Abstract: OBJECTIVE: We reviewed the literature and examined the issues surrounding the use of glutamine in pediatrics and neonatology. METHODS: We reviewed the literature using Medline, Embase, Current Contents, and International Pharmaceutical Abstracts. Additional information was obtained from bibliographic citations and personal communications. RESULTS: Evidence showed that glutamine levels are affected in a number of life stages and conditions. Useful, indicative studies are emerging but many fail to demonstrate significant differences. The problems of researching in this patient population were demonstrated. CONCLUSION: A need for a great deal of further research in this area, including larger multicenter trials, clearly emerged.

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GLUTAMINE IN PAEDIATRICS: Where Next?

Running Head: Glutamine in Paediatrics – review.

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Abstract

Objective
To review the literature and examine the issues surrounding the use of glutamine in paediatrics and neonatology.

Method

Literature review using Medline®, Embase®, Current Contents® and International Pharmaceutical Abstracts®. Additional information was obtained from bibliographic citations and personal communications.

Findings

Evidence is growing that glutamine levels are affected in a number of life stages and conditions. Useful, indicative studies are emerging but many fail to demonstrate significant differences. The problems of researching in this patient population are demonstrated. A need for a great deal of further research in this area, including larger multi-centre trials clearly emerges.
Introduction

The purpose of this article is to review the literature on studies relating to the effects and clinical application of glutamine (Gln) supplementation in paediatrics and neonatology.

The paramount importance of maintaining normal growth and development wherever possible in paediatrics and neonatology has ensured a close relationship between the development of these specialities and that of clinical nutrition. The ethical and practical constraints of conducting research on children and infants means that many medical innovations that rapidly become established practice in adults, take longer to trickle down into paediatric practice. Children are not small adults and neonates are not small children, so specific research in these age groups is required. Nevertheless, the physiological differences are mostly quantitative or qualitative rather than absolute, so parallels can often be drawn from the adult literature.

In paediatrics, the published literature suggests that interest in glutamine has been focused in several areas. The largest single body of literature focuses on the neonate, particularly those born preterm. The aforementioned constraints on research in newborn infants mean that much of the data to date has been generated from studies in newborn and weaning animals. As in adult medicine, it was found that attention has also focused on certain specific conditions including critical injuries, inflammatory bowel disease, short bowel syndrome, Duchenne muscular dystrophy and interesting isolated reports in falciparum malaria and meningitis.
Methods

A literature review was undertaken using Medline®, Embase®, Current Contents® and International Pharmaceutical Abstracts®. Further material was obtained through the bibliographies of papers accessed and through personal communications. Animal and human studies were grouped when appropriate and examined for Gln dosage, use of isonitrogenous or isocaloric controls, outcome and recommendations. The major findings were then summarised.

Metabolic background

Gln, originally classified as ‘non-essential’ but now commonly regarded as ‘conditionally essential’, is the most abundant amino acid, especially in the free amino acid intracellular pool. Gln is involved in the pathway of de novo purine biosynthesis where the amide amino group provides two of the nitrogen atoms of the purine ring for the nucleotides that are important fuels for cells of the immune system. The metabolism of Gln is of special significance in the developing infant because of its unique role in ammoniagenesis and gluconeogenesis in the kidney. This involves mitochondrial deamination by glutaminase to glutamate (Glu), followed by subsequent oxidative deamination. In view of the limitation on hepatic gluconeogenesis from amino acids during suckling, the activity of renal gluconeogenesis with respect to Gln becomes important; and may be restricted until about the time of weaning. For the growing neonate to achieve net body protein deposition without an increase in protein intake there must either be a reduction in
amino acid catabolism and N excretion or a decrease in protein degradation with concurrent contraction of the free amino acid pool. Skeletal muscle, especially during the suckling period accounts for 60-80% of total body protein synthesis. However, there is no clear evidence for a reduction in protein turnover in the rapidly growing animal. In fact the rate of protein degradation in skeletal muscle is increased in young rats (1). The increased utilisation of the amino acid pool for protein synthesis and growth in the face of limited dietary protein supply is achieved by a decreased rate of amino nitrogen excretion as urea and increased re-utilisation of amino acid derived ammonia.

Despite large deposits of Gln in adult muscle, endogenous stores may become depleted in the course of catabolic insults such as injury or infection. VLBW infants already have sparse energy reserves and relatively small amounts of skeletal muscle. Protein flux and turnover may also be different in the growing child so that protein synthesis and hence Gln utilisation is invariably higher. Consequently at a time of very rapid growth they generally receive little protein (or Gln) during the first weeks of life. Supplementation however results in a decrease in endogenous protein breakdown, as demonstrated by stable isotope kinetic studies with Leucine. Gln failed to enhance the rate of protein synthesis but did have a protein sparing effect (2) in VLBW infants and reduced whole body protein breakdown by 15% in surgical neonates (3).

For many years researchers have linked protein-energy malnutrition (PEM) to low birth weights and poor brain development. The stress response to PEM, as demonstrated by increased cortisol levels, could contribute to long term learning and behavioural differences. Certainly malnourished children receiving high protein
supplements have scored higher marks than controls for reading, vocabulary and numeracy, suggesting that specific nutrient deficiencies may effect the brain’s anatomy or function (4). Maturation of the developing brain is associated with the formation of metabolic processes responsible for neuronal activity. Thus, Gln is no longer in equilibrium with the bulk of its Glu precursor but a discreet pool of Glu is converted to Gln, predominantly in the glia (rather than the neurones). If insufficient Glu and/or Gln are available from the already small skeletal muscle stores then supplementation may be necessary. In such a rapid period of brain growth, childhood Gln deficiencies could have life long consequences.

**Glutamine and the newborn**

Evidence of the potential importance of Gln to the newborn is found in observational studies by Agostoni et al (5) on the free amino acid (FAA) content of breast milk from healthy lactating women after delivery of full term infants. They demonstrated that Gln content increased 20 fold and Glutamic acid (Glu), the most prevalent FAA in milk and colostrum increased 2.5 times, as lactation progressed over 3 months. Since the concentration of taurine and all other FAA remained stable, the changes in total FAA are almost entirely due to the massive increase in Gln. Breast-fed infants therefore appear to be supplied with increasing amounts of Gln and Glu throughout lactation, not only as sources of nitrogen but possibly as neurotransmitters and also to help protect the gut mucosa (5). Secretion of these two “non-essential” amino acids into breast milk could be a way of sparing the essential amino acids during this rapid growth phase. Since both Gln and Glu concentrations are low or non-existent in
artificial milk formulas there is a clear need to investigate the potential benefits of enrichment.

If an infant is born prematurely, the supply of Gln from the mother is interrupted, so the infant is dependent on endogenous synthesis (or exogenous supply) to meet the high metabolic demands for tripling of body weight during the first few months of life. Gln serves as a major fuel and nitrogen source for nucleotides in rapidly replicating cells and amino sugars which protect the gut mucosa. Moreover, data from developing animals suggest that Gln may not only supply energy for the infant small intestine but can also promote electrolyte absorption (6), reverse intestinal atrophy and support the immune function (7,8,9).

A child has proportionally more gut associated lymphoid tissue (GALT) than an adult, possibly as much as 60% of total immune tissue. Loss of such a large amount of immune cells following extensive bowel resection would then be expected to radically alter whole body Gln kinetics. Regardless of intestinal status, Hankard et al (10) observed both Leucine and Gln fluxes were always higher than values previously reported for adults. In contrast, small bowel resection in 11 infants was associated with reduced Gln appearance (increased utilisation) rate, whereas Leucine kinetics were unaltered. The high Gln utilisation rate in infants could be due to enhanced oxidation by the developing enterocytes and/or increased uptake by the immune system. This suggests that the small intestine may be a preferential user of Gln in human infants, and play a prominent role in their developing immune system.
Glutamine and the gut

Transmission electron and light microscopy have revealed significant mucosal disruption in rat pups with Gln deprivation, suggesting that dietary Gln may help prevent intestinal-breakdown mediated proinflammatory responses in the developing infant. Studying tissue stripped from the intestinal mucosa of newborn and weaning animals, Smith et al (11) concluded: 1) The newborn mucosal barrier has uniquely different electrophysiologic characteristics. 2) Gln improves the metabolic activity as measured by potential difference in both newborn and weaning animals. 3) The newborn mucosal barrier allows increased transmucosal passage of bacteria. Extrapolating the work of Sondheimer (12), this may also be a factor in PN associated cholestasis. Studies reported earlier in these proceedings by Vejchapipat et al have looked at changes in Gln metabolism in endotoxaemia in neonatal rats. In neonatal pigs, high dose IV supplementation with 11 g/kg/day or 25 g/kg/day Gln did not prevent PN associated villous atrophy but did improve villous morphology (13). More recent animal work by Madej et al (14) working with weaning piglets suggested ‘the utilization of substrates for energy production differs markedly between the stomach, the small intestine and the colon, and that alpha-ketoglutarate may also be important as a precursor of glutamine

Necrotising enterocolitis (NEC) is a serious condition of unknown origin, that is associated with pre-term and low birth weight infants, premature rupture of membranes and hypoxia or respiratory distress (15). Becker et al (16) studied plasma amino acid levels in all the infants admitted to their Newborn Critical Care Centre, who did not have major congenital abnormality, liver dysfunction or inborn errors of metabolism. A total of 45 infants met the study criteria and were admitted. Of those,
13 subsequently developed NEC. Amino acid and urea levels were determined on days 3, 7, 14 and 21 of life. Although the infants who developed NEC were diagnosed between days 11 and 14 of life, median values for plasma glutamine and arginine were significantly lower from day 7. The authors concluded that infants who have NEC have selective amino acid deficiencies including reduced levels of glutamine and arginine that may predispose to the development of the condition.

**Monitoring Glutamine concentrations in vivo**

Lepage *et al* (17) showed changes in plasma concentrations of a range of amino acids with age. Plasma Gln concentrations are seen to decrease during severe burn stress as a result of deficits in peripheral Gln release in conjunction with increased central consumption. This has been confirmed in an elegant study by Gore and Jahoor in Texas who quantitated Gln kinetics using $^{5N^{15}}$Gln in children with severe burns who had received enteral nutrition without Gln supplementation and healthy controls (previously burned) (18). Arterial Gln and the rate of Gln turnover were significantly reduced after burns, whereas the net efflux of Gln was unchanged. The authors concluded that this inadequate skeletal muscle response to severe injury makes Gln supplementation essential to minimise this acquired metabolic deficiency. In a further report of Gln kinetics in 5 children with severe burns, Gln concentrations decreased during severe stress, supporting the notion that exogenous glutamine supplementation may be appropriate in this group (19). Plasma Gln was also found to decrease markedly in children with acute falciparum malaria (20).
Tucci et al 1998 (21) describe a high resolution, specific method for determining Gln in cerebrospinal fluid (CSF) of children with meningitis. The small size (100μL) of sample and relatively short analytical procedure makes Capillary Zone Electrophoresis and Laser Induced Fluorescence Detection (CZE-LIFD) a potentially useful technique for clinical monitoring. CSF-Gln levels were lower than normal in a group of 152 children with suspected viral or bacterial meningitis and Gln concentration improved as the patients clinical condition improved. The authors speculate that the depressed Gln levels (below 2mmol/L) could be due to the amino acid being used for bacterial growth and that this data, coupled with more widespread use of CZE-LIFD, could have diagnostic potential.

**Glutamine and Glutathione**

The importance of Gln to the intestine may lie in its role as a precursor for glutathione (GSH) via glutamate. Quantitatively GSH is the most important free radical scavenger and may help prevent oxidative damage to the gut. Parenteral or enteral infusions of Gln stimulate gut GSH synthesis and preserves GSH in the immune system (22).

Research from the Paediatric Surgical Group at the Institute of Child Health, London, reported elsewhere in these proceedings has demonstrated the dramatic effect of Gln in increasing fatty acid oxidation in endotoxaemia. Gln (or its dipeptides) are unique in reversing the effects of the septic mediators, hydrogen peroxide or nitric oxide. During sepsis, neutrophil and macrophage activation can lead to excessive production of these reactive oxygen species (ROS) that can cause DNA breakdown, inhibition of glycolysis and ATP synthesis with activation of the GSH redox cycle.
Working with hepatocytes from a neonatal rat model, Professor Pierro and colleagues have elegantly shown that Gln (and no other amino acid) reverses this inhibition of liver oxidative metabolism. It is thought that this action is mediated via GSH synthesis, for which Gln may be rate limiting. In neonatal sepsis and critical illness Gln specifically neutralises ROS, so that supplementation with Gln or its dipeptides may be an important adjunct to the management of sepsis (23).

**Parenteral Supplementation**

Most Gln supplementation studies in adults have used the parenteral route and this was initially the case in the paediatric field. Wilmore’s group have reported a complex dose ranging study in parenteral nutrition (PN)(24). A total of 78 infants were enrolled. Varying doses of L-glutamine were added to parenteral nutrition that was maintained isocaloric and isonitrogenous. The concentrations of Gln added were 15% (n=4), 20% (n=15) or 25% (n=3) of total amino acid (TAA) according to their PN protocol. Actual doses received by individual patients varied but there were slightly more episodes of elevated BUN (>8.9mmol/L) at the higher dose level. Consequently the dose of TPN containing Gln as 20% of TAA seemed to be the most appropriate for patients to subsequently receive throughout their stay in NICU. PN was discontinued when fluid needs were met by the enteral route or ran below 1.5ml/hr. The study was completed in 44 patients and was concluded to be non-toxic and safe for more extensive use. In subsequent analysis of the data they split their patients into two cohorts, those weighing <800g and those above. Clear benefits were demonstrated in the <800g cohort only.
Reduced time on TPN \( p = 0.02 \)
Reduced time to full enteral \( p = 0.03 \)
Reduced time on ventilator \( p = 0.04 \)

There are case reports describing the use of parenteral Gln in Short Bowel Syndrome infants (25,26). No improvement in gut adaptation was observed but the prognosis was better than originally predicted. Practically, an aqueous solution of L-Gln was cycled with the standard PN regimen to provide 20% of the total amino acids at 3 hourly intervals, during the several months of parenteral nutrition support.

**Enteral Supplementation**

Two multicentre trials in maternal-foetal-placental units are currently assessing the benefits of enteral Gln supplementation in very low birth weight infants (27). In an earlier, much-studied cohort of 68 very-low-birthweight infants (28,29,30,31), with gestational ages between 25wk and 32 wk, and birthweights between 500g and 1250g, the infants were randomised into two groups. The control group (n=33) received a standard enteral feeding regimen whilst the investigation group (n=35) received an isocaloric regimen supplemented with up to 0.3g/kg/day of glutamine. Reported benefits were:

- Reduced sepsis (28,29,30)
- Increased tolerance (29,30)
- Reduced costs (29,30)
  - Hospital costs \( p = 0.001 \)
  - Radiology costs \( p < 0.001 \)
Faintuch and colleagues at Hospital das Clinicas, Sao Paulo Brazil, recently carried out a prospective randomised pilot study of enteral glutamine with nine seriously ill infants (32). One group was treated with 0.3g/kg Gln and the controls received an equivalent dose of casein over 5 days. Septic complications occurred in 75% of the controls and 2/4 patients died of bacterial infections. Only 20% of the Gln group experienced septic complications with no deaths. Days in ICU, in hospital and on ventilators were improved with Gln but were not statistically significant.

**Oral Supplementation**

Other positive findings with oral supplementation include the condition Duchenne Muscular Dystrophy (DMD), where there is progressive loss of up to 50% muscle mass which is likely to lead to increased risk of Gln deficiency during surgery, infection or other stressful situations. Six boys with DMD were given an enteral glutamine intake of 800 µmol/kg/hr (equivalent to 3g/kg/d) with an apparent decrease in whole body protein degradation and reduced glutamine synthesis(33). In a subsequent study this group (34) demonstrated increased negative leucine balance and lower post-absorbtive Gln availability leading to the suggestion that Gln could be considered ‘conditionally essential’ in patients with this condition.
Stomatitis is a severe and debilitating complication of cytotoxic chemotherapy, particularly in children, leading to reduced nutritional intake and poor recovery. In 24 patients with chemotherapy-induced stomatitis (16 children and eight adults), an oral swish and swallow solution providing glutamine 2g/m²/twice daily (equivalent to 0.13g/kg/d) reduced the severity and duration (p=0.02)(35) of the oral mucositis. Palatability of oral Gln in a 500mg/ml aqueous suspension was well accepted by the adults, but young children preferred a sweeter version incorporating sucrose. For longer-term treatment a thicker suspension to prolong contact with the mouth may be more effective.

Comments and Conclusions

Newborns appear to have a large requirement for Gln that under normal circumstances is provided from maternal breast milk. Premature babies, on the other hand, are deprived of this source, just at the crucial time when they have their highest metabolic demands. Moreover, complications such as NEC, small bowel resection or other insults to the mucosal barrier can all lead to a reduction in endogenous synthesis and will increase the Gln requirement.

Gln levels also decrease in burns, infection, stress and sepsis, but at the same time it seems that Gln is required to neutralise the ROS acting as septic mediators. Almost all authors in the paediatric literature conclude that supplementation of the bodies natural Gln stores could be a powerful adjunct to the management of these debilitating clinical conditions.
PN support at a dose of 0.3g/kg/d has benefited VLBW babies and enteral feeding at the same dosage can reduce septic rates and some hospital costs. Higher oral doses (up to 3g/kg/d) may alleviate chemotherapy induced stomatitis and DMD symptoms.

Optimal outcomes will likely require more than merely the addition of Gln to nutritional support regimens and clinical results may be further improved by concurrent optional use of fatty acids, trace elements such as selenium, and in the case of specific conditions, disease controlling therapies.

Potential obstacles to fully understanding/use of glutamine in paediatrics are set out clearly and concisely at the end of her article by Janet Lacey. The major barriers to progress in the application of Gln to paediatrics and neonatal practice (24) are the factors common to all studies in paediatrics;

1) The difficulties in recruiting sufficient subjects in a single unit or under common patient management strategies. 2) Balancing the volume of sampled blood ideally required for the research project with the amount that can clinically and/or ethically be drawn from the patient. 3) The difficulties in formulating Gln supplemented nutrition within volume and osmolarity constraints. 4) That many therapeutic decisions are taken on subjective rather than objective criteria, particularly in relation to nutrition support techniques employed. 5) The unknown significance of factors such as maternal risk factors. 6) Possible long-term sequelae to interventions made. Further possible problems come from extrapolation of the work of Madej (6) who suggests that the optimal dosage of Gln may prove to be gestation, postnatal age and condition dependant.
Before the full benefits of glutamine use in paediatric patients can be realised, a great deal of further study will be required. This is likely to require large multi-centre clinical trials in order to achieve the necessary patient numbers, with all the difficulties and expense involved in such studies. Further advances in biochemistry reducing the blood volumes required for study would facilitate such research.
References

1. Snell K. Protein, amino acid and urea metabolism in the neonate. Biochem Devel of the Fetus and Neonate. 1982 651-695


