rates were substantially lower in CLP animals than in shamoperated controls (76). This impairment of protein synthesis was associated with reductions in RNA activity (protein synthesis per unit RNA) and cellular efficiency (protein synthesis per unit DNA). In contrast, protein synthesis was increased by more than 65% in enterocytes isolated from all intestinal mucosal layers of CLP-induced septic rats (49) but was markedly reduced in the heart of septic rats (77).

Sepsis is also induced by turpentine, endotoxins, or protracted peritonitis. Subcutaneous injections of turpentine produce many of the features of the acute-phase response to injury, and, therefore, this technique is used for studying various aspects of the protein metabolic response to injury (78). Induction of sepsis by subcutaneous injection of turpentine resulted in decreases in (a) glutamine concentration in plasma and skeletal muscle; (b) concentrations of branched-chain amino acids (BCAA) in the liver and jejunum; and (c) protein synthesis in skeletal muscle, liver, and jejunum (79). Additionally, a loss of muscle protein occurred together with similar (45-50%) reductions in intramuscular glutamine concentrations and protein FSR in turpentine-treated rats, whereas hepatic weights, protein content, and FSR were all increased (78). Similarly, turpentine-treatment reduced glutamine concentration and protein FSR in skeletal muscle by 39-45% and 41-49%, respectively (80,81). Given such impressive responses to glutamine, it is surprising that some authors suggested that reduced muscle protein synthesis and increased myofibrillar protein breakdown during sepsis may not be caused by low intramuscular glutamine levels (74,82-84). One possible explanation is the studied muscles which may have various levels of glutamine before glutamine supplementation was initiated. Clearly, factors other than glutamine can regulate intracellular protein turnover in muscle and other tissues.

Liver responds to sepsis differently than muscle, likely because of the hepatic synthesis of heat-shock proteins and glucose. Glutamine uptake by the portal-drained viscera fell in the endotoxin-treated animals while glucose uptake doubled (84). Simultaneously, hepatic glutamine uptake was augmented ten-fold owing to an increase in hepatic blood flow and glutamine extraction from the bloodstream. The enhancement of hepatic glutamine utilization was associated with increases in (a) parenchymal DNA and glutathione levels; and (b) glutathione and urea release into the systemic circulation. During endotoxemia, the liver becomes the major organ of glutamine consumption. This accelerated utilization provides carbons for (a) ATP production and gluconeogenesis; (b) nitrogen for ureagenesis; and (c) substrate for nucleotide and glutathione biosynthesis to support cell repair and detoxification reactions. Other studies showed that endotoxin treatment induced negative protein balance by increasing whole-body proteolysis in rats (85). Additionally, in a protracted-peritonitis rat model, serum glutamine concentrations correlated positively with protein FSR in the liver (86).

Available evidence overwhelmingly supports the conclusion that supplementation with free glutamine, Ala-Gln or its precursor is beneficial for improving protein balance in the whole body, muscle, liver or gut of septic animals (Table 2). For example, Ala-Gln-supplemented TPN, in comparison with standard glutamine-free TPN, enhanced whole-body protein synthesis in the liver and skeletal muscle, protected the intestinal mucosa against injury, and improved survival in septic rats with protracted bacterial peritonitis (86). The dramatic effects of Ala-Gln were associated with decreases in plasma BCAA levels and leucine oxidation, as well as increased protein balance and attenuated whole-body proteolysis (85). Similar changes in leucine and protein metabolism were induced by the infusion of glutamine but not glycine. Furthermore, feeding a glutamine-rich (3.6% glutamine by weight) diet for 4 days increased villus height and crypt depth of small intestine in rats before and during an acute-phase response to injury induced by subcutaneous injections of turpentine (87). Of note, ornithine alpha-ketoglutarate (OKG) exerted a dose-dependent effect on concentration of glutamine in muscle, jejunum mucosa and liver tissue and nitrogen balance, but only the highest dosage (4.5g/kg/d) counteracted myofibrillar hypercatabolism and caused a positive nitrogen balance in septic rats (88).

We would like to bring into attention that a few studies showed that glutamine supplementation had no effect on protein metabolism in the whole body, muscle and heart of septic rats. For example, in turpentine-treated rats, the reduction in intramuscular glutamine concentration was

**Table 2.** Effects of exogenous glutamine supplementation on protein metabolism in rats with sepsis induced by CLP, turpentine or endotoxin[1]

| Ref | Route of GLN supply | GLN source | Dose and duration of GLN supplementation | Whole body, tissues, or cells | [GLN] in tissue | PS in whole body or tissue | PD in whole body or tissue | Whole-body N balance |
|---|---|---|---|---|---|---|---|---|
| 80 [2] | I.V. | GLN | 1 mL of 0.22 M GLN•100 g $BW^{-1}$•$h^{-1}$, 5 h | Muscle | ↑ | NS | ND | ND |
| 89 [3] | TPN | ALA-GLN | 2% TPN, 5 d | Whole body | ND | ND | NS | NS |
| 49 [4] | Culture medium | GLN | ND | Enterocytes (isolated from septic rats) | ND | ↑ | ND | ND |
| 81 [2] | I.V. | GLN | 1 mL of 0.22 M GLN •100 g $BW^{-1}$ •$h^{-1}$, 5 h | Muscle | ↑ | NS | ND | ND |
| 86 [5] | TPN | ALA-GLN | 4.36 g•kg $BW^{-1}$•$d^{-1}$, 5 d | Whole body, liver, and muscle | ND | ↑ | ND | ND |
| 85 [3] | I.V. | GLN or ALA-GLN | 200 mg/ml, 30 min | Whole body | ND | ND | ↓ | ↑ |
| 75 [4] | TPN | GLN | 15 g•$L^{-1}$, 2 mL•$h^{-1}$, 6 h | Muscle and liver | NS | NS | ND | ND |
| 77 [4] | TPN | GLN | 15 g•$L^{-1}$, 2 mL•$h^{-1}$, 6 h | Myocardial muscle | ND | NS | ND | ND |
| 88 [3] | Enteral | OKG | 0.5-4.5 g•kg $BW^{-1}$• $d^{-1}$, 48 h | Whole body, muscle, liver, and jejunum | ↑ | ND | Myofibrillar protein (↓) | Whole body and muscle (↑) |
| 79 [2] | Infusion | GLN | 5 mL of 1.75% GLN, 6 h | Muscle, liver, and jejunum | ↑ (only in muscle) | ↑ (only in liver) | ND | ND |

[1]Abbreviations: BCAA, branched-chain amino acids; BW, body weight; CLP, cecal ligation and puncture; GLN, glutamine; I.V., intravenous; N, nitrogen; ND, not determined; NS, no change; OKG, ornithine alpha-ketoglutarate; PD, protein degradation; PS, protein synthesis; TPN, total parental nutrition; ↑, increase; ↓, decrease. [2]Sepsis was induced by injection of turpentine. [3]Sepsis was induced by injection of endotoxins.[4]Sepsis was induced by CLP. [5]Sepsis was induced by protracted peritonitis model.

completely reversed by 5-h intravenous infusion of a 220 -mM glutamine solution, but there was no concomitant increase in muscle protein synthesis (81). This result does not support the proposition that an acute change in intramuscular glutamine concentration is a major factor in regulating muscle protein synthesis. Other studies have found that the short-term administration of standard TPN attenuated the reduction in muscle protein synthesis associated with sepsis, but the use of an isocaloric isonitrogenous glutamine-containing TPN solution was no more effective than TPN alone (75). Okuma *et al.* (89) investigated the effects of administering TPN supplemented with 2% of Ala-Gln on gut structure, barrier function, and protein metabolism in septic rats induced by the continuous intraperitoneal administration of endotoxin. These authors observed that TPN supplemented with Ala-Gln did not affect nitrogen balance, urinary excretion of 3-methylhistidine (as an indicator of myofibrillar protein catabolism), plasma concentration of endotoxin in the portal vein, or the incidence of bacterial translocation from the gut to the mesenteric lymph nodes.

### 5.3. Critical illness

Progressive muscle wasting is a characteristic feature of patients treated at the intensive care unit (ICU). As a consequence, endogenous glutamine production by skeletal muscle may be compromised (90). Marked changes in glutamine and protein metabolism occur in the whole body and skeletal muscle of critically ill subjects. For example, in patients under critical care, glutamine concentrations in plasma and skeletal muscle were decreased (41,91,92). These patients also exihibit increases in (a) the proportion of glutamine Ra arising from protein breakdown; (b) glutamine metabolic clearance rate (MCR) (91,92); (c) leucine Ra (whole body proteolysis); (d) whole-body protein synthesis and amino acid oxidation (91); and (e) negative protein balance, but no change in whole-body glutamine Ra (91,92). Another study revealed that the net release of glutamine from skeletal muscle was not decreased in stabilized critically ill patients with multiple organ failure over the initial 2 weeks of ICU stay, whereas a progressive net loss of muscle protein occurred in these patients (90). On balance, available evidence shows that critical illness is associated with a major increase in whole-body and muscle protein turnover, as well as a negative nitrogen balance. Interestingly, critical illness is also associated with alterations in muscle glutathione metabolism. Thus, in critically ill patients, glutathione and glutamine concentrations, as well as the ratio of reduced glutathione to total glutathione, were decreased in skeletal muscle, likely due to oxidative stress in this tissue (93). In addition, there were positive correlations between glutamine concentrations and total muscle glutathione, as well as between glutamine and the ratio of reduced glutathione to total glutathione in skeletal muscle (93). Collectively, these results suggest that glutamine may play an important role in intramuscular glutathione synthesis and oxidative defense.

A large body of literature shows that glutamine supplementation has beneficial effects on protein metabolism in critically ill patients (Table 3). For example, parenteral or enteral administration of glutamine increased plasma glutamine concentration (41,92,94,95), enhanced glutamine uptake, and decreased protein breakdown (94), without altering glutamine production rate, glutamine MCR (92), muscle glutamine concentration and kinetics (incorporation into muscle, release from muscle, and rate of de novo glutamine synthesis in muscle), or muscle protein synthesis in critically ill patients (94,95). Also, glutamine supplementation increased net protein deposition in the skeletal muscle of these subjects (41). Moreover, TPN containing both glutamine and growth hormone/insulin-like growth factor-1 (GH/IGF-I) shifted protein balance from

**Table 3.** Effects of exogenous glutamine supplementation on protein metabolism in humans or rats with critical illness [1]

| Ref | Species | Route of GLN supply | GLN source | Dose and duration of GLN supplementation | Whole-body or tissue | Plasmas [GLN] | [GLN] in tissue | PS in whole body or muscle | PD in whole body or muscle | Whole-body N balance |
|---|---|---|---|---|---|---|---|---|---|---|
| 42 | Rats injected i.p. with zymosan | Diet | GLN-rich protein | 50.5 g•kg diet$^{-1}$, 2 weeks | Muscle | NS | NS | ND | ND | Muscle protein wasting (NS) |
| 70 | Critically ill patients | Enteral | GLN | 0.35 g• kg BW$^{-1}$•d$^{-1}$, 3 d | Whole body | ND | ND | NS | NS | NS |
| 96 | Severe ill patients | TPN | GLN dipeptide | 60 µmol•kg BW$^{-1}$•h$^{-1}$, 8-10 d | Whole body | NS | ND | ND | ND | ND |
| 41 | Critically ill patients | Enteral | GLN | 24 g, 10% solution, 3h | Muscle | ↑ | NS | NS | NS | ND |
| 92) | Critically ill patients | TPN | GLN alone or GLN+GH+IGF-1 | 0.4 g• kg BW-$^{1}$•d$^{-1}$, 3 d | Whole body | ↑ | ND | ND | ND | GLN alone (NS); Gln+GH +IGF-1 (↑) |
| 94) | ICU patients | TPN | GLN alone or GLN+GH+IGF-1 | 0.4 g•kg BW$^{-1}$•d$^{-1}$, 3 d | Muscle | ↑ | ND | ND | ↓ | GLN+ GH+ IGF-1 (↑) |
| 95) | ICU patients | I.V. | GLN | 0.28 to 0.86 g•kg BW$^{-1}$•d$^{-1}$ | Muscle | ↑ | NS | NS | ND | ND |

[1]Abbreviations: BW, body weight; GH, growth hormone; GLN, glutamine; ICU, intensive care unit; IGF-1, Insulin-like growth factor; N, nitrogen; ND, not determined; NS, no change; PD, protein degradation; PS, protein synthesis; TPN, total parental nutrition; ↑, increase; ↓, decrease.

negative to positive (92,94). In contrast, there is evidence that oral administration of glutamine did not attenuate the decrease of intramuscular glutamine in critically ill rats and did not counteract the wasting of skeletal muscle in zymosan-treated rats (41,42). It is possible that enterally administered glutamine is utilized primarily by the gut-associated lymphoid organ and splanchnic tissues and, therefore, is not available substantially for peripheral tissues. It should also be borne in mind that the time of tissue sampling relative to feeding is a critical factor in assessing circulating levels of glutamine.

### 5.4. Severe burn

Major burn injury results in the efflux of amino acids from peripheral tissues to the abdominal viscera (98-100). For example, thermal injury causes activation of proteolysis, release of glutamine from skeletal muscle, and a depletion of muscle glutamine (100). Due to enhanced utilization of glutamine by cells of the immune system and other cell types, circulating levels of glutamine decline in response to burns. Many studies have also demonstrated that burn injury increases plasma levels of diamine oxidase, endotoxin, intestinal mucosal permeability, and urine excretion of nitrogen (99). Moreover, in burned rats, glutamine synthetase (GS) mRNA levels were increased 2.3-fold in the lung at 8 h and 7.3-fold in muscle at 24 h after burn injury, but there was no increase in GS mRNA level the kidney or liver (101). These results indicate tissue-specific responses of GS gene expression to thermal stress. In addition, the maximal activity of phosphate-dependent glutaminase, glutamine utilization and the formation of glutamate and alanine were enhanced in enterocytes from rats with burn injury (103). To further elucidate the underlying mechanisms, Pietsch *et al.* (103) determined the kinetics of glutamine transport across basolateral membrane vesicles of enterocytes from control rats and rats subjected to 20% full-thickness scald burn for 48 h. Their results indicated that initial rates of glutamine uptake were depressed in thermal injury. Kinetic analysis of glutamine uptake showed a marked decrease in transport Vmax and transport Km in burned rats. Similarly, in the post-absorptive state, patients with severe burns exhibited accelerated muscle loss and leucine oxidative decarboxylation, and depletion of the intramuscular free glutamine pool, when compared with healthy control subjects (104). To further contribute to glutamine deficiency, the rate of glutamine synthesis was decreased by 48%, whereas net alanine synthesis was increased by 174%, in skeletal muscle of burned patients (104). Thus, glutamate is channeled to the synthesis of alanine through transamination rather than the production of glutamine via amidation despite an elevation of GS expression. It is possible that high levels of glycolysis-derived pyruvate drive the formation of alanine from glutamate, which can function to remove both $H^+$ and ammonia as an adaptive mechanism for survival. We propose that, in severely hypercatabolic burned patients, alanine is the major vehicle for inter-organ nitrogen transport.

Most of the published work shows that enteral or oral supplementation of glutamine for 7 or 14 days could increase plasma concentrations of glutamine, promote protein synthesis, inhibit protein catabolism, and ameliorate intestinal mucosal injury, therefore improving wound healing and reducing hospital stay in severely burned patients (99,100). Further, combined administration of glutamine and recombinant human growth hormone (rhGH) to severely burned patients could have additive benefits on increasing plasma glutamine levels, whole-body protein synthesis, and wound healing, therefore shortening total hospital stay (105). However, there is some evidence that enriched glutamine feeding for 48 h reduced leucine flux and leucine oxidation rate without altering net protein synthesis in stressed pediatric burn children (106). Specifically, enriched glutamine feeding for a short period of time did not result in a detectable gain of whole-body protein in these patients.

It is possible that a concomitant deficiency of other key amino acids (e.g., arginine and proline) limits a stimulatory effect of glutamine on tissue protein synthesis in burn patients. In support of this hypothesis, supplementation with ornithine alpha-ketoglutarate (OKG; as a precursor of glutamine and proline) increased plasma

glutamine concentration and decreased whole-body proteolysis in burned rats refed after fasting (107). Compared with AKG, OKG supplementation induced a greater increase in intramuscular glutamine. Interestingly, only OKG led to an increase in hepatic glutamine concentration (107). Moreover, administration of precursors of glutamine plus arginine (e.g., OKG and arginine alpha-ketoglutarate) is highly effective in enhancing glutamine concentrations in plasma, muscle and liver, as well as nitrogen balance (107). Because ornithine is converted into glutamine through AKG as an intermediary metabolite, it is not clear why administration of OKG, but not AKG, leads to an increase in circulating levels of glutamine. This phenomenon may be explained by compartmentalization of AKG and OKG metabolism in cells and tissues. However, before much effort is directed to test this hypothesis, it would be prudent to confirm the previous findings (107) by independent researchers.

### 5.5. Hypercatabolism induced by high levels of glucocorticoids

Catabolism occurs under stressful conditions associated with elevated levels of circulating glucocorticoids (108). Thus, administration of natural or synthetic glucocorticoids is used as a hypercatabolic model to study therapeutic effects of glutamine on reducing muscle loss and negative nitrogen balance (108-110). Muscle glutamine concentrations as well as protein synthesis in skeletal muscle and the small intestine were decreased (108-111), while the synthesis of proteins in the liver (including inflammatory proteins and acute-phase proteins) was consistently enhanced (110,111), in glucocorticoid–treated rats. Other studies with rats demonstrated that, following administration of glucocorticoids, GS enzyme activity and mRNA levels were increased by 2 - to 4 - fold in plantaris muscle consisting of fast-twitch white and fast-twitch red fibers (112). This work established an important role for glucocorticoids in upregulating glutamine synthesis in muscle cells. Furthermore, results of *in vitro* study demonstrated that dexamethasone increased the availability of glutamine and its release from soleus muscle, while decreasing the concentrations of glutamine from both gastrocnemius and extensor digitorum longus (EDL) muscles (113). Interestingly, the glucocorticoid treatment did not affect the rate of glutamine release from EDL muscle, which primarily consists of glycolytic fibers (113). These findings suggest that skeletal muscles differentially respond to glucocorticoids in a fiber type-dependent manner.

Based on animal studies, interesting work has been published on glutamine and protein metabolism in patients with a hypercatabolic state. For example, Lofberg *et al.* (114) found that, after 3 days of prednisolone treatment, the rate of protein degradation was enhanced in human muscle despite no increase in mRNAs' encoding components of the ubiquitin-proteasome pathway, while the rate of protein synthesis was unaltered. These findings indicate that high doses of prednisolone lead to net protein catabolism, mainly due to a greater rate of protein breakdown than the rate of protein synthesis in skeletal muscle. Therefore, administration of glucocorticoids can increase the release of glutamine from human skeletal muscle (114). In contrast, gut mucosal protein synthesis was increased in healthy humans treated with glucocorticoids for 2 days (115). Thus, the gut and muscle of adults have different responses to the prednisolone treatment.

Effects of glucocorticoids on glutamine and protein metabolism vary greatly with nutritional and hormonal states. In dogs, protein restriction combined with a 7-day course of dexamethasone treatment resulted in a 32% increase in leucine Ra (whole-body proteolysis), a 186% increase in leucine oxidation, but no change in whole-body protein synthesis, compared with animals subjected to protein restriction alone (116). In volunteers, the infusion of catabolic hormones (epinephrine, cortisol, and glucagon) led to an increase in whole-body glutamine flux and induced a large efflux of glutamine from the leg muscle (117). Moreover, decreases in muscle glutamine concentration and protein synthesis were noted in healthy male volunteers receiving a short-term infusion of the stress hormone (118). Thus, under acute stress conditions, skeletal muscle preferentially releases glutamine from its free intracellular pool likely due to enhanced expression of glutamine transporters and alterations in membrane function.

Translating molecular and cellular research into nutritional practices, glutamine supplementation greatly impacts protein metabolism in the whole body and specific tissues of animals and humane with elevated levels of circulating glucocorticoids (Table 4). For example, intravenous infusion of glutamine to glucocorticoid-treated rats attenuated the decline of intramuscular glutamine concentration, prevented muscle mass loss, and prevented decreases in total protein synthesis and the FSR of the myosin heavy chain (119,120). Another study demonstrated that intravenous administration of Ala-Gln increased serum glutamine levels, prevented the losses of total body weight and fast-twitch muscle mass by more than 70% (112). Thus, muscle atrophy can be prevented in animals with a catabolic state through supplementation of glutamine or its dipeptides. Further, administration of glutamine, but not leucine, was able to prevent the 3,5 dimethylpyrazole (DMP)-induced increase in valine release from the perfused liver (123). Similarly, other workers noted that OKG (a precursor of glutamine) exerted a dose-dependent effect on increasing concentrations of glutamine in muscle, jejunum mucosa and liver as well as whole-body nitrogen balance in endotoxins-challenged rats (88). However, a moderate enteral supply of glutamine failed to yield a significant effect on gut mucosal protein synthesis in healthy volunteers receiving 2-day administration of glucocorticoids (115), likely due to extensice catabolism of glutamine by the small intestine (3).

Careful examination of the literature reveals that glucocorticoids and dietary protein interact to regulate the response of animals to glutamine supplementation. First, in glucocorticoid-treated rats fed a whey protein-based diet, supplementation with free glutamine enhanced its

**Table 4**. Effects of exogenous glutamine supplementation on glutamine concentrations in protein metabolism in humans and animals under hypercatabolic conditions [1]

| Ref | Species | Route of GLN supply | GLN source | Dose and duration of GLN supple-mentation | Whole-body or tissue | Plasmas [GLN] | [GLN] in tissue | PS in whole body or tissue | PD in whole body or tissue | Whole-body or tissue N balance |
|---|---|---|---|---|---|---|---|---|---|---|
| 123[2] | Rat | IP injection | GLN | 12.5 mg•kg $BW^{-1}$ | Liver | ND | ND | ND | ↓ | ND |
| 119[3] | Rat | Infusion | GLN | 0.75 mL of 240 mM GLN per h, 7 d | Muscle | ND | ↑ | Prevent decline | ND | Prevent muscle wasting |
| 112[3] | Rat | Infusion | ALA-GLN | 1.15 µmol• $min^{-1}$•100 g $BW^{-1}$, 0.75 mL•$h^{-1}$, 7 d | Muscle | ↑ | NS | ND | ND | Prevent muscle mass losses |
| 120[3] | Rat | Infusion | GLN | 0.75 mL of 240 mM GLN per h, 7 d | Muscle | ↑ | NS | ND | ND | ND |
| 122[3] | Rat | Infusion | GLN | 0.75 mL of 240 mM GLN per h, 7 d | Muscle | ND | ND | ND | ND | Prevent skeletal muscle mass loss |
| 115[3] | Human | Enteral | GLN | 0.02 g•kg $BW^{-1}$• $h^{-1}$, 5 h | Gut mucosa | ND | ND | NS | ND | ND |
| 110[3] | Rat | Enteral | GLN | 17 g•100 g dietary $protein^{-1}$, 4 d | Muscle, jejunum, and liver | ↑ | Muscle and liver (↑) | Muscle and jejunum (↑) | ND | ND |
| 116[3] | Dog | I.V. | Gln | 4 mL of 200 mM GLN• kg $BW^{-1}$ •$h^{-1}$, 7 h | Whole body | ↑ | ↑ | NS | NS | NS |
| 121[3] | Dog | Enteral | GLN | 1.15 mmol• kg $BW^{-1}$•$h^{-1}$ | Whole body and duodenum | ↑ | ND | Whole body (NS), duodenum (↑) | Whole body (NS) | Whole body (↑) |

[1]Abbreviations: ALA-GLN, alanyl-glutamine; BW, body weight; DMP, 3,5 dimethylpyrazole (antilipolytic drug); GLN, glutamine; IP injection, intraperitoneal injection; I.V., intravenous; N, nitrogen; ND, not determined; NS, no change; PD, protein degradation; PS, protein synthesis; TPN, total parental nutrition; ↑, increase; ↓, decrease. [2]Protein degradation was stimulated by the injection of the antilipolytic drug DMP. [3]Hypercatabolic models were induced by administration of glucocorticoids.

concentrations in plasma, muscle, and liver, as well as protein synthesis in the gut mucosa and muscle (110). However, no effects of glutamine on tissue protein synthesis could be observed under fasting conditions. Second, in hypercatabolic adult dogs adapted to a normocaloric, low-protein diet and received intramuscular dexamethasone in the fed state, enteral glutamine supplementation decreased leucine oxidation and improved leucine balance (121). Third, glutamine enhanced intestinal protein FSR by 22% in the glucocorticoid-treated dogs fed a protein-adequate diet (121), but did not affect leucine Ra, leucine oxidation, or protein synthesis in dexamethasone-treated dogs fed a low-protein diet (116). Interestingly, the attenuation of glucocorticoid-induced muscle atrophy by glutamine infusion was not associated with changes in circulating levels of IGF-I or insulin-like growth factor binding protein (IGFBPs) (122). Thus, the availability of amino acids is a major factor limiting beneficial effects of glutamine on whole-body nitrogen balance. This notion has important implications for both enteral and parenteral nutrition involving supplementation of glutamine to animals and humans.

### 5.6. Duchenne muscular dystrophy

Human Duchenne muscular dystrophy (DMD) appears to be the result of a single gene mutation**.** Among a variety of animal models, the hereditary mouse muscular dystrophy model (129) is one of the most widely studied**.** Other models include the C57BL/6Jdy'J mouse as well as the dystrophipcrocess which develops spontaneously in chickens (124). DMD is associated with a dramatic loss of muscle mass. Although net muscle wasting is the ultimate consequence of the dystrophic phenomenon, it is unfortunate that published data on protein metabolism and glutamine kinetics in dystrophic muscle were inconsistent in many studies due to differences in experimental designs and biochemical analysis. For example, some workers observed that concentrations of glutamine in skeletal muscle and whole-body glutamine turnover were reduced in patients with DMD, as compared to normal subjects (125,126). However, other investigators found that intramuscular concentrations of free glutamine and glutamate were higher in mdx mice (an animal model for DMD) versus C57BL/10 (normal mice) (127). Additionally, both glutamine synthesis and release were increased in hereditary mouse muscular dystrophy (124), but were not observed in some models of DMD (124-126). Because glutamine is hydrolyzed under acidic conditions, analysis of glutamine in an acidified sample (without neutralization) will surely lead to its loss and accumulation of glutamate, resulting in unreliable results.

A chronic imbalance between protein synthesis and proteolysis in favor of protein breakdown leads to muscle atrophy. Thus, much effort has been directed to identifying means to inhibit proteases in skeletal muscle of DMD patients. Disappointingly, this field has been beset with controversies. For example, protein synthesis and degradation have been reported to be either increased or unchanged, compared to appropriate controls (124-126,128-130). Further, there was no difference in protein degradation and synthesis between DMD boys and controls, whereas leucine oxidation rate was higher in DMD boys (125,126). While these findings suggest that the dramatic muscle mass loss observed in DMD boys might be associated with significant protein wasting (125,126), direct measurements of muscle proteolysis are lacking. Nonetheless, emerging evidence from animal studies suggests that proteolysis and protein synthesis were enhanced in gastrocnemius and soleus muscles from the 129 ReJ and C57BL mice with hereditary muscular dystrophy (124). Similarly, turnover rates of myofibrillar as well as sarcoplasmic proteins were accelerated in dystrophic male mice (128). In view of these results,

increases in both protein synthesis and protein degradation likely occur in animals with hereditary muscular dystrophy. However, evidence on enhanced synthesis of muscle proteins in DMD subjects is not particularly compelling.

Most of studies have identified an important role for glutamine supplementation in regulating intramuscular glutamine kinetics and protein metabolism in dystrophic subjects. For example, in boys with DMD, oral glutamine administration resulted in an 8% decrease in leucine release from protein breakdown and a 35% decrease in leucine oxidation rate, without affecting protein synthesis. Whole-body glutamine flux in plasma doubled, but both glutamine production from protein degradation and de novo synthesis of glutamine were decreased, suggesting that acute oral glutamine administration may have a protein-sparing effect in children with DMD (125). There is also evidence that oral glutamine (0.5 g/kg body weight per day) or amino acid supplementation over 10 days equally inhibited whole-body protein degradation in DMD boys (131). Moreover, intraperitoneal administration of L-glutamine (0.5 g/kg body weight per day) to young DMD mice for 3 consecutive days reduced the ratio of reduced glutathione to oxidized glutathione and extracellular signal-regulated kinase 1/2 activation in dystrophic skeletal muscle. This antioxidant protective mechanism provides a molecular basis for glutamine's antiproteolytic effect in DMD children (127). However, additional benefits of long-term (4 month) oral glutamine (0.5 g/kg/d) over placebo on muscle mass or function in ambulatory DMD boys were not observed, although glutamine was safe and well-tolerated (132). It is imperative that data on the supply of glutamine and other amino acids in the basal diet be provided to interpret these clinical results.

**5.7. Malnutrition and starvation**

Malnutrition may occur under a variety of clinical conditions and the settings of livestock production (133,134). For example, women suffering from hyperemesis gravidarum during pregnancy often experience severe malnutrition. Additionally, malnutrition is manifested in mothers (e.g., cows and sows) during early gestation (2) and in neonates immediately after weanling (135,136). A reduced supply of proteins and energy resulted in reduction of intramuscular glutamine and protein synthesis as well as a negative nitrogen balance in animals and humans (78,133,134). Of particular interest, intramuscular glutamine concentration can be preserved in starving individuals receiving only 400 kcal/day in the form of glucose alone (137,138). The underlying mechanisms are not known but may involve the use of glucose as the AKG source for intramuscular glutamine synthesis. In addition to suppressed protein synthesis, undernutrition results in increased protein catabolism in skeletal muscle and the whole body of organisms (116,139,140). Notably, dietary supplementation with glutamine for 3 weeks increased intestinal protein synthesis in rats (141). Similarly, supplementing 1% glutamine to a corn- and soybean meal-based diet for 7 days prevented intestinal atrophy and improved growth performance in weanling piglets (8).

Starvation induced significant changes in glutamine kinetics and protein turnover in humans and animals. An extended period of fasting (a 3-day fast) decreased concentrations of glutamine in skeletal muscle and plasma of young healthy male volunteers (142). In healthy adults subjected to 18-24 h food deprivation, the relative contribution of protein breakdown to glutamine production was enhanced, while *de novo* synthesis of glutamine declined due to a limited availability of both BCAA and glucose (143). Starvation for two days lead to reductions in intramuscular glutamine concentrations, muscle protein synthesis, as well as body and muscle weights, but a transient increase in plasma glutamine concentrations due to net protein degradation in skeletal muscle (144,145). Moreover, protein synthetic capacity decreased approximately by 12% in rats after 18 h of fasting, regardless of age and muscle type (146).

Interestingly, in some (50,147,148), but not all (149-151), studies involving animal models, protein metabolism in the small intestine has been found to be sensitive to the nutritional state. For example, intestinal protein synthesis did not differ between 13 and 36 h of fasting in humans (152), but a 48-h fast starvation resulted in a decrease in both glutamine content and rate of protein synthesis in chicken leg muscle (15). Such observations are not surprising on the basis of the known roles for the gut in regulating whole-body protein homeostasis (3). Because of the complex interplays among potential factors (e.g., hormones, amino acids, glucose, and fatty acids) *in vivo*, *in vitro* studies involving Caco-2 cells have been conducted to define the specificity of glutamine's actions on the gut (153). Le Bacquer *et al.* (153) demonstrated that a 24-h apical nutrient deprivation (luminal fasting) was associated with (a) a decline in intracellular concentrations of glutamine, glutamate, and glutathione; (b) reduced protein FSR (-20%); and (c) a rise in transepithelial permeability (153). In these cells, basolateral or luminal glutamine supplementation to a low-glutamine culture medium restored protein FSR to normal values (153). Furthermore, such effects of glutamine were abolished by addition of 6-diazo-oxo-l-norleucine (an inhibitor of glutaminase) and were mimicked by glutamate. Therefore, in intestinal cells, protein synthesis depends on nutrient supply on the apical side, and glutamine prevents some of the deleterious effects of malnutrition regardless of the route of administration (153).

**4.8. Cancer**

Major disturbance in protein and glutamine metabolism occurs at various stages of cancer (156-161). For example, with progressive growth of the MCA sarcoma, the liver and the tumor itself become the predominant consumers of glutamine (154). Under this diseased condition, the release of glutamine from skeletal muscle is accelerated at a rate greater than glutamine synthesis, resulting in a progressive decrease in intramuscular concentrations of glutamine over time (154,156). Many studies have shown that plasma glutamine concentration is markedly decreased in tumor-bearing rats, likely due to enhanced consumption of glutamine by tumor cells and activation of the immune system (155-157). Interestingly, whole-body glutamine turnover remained unchanged in rats with small tumors but increased by 25%

in animals with large tumors (158). Thus, increases in both production and consumption of glutamine in tumor-bearing subjects contribute to an irreversible loss of body protein, because protein-derived BCAA (essential amino acids and the donors of the amino group) and amino acids-derived glucose (major sources of AKG) are used for glutamine synthesis (3). Unfortunately, this is a characteristic of cancer cachexia. Eventually, skeletal muscle is exhausted in rats with large tumors (158-164), leading to multiple organ dysfunction and death.

Given a severe depletion of glutamine in tumor-bearing subjects, exogenous provision of this amino acid could be highly effective in for improving nitrogen balance and immune function in the host. In support of this proposition, glutamine supplementation increased muscle protein synthesis (161,166,168) and decreased body protein breakdown (166,168), therefore attenuating body-weight loss (159,166) in tumor-bearing rats. Also, glutamine supplementation increased concentrations of glutamine (167), DNA (169,170), RNA (170), protein synthesis (166), and glutathione (165,167,170) in the gut mucosa of tumor-bearing animals. However, some authors reported that the administration of glutamine-supplemented enteral or parenteral nutrition to tumor-bearing rats did not affect tumor weight or size (159,165,171), DNA content or synthesis (159,165,170,171), RNA content (170), protein content or synthesis (166,170,171), concentrations of glutamine and glutathione, bromodeoxyuridine-labeling index (166), tumor glutaminase activity, the number of metaphase mitoses/high power field (159), or tumor glutamine metabolism, but the ratio of tumor cells to host infiltrating cells within the tumor mass was increased (165). On balance, a majority of published work shows that glutamine supplementation is beneficial for the tumor-bearing host by maintaining intramuscular glutamine concentration, supporting muscle and whole-body protein synthesis, and preventing gut glutathione deficiency in tissues without stimulating tumor growth or metastasis.

### 4.9. Low birth weight infants

Very low birth weight (VLBW) infants are subjected to severe stress (172,173). There are only limited studies to investigate glutamine and protein metabolism in VLBW infants because of ethical concerns and technical difficulties. The very preterm infants, either because of immaturity or because of the intercurrent illness, are deficient in glutamine (172). Additionally, these compromised neonates have a high rate of protein breakdown (173). Intervention strategies aimed at promoting nitrogen accretion, such as administration of glutamine, insulin or human growth hormone, have not so far resulted in enhanced protein accretion or whole-body growth (173). However, the design of this published study may be suboptimal, because the supplemental dose of glutamine could not substantially increase circulating levels of glutamine in the infants for a prolonged period of time. It is also possible that a deficiency of other amino acids (e.g., arginine) (174) limits a beneficial effect of glutamine supplementation on preterm infants because arginine is a major factor regulating muscle protein synthesis in neonates (3). This proposition may explain the previous finding that a short-term intravenous infusion of glutamine enhanced plasma glutamine levels and inhibited whole-body protein breakdown, but had no effect on protein balance, in parenterally fed VLBW infants in the first few days of life (175). Nonetheless, glutamine supplementation decreased infections (176), as well as morbidity and hospital costs in VLBW neonates from postnatal days 3 to 30 (177). These are significant outcomes for glutamine supplementation to VLBW infants.

### 4.10. Other pathological conditions

Immunological challenges are associated with reduced concentrations of glutamine in plasma due to elevated catabolism of this amino acid by activated lymphocytes and macrophages (98). Glutamine supplementation can enhance immunity in the host under a wide array of conditions, including sepsis, inflammation, and injury (9). In patients with active celiac disease (another inflammatory condition), whole-body leucine and glutamine fluxes are enhanced, reflecting a dramatic increase in protein breakdown (178). Administration of glutamine is beneficial in ameliorating the autoimmune gut damage through multiple signaling pathways (18).

Glutamine metabolism is altered in obese or diabetic patients (179-182). Interestingly, plasma concentrations of glutamine are elevated in both obese subjects and insulin-dependent diabetes mellitus (IDDM) patients with poor hyperglycemic control (179,180). At present, little is known about whole-body glutamine synthesis in obesity. However, available evidence suggests that *de novo* synthesis of glutamine is not increased in IDDM subjects (182). Thus, impaired synthesis of protein (incorporation of glutamine into protein) and enhanced proteolysis (release of glutamine from protein) in skeletal muscle contribute to a rise of circulating glutamine in diabetics. In contrast to diabetic patients, the contribution of glucose to the glutamine carbon skeleton increased in response to intravenous infusion of 7.5% glucose in healthy volunteers (182). These results indicate that glutamine synthesis critically depends on the action of insulin to stimulate glycolysis and AKG formation. Because glutamine is a substrate for the synthesis of glucosamine [which induces insulin resistance in skeletal muscle and the vasculature (179)], care should be taken when glutamine is supplemented to obese or diabetic subjects.

Given a potentially important role for the lung in glutamine metabolism (6) and large numbers of patients with cystic fibrosis in North America and the Europe, there has been interest in using glutamine to modulate whole-body protein balance. Evidence from limited studies suggest that, in children with cystic fibrosis who were either malnourished or growing poorly, oral administration of glutamine had no effect on protein balance, whereas rhGH promoted muscle protein synthesis and growth without altering glutamine kinetics (183,184). These data suggest that oral administration of glutamine alone may not be beneficial for treating chronic debilitating conditions.

Irradiation reduces intramuscular glutamine concentration, as well as protein synthesis in the jejunum,

colon and heart, while increasing the synthesis of blood and splenic proteins and whole-body leucine oxidation (185). Because glutamine has an anti-oxidative function (3), some researchers have used this amino acid to reduce irradiation-induced injury. Notably, administration of Ala-Gln to rats inhibited whole-body proteolysis and leucine oxidation, while stimulating the incorporation of leucine into body proteins (185).

## 6. CONCLUDING REMARKS AND PERSPECTIVES

Glutamine displays remarkable diversity in cell nutrition and metabolism. Activation of the mTOR signaling pathway by glutamine is the biochemical basis for this amino acid to stimulate protein synthesis in multiple cell types, including myocytes and enterocytes. A wide array of physiological and pathological states is associated with glutamine depletion in tissues, particularly blood and skeletal muscle. Most of the published studies have demonstrated that enteral or parenteral supplementation with appropriate doses of glutamine is beneficial for improving protein balance in organisms under such conditions as infection, sepsis, severe burn, and cancer. The trophic effects of glutamine provide strong evidence for supporting glutamine supplementation to low-birth-weight neonates, rapidly growing animals, and catabolic patients.

Despite much progress in glutamine research, relatively little is known about the effects of glutamine on the developing fetus and neonates who do not exhibit a marked reduction in the circulating level of glutamine. Excitingly, this new line of research is now gaining much attention (3). It is noteworthy that supplementing the gestation diet with 0.6% L-glutamine enhanced the efficiency of nutrient utilization, reduced variation in piglet birth weight, and increased litter birth weight in gilts (5). Additionally, like arginine (186-187), oral administration of glutamine effectively promoted the growth of sow-reared piglets (24). Thus, glutamine can be considered as a functional amino acid to beneficially regulate metabolic pathways during fetal and neonatal development.

The traditional approaches to study glutamine nutrition include digestibility trials, nitrogen balance, assessments of growth and reproductive performance, and isotope tracer techniques (quantification of protein turnover, as well as glutamine synthesis, catabolism and flux) (8,135,188-192). While much has been learned about glutamine biochemistry and nutrition using these techniques, we are now fortunate to have advanced tools, such as genetics, epigenetics, genomics, transcripteomics, proteomics and metabolomics, to determine how dietary glutamine influences protein expression and the physiological pattern of metabolites (27,193,194). Elucidation of the complex mechanisms responsible for the actions of glutamine on cells is expected to expand its applications to solve major problems associated with protein losses in animals and humans.

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**Abbreviations:** AKG: alpha-ketoglutarate; Ala-Gln: L-alanyl-L-glutamine; BCAA: branched – chain amino acids; CLP: Cecal ligation and puncture; DMD: Duchenne muscular dystrophy; DMP: 3, 5-dimethylpyrazole (antilipolytic drug); EDL muscle: extensor digitorum longus; FSR: fractional synthesis rate; GH: growth hormone; GS: glutamine synthetase; ICU: Intensive care unit; IDDM: insulin-dependent diabetes mellitus; IGF-1: insulin-like growth factor-1; IGFBPs: insulin-like growth factor binding protein; LPS: lipopolysaccharide; MCR: metabolic clearance rate; mTOR: mammalian target of rapamycin; NOLD: nonoxidative leucine disposal; OKG: ornithine alpha-ketoglutarate; Ra: appearance rate; rhGH: recombinant human growth hormone; TPN: total parenteral nutrition; VLBW: very low birth weight.

**Key Words:** Glutamine, Protein turnover, Health, Disease, Nutrition, Metabolism, Catabolism, Review

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