Glutamine supplementation in the critically ill – an update on translational research

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Abstract - In critically ill patients, glutamine supplementation is essential because the body consumes more glutamine than it produces and glutamine associated functions become dependent on its delivery. A Meta-analysis on trials using glutamine-enriched nutrition in the critically ill showed a positive effect on infectious morbidity, length of ICU stay and mortality. Therefore, as several guidelines recommend, glutamine supplementation should be considered for these patients. The precise workings of glutamine are still under investigation; recent translational research has revealed that glutamine can be recognized as a modulator of the expression of many genes associated with metabolism, cell defence and repair, cytokine production and intracellular signalling pathways. This review ends with a recommendation on glutamine supplementation in critically ill patients. According to the ESPEN guidelines, burn and trauma patients on enteral nutrition should be given glutamine supplements. The ESPEN guidelines appropriately state that all ICU patients should receive glutamine parenterally in an amino acid solution. We recommend supplying glutamine in a total dose of 0.65 g/kg/day of the dipeptide (0.5 g glutamine/kg/day). Furthermore, although hard evidence is lacking, we would like to recommend both administration routes to supplement glutamine levels. Finally, since the glutamine level in critically ill patients depletes within 24 hrs, we recommend starting with supplements as early as possible.

Introduction
Glutamine, the most abundant free amino acid, regulates many biological functions in preserving cellular function [1]. In the critically ill, rapid depletion of glutamine occurs in plasma and muscle. Plasma levels tend to drop for several weeks and are related to increased ICU mortality [2]. This state of glutamine depletion is mainly caused by the high consumption of glutamine by rapidly dividing cells such as enterocytes, bone marrow cells, and lymphocytes. In the absence of adequate glutamine intake, compensatory release from muscle stores is unable to restore plasma levels. As a result, glutamine becomes essential in the critically ill because its availability relies on exogenous administration.

A number of trials using glutamine-enriched nutrition revealed a positive effect on infectious morbidity, length of ICU stay, and mortality in the critically ill [1,3,4]. However, some trials failed to show beneficial effects [5-7]. Traditionally, beneficial effects were explained as glutamine acting as a key respiratory fuel and nitrogen donor for rapidly dividing cells. However, recent translational research has revealed that this explanation is incomplete and oversimplified. Glutamine is now recognized as a modulator of the expression of many genes associated with metabolism, cell defence and repair, cytokine production and intracellular signalling pathways [8].

In this review, we focus on the newly-discovered effects of glutamine relevant to the critically ill. Also, an update of a meta-analysis on glutamine in the critically ill and considerations on the dosage and routes of administration are given.

Glutamine in cellular protection
Heat shock proteins
Heat shock proteins (HSPs) protect cells by preventing apoptosis and by acting as molecular chaperones to correct stress-related misfolding of proteins. A heat shock transcription factor (HSF-1) is activated in response to several stressors and increases the transcription of heat shock protein genes [9,10]. The availability of glutamine in cell cultures is necessary for the expression of HSPs [11-13]. Furthermore, glutamine supplementation enhances the expression of HSPs in in vitro and in vivo studies [14]. In an in vitro experiment, glutamine supplementation increased HSF-1 activation to protect cells against heat stress; this effect was lost in HSF-1 gene knockout cells [15]. In a mice model of abdominal sepsis, glutamine increased plasma levels of HSPs, decreased nuclear factor kappa B (NFκB) activation and reduced mortality; these effects were lost in HSF-1 gene knockout mice [16].

In trauma patients, an early increase of HSP plasma levels has been associated with survival [17]. In surgical intensive care patients, glutamine-enriched parenteral nutrition increased HSP levels after 7 days, a finding that correlated with a decrease in ICU length of stay [18]. This effect of glutamine may be time dependent, since infusion of alanly-glutamine in human experimental endotoxemia fails to increase early HSP expression within 8 hours [19].

Apoptosis
Apoptosis or programmed cell death probably influences cellular survival in the critically ill, since anti-apoptotic treatments improve
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Survival in septic mice [20,21]. Glutamine deprivation in mammalian gut and immune cell lines elicits apoptosis through cell type specific signalling mechanisms, whereas glutamine supplementation prevents tumour necrosis factor alpha (TNF-α) induced apoptosis in a human colonic cell line [22,23]. This glutamine effect may be regulated by the activation of extra-cellular signal-related kinase (ERK) pathways that have an anti-apoptotic effect. In rat intestinal cells, glutamine supplementation does indeed activate ERK pathways [24]. Also, in other cell lines, like Jurkat T cells (human T-lymphocyte cell line) and neutrophils (in rats and humans) glutamine supplementation increases anti-apoptotic protein Bcl-2 expression [8]. Glutamine modulates intracellular signalling pathways involved in apoptotic changes. This may become a target of treatment in human sepsis.

Gut barrier dysfunction

Intestinal integrity is secured by intact cellular linings and their tight junctions. It is jeopardized by glutamine depletion, oxidative stress, increased nitric oxide (NO) production, pro-inflammatory cytokines and prolonged parenteral nutrition (for a review see De Souza & Greene [25]). Gut barrier dysfunction may promote bacterial and endotoxin translocation, which induces systemic inflammatory response [26].

Recent in vitro and animal experimental studies indicate that the gut protective function of glutamine involves more than just providing oxidative fuel to the intestinal epithelium [26,27]. It includes the release and preservation of glutathione (GSH), attenuation of inducible NO synthase (iNOS) expression maintaining tight junction functionality, rapid reduction of protein kinase C mediated hyperpermeability in intestinal cells, attenuation of cytokine release, increased HSP expression and reduced gut mucosal apoptosis [28-30]. Some clinical trials on glutamine supplementation show benefits in terms of reduced gut permeability and lower rates of gram negative bacteria in trauma and burn patients [31]. Unfortunately, studying bacterial translocation in humans is very difficult due to methodological limitations, which may explain the lack of hard evidence for an effect of glutamine [32].

Anti-inflammatory action and immune modulation

Cytokine production

One of the first indications that glutamine could be a modulator of inflammatory cytokine production was found by Houdijk et al. in trauma patients [33]. For patients in this trial, glutamine-enriched enteral nutrition reduced levels of soluble TNF receptors. More recent studies have focussed on the effect of glutamine on NFκB signalling pathways, which are involved in the initiation of inflammatory cytokine response. In a septic rat model, e.g., a single dose of glutamine attenuated nuclear binding of NFκB and prevented the degradation of its inhibitory protein IκBα [30]. This coincided with reduced levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-18) and improved survival. These results were lost in HSP gene knockout mice, which suggests a role for HSPs in the inflammatory response [16]. In mice with abdominal sepsis, Yeh et al. showed that glutamine supplementation reduced IL-6 expression in the lung, kidney and gut [34]. Combined, these data suggest that glutamine is able to induce an anti-inflammatory response that may

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

<table>
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<th>Study or sub-category</th>
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<th>Control n/R</th>
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prove beneficial to the critically ill. However, until now this could not be shown in human studies. Luo et al. showed that 0.5g/kg/day of the dipeptide alanyl-glutamine, whether enterally or parenterally administered, failed to affect the T-lymphocyte subset (CD-3, CD-4, CD-8) number, compared to unsupplemented ICU patients [5]. In agreement with, van den Berg et al. did not detect differences in IFNγ, TNFα, IL-2, IL-4, IL-5 and IL-10 in very low birth weight infants [35]. Furthermore, perioperative supplementation of glutamine to cardiopulmonary bypass patients did not appear to affect IL-1, IL-6, TNF-alpha, IL-8 [36].

Cellular immunity
The expression of major histocompatibility complex class II antigens (HLA-DR) on monocytes is a prerequisite for effective antigen presentation to CD4+ T cells, which is an important component of immune response to infection [37]. Surgery, trauma and inflammation reduce HLA-DR expression on monocytes [38]. Spittler et al. showed that postoperative administration of glycyl-glutamine was accompanied by a smaller reduction in HLA-DR expression compared to a control group [38]. The preservation of HLA-DR expression on monocytes was also found in trauma patients receiving glutamine-enriched enteral nutrition [39]. Both parenteral and enteral glutamine administration seem to preserve HLA-DR expression on monocytes.

Glutamine and oxidative stress
In the critically ill, oxidative stress contributes to tissue injury and influences clinical outcome [40]. The redox potential influences the cellular capability to scavenge free radicals derived from oxygen and also influences several intracellular mechanisms [41,42]. A regulator of cellular redox potential is GSH, a potent anti-oxidant. During critical illness, plasma GSH levels decrease [42]. Glutamine is essential for GSH synthesis and may therefore help to reduce oxidative stress [8]. In trauma patients, parenteral glutamine supplementation has been found to attenuate GSH depletion in skeletal muscle [43]. Moreover, early enteral nutrition with high dose glutamine (50-60g) and antioxidants improved the sequential organ failure assessment score in a small group of medical patients with sepsis [44]. In contrast, however, another study found no effect of enteral and parenteral glutamine (alanyl-glutamine dipeptide) on antioxidant capacity or oxidative stress markers [5].

Glutamine may also attenuate oxidative stress by increasing plasma taurine levels. Taurine, an amino acid with anti-oxidative properties, is present in large amounts within the cell itself [45]. It has been found that trauma and sepsis plasma taurine levels decrease in response to surgical injury [46]. Houdijk et al. showed that by using enteral glutamine supplements, an increase in plasma taurine levels could be achieved in trauma patients and stressed rats [45]. However, the exact mechanism underlying this relation is yet to be elucidated.

Attenuation of inducible nitric oxide synthase expression
NO is a potent vasodilator and free radical that enhances the inflammatory response [47] and iNOS can produce large quantities of NO through the stimulation of cytokines. In sepsis, iNOS is seen as an important pathogenic factor [48,49]. In rats, enteral glutamine supplementation reduced NO production by modulating iNOS gene expression [50]. Another study in rats undergoing cardiopulmonary bypass, showed that parenteral glutamine decreased iNOS activity, reduced IL-6 and IL-8 levels, increased HsP expression and reduced myocardial and lung damage [51]. In rats with abdominal sepsis, parenteral glutamine reduced iNOS expression, inflammatory response and mortality [30]. The mechanisms by which glutamine reduces iNOS expression are not yet clear, but it seems that the glucosamine pathway and inhibition of citrulline mediated NO production may be involved [52].

Figure 2. Effect of enteral and parenteral glutamine on infectious morbidity in critical illness
Preservation of insulin sensitivity
Glutamine has been found to preserve insulin sensitivity in animal studies and critically ill patients. Severe illness is accompanied by reduced insulin sensitivity, which leads to hyperglycaemia. Van den Berghe et al. showed that tight glucose control through the administration of insulin, reduced morbidity and mortality in ICU patients [53].

In dogs, Borel et al. showed that glutamine blunted the effect of insulin on glucose production and enhanced insulin-mediated glucose utilization [54]. In rats on a high fat diet, glutamine prevented weight gain by inducing insulin resistance in adipose tissue, thus preventing lipogenesis, while it improved liver and peripheral insulin sensitivity. Furthermore, decreased TNF-α production and NFκB activation were observed, suggesting that glutamine influences glucose and fat metabolism by reducing pro-inflammatory pathways [55].

Bakalar showed that in multiple trauma patients, parenteral nutrition enriched with glutamine preserved insulin sensitivity [56]. In a multicenter randomized controlled trial in 114 surgical and trauma ICU patients, glutamine supplementation resulted in reduced hyperglycaemic events [57]. The exact mechanism by which glutamine acts on insulin sensitivity is not known and is currently under investigation.

Update on meta-analysis of glutamine in critical illness
In 2002, Novak et al. published a comprehensive review of all trials on glutamine supplementation in critically ill patients to date [1]. The Critical Care Nutrition Team makes an effort to update this meta-analysis with new randomized clinical trials on glutamine as these are published. The latest updated version of this meta-analysis (January 31st 2009) is available at www.criticalcarenutrition.com (search “Nutrition CPGs”, pdf’s “Composition of EN, 1c. Glutamine” and “Composition of PN, 4. Glutamine”). This analysis reveals that glutamine given by either the enteral or the parenteral route reduces mortality (Figure 1). Also, glutamine reduces infectious morbidity and the length of ICU stay (Figure 2; Figure 3). The updated meta-analysis revealed that the greatest benefit was seen in patients receiving high dose (>0.2 g/kg/day) parenteral glutamine.

Enteral versus parenteral glutamine administration
Recommendations on nutrition in the critically ill suggest that enteral feeding should be started as early as possible. One disadvantage of enteral support is that insufficient energy and protein coverage may occur, especially in the early phase. This makes it more complicated to provide sufficient glutamine supply via the enteral route. Therefore, combining both enteral and parenteral routes for the supply of glutamine is advocated. The ESPEN guidelines on enteral nutrition state that glutamine should be added to standard enteral formula in burn and trauma patients (Grade A recommendation) [58]. The ESPEN guidelines on parenteral nutrition state that when parenteral nutrition is indicated in ICU patients, a balanced amino acid mixture should be given (Grade B recommendation) containing 0.2-0.4 g/kg/day of L-glutamine (Grade A recommendation) [59]. New insights on the route and dosage of glutamine will be described in the following paragraphs.

Route of administration
Intestinal cells take up glutamine whether it is provided via the parenteral or the enteral route. The brush border enzyme glutaminase converts glutamine that is administered enterally into glutamate, which, in turn, can be metabolized to α-ketogluataat, alanine and citrulline. Citrulline is a precursor for arginine synthesis in the kidneys, which is an amino acid with important immune modulating effects. The gut preferably metabolizes enterally administered glutamine, which is demonstrated by higher conversion into citrulline [60]. In humans, parenteral administration of glutamine and alanyl-glutamine raises glutamine to higher plasma levels than enteral administration [5,60]. Plasma alanyl-glutamine levels were

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

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only observed when alanyl-glutamine was supplied parenterally and not when it was supplied enterally. This suggests that enterally supplied alanyl-glutamine is immediately hydrolysed into alanine and glutamine by the gut [60]. These data indicate that the route of administration may affect glutamine supply to the rest of the body and may lead to differences in biologically active metabolites.

**Dose of administration**

Ziegler et al. [61] showed that when administered parenterally, glutamine, is safe up to a dose of at least 0.025 g/kg/h (solely glutamine) or up to 0.570 g/kg/day when combined with normal parenteral formula. In the ICU, Heyland et al. [62] studied the safety of high dosages of alanyl-glutamine (up to 41.1 ± 15.2 g/day glutamine) and did not find any adverse effects. Dosages were studied parenterally or in combination with an enteral supply. A meta-analysis by Novak et al. [1] showed that high dose supplements (>0.2 g/kg/day) appear to be more effective than low dose supplements.

**Recommendation**

Combining the knowledge described in this review, we would like to give the following recommendation when assessing glutamine supplementation in the critically ill. The principle behind our recommendation lies in the concept that glutamine should be considered as a drug and not as a nutrient.

First, which patients should be supplemented? The ESPEN guidelines state that when enteral nutrition is given to burn and trauma patients it should contain glutamine. However, in these patients, reaching the caloric targets is very often frustrated by gastro-paresis or intestinal failure which compromises glutamine administration. We suggest that these patients are supplied with the desired amount of glutamine by combining the enteral and the parenteral routes of administration. For ICU patients, in general, only results from parenteral studies are available and therefore no recommendations can be made on enteral glutamine supply. The ESPEN guidelines appropriately state that all ICU patients should receive glutamine parenterally in an amino acid solution.

Secondly, which dose should be given? A total dose of 0.65 g/kg/day of the dipeptide (0.5 g glutamine/kg/day) seems safe and efficient.

Thirdly, via which route should we administer glutamine? As stated earlier in this review, the route of administration may affect the glutamine supply to the rest of the body and may lead to differences in biologically active metabolites. Although hard evidence on this point is lacking, we would like to recommend both administration routes for supplementing glutamine to ICU patients.

Fourthly and finally, when should glutamine supplements be started? Since glutamine depletes within 24 hrs in critically ill patients, we recommend that supplements are started as early as possible.

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