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Review

Muscle glutamine depletion in the intensive care unit

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Abstract

Glutamine is primarily synthesized in skeletal muscle and enables transfer of nitrogen to splanchnic tissues, kidneys and immune system. Discrepancy between increasing rates of glutamine utilization at whole body level and relative impairment of de novo synthesis in skeletal muscle leads to systemic glutamine deficiency and characterizes critical illness. Glutamine depletion at whole body level may contribute to gut, liver and immune system disfunctions, whereas its intramuscular deficiency may directly contribute to lean body mass loss. Severe intramuscular glutamine depletion also develops because of outward transport system upregulation, which is not counteracted by increased de novo synthesis. The negative impact of systemic glutamine depletion on critically ill patients is suggested both by the association between a lower plasma glutamine concentration and poor outcome and by a clear clinical benefit after glutamine supplementation. Enteral glutamine administration preferentially increases glutamine disposal in splanchnic tissues, whereas parenteral supplementation provides glutamine to the whole organism. Nonetheless, systemic administration was ineffective in preventing muscle depletion, due to a relative inability of skeletal muscle to seize glutamine from the bloodstream. Intramuscular glutamine depletion could be potentially counteracted by promoting de novo glutamine synthesis with pharmacological or nutritional interventions.

Keywords: Glutamine; Kinetics; Skeletal muscle; Critical illness

Contents

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1. Introduction

Following tissue injury or severe infections, critically ill patients experience metabolic alterations leading to muscle proteolysis activation, enhanced liver gluconeogenesis and tissue insulin resistance (Griffiths, 2003). In addition, these patients exhibit marked reductions in plasma and tissue concentrations of glutamine, the most abundant free amino acid in body compartments (Melis, ter Wengel, Boelens, & van Leeuwen, 2004). Glutamine is primarily synthesized in skeletal muscle and enables transfer of nitrogen to splanchnic tissues, kidneys and immune system. Furthermore, glutamine plays a regulatory role in several specific cell processes. In critically ill patients, glutamine depletion is proportional to severity of illness (Oudemans-van Straaten, Bosman, Treskes, van der Spoel, & Zandstra, 2001) and is not readily reversible by nutrition or other therapeutical approaches (Tjader et al., 2004). Evidence indicates that supplementation with exogenous glutamine in order to enhance its inter-organ flux is often associated with improved outcome of patients (Novak, Heyland, Avenell, Drover, & Su, 2002). In this paper, we will review recent data on glutamine metabolism both in health and disease states, aiming at defining kinetic mechanisms that lead to glutamine depletion.

2. Inter-organ glutamine kinetics

2.1. Glutamine appearance

Tracer kinetic studies in normal volunteers indicate that approximately 90 g of glutamine appear every day in the bloodstream, 80 g from body tissues and 10 g from nutrient intake (Biolo, Fleming, Maggi, & Wolfe, 1995) (Fig. 1). Thus, approximately one-third of all nitrogen derived from protein metabolism is transported in the form of glutamine. The bulk of glutamine entering plasma is derived from skeletal muscle (Biolo et al., 1995). All together, lung, liver and adipose tissue do not therefore account for more than 50% of whole body glutamine appearance. Lung glutamine release has been directly assessed in humans by determining the pulmonary artero-systemic arterial concentration

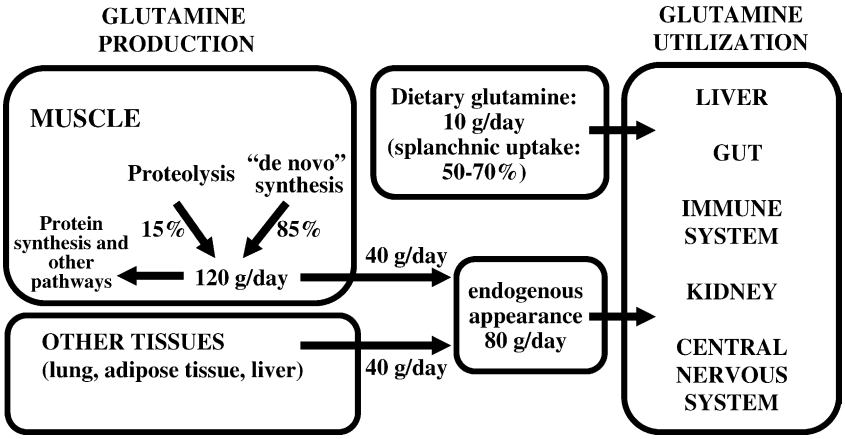

Fig. 1. Body glutamine kinetics assessed by stable isotopes in physiological conditions, numerical data are calculated from Biolo et al. (1995). CNS, central nervous system; GABA, gamma-aminobutyric acid.

difference with the Fick principle (Herskowitz et al., 1991). Relatively healthy subjects were studied before elective surgery to determine lung glutamine exchange (Herskowitz et al., 1991). In these conditions, lung glutamine release appeared to be lower than that observed in skeletal muscle in a different study (Biolo et al., 1995). When turnover of free glutamine was assessed in muscle (Fig. 1), the rate of appearance in cell cytoplasm was three times greater than its release into the bloodstream (Biolo et al., 1995). Glutamine de novo synthesis accounted for 85% of intramuscular appearance, whereas only 15% of the intracellular amino acid was derived from proteolysis (Biolo et al., 1995).

2.2. Storage in skeletal muscle

In skeletal muscle, after de novo synthesis, free glutamine is largely stored in the cytoplasm. In muscle, glutamine is the most abundant free amino acid: its concentration is infact 50–200 times greater than that of the all essential amino acids (Biolo et al., 1995). In physiological conditions, intramuscular free glutamine concentrations range from 10 to 20 mmol/l of tissue water. Thus, this amino acid significantly contributes to total cell osmolarity and hydration (Haussinger, Roth, Lang, & Gerok, 1993). Osmo-sensing structures located inside cells, allow transduction of information depending on the extent of cell hydration, and therefore, allow enzyme activity and gene expression modifications. Indeed, through this mechanism, changes in glutamine muscle cell concentrations may directly regulate protein synthesis and degradation (Table 1), since cell swelling and shrinking are major anabolic and catabolic signals, respectively (Haussinger, Graf, & Weiergraber, 2001). The difference between intracellular and interstitial concentrations of glutamine is determinant of muscle cell hydration. Such transmembrane concentration gradient is largely dependent on cell membrane transport system activity.

Table 1
Organ-specific regulative potential of glutamine

Immune cells
Nucleotide synthesis precursor
Fuel for proliferating immune cells
Modulation of cytokine secretion
Enhances T-lymphocyte response
Synthesis of immunoglobulins A
Modulation of heat shock proteins
Attenuation of NO formation
Supports neutrophil and macrophage functions
Gastrointestinal tract
Nucleotide synthesis precursor
Fuel for proliferating enterocytes
Maintenance of gut-associated lymphoid tissue
Maintenance of gut barrier
Modulation of heat shock proteins
Attenuation of NO formation
Liver
Substrate for ureagenesis
Gluconeogenetic precursor
Glutathione synthesis (antioxidant)
Osmotic signaling mechanism in regulation of protein synthesis and degradation
Precursor of taurine
Modulation of heat shock proteins
Attenuation of NO formation
Precursor of taurine
Kidney
Substrate for renal gluconeogenesis
Acid/base regulation
Ammoniogenesis
Skeletal muscle
Ammonia scavenger
Nitrogen transport (one third of circulating nitrogen)
Osmotic signaling in regulation of protein synthesis and degradation
Lung
Fuel for proliferating endothelial cells
Nitrogen transport
Modulation of heat shock proteins
Central nervous system
Shuttle for glutamate
GABA synthesis

NO, nitric oxide; GABA, gamma-aminobutyric acid.

2.3. Transmembrane glutamine transport

In physiological conditions, transport systems maintain a large transmembrane gradient because intracellular glutamine concentration is 25–30 times greater than extracellular values. Several glutamine transporters have been described in human skeletal muscle (Bode, 2001). Na^+-dependent glutamine transporters include ASC, $B^{0,+}$, y^1L, A and N systems, while Na^+-independent transporters include L, $b^{0,+}$ and n systems. Every glutamine transporter exhibits overlapping affinity for the transport of several other amino acids. Transmembrane Na^+ electrochemical gradient, maintained by the Na^+/K^+-ATPase, drives the uptake of amino acids against their concentration gradient through Na^+-dependent transporters. This accounts

for the maintenance of glutamine cytoplasmatic levels above their transmembrane equilibrium distribution (Bode, 2001).

By utilizing a new technique based on amino acid isotopic tracers in combination with the leg arterovenous technique and muscle biopsies, we were able to determine the bi-directional kinetics of membrane amino acid transport in humans (Biolo et al., 1995). We found that the relative inward transmembrane transport contribution to the intracellular turnover rate of each individual free amino acid was extremely variable. Transport from blood accounted for only 25% of the intramuscular glutamine pool turnover. In contrast, the intracellular pools of most essential amino acids, such as phenylalanine or leucine, derived largely from the extracellular space. Thus, exogenous administration of amino acids that are readily taken up by membrane transport systems will rapidly lead to increases in their intracellular concentration, while free glutamine intracellular concentration will only change slowly after exogenous supplementation (Hammarqvist, Wernerman, Ali, von der Decken, & Vinnars, 1989; Petersson, Waller, Vinnars, & Wernerman, 1994; Tjader et al., 2004).

Transmembrane amino acid transport systems have been mostly investigated focusing on their inward direction activity. Nonetheless, evidence indicates that skeletal muscle is, in all circumstances, a net exporter of glutamine (Ahmed, Peter, Taylor, Harper, & Rennie, 1995). We may predict, therefore, that outward transport systems may play an important role in the inter-organ glutamine exchange regulation. In the last years, attention was focused on the role of system N in the regulation of glutamine efflux from skeletal muscle to the bloodstream (Bode, 2001). In a previous study, we investigated the outward transport kinetics of different amino acids (Biolo et al., 1995). Outward transport rates were normalized per unit of intracellular concentration of free amino acids in order to express the ability of outward transporters to release amino acids at any given concentration. Glutamine outward transport activity of was 30–40 times lower than leucine and phenylalanine and almost eight times lower than lysine. Such relative inability of skeletal muscle to release free glutamine explains its large transmembrane gradient.

It is now evident that several factors may regulate system N activity. Cytokines, cortisol and other hormones activate this transport system in vitro (Watkins, Dudrick, Copeland, & Souba, 1994). In addition, system N-mediated glutamine transport is highly dependent on membrane electrical potential: its progressive depolarization within physiologic ranges results in a switch from glutamine uptake to glutamine release (Fei et al., 2000). Experimental evidence indicates that there is an early transmembrane potential decrease in skeletal muscle during sepsis and after trauma, possibly due to the action of a high molecular weight circulating plasma protein complex (Button et al., 2001). Critically ill patients are characterized by reduced ability to generate action potentials in muscle fibers, and this often leads to a well defined clinical myopathy (Rich & Pinter, 2003). Thus, upregulation of outward transport systems by humoral factors or electrical transmembrane potential changes may play a key role in the rapid mobilization of muscle free glutamine large reservoirs, leading to the release of the amino acid into the bloodstream when its requirements increase in tissues other than skeletal muscle.

2.4. Splanchnic metabolism of dietary glutamine

Glutamine accounts for about 8–12% of total amino acid content of dietary proteins. The total oral intake and absorption of the free amino acid is, therefore, about 10 g per day, but this represents only 12% of the whole body glutamine appearance (Fig. 1). In addition, systemic availability of enteral glutamine is further reduced by first pass splanchnic metabolism. Studies involving oral ingestion of stable isotope-labelled glutamine indicate that 50–70% of enterally administered glutamine is taken up during first pass by splanchnic organs (gut and liver) (Matthews, Marano, & Campbell, 1993) where it is largely oxidized (Haisch, Fukagawa, & Matthews, 2000). Thus, kinetic data indicate that whole body glutamine availability largely depends on the rate of endogenous synthesis in skeletal muscle.

2.5. Whole body glutamine utilization

In physiological conditions, approximately 90 g of glutamine appear daily in the bloodstream. Eighty grams are derived from endogenous sources, mainly skeletal muscle and lung, whereas 10 g are deriving from oral intake. Thereafter, circulating glutamine is preferentially taken up by liver, kidneys, gut mucosa, central nervous system and immune cells (Fig. 1).

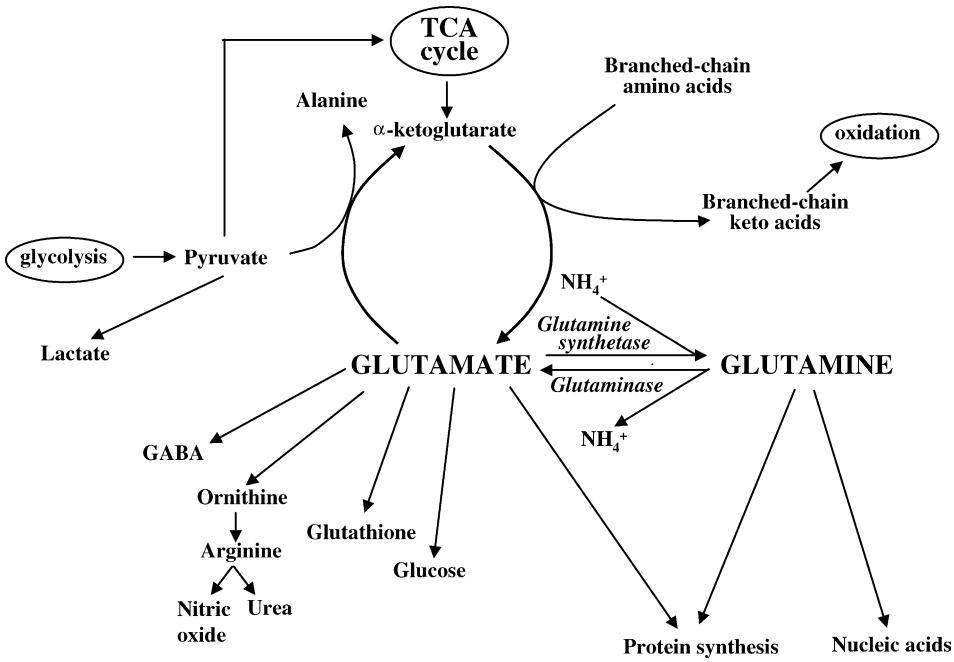

Fig. 2. Overview of glutamine and glutamate metabolism. Glutamate is produced from glutamine through glutaminase activity. Glutamate can be converted into glutamine through glutamine synthetase activity. TCA, tricarboxylic acid; GABA, gamma-aminobutyric acid.

Evidence indicates that these tissues utilize glutamine at high rates and that glutamine utilization is essential for their function (Table 1) (Newsholme, Procopio, Lima, Pithon-Curi, & Curi, 2003). Glutamine tracer kinetic studies (Perriello et al., 1997) indicate that 40–60% of plasma glutamine disappearance is due to oxidation, 10–20% to gluconeogenesis and most of the remainder to protein synthesis and incorporation into macromolecules. The immediate product of glutamine metabolism is glutamate, generated by the glutaminase enzyme (Fig. 2). Glutamine and glutamate are precursors of many compounds, including hepatic and renal glucose, urinary ammonia, intracellular glutathione, nitric oxide and nucleic acids (Fig. 2; Table 1). Furthermore, glutamine is a major fuel for rapidly dividing cells of intestinal mucosa and immune system (Table 1) (Newsholme et al., 2003). We may predict, therefore, that in pathological conditions characterized by activation of immune system, gut dysfunction, increased oxidative stress or metabolic acidosis, glutamine requirements and disposal may be increased (Newsholme, 2001). Kinetic studies in critically ill patients have shown that whole body glutamine disposal is increased both in absolute terms (Gore & Jahoor, 1994) and as metabolic amino acid clearance rate (i.e., rate of disposal divided by the prevailing glutamine concentration in plasma) (Jackson et al., 1999).

3. Regulation of muscle glutamine synthesis

The described kinetic studies indicate that skeletal muscle is the main site of glutamine production and release because of its large free glutamine pool and its capacity for de novo synthesis. Immediate precursors of glutamine synthesis are glutamate and free ammonia (Fig. 2); the process is catalyzed by glutamine synthetase. Glutamate is synthesized by transamination of the branched chain amino acids leucine, isoleucine and valine, which are extensively decarboxylated in skeletal muscle. In this reaction, the branched chain amino acids react with α-ketoglutarate to produce α-ketoacids and glutamate in presence of the branched chain amino acid aminotransaminase enzyme. We may,

therefore, predict that muscle glutamine synthesis does not only require intramuscular availability of branched chain amino acids for transamination but also an adequate flux of energy substrates through the pyruvate dehydrogenase and the tricarboxylic acid cycle as well as an adequate source of carbon skeletons. Several lines of evidence support the key role of these regulatory mechanisms for glutamine synthesis in vivo. First, studies using infusion of leucine, labelled with nitrogen stable isotopes, demonstrated direct tracer incorporation into glutamine (Darmaun & Dechelotte, 1991). Second, in contrast to most amino acids, glutamine release from skeletal muscle is not decreased in the postprandial state: enteral and parenteral nutrient administration infact lead to significant stimulation of glutamine de novo synthesis (Darmaun et al., 1994). Third, when dicholoracetate, a potent activator of the pyruvate dehydrogenase complex through inhibition of the pyruvate dehydrogenase kinase, was infused in burnt patients, muscle glutamine concentrations increased significantly (Ferrando et al., 1998). Fourth, hyperinsulinemia, in combination with glucose infusion to maintain euglycemia, increased muscle glucose uptake with subsequent conversion to glutamine and accelerated release of the amino acid (Meyer, Woerle, & Gerich, 2004). Finally, supplementation with α-ketoglutarate was able to increase muscle free glutamine (Hammarqvist, Wernerman, von der Decken, & Vinnars, 1991). Glutamine synthesis is also regulated by glutamine synthetase activity. Hormones and substrates may directly modulate enzyme activity through transcriptional and post-transcriptional mechanisms. Glucocorticoids can up-regulate glutamine synthetase mRNA levels in muscle cells through a glucocorticoid receptor-dependent process (Max et al., 1987). In vivo, glutamine synthetase mRNA levels can increase roughly 10-fold in response to glucocorticoid administration (Max et al., 1988). In healthy humans, physiological elevations of plasma cortisol levels lead to significant stimulation of glutamine production, primarily because of an increase in glutamine de novo synthesis (Darmaun, Matthews, & Bier, 1988). Growth hormone may also potentially increase the glutamine synthetase gene expression (Nolan, Masters, & Dunn, 1990). Nonetheless, we recently found that the rate of glutamine release from leg muscle decreased after administration of growth hormone to trauma patients despite the increased enzyme gene expression (Biolo, Iscra et al., 2000). Also catecholamines are indeed important regulators of glutamine production. Effects on glutamine metabolism were studied in isolated skeletal muscle. Physiological levels of epinephrine reduced glutamine formation and release from skeletal muscle, via a beta-adrenergic receptor pathway and the adenylate cyclase system (Nie, Wallberg-Henriksson, Johansson, & Henriksson, 1989). In addition to these hormone-dependent effects, glutamine synthetase expression is directly induced in rat skeletal muscle cells by treatment with inflammatory cytokines, such as tumor necrosis factor alpha and interleukin-1 beta (Huang & O'Banion, 1998). Glutamine synthetase activity is also directly regulated by glutamine concentrations through a post-transcriptional mechanism that increases enzyme activity when tissue glutamine levels are low (Feng, Shiber, & Max, 1990).

Levels of physical activity may also directly regulate muscle glutamine production. During exercise, substrate flux through the TCA and branched chain amino acid oxidation greatly increases (Gibala, MacLean, Graham, & Saltin, 1998). Glutamine synthesis and release are, therefore, significantly stimulated (Van Hall, Saltin, & Wagenmakers, 1999) leading to increased plasma glutamine concentrations, as observed following short-term moderate exercise or during endurance training (Hood & Terjung, 1994). Glutamine concentrations increase despite the fact that exercise in rats decreases the activity of glutamine synthetase and prevents glucocorticoid-mediated enzyme induction (Falduto, Young, & Hickson, 1992). These results suggest that after exercise, as after growth hormone administration, glutamine synthesis regulation by precursor availability may overcome the effects of changes in glutamine synthetase expression or activity. Glutamine concentration is decreased after prolonged, exhaustive training, in contrast to moderate exercise, possibly due to increased glutamine demand as a consequence of immune system activation (Rowbottom, Keast, & Morton, 1996). Athletes with overtraining syndrome also have low plasma glutamine. Glutamine levels remain low even after several weeks of rest (Hiscock & Mackinnon, 1998). It has been hypothesized that there could be a link between exercise-mediated changes in glutamine metabolism and the epidemiological data showing that athletes are at increased risk for upper respiratory tract infections during periods of heavy training and following race

events (Nieman, 1997). In contrast to physical exercise, the effects of immobility on glutamine metabolism have been poorly investigated. In animal models of muscle unloading or denervation, glutamine synthesis rates and intracellular levels are decreased, despite a greater activity of glutamine synthetase (Feng, Konagaya et al., 1990; Jaspers, Jacob, & Tischler, 1986). In addition, rats exposed to 7 days of weightlessness during the Spacelab-3 shuttle flight exhibited decreased muscle levels of free glutamine (Steffen & Musacchia, 1986). In agreement with these animal studies, in a recent short-term bed rest study in normal volunteers (Biolo et al., 2004), we observed that de novo glutamine synthesis rate at the whole body level significantly decreased by about 10% following 15 days of muscle unloading (Biolo et al., unpublished). Previous evidence indicates that during muscle unloading, activities of the tricarboxylic acid cycle enzymes are reduced in both animals and humans (Berg, Dudley, Hather, & Tesch, 1993). This alteration may lead to decreased glutamate and glutamine synthesis.

4. Systemic and muscle glutamine depletion in critical illness

Critical illness is characterized by increased production of cytokines and stress hormones, such as glucocorticoids and catecholamines. These mediators interact with bed rest and, possibly, with hyponutrition to produce a cluster of metabolic abnormalities such as muscle wasting, insulin resistance and glutamine kinetic alterations. Systemic glutamine depletion results from discrepancies between rates of skeletal muscle release and uptake in other tissues. In addition, severe intramuscular glutamine depletion develops because transport systems upregulation, accelerating outflux from muscle, is not adequately matched by an increase in de novo synthesis. Muscle and plasma glutamine concentrations decrease proportionally to severity of diseases. In addition, the ratio between muscle cells and plasma glutamine concentrations decreases progressively from physiological values of 20–30 to values of 10–15 in more severe conditions (Biolo, Fleming et al., 2000; Flaring, Rooyackers, Wernerman, & Hammarqvist, 2003; Wernerman, 2003), clearly showing an upregulation of outward transport systems. Regarding muscle glutamine production, critical illness may be associated with either increased or decreased de novo synthesis. While tumor necrosis factor-alpha directly induces glutamine synthetase, the two major stress hormones, cortisol and epinephrine, have opposite effects on its synthesis. In addition, bed rest and hyponutrition may further decrease glutamine synthesis. In a recent study, we have determined, in skeletal muscle and during the post-absorptive state, glutamine synthesis de novo rate both in normal controls and in patients with severe burns during the “flow” phase after injury. We found that in burn patients, muscle glutamine synthesis was 50% lower than in healthy controls (Biolo, Fleming et al., 2000). Nonetheless, glutamine appearance rate was found either decreased (Jackson et al., 1999) or accelerated (Gore & Jahoor, 1994) when determined by stable isotopes at the whole body level. We may, therefore, speculate that, while glutamine synthesis is suppressed in muscle, it may be accelerated in other tissues. Evidence indicates infact that in lungs, glutamine synthesis is greatly accelerated after surgical stress (Herskowitz et al., 1991).

5. Clinical consequences of glutamine depletion

Glutamine depletion at whole body level may impair physiological functions of gut, liver and immune system (Newsholme et al., 2003), whereas intramuscular glutamine depletion may directly contribute to lean body mass wasting (Jepson, Bates, Broadbent, Pell, & Millward, 1988) (Table 1) and delayed recovery from illness.

6. Glutamine supplementation

Critical illness is characterized by increased glutamine utilization leading to depletion of the amino acid. Nonetheless, standard nutrition support solutions contain none (standard parenteral formulas) or very little glutamine (polymeric casein-derived enteral formulas). A lower plasma glutamine concentration (i.e., <0.420 mmol/l) is associated with a higher ICU mortality (60% versus 29%), supporting the hypothesis of a negative impact of glutamine depletion on clinical outcome of critically ill patients (Oudemans-van Straaten et al., 2001). In addition, there is evidence for a clear clinical benefit of glutamine supplementation in critically ill patients. A recent meta-analysis evaluated

Table 2
Randomized studies evaluating glutamine supplementation in critically ill patients

Study (reference)	ICU patient population	Route of delivery, dose (g/(kg day))	Infections	Mortality no./*n* (%)	
				Control	Experiment
Griffiths et al. (1997)	Unselected	TPN, 0.26	ns	28/42 (67)	18/42 (43), $p < 0.04$[a]
Houdijk et al. (1998)	Trauma	EN, 0.25	$p < 0.005$[b] $p < 0.02$[c]	2/29 (7)	2/27 (7)[ns]
Powell-Tuck et al. (1999)	Unselected	TPN, 0.26	–	20/83 (24)	14/85 (16)[ns]
Jones et al. (1999)	Unselected	EN, 0.16	–	12/24 (50)	10/26 (38)[ns]
Brantley and Pierce (2000)	Trauma	EN, 0.50	–	0/41 (0)	0/31 (0)[ns]
Wischmeyer et al. (2001)	Burns	TPN, 0.57	$p < 0.04$[d]	4/14 (29)	1/12 (8)[ns]
Goeters et al. (2002)	Trauma	TPN, 0.30	ns	21/35 (60)	11/33 (33), $p < 0.05$
Conejero et al. (2002)	Unselected	EN, 0.43	$p = 0.04$[c] $p = 0.001$[d]	9/33 (27)	14/43 (33)[ns]
Garrel et al. (2003)	Burns	EN, 0.30	$p < 0.01$[b]	8/22 (36)	0/19 (0), $p < 0.01$
Hall et al. (2003)	Unselected	EN, 0.28	ns	30/182 (16)	27/179 (15)[ns]

Levels of significance are shown for incidence of infectious complications and mortality rates. ICU, intensive care unit; TPN, total parenteral nutrition; EN, enteral nutrition; –, not available; ns, no significant difference.
[a] Six-month mortality.
[b] Bacteremia.
[c] Pneumonia.
[d] Gram-negative bacteremia.

six randomized studies on glutamine supplementation in serious illness (Novak et al., 2002). The authors conclude that glutamine supplementation is associated with a strong trend toward a reduction in mortality, a lower infectious complication rate and a shorter hospitalization. More recently, four other randomized trials have been published. The results of the ten studies are summarized in Table 2 (Brantley & Pierce, 2000; Conejero et al., 2002; Garrel et al., 2003; Goeters et al., 2002; Griffiths, Jones, & Palmer, 1997; Hall et al., 2003; Houdijk et al., 1998; Jones, Palmer, & Griffiths, 1999; Powell-Tuck et al., 1999; Wischmeyer et al., 2001). These studies show that survival is improved by glutamine supplementation only in high mortality rate settings. The effective daily supplementation dose ranged from 20 to 40 g. These glutamine administration rates approximately match the extent of the decrease in muscle glutamine de novo synthesis observed in burn patients (Biolo, Fleming et al., 2000). There is clear evidence that both enteral or parenteral supplementation can be effective in counteracting the systemic depletion glutamine. Enteral glutamine administration preferentially increases glutamine disposal in splanchnic tissues, whereas parenteral supplementation provides glutamine to the whole organism.

Glutamine supplementation was mostly ineffective in preventing muscle glutamine depletion in critically ill patients, because of the relative inability of this tissue in the uptake of the circulating amino acid. Intramuscular glutamine depletion should, therefore, be preferentially counteracted by promoting de novo glutamine synthesis. Clinical studies have shown that stimulation of pyruvate oxidation by dichloroacetate administration in burnt patients (Ferrando et al., 1998) and supplementation of ketoglutarate in post-surgical patients (Hammarqvist et al., 1991) may improve glutamine synthesis in skeletal muscle. In addition, branched chain amino acid administration may affect de novo muscle glutamine production rate by increasing nitrogen availability for ketoglutarate transamination (Darmaun & Dechelotte, 1991).

References

Ahmed, A., Peter, G. J., Taylor, P. M., Harper, A. A., & Rennie, M. J. (1995). Sodium-independent currents of opposite polarity evoked by neutral and cationic amino acids in neutral and basic amino acid transporter cRNA-injected oocytes. *J. Biol. Chem.*, *270*(April (15)), 8482–8486.

Berg, H. E., Dudley, G. A., Hather, B., & Tesch, P. A. (1993). Work capacity and metabolic and morphologic characteristics of the human quadriceps muscle in response to unloading. *Clin. Physiol.*, *13*(July (4)), 337–347.

Biolo, G., Fleming, R. Y., Maggi, S. P., & Wolfe, R. R. (1995). Transmembrane transport and intracellular kinetics of amino acids in human skeletal muscle. *Am. J. Physiol.*, *268*(January (1 Pt 1)), E75–E84.

Biolo, G., Fleming, R. Y., Maggi, S. P., Nguyen, T. T., Herndon, D. N., & Wolfe, R. R. (2000). Inhibition of muscle glutamine formation in hypercatabolic patients. *Clin. Sci. (Lond.)*, *99*(September (3)), 189–194.

Biolo, G., Iscra, F., Bosutti, A., Toigo, G., Ciocchi, B., Geatti, O., et al. (2000). Growth hormone decreases muscle glutamine production and stimulates protein synthesis in hypercatabolic patients. *Am. J. Physiol. Endocrinol. Metab.*, *279*(August (2)), E323–E332.

Biolo, G., Ciocchi, B., Lebenstedt, M., Barazzoni, R., Zanetti, M., Platen, P., et al. (2004). Short-term bed rest impairs amino acid-induced protein anabolism in humans. *J. Physiol.*, *558*(July (Pt 2)), 381–388.

Bode, B. P. (2001). Recent molecular advances in mammalian glutamine transport. *J. Nutr.*, *131*(September (9 Suppl.)), 2475S–2485S.

Brantley, S., & Pierce, J. (2000). Effects of enteral glutamine on trauma patients. *Nutr. Clin. Pract.*, *15*, S13.

Button, B., Baker, R. D., Vertrees, R. A., Allen, S. E., Brodwick, M. S., & Kramer, G. C. (2001). Quantitative assessment of a circulating depolarizing factor in shock. *Shock*, *15*(March (3)), 239–244.

Conejero, R., Bonet, A., Grau, T., Esteban, A., Mesejo, A., Montejo, J. C., et al. (2002). Effect of a glutamine-enriched enteral diet on intestinal permeability and infectious morbidity at 28 days in critically ill patients with systemic inflammatory response syndrome: A randomized, single-blind, prospective, multicenter study. *Nutrition*, *18*(September (9)), 716–721.

Darmaun, D., Matthews, D. E., & Bier, D. M. (1988). Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. *Am. J. Physiol.*, *255*(September (3 Pt 1)), E366–E373.

Darmaun, D., & Dechelotte, P. (1991). Role of leucine as a precursor of glutamine alpha-amino nitrogen in vivo in humans. *Am. J. Physiol.*, *260*(February (2 Pt 1)), E326–E329.

Darmaun, D., Just, B., Messing, B., Rongier, M., Thuillier, F., Koziet, J., et al. (1994). Glutamine metabolism in healthy adult men: Response to enteral and intravenous feeding. *Am. J. Clin. Nutr.*, *59*(June (6)), 1395–1402.

Falduto, M. T., Young, A. P., & Hickson, R. C. (1992). Exercise interrupts ongoing glucocorticoid-induced muscle atrophy and glutamine synthetase induction. *Am. J. Physiol.*, *263*(December (6 Pt 1)), E1157–E1163.

Fei, Y. J., Sugawara, M., Nakanishi, T., Huang, W., Wang, H., Prasad, P. D., et al. (2000). Primary structure, genomic organization, and functional and electrogenic characteristics of human system N1, a Na^+- and H^+-coupled glutamine transporter. *J. Biol. Chem.*, *275*(August (31)), 23707–23717.

Feng, B., Shiber, S. K., & Max, S. R. (1990). Glutamine regulates glutamine synthetase expression in skeletal muscle cells in culture. *J. Cell. Physiol.*, *145*, 376–380.

Feng, B., Konagaya, M., Konagaya, Y., Thomas, J. W., Banner, C., Mill, J., et al. (1990). Neural control of glutamine synthetase activity in rat skeletal muscles. *Am. J. Physiol.*, *258*(May (5 Pt 1)), E757–E761.

Ferrando, A. A., Chinkes, D. L., Wolf, S. E., Matin, S., Herndon, D. N., & Wolfe, R. R. (1998). Acute dichloroacetate administration increases skeletal muscle free glutamine concentrations after burn injury. *Ann. Surg.*, *228*(August (2)), 249–256.

Flaring, U. B., Rooyackers, O. E., Wernerman, J., & Hammarqvist, F. (2003). Glutamine attenuates post-traumatic glutathione depletion in human muscle. *Clin. Sci. (Lond.)*, *104*(March (3)), 275–282.

Garrel, D., Patenaude, J., Nedelec, B., Samson, L., Dorais, J., Champoux, J., et al. (2003). Decreased mortality and infectious morbidity in adult burn patients given enteral glutamine supplements: A prospective, controlled, randomized clinical trial. *Crit. Care Med.*, *31*(October (10)), 2444–2449.

Gibala, M. J., MacLean, D. A., Graham, T. E., & Saltin, B. (1998). Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. *Am. J. Physiol.*, *275*(August (2 Pt 1)), E235–E242.

Goeters, C., Wenn, A., Mertes, N., Wempe, C., Van Aken, H., Stehle, P., et al. (2002). Parenteral L-alanyl-L-glutamine improves 6-month outcome in critically ill patients. *Crit. Care Med.*, *30*(September (9)), 2032–2037.

Gore, DC., & Jahoor, F. (1994). Glutamine kinetics in burn patients, comparison with hormonally induced stress in volunteers. *Arch. Surg.*, *129*(December (12)), 1318–1323.

Griffiths, R. D., Jones, C., & Palmer, T. E. (1997). Six-month outcome of critically ill patients given glutamine-supplemented parenteral nutrition. *Nutrition*, *13*(April (4)), 295–302.

Griffiths, R. D. (2003). Nutrition support in critically ill septic patients. *Curr. Opin. Clin. Nutr. Metab. Care*, *6*(March (2)), 203–210.

Haisch, M., Fukagawa, N. K., & Matthews, D. E. (2000). Oxidation of glutamine by the splanchnic bed in humans. *Am. J. Physiol. Endocrinol. Metab.*, *278*(April (4)), E593–E602.

Hall, J. C., Dobb, G., Hall, J., de Sousa, R., Brennan, L., & McCauley, R. (2003). A prospective randomized trial of enteral glutamine in critical illness. *Intensive Care Med.*, *29*(October (10)), 1710–1716.

Hammarqvist, F., Wernerman, J., Ali, R., von der Decken, A., & Vinnars, E. (1989). Addition of glutamine to total parenteral nutrition after elective abdominal surgery spares free glutamine in muscle, counteracts the fall in muscle protein synthesis, and improves nitrogen balance. *Ann. Surg.*, *209*(April (4)), 455–461.

Hammarqvist, F., Wernerman, J., von der Decken, A., & Vinnars, E. (1991). Alpha-ketoglutarate preserves protein synthesis and free glutamine in skeletal muscle after surgery. *Surgery*, *109*(January (1)), 28–36.

Haussinger, D., Roth, E., Lang, F., & Gerok, W. (1993). Cellular hydration state: An important determinant of protein catabolism

in health and disease. *Lancet*, *341*(May (8856)), 1330–1332.

Haussinger, D., Graf, D., & Weiergraber, O. H. (2001). Glutamine and cell signaling in liver. *J. Nutr.*, *131*(September (9 Suppl.)), 2509S–2514S.

Herskowitz, K., Plumley, D. A., Martin, T. D., Hautamaki, R. D., Copeland, E. M., 3rd, & Souba, W. W. (1991). Lung glutamine flux following open heart surgery. *J. Surg. Res.*, *51*(1 (July)), 82–86.

Hiscock, N., & Mackinnon, L. T. (1998). A comparison of plasma glutamine concentration in athletes from different sports. *Med. Sci. Sports Exerc.*, *30*(December (12)), 1693–1696.

Hood, D. A., & Terjung, R. L. (1994). Endurance training alters alanine and glutamine release from muscle during contractions. *FEBS Lett.*, *340*(March (3)), 287–290.

Houdijk, A. P., Rijnsburger, E. R., Jansen, J., Wesdorp, R. I., Weiss, J. K., McCamish, M. A., et al. (1998). Randomised trial of glutamine-enriched enteral nutrition on infectious morbidity in patients with multiple trauma. *Lancet*, *352*(September (9130)), 772–776.

Huang, T. L., & O'Banion, M. K. (1998). Interleukin-1 beta and tumor necrosis factor-alpha suppress dexamethasone induction of glutamine synthetase in primary mouse astrocytes. *J. Neurochem.*, *71*(October (4)), 1436–1442.

Jackson, N. C., Carroll, P. V., Russell-Jones, D. L., Sonksen, P. H., Treacher, D. F., & Umpleby, A. M. (1999). The metabolic consequences of critical illness: Acute effects on glutamine and protein metabolism. *Am. J. Physiol.*, *276*(January (1 Pt 1)), E163–E170.

Jaspers, S. R., Jacob, S., & Tischler, M. E. (1986). Metabolism of amino acids by the atrophied soleus of tail-casted, suspended rats. *Metabolism*, *35*(March (3)), 216–223.

Jepson, M. M., Bates, P. C., Broadbent, P., Pell, J. M., & Millward, D. J. (1988). Relationship between glutamine concentration and protein synthesis in rat skeletal muscle. *Am. J. Physiol.*, *255*(August (2 Pt 1)), E166–E172.

Jones, C., Palmer, T. E., & Griffiths, R. D. (1999). Randomized clinical outcome study of critically ill patients given glutamine-supplemented enteral nutrition. *Nutrition*, *15*(February (2)), 108–115.

Matthews, D. E., Marano, M. A., & Campbell, R. G. (1993). Splanchnic bed utilization of glutamine and glutamic acid in humans. *Am. J. Physiol.*, *264*(June (6 Pt 1)), 848–854.

Max, S. R., Thomas, J. W., Banner, C., Vitkovic, L., Konagaya, M., & Konagaya, Y. (1987). Glucocorticoid receptor-mediated induction of glutamine synthetase in skeletal muscle cells in vitro. *Endocrinology*, *120*, 1179–1183.

Max, S. R., Mill, J., Mearow, K., Konagaya, M., Konagaya, Y., Thomas, J. W., et al. (1988). Dexamethasone regulates glutamine synthetase expression in rat skeletal muscles. *Am. J. Physiol.*, *255*, E397–E402.

Melis, G. C., ter Wengel, N., Boelens, P. G., & van Leeuwen, P. A. (2004). Glutamine: Recent developments in research on the clinical significance of glutamine. *Curr. Opin. Clin. Nutr. Metab. Care.*, *7*(January (1)), 59–70.

Meyer, C., Woerle, H. J., & Gerich, J. (2004). Paradoxical changes of muscle glutamine release during hyperinsulinemia euglycemia and hypoglycemia in humans: Further evidence for the glucose–glutamine cycle. *Metabolism*, *53*(September (9)), 1208–1214.

Newsholme, P. (2001). Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? *J. Nutr.*, *131*(September (9 Suppl.)), 2515S–2522S.

Newsholme, P., Procopio, J., Lima, M. M., Pithon-Curi, T. C., & Curi, R. (2003). Glutamine and glutamate—Their central role in cell metabolism and function. *Cell. Biochem. Funct.*, *21*(March (1)), 1–9.

Nie, Z. T., Wallberg-Henriksson, H., Johansson, S., & Henriksson, J. (1989). Effects of adrenaline and prior exercise on the release of alanine, glutamine and glutamate from incubated rat skeletal muscle. *Acta Physiol. Scand.*, *136*(July (3)), 395–401.

Nieman, D. C. (1997). Immune response to heavy exertion. *J. Appl. Physiol.*, *82*(May (5)), 1385–1394.

Nolan, E. M., Masters, J. N., & Dunn, A. (1990). Growth hormone regulation of hepatic glutamine synthetase mRNA levels in rats. *Mol. Cell. Endocrinol.*, *69*(March (2–3)), 101–110.

Novak, F., Heyland, D. K., Avenell, A., Drover, J. W., & Su, X. (2002). Glutamine supplementation in serious illness: A systematic review of the evidence. *Crit. Care Med.*, *30*(September (9)), 2022–2029.

Oudemans-van Straaten, H. M., Bosman, R. J., Treskes, M., van der Spoel, H. J., & Zandstra, D. F. (2001). Plasma glutamine depletion and patient outcome in acute ICU admissions. *Intensive Care Med.*, *27*(January (1)), 84–90.

Perriello, G., Nurjhan, N., Stumvoll, M., Bucci, A., Welle, S., Dailey, G., et al. (1997). Regulation of gluconeogenesis by glutamine in normal postabsorptive humans. *Am. J. Physiol.*, *272*(March (3 Pt 1)), E437–E445.

Petersson, B., Waller, S. O., Vinnars, E., & Wernerman, J. (1994). Long-term effect of glycyl-glutamine after elective surgery on free amino acids in muscle. *JPEN J. Parenter. Enteral Nutr.*, *18*(July–August (4)), 320–325.

Powell-Tuck, J., Jamieson, C. P., Bettany, G. E., Obeid, O., Fawcett, H. V., Archer, C., et al. (1999). A double blind, randomised, controlled trial of glutamine supplementation in parenteral nutrition. *Gut*, *45*(July (1)), 82–88.

Rich, M. M., & Pinter, M. J. (2003). Crucial role of sodium channel fast inactivation in muscle fibre inexcitability in a rat model of critical illness myopathy. *J. Physiol.*, *547*(March (Pt 2)), 555–566.

Rowbottom, D. G., Keast, D., & Morton, A. R. (1996). The emerging role of glutamine as an indicator of exercise stress and overtraining. *Sports Med.*, *21*(February (2)), 80–97.

Steffen, J. M., & Musacchia, X. J. (1986). Spaceflight effects on adult rat muscle protein, nucleic acids, and amino acids. *Am. J. Physiol.*, *251*(December (6 Pt 2)), R1059–R1063.

Tjader, I., Rooyackers, O., Forsberg, A. M., Vesali, R. F., Garlick, P. J., & Wernerman, J. (2004). Effects on skeletal muscle of intravenous glutamine supplementation to ICU patients. *Intensive Care Med.*, *30*(February (2)), 266–275.

Van Hall, G., Saltin, B., & Wagenmakers, A. J. (1999). Muscle protein degradation and amino acid metabolism during prolonged knee-extensor exercise in humans. *Clin. Sci. (Lond.)*, *97*(November (5)), 557–567.

Watkins, K. T., Dudrick, P. S., Copeland, E. M., & Souba, W. W. (1994). Interleukin-6 and dexamethasone work coordinately to augment hepatic amino acid transport. *J. Trauma*, *36*(April (4)), 523–528.

Wernerman, J. (2003). Glutamine and acute illness. *Curr. Opin. Crit. Care*, *9*(August (4)), 279–285.

Wischmeyer, P. E., Lynch, J., Liedel, J., Wolfson, R., Riehm, J., Gottlieb, L., et al. (2001). Glutamine administration reduces Gram-negative bacteremia in severely burned patients: A prospective, randomized, double-blind trial versus isonitrogenous control. *Crit. Care Med.*, *29*(November (11)), 2075–2080.