Abstract
Over the past couple of decades, glutamine (GLN) has emerged as important metabolic intermediate, signaling molecule and nutrient that becomes rapidly depleted and therefore critically important during stress (fig. 1). Studies in critically ill adults indicate that GLN may be potentially lifesaving in critical illness [1] particularly when administered in doses greater than 0.3 g/kg per day, when given as a pharmacologic agent, rather than as nutritional replacement [2, 3].

Introduction
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Several trials of GLN supplementation for critically ill and postsurgical patients have been completed in various age groups. Recommendations based on these studies have only recently begun to emerge. The Canadian Clinical Practice Guidelines for Nutrition Support Team and the European Society for Clinical Nutrition and Metabolism have concluded that GLN should be added to a standard enteral formula in burn and trauma patients [4, 5].

In very low-birthweight (VLBW) infants, a population of patients in whom supplementation of GLN should provide major benefits, GLN is provided in subnutritional quantities because standard parenteral nutrition solutions do not contain GLN, and most of these babies do not receive full enteral feedings until several weeks after birth. Studies in VLBW infants have investigated the effect of parenteral or enteral GLN supplementation on morbidity, mortality, and outcome in the neonatal period [6–12]. No evidence of toxicity of GLN supplementation was found in these clinical trials, but the results for efficacy on a limited number of outcomes have been mixed. The use of GLN supplementation in VLBW infants has therefore not become routine. Some authors suggest that further study in this area is no longer warranted [13]. The purpose of this review is to provide an update in the area of GLN supplementation for preterm infants in order to determine whether GLN supplementation is unwarranted and/or additional investigations are needed.

Fig. 1. GLN is a very versatile amino acid and has many functions including synthesis to other highly bioactive molecules, energy metabolism, signaling and mediation of inflammatory responses.
Basis for Glutamine Supplementation in Preterm Infants

In premature infants, there is a strong theoretical rationale for supplementation with GLN. Premature birth leads to a sudden cessation of a special combination of nutrients, including GLN, specifically suited for the rapidly developing fetus [14]. In the first weeks of life, these infants frequently derive most of their nutrition from the parenteral route, which contains no GLN, and are deprived of luminal nutrients, which contain GLN, because of the reluctance of neonatologists to use the enteral route. Furthermore, these infants are highly stressed and have an increased utilization of GLN during their first several weeks of life [15]. Although GLN is considered a nonessential amino acid, its synthesis may not keep up with requirements during times of stress, thus making it a ‘conditionally essential’ amino acid [15]. In addition, other precursors involved in the pathway of GLN synthesis may not be provided during this time.

Clinically Relevant Mechanisms for Glutamine Action

In preterm infants, the loss of intestinal barrier function is likely to result in systemic infections and inflammation (fig. 2). An imbalance between pro- and anti-inflammatory responses may present as systemic inflammatory response syndrome leading to multiple organ failure. GLN has been recognized to prevent translocation of microorganisms and an output of inflammatory mediators from the intestine after stimulation by proinflammatory agents. In an adult rodent model of endotoxemia, GLN was found to decrease various proinflammatory mediators [16]. Likewise, GLN provided to adult burn patients markedly decreased Gram-negative sepsis, suggesting decreased intestine-derived translocation [17]. Adult trauma patients given supplemental GLN by the enteral route have decreased rates of infection, including pneumonia, and this was associated with decreased soluble proinflammatory agents in the patients’ plasma [18]. In an infant rodent model (‘pup in the cup’) GLN significantly decreased lipopolysaccharide-induced inflammatory mediators in the intestine, liver and lung [19]. Likewise, studies of enteral GLN supplementation of formula in VLBW infants demonstrated a decrease in hospital-acquired sepsis along with a decrease in the stimulation of CD16 and HLA-DR markers on peripheral blood lymphocyte populations, consistent with decreased stimulation of the inflammatory response secondary to decreased translocation of proinflammatory antigens [6]. Thus, there appears to be a strong relationship between amelioration of gut-derived systemic inflammation by GLN and the notion that GLN is able
to ‘turn off the motor that drives systemic inflammation’. This is supported by numerous investigations [20].

Another mechanism of GLN modulation of inflammation relates to interepithelial junction integrity. Breakdown of the paracellular barrier can allow for passage of various agents that can stimulate the highly immunoreactive submucosa. In vitro studies of GLN deprivation in intestinal epithelial cells show decreased tight junction claudin 1, occludin and ZO-1 protein expression, and disappearance of perijunctional claudin 1 and a reduction of occludin [21]. These proteins play a critical role in paracellular apical tight junction integrity. Transmission electron microscopy revealed that GLN-deprived cells formed irregular junctional complexes between the apical lateral margins of adjoining cells. These findings indicate that tight junction protein expression and cellular localization in Caco-2 cell monolayers rely on GLN for optimal maintenance of barrier function, especially during times of stress.

In addition to the maintenance of intercellular junctions, direct stimulation of the intestinal epithelium by proinflammatory agents can also be modulated by GLN. These mechanisms relate to GLN-mediated down-regulation of inflammation via its interaction with the IκB/NF-κB signaling pathway. Using fetal-derived (H4) and adult (Caco-2) enterocytes, it was sug-

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**Fig. 2.** GLN stabilizes intestinal barrier function via the tight junction. NEC = Necrotizing enterocolitis; SIRS = systemic inflammatory response syndrome.
gested that a reduced capacity of the immature enterocyte (H4) to respond to GLN deprivation with increased synthesis of IκB rather than increased proteolysis as seen in the adult derived Caco-2 cells is the underlying mechanism of the immature cells (H4) having a greater response to lipopolysaccharide and other proinflammatory mediators than the adult (Caco2) cells [22].

Critical Illness in Neonates

An early study of enteral GLN supplementation of formula in VLBW infants demonstrated a decrease in hospital-acquired sepsis with evidence of a blunted inflammatory response secondary to decreased translocation of proinflammatory antigens [6]. Another trial of enteral GLN supplementation in low-birthweight infants showed decreased sepsis [8] in the GLN-supplemented group. In this latter study, GLN-enriched enteral nutrition did not improve feeding tolerance. Infectious morbidity was, however, significantly lowered in infants who received GLN-enriched enteral nutrition [8]. A multicenter trial that used enteral GLN administration (separate from formula) in 649 VLBW infants did not show differences in sepsis (the primary outcome), but showed significant improvement in secondary outcomes, which included intestinal function and a decrease in grades 3/4 intraventricular hemorrhages or periventricular leukomalacia in survivors [10]. Inflammatory mediators were not measured in this large trial of enteral supplementation.

Whether a higher supply of other amino acids may spare GLN or vice versa has recently been addressed by tracer studies showing that endogenous synthesis of GLN via GLN synthetase is dependent on the availability of other amino acids [23]. Conversely, parenteral GLN supplements in premature infants have been demonstrated to decrease whole-body protein breakdown [24]. As the decrease in whole-body proteolysis is associated with protein accretion, parenteral GLN supplementation may be beneficial in selected populations of premature infants. A multicenter trial of intravenous GLN supplementation, however, did not show benefit in terms of decreased sepsis or growth parameters [9]. Despite having large patient numbers, multicenter trials may still be flawed. This multicenter trial of parenteral GLN administration involved addition of 20%GLN, but the withholding of other amino acids including essentials in the GLN-supplemented solution in order to make the experimental solution isonitrogenous to the control solution. Not only was this a GLN supplementation trial, but also a trial of amino acid deprivation in the group receiving the GLN supplement.
Long-Term Follow-Up Studies of Glutamine-Supplemented Infants

In a follow-up study from the study of enteral GLN administration in preterm infants from the Amsterdam group [8], it was found that GLN-enriched nutrition decreases the risk of allergic and infectious disease at 6 years of age [25]. A decreased risk of gastrointestinal tract infections was also found in the GLN-supplemented group.

Another study using the same cohort utilized MRI volumetric measures of brain structures as well as fractional anisotropy of major white matter tracts when these children reached school age [26]. They found that the GLN-supplemented infants had increased white matter, hippocampus, and brain stem volumes. The authors surmised this was due to the decreased infections seen in infancy in the GLN-supplemented infants.

Dipeptides of Glutamine

In addition to deprivation of GLN, premature infants undergoing intensive care also frequently have low blood levels of arginine [27]. They are unable to maintain endogenous synthesis of these conditionally essential amino acids, making these infants highly vulnerable to GLN and arginine deprivation.

A randomized study of arginine supplementation in low-birthweight infants demonstrated a decreased incidence of necrotizing enterocolitis [28]. It has been speculated that this effect is secondary to improved blood flow to the microvasculature of the intestine via increased local nitric oxide production through the L-arginine-nitric oxide synthase pathway.

The concept of combining amino acids to improve stability and solubility of GLN was conceived over 20 years ago and utilized the combination of alanine and GLN [29–31]. The newer concept of combining arginine and GLN as a dipeptide (Arg-Gln) stems from two lines of reasoning. First, this dipeptide combination obviates the decomposition of aqueous GLN into the cyclic product associated with ammonia liberation and improves its limited solubility in water. Second, dipeptides are better absorbed than single amino acids [32]. The combination of arginine and GLN as a dipeptide should thus offer the benefits of both of these amino acids in addition to better solubility in aqueous solutions, less breakdown of GLN and better intestinal absorption. In a study conducted to examine the effect of the dipeptide Arg-Gln on vascular endothelial cell growth factor (VEGF) levels in primary human retinal pigment epithelial cell cultures and on inhibition of neovascularization in a mouse model of oxygen-induced retinopathy, treatment of human retinal pigment epithelial cells with Arg-Gln de-
creased VEGF levels in a dose-dependent manner. Arg-Gln also significantly reduced preretinal neovascularization when compared with the control dipeptide Ala-Gly, and it significantly reduced VEGF mRNA. Arg-Gln in these models appeared to be safe and, with future studies in human infants, may prove beneficial in the prevention of retinopathy associated with prematurity. Studies evaluating the effect of this dipeptide in the intestine [33] and lungs [34] of these animals exposed to toxic levels of oxygen also demonstrated protective effects.

**Dilemmas in Trials of Glutamine Supplementation in Preterm Infants**

There are several inherent dilemmas common to trials of GLN supplementation (and perhaps many other types of nutritional supplementation studies):

1. **Should isonitrogenous controls be used?** The issue and necessity of having an isonitrogenous control group requires debate. The desire to have such a control group to ensure specificity of the effect of the nutrient may have led to a significant shortcoming in the large multicenter trial of intravenous GLN supplementation. It is apparent that amino acids, including essential amino acids, were removed from the GLN-containing solution to make the GLN-containing solution isonitrogenous to the control solution. This design produces not just a GLN supplementation study, but also an amino acid deprivation study.

2. **How long should the GLN supplementation last, and what is the correct amount that should be supplemented?** Previous studies have utilized levels that reflect approximately 10% of the amino acids the baby should be receiving in the form of GLN, i.e. 0.3 g/kg per day based on a 3 g/kg per day requirement of protein. Studies in adults are suggesting that much higher ‘pharmacologic’ quantities than 0.3 g/kg per day should be provided for optimal effects [2]. The dosages given to preterm infants in the previous studies are likely to have been relatively low based on the fact that preterm infants require approximately 4–5 times the quantity of protein than adults on a per weight basis.

3. **Is it better to supply GLN via the enteral or the parenteral route?** The advantage of using the enteral route is that more GLN can be delivered to the intestine, where it may reach a concentration of >20 mM and can be rapidly used. Intravenous infusions rarely result in plasma (basolateral) concentration of more than 1 mM.

4. **What outcomes should be evaluated in future studies?** The primary outcome in many of the studies was sepsis. Is hospital-acquired sepsis a good outcome to evaluate in multicenter trials?
Conclusions and Future Directions

There is a recent reluctance to continue research on GLN-mediated amelioration of morbidity in premature infants. Based on the large body of evidence available from studies in animals and adults, as well as several of the recent studies in preterm infants, the notion that additional GLN research in premature infants should not be a priority is probably injudicious. As with the studies in adults, trials encompassing a large variety of premature infants with an array of problems may dilute effects. A variety of dosages have never been evaluated. If one extrapolates the dosages used in studies of enteral GLN administration in adults normalized to total protein requirements, the dosages used in the infant studies were relatively low. The route of administration (enteral vs. parenteral) may be critical. Downregulation of intestine-derived inflammation, apoptosis and stabilization of heat shock responses would theoretically occur to a greater degree with direct enteral application than if administered by the intravenous route, and this has not been investigated in preterms. The use of dipeptides of GLN also provides new avenues of research where the dosages can be increased and absorption improved. A thoughtful reevaluation of future applications and trials of GLN in premature infants is warranted.

Disclosure Statement

J. Neu has served on the scientific advisory boards of Mead Johnson, Medela, and consulted for the Fonterra company (New Zealand). His is funded by the National Institutes of Health for his research on the Microbiome and Necrotizing Enterocolitis.

References


