

Glutamine: role in critical illness and ongoing clinical trials

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Purpose of review

This review will assess recent clinical and mechanistic data examining glutamine's ability to reduce morbidity and mortality in critical illness.

Recent findings

Updated metaanalysis data reveal a significant benefit of glutamine supplementation on mortality, length of stay, and infectious morbidity in critical illness. Recent data support glutamine's use in critically ill patients requiring parenteral nutrition and new data reveal safety and efficacy in head-injured patients. Further, new findings on glutamine's beneficial effect on insulin resistance in critical illness will be reviewed. Recent laboratory data have clarified a number of key mechanistic pathways by which glutamine may improve outcome in critical illness.

Summary

Severe glutamine deficiencies occur rapidly in critical illness. The magnitude of glutamine deficiency is correlated with ICU mortality. Further, metaanalysis reveals glutamine reduces morbidity and mortality in critical illness. It is likely that our new understanding of the molecular pathways by which glutamine acts will lead to insight on how best to utilize glutamine as a nutritional therapy. Presently, randomized, multicenter clinical trials utilizing glutamine as both nutritional replacement and pharmacologic intervention, independent of nutritional needs, are ongoing.

Keywords

head injury, heat shock protein, insulin resistance, molecular mechanism, mortality, nutritional pharmacology

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Introduction

The debate over whether glutamine supplementation should be standard of care in critically ill patients will soon be clarified as a number of large, multicenter trials from around the world will be completed in the next 2–3 years. Many millions of dollars from government funding agencies have recently been committed to answering this question. The question this review will attempt to address is: How did we get here? This review will examine the data that have driven this ongoing debate and new findings revealing the mechanistic pathways potentially responsible for glutamine's effect in critical illness. These translational data will assist us in targeting our therapy with glutamine to optimize its benefit.

Clinical data over the past 15–20 years reveal glutamine may be beneficial in improving outcome in numerous small clinical trials of critically ill patients with virtually no risk to the patient and at a very low cost [1]. A major advantage of glutamine as a nutritional therapeutic is that it has received extensive worldwide study and application for many years. Thus, we have extensive safety data and

postapproval marketing, particularly in Europe, where it has been used in many thousands of patients. Further, as a nutritional therapeutic agent it is this author's hope that US Food and Drug Administration (FDA) approval will be achieved in the US using the extensive European safety data at a much lower cost than a typical new drug. This is vital as profits from nutritional therapeutics will always be lower than from a new synthetic pharmaceutical agent, as patent protection of these naturally occurring agents is limited. The issues of risk and cost must be paramount to any discussion of critical care interventions as the cost of critical care medicine in the US alone was \$55 billion in 2000, which made up 0.56% of the gross domestic product at that time [2]. We must as clinical researchers develop therapies that are clinically effective, but also fiscally responsible. The issues of clinical efficacy, risk, and cost are exemplified well by the story of activated protein C (APC). APC, a drug with significant cost and potential risk to the patient, will enter its third major worldwide trial this year in an attempt to refine clinical indications and efficacy.

A number of trials of glutamine in critical illness have revealed that it improves infectious morbidity and

mortality in critically ill patients [3–7]; there are, however, negative trials of glutamine in critical illness as well [8,9]. Key methodological differences between the trials showing benefit versus the trials that did not show benefit relate to dose, route of administration, and potential patient selection. The beneficial trials consistently administered larger doses of glutamine (typically >0.5 g/kg/day) and most gave it intravenously. This trend towards larger parenteral doses of glutamine being beneficial is supported by the updated metaanalysis of glutamine in critical illness available at <http://www.criticalcarenutrition.com> [1].

To fully understand the recent focus on this ‘nutrient therapy’, the history of the explanations for glutamine supplementation in critical illness must be examined. Glutamine is now considered by many investigators to be ‘conditionally essential’ in critical illness [10]. In catabolic states large amounts of glutamine are released from muscle tissue [11]. One described hypothesis for the release of glutamine following stress is that it provides a vital fuel source for rapidly dividing cells; it is a precursor for nucleic acid synthesis; and it is a key precursor for acid–base homeostasis in the kidney [12,13]. Despite this significant release of glutamine, it is well known that plasma levels decrease significantly following critical illness and remain decreased for over 21 days [14]. This deficiency is associated with increased mortality in critically ill patients [15].

Recent translational research reveals long-held beliefs about glutamine acting primarily as a metabolic fuel for rapidly dividing cells were erroneous and oversimplified. These data reveal that glutamine may serve as a vital cell signaling molecule in states of illness and injury [16••]. Glutamine has been shown to regulate the expression of many genes related to metabolism, signal transduction, cell defense and repair, and to activate intracellular signaling pathways [16••]. It is possible that the release of glutamine from muscle and other sources serves as a ‘stress signal’ to the organism to turn on genes vital to cellular protection and immune regulation. Adequate plasma and cellular glutamine levels appear vital to the cell’s response to stress and injury. These new data are beginning to provide attractive explanations for the mechanisms by which glutamine exerts a beneficial effect in critical illness.

Recent clinical trials of glutamine in critical illness

A multicenter randomized controlled trial of 114 surgical and trauma ICU patients who required parenteral nutrition has recently been completed [17••]. The study examined parenteral nutrition supplemented with alanyl-glutamine (a stable, soluble form of intravenous

glutamine used worldwide, however not currently available in the US). Via intention-to-treat analysis, glutamine led to a significant reduction in complicated clinical outcomes (41.4% versus 60.7%; $P < 0.05$). This effect was predominately driven by reduced infectious rate and pneumonias. There was no difference in survival in this group of patients in whom the age and predicted risk of death were low. The other interesting finding of this study was that the investigators found glutamine supplementation led to a significant reduction of hyperglycemia and a significant reduction in the number of patients requiring insulin.

Another recent study from a Chinese research group [18•] examined the effect of intravenous alanyl-glutamine dipeptide on the outcome of severe traumatic brain injury. This randomized study of 46 patients revealed glutamine supplementation led to a statistically significant reduction in mortality, lung infection, and gastrointestinal hemorrhage. This study is seminal because it is the first to utilize glutamine to study outcome in a head-injured patient group. Concerns over glutamine crossing the blood–brain barrier and leading to increases in intracerebral levels of the excitatory neurotransmitter glutamate have recently been dispelled. Berg *et al.* [19••] demonstrated infusion of clinically relevant doses of glutamine to head-injured patients does not increase intercerebral glutamate levels. This study examined intercerebral microdialysate fluid and found significant changes in plasma glutamine levels. No changes in intracerebral glutamate levels, however, were observed in the group as a whole or in any individual patient.

A final trial highlights the beneficial effect of glutamine on insulin resistance and hyperglycemia observed following critical illness or injury. It is well known that hyperglycemia contributes to morbidity and mortality in critical illness [20,21]. An elegant study [22••] specifically examined insulin resistance in trauma. The authors randomized 40 patients with multiple trauma to receive either 0.4 g of glutamine per kg of body weight per day or isocaloric and isonitrogenous control. To assess insulin sensitivity, euglycemic clamp was performed on days 4 and 8. The investigators found that improved insulin sensitivity in multiple trauma patients was positively associated with parenteral glutamine supplementation. In light of this study and the aforementioned study by Dechelotte *et al.* [17••], it appears reasonable to accept that glutamine exerts a beneficial effect on insulin-dependent glucose metabolism.

Update of metaanalysis of glutamine in critical illness

In order to analyze the overall effect of glutamine in the critically ill, Novak and colleagues [1] performed a

comprehensive review of all published trials of glutamine therapy in critical illness. This metaanalysis has been updated with the most recent randomized trials of glutamine in critical illness. The new analysis, which is available on <http://www.criticalcarenutrition.com>, reveals glutamine given by either enteral or intravenous route leads to a statistically significant reduction in mortality in critical illness [relative risk (RR) 0.75, 95% confidence interval (CI) 0.59–0.96, $P=0.02$] (Fig. 1). The new analysis also revealed glutamine significantly reduces infectious morbidity in critically ill patients (RR 0.79, 95% CI 0.63–0.98, $P=0.04$) (Fig. 2). Further, glutamine administration leads to a dramatic 4.5-day (95% CI –8.28, –0.72, $P=0.02$) reduction of length of ICU stay (Fig. 3). These data continue to support that higher dose glutamine (>0.3 g/kg/day) and parenteral route of administration are most beneficial. This is best illustrated by data from this analysis indicating the largest effect of glutamine on mortality is in critically ill patients requiring total parenteral nutrition (TPN; receiving only intravenous glutamine supplementation). In this setting, glutamine led to a 31% reduction in the risk of death (RR 0.69, 95% CI 0.48–0.92, $P=0.01$) (Fig. 4). These data, when taken with the minimal risk and cost to the patient of glutamine treatment, advocates that glutamine supple-

mentation of critically ill patients requiring TPN should be standard of care unless new trials refute this clear evidence of benefit. This is not surprising as standard parenteral nutrition solutions do not contain glutamine and critically patients exhibit the greatest clinical glutamine deficiencies.

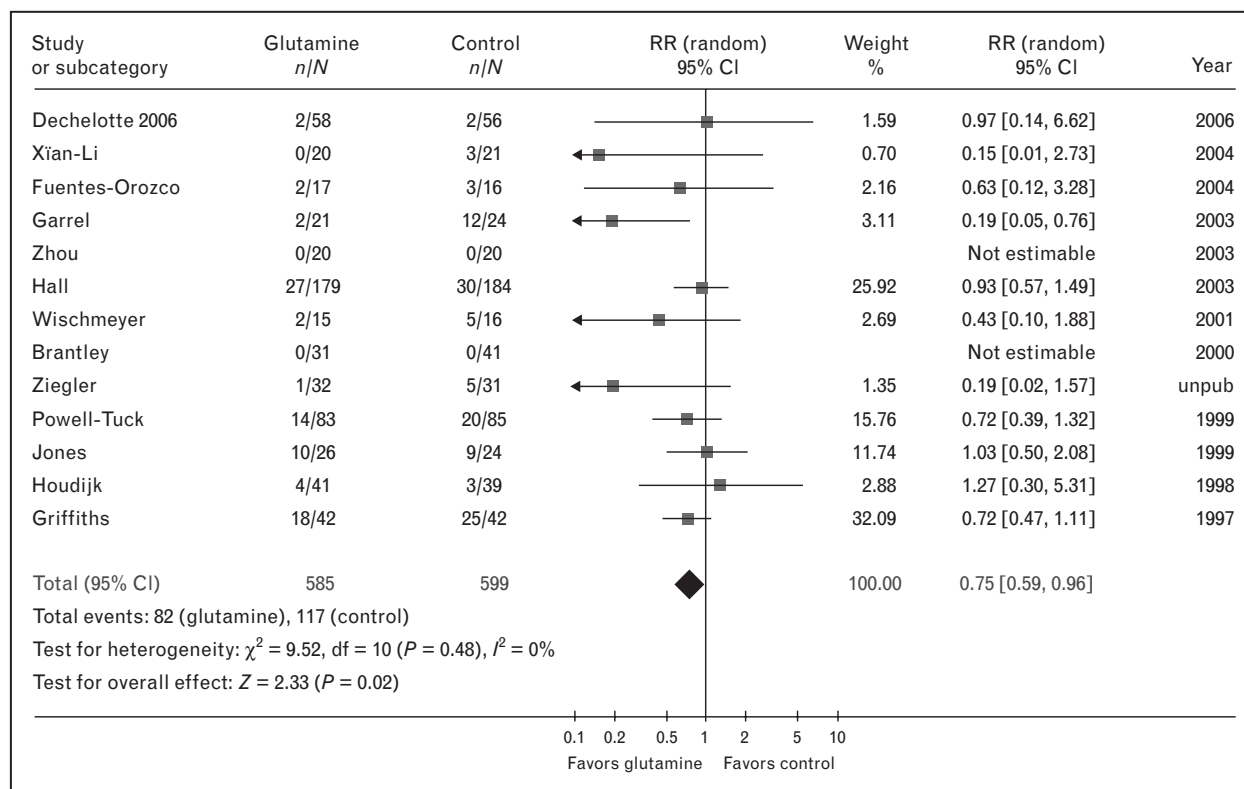
Recent mechanistic studies of glutamine in critical illness

In an editorial ‘Glutamine, a life-saving nutrient, but why?’ [23], potential mechanisms were proposed by which glutamine may be acting to improve outcome in critical illness. Our laboratory and others have recently published data on these possible mechanisms. Based on these recent data, we will attempt to briefly cover the recent literature supporting each of these mechanisms: glutamine effects on tissue protection, anti-inflammatory/immunologic, metabolic, and antioxidant/inducible nitric oxide synthase (iNOS) attenuation.

Tissue protection

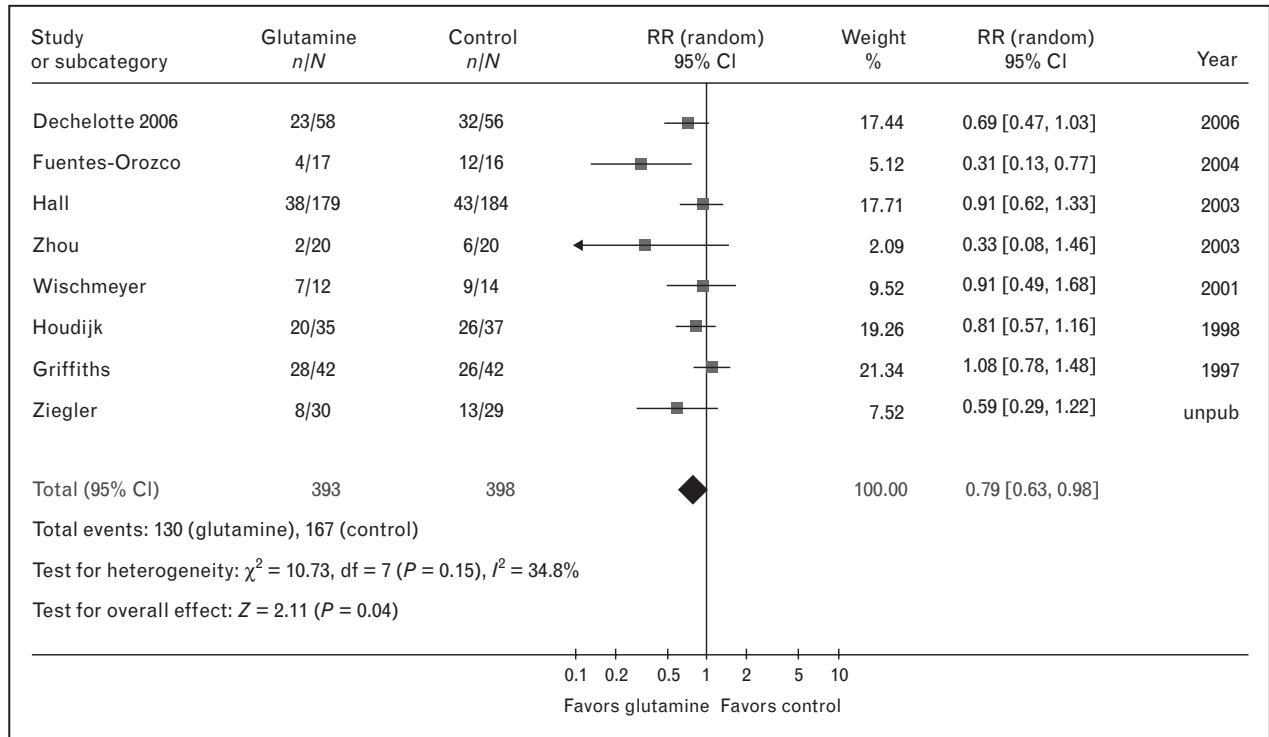
Glutamine appears to have effects on a number of cell-protective pathways vital to cell survival following stress and injury.

Figure 1 Effect of enteral and parenteral glutamine on overall mortality in critical illness



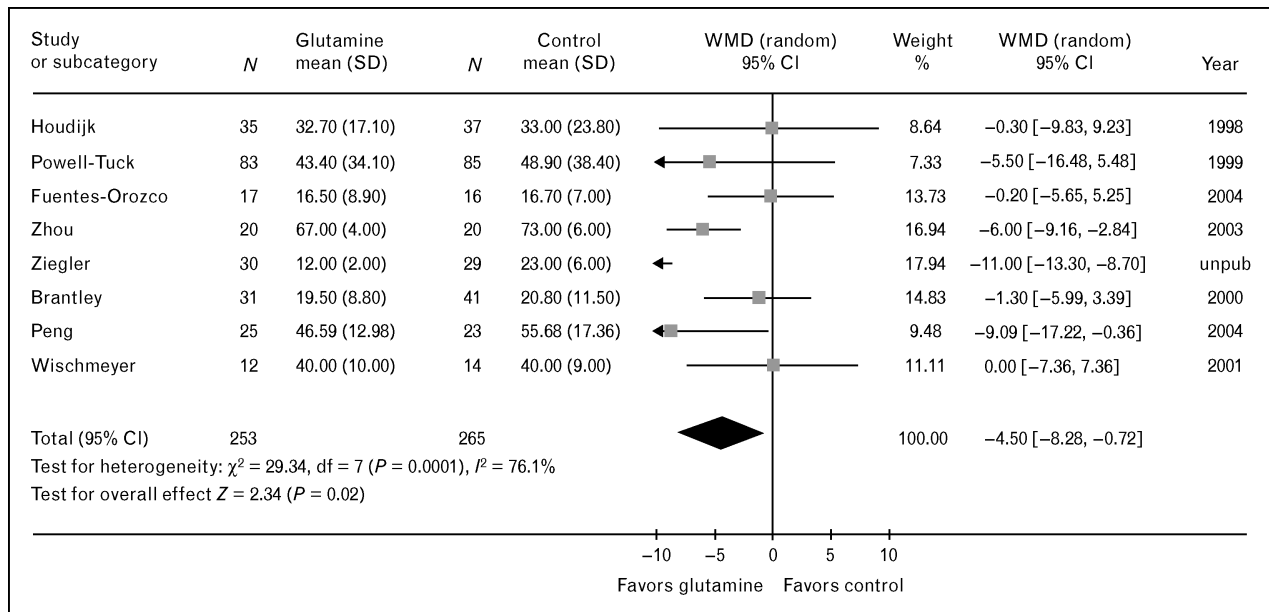
For complete details, see <http://www.criticalcarenutrition.com>. CI, confidence interval; RR, relative risk.

Figure 2 Effect of enteral and parenteral glutamine on infectious morbidity in critical illness



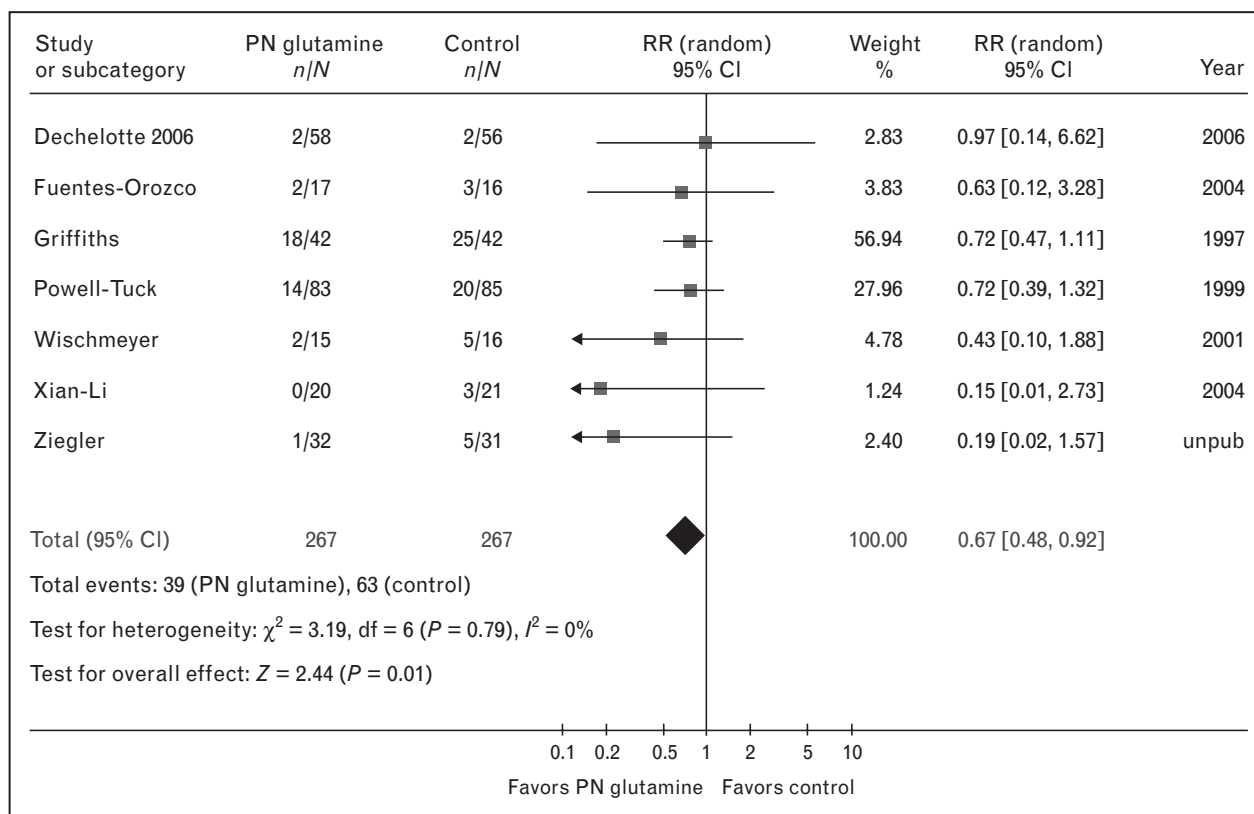
For complete details, see <http://www.criticalcarenutrition.com>. CI, confidence interval; RR, relative risk.

Figure 3 Effect of enteral and parenteral glutamine on hospital length of stay in critical illness



For complete details, see <http://www.criticalcarenutrition.com>. CI, confidence interval; RR, relative risk.

Figure 4 Effect of parenteral glutamine on mortality in critical illness



For complete details, see <http://www.criticalcarenutrition.com>. CI, confidence interval; PN, parenteral nutrition; RR, relative risk.

Enhanced heat shock protein expression

The initial data that led to the discovery of this mechanism were generated by our laboratory in cellular models of injury [24] and animal models of shock [25–27].

Given our initial data showing glutamine enhances tissue heat shock protein (HSP) expression in sepsis and this correlated with reduced organ injury and mortality [28], we utilized cells and animals with specific genetic deletions of the HSP pathway. We recently published data [29••] indicating that in heat shock factor 1 (HSF-1) knockout cells glutamine's ability to generate an HSP response is lost and the protection conferred by glutamine is also completely abrogated. Utilizing HSP-70 knockout mice we found HSP-70 expression is vital for rodent survival following sepsis [30•]. Utilizing these mice we have determined glutamine protects HSP-70 wild type mice following sepsis, but is unable to provide a survival advantage in mice with a gene deletion of HSP-70 from experimental sepsis [31••]. A newly published study [32•] reveals that glutamine can reduce vascular hyporeactivity in lipopolysaccharide-treated rats and this may be related to enhanced vascular HSP expression.

Prevention of enhanced gut permeability

A recent metaanalysis [33] examined the question of glutamine's effect on gut permeability. It is difficult to make any inferences from these limited data. The role of glutamine in preventing injury to the gut barrier still requires further study.

Prevention of apoptosis

A number of recent in-vitro studies [34,35] have examined the role of glutamine in preventing apoptosis following stress or injury. This has been expanded on by Larson *et al.* [36••] who demonstrated a crucial role for the extracellular signal regulated kinase (ERK) signaling pathway in glutamine-mediated prevention of cellular apoptosis. Furthermore, these authors found that the phosphoinositide-3 kinase (PI3K)/Akt pathway appears to be activated during periods of glutamine starvation, which likely serves as a protective mechanism to limit apoptosis associated with cell stress. This finding further supports that the cell utilizes intracellular glutamine levels as a sensor of stress.

Anti-inflammatory/immune regulation

The focus of recent studies has been on glutamine's ability to attenuate the inflammatory response via effects

on the nuclear factor κ B (NF κ B) signaling pathways. Our laboratory [37] found in experimental sepsis a single dose of glutamine (0.75 g/kg) can attenuate nuclear binding/activation of NF κ B and prevent the degradation of its inhibitory protein I κ B α [37]. These findings have been confirmed in a recently published rodent model of hemorrhagic shock [38^{*}]. These data reveal that glutamine-based resuscitation can attenuate cytokine expression in the liver following shock.

This anti-inflammatory effect may, in part, depend on HSP expression. Utilizing the HSP-70 knockout mice described previously, we have shown that mice with a deletion of the HSP-70 gene do not demonstrate attenuated nuclear binding/activation of NF κ B following glutamine treatment. Furthermore, glutamine-mediated attenuation of tumor necrosis factor α and interleukin-6 expression are lost in the HSP-70 knockout mice [31^{**}]. Recently, glutamine's anti-inflammatory effect was confirmed in a hemorrhagic shock model which used glutamine as a resuscitation fluid postshock [38^{*}].

Specific to the gut, Sato *et al.* [39^{**}] have found that following gut (ischemia/reperfusion) injury glutamine can activate peroxisome proliferator-activated receptor (PPAR) DNA binding. PPAR- γ is a vital transcription factor known to attenuate the inflammatory response by interference with proinflammatory pathways (such as NF κ B and activator protein-1). This activation was correlated with attenuation of injury via gut histology and myeloperoxidase activity.

Preservation of tissue metabolic function

A recent hypothesis indicates that multisystem organ failure following shock and sepsis may be due to tissue metabolic dysfunction [40]. We have shown that glutamine can preserve tissue level metabolic function in the face of sepsis, shock, and ischemia/reperfusion injury [27,28,41]. This effect appears to be tied to enhanced HSP expression [26,27]. A recently published study [42^{*}] reveals that glutamine resuscitation in hemorrhagic shock can restore hepatic ATP levels, reduce cellular apoptosis, and improve survival.

Antioxidant/attenuation of inducible nitric oxide synthase expression

Glutamine has long been known to be an important precursor of glutathione, a vital antioxidant molecule, during ischemia/reperfusion injury [41,43]. Recent data reveal that glutamine can attenuate myocardial iNOS expression following ischemia/reperfusion injury and lung iNOS expression following sepsis [37,44].

Molecular mechanism becomes clinical reality? Bench–bedside effect of glutamine on heat shock protein expression and its relationship to outcome in critical illness

Critically ill patients who are glutamine deficient (virtually all patients are) appear to be unable to generate an adequate HSP response, placing them at great risk for organ failure and a deregulated inflammatory response. In an attempt to translate these findings to critically ill patients, we recently completed a pilot trial examining the potential for glutamine to enhance HSP-70 expression in critically ill patients. This double-blind trial examined patients in the surgical ICU who required TPN for over 5 days and were randomized to receive glutamine (given as alanyl-glutamine, 0.5 g/kg/day) or an iso-nitrogenous control. This subtrial specifically examined the expression of HSP-70 at 7 days following initiation of glutamine or iso-nitrogenous control and correlated this level with outcome. Glutamine treatment enhanced serum HSP-70 expression (3.7-fold increase versus iso-nitrogenous control, 95% CI 1.5–11.9, $P=0.029$) and this enhanced HSP-70 expression correlated with a decrease in ICU length of stay ($P<0.009$ versus control) [45]. This study demonstrated that glutamine was the first nontoxic, clinically relevant enhancer of HSP-70 expression in critically ill patients.

These data were recently confirmed by a study of 44 critically ill patients admitted to a trauma/neurosurgical ICU [46^{*}]. This study randomized patients to receive parenteral glutamine as a separate pharmacologic supplement to enteral feeding versus standard feeding alone. The results revealed that pharmacologic glutamine supplementation led to increases in serum HSP-70 levels 7 days following ICU admission. HSP-70 level was positively correlated with glutamine levels in these patients ($r=0.65$, $P=0.001$). The patients receiving glutamine therapy had decreased liver dysfunction and length of mechanical ventilation.

Conclusion

Critical illness and sepsis continue to be major health problems in this country and around the world. An annualized increase in hospital sepsis incidence rates of 8.7% occurred, from approximately 164 000 cases in 1979 to nearly 660 000 cases in 2000 [47]. Furthermore, the total number of sepsis-induced hospital deaths has continued to increase over time [47], increasing more than 90% in the last 20 years [48]. Very recent (2007) data indicate that severe sepsis continues to increase in incidence and lethality in the US. The percentage of severe sepsis cases among all sepsis cases increased continuously from 25.6% in 1993 to 43.8% in 2003 ($P<0.001$). Age-adjusted rate of hospitalization for severe sepsis grew

from 66.8 ± 0.16 to 132.0 ± 0.21 per 100 000 population ($P < .001$). Age-adjusted, population-based mortality rate within these years increased from 30.3 ± 0.11 to 49.7 ± 0.13 per 100 000 population ($P < 0.001$) [49]. Severe sepsis now causes as many deaths as acute myocardial infarction in the US (National Center for Health Statistics). Sepsis and inflammation commonly lead to the occurrence of multiple organ dysfunction syndrome (MODS) [50]. MODS is often the ultimate cause of death in the ICU. Sepsis-induced organ dysfunction, such as ARDS, is thought to be associated with unchecked inflammation and a failure of cellular and tissue metabolism [40]. A low-risk, low-cost therapeutic intervention such as glutamine that could protect cells and tissues against injury, attenuate inflammation, and preserve metabolic function may be an ideal intervention in the prevention/treatment of MODS following sepsis or other injuries.

As a result of this large body of supportive clinical and translational evidence, a number of high-profile clinical trials are currently underway. The Reducing Oxidant Stress (REDOXS) trial group recently published its pilot dosing trial [51^{••}] in preparation for the 1200 patient multinational (Canada, US, and European) trial. This trial will examine the effect of glutamine and antioxidants in a factorial design with mortality as the primary endpoint. The trial will administer glutamine both parenterally and enterally. A National Institutes of Health funded multicenter trial of glutamine in parenteral nutrition patients requiring surgical ICU is already underway in the US. Information may be found at <http://www.clinicaltrials.gov>. The Scottish Intensive care Glutamine or selenium Evaluative Trial (SIGNET) group is currently enrolling [52[•]]. This trial is studying the effect of parenteral glutamine and selenium in critically ill patients requiring parenteral nutrition and will utilize a factorial design. The trial has primary endpoints of ICU infection and mortality. This study has a target completion date of August 2008. Finally, a Swedish multicenter trial of parenteral glutamine in critical illness is also ongoing. From a mechanistic standpoint, many of these trials are collecting samples for mechanistic and translational endpoints. It is likely the results of these trials will begin to confirm or refute the importance of the aforementioned mechanistic pathways in glutamine's clinical effects.

Given the enormous amount of data we currently have from small clinical trials and mechanistic bench research it is clear why glutamine has become a 'hot' funding and research target for major funding sources worldwide. It is exciting to look forward to finally having data from large, multicenter, multinational clinical trials to answer the following question: should glutamine administration be the standard of care in critically ill patients?

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 259–260).

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