Glutamine in the Fetus and Critically Ill Low Birth Weight Neonate: Metabolism and Mechanism of Action

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ABSTRACT Of all the amino acids, glutamine is the most versatile. Studies in the maternal-fetal-placental unit demonstrate that both glutamine and glutamate play an important role in fetal and placental metabolism. If an infant is born very prematurely, the supply of glutamine from the mother is suddenly interrupted. The infant is dependent on endogenous synthesis or an exogenous supply of glutamine to meet the challenges of the external environment and a tripling of body weight in the first 3–4 mo of life. Studies of glutamine supplementation in low birth weight infants and critically ill adults suggest benefits, especially in terms of decreased nosocomial infections. Two large multicenter trials are currently underway that are designed to address these potential benefits in very low birth weight infants. These trials will not explain the mechanism of action. This review raises hypotheses about the role of the amide nitrogen of glutamine for nucleotide and glucosamine synthesis in the small intestine and how this might relate to greater integrity of the intestinal mucosa, hence preventing bacterial translocation and/or the subsequent proinflammatory response that might lead to multiorgan failure. J. Nutr. 131: 2585S-2589S, 2001.

KEY WORDS: glutamine • intestine • mechanism • placenta • neonate

The importance of glutamine for optimal growth of cells in culture has been known since the 1950s when Eagle (1955) published his landmark article in Science. His work demonstrated that the requirement of cells for growth in culture related more on glutamine than on any other amino acid. Since the studies of Eagle in the 1950s, numerous reports have established the importance of glutamine for a variety of metabolic processes in the whole organism and suggest that nutritional supplementation of this amino acid, under certain circumstances, may be highly beneficial.

The goals of this review are to present basic biochemical and metabolic interactions of glutamine, discuss how glutamine relates to fetal physiology, present some of the key aspects of glutamine as a nutritional supplement for very low birth weight infants, and to speculate on mechanisms of action using studies of the intestinal epithelium as a model.

Overview of glutamine metabolism

One can begin to appreciate the array of metabolic processes in which glutamine is involved by examining the relationship of this amino acid to its metabolites and the citric acid cycle (Fig. 1). Glutamine, via glutamate, is readily converted to α-ketoglutarate, an integral component of the citric acid cycle. As seen in this figure, several important metabolic products are derived from glutamine. Deamidation of glutamine via glutaminase produces glutamate, a precursor of γ-aminobutyric acid, a neurotransmission inhibitor. Proline is also produced by the cyclization of glutamate. Proline is an important amino acid component of collagen and connective tissue. The transamination and deamidation of glutamine is involved in ammonia transfer between various tissues. The transfer of amide nitrogen from glutamine via the amidotransferase reaction is also involved in the biosynthesis of purines and pyrimidines. The amide group derived from glutamine is important in the production of hexosamines, which are vital components for maintaining integrity and function of mucosal surfaces. The antioxidant glutathione, which protects against free radical damage, is composed of glutamate, cysteine, and glycine. Glutamate is also a component of polyglutamated folate acid, a cofactor in many enzymatic processes. Glutamine entrance into the citric acid cycle via α-ketoglutarate is an important pathway for energy production and anapleurosis.1

Glutamine in the fetus

Much of what we know about fetal amino acid metabolism comes from studies in pregnant sheep. Approximately 25 yr ago, a key observation was made by Lemons et al. (1976). There was no umbilical uptake of glutamate in the sheep fetus. In fact, there was a net flux of glutamate from the fetus into the placenta. This indicated that glutamate was formed within the fetus. However, of all the amino acids transferred from the mother and placenta to the fetus, the concentration of glutamine was the highest. Several subsequent studies, all of which were performed in the laboratories of Dr. Giaccomio Meschia and Fred Battaglia (Battaglia 2000) at the University of Pennsylvania.
of Colorado, support the existence of a glutamine-glutamate shuttle illustrated in Figure 2.

In this shuttle mechanism, there is a net flux of glutamine from the maternal circulation that is amplified by the addition of more glutamine from synthesis in the placenta. The enzyme responsible for glutamine synthesis in the placenta is glutamine synthetase, which was demonstrated, using immunohistochemistry, to be present largely in the cytotrophoblast and to a lesser extent in mesenchymal cells (DeMarco et al. 1997). In this shuttle, glutamine from the mother travels to the placenta where augmentation by placental glutamine synthesis significantly increases the concentration of glutamine reaching the fetus. In the fetus, glutamine is taken up by the fetal liver. Battaglia (2000) estimated that for the 130-d sheep fetus, the fetus. In the fetus, glutamine is taken up by the fetal liver. Battaglia (2000) estimated that for the 130-d sheep fetus, the fetal liver fell to 25% of the preinfusion levels. This led to a significant fall in fetal plasma glutamate concentrations and placental glutamate uptake from the fetal plasma. At the same time, a 60% drop in progesterone output from the pregnant uterus was evidenced. This dexamethasone-induced drop in progesterone output confirmed previous findings of Liggins and colleagues performed in the late 1960s and early 1970s (Liggins 1969, Liggins et al. 1972), but added to our knowledge by demonstrating the role that the glutamine/glutamate pathway plays in this process. A likely mechanism underlying this was described by Klimek et al. (1993), who demonstrated the relationship between placental mitochondrial NADPH reduction, the malate dehydrogenase reaction and glutamate concentration in the incubation medium. A decline in placental glutamate results in a decrease in the synthesis of NADPH, which is necessary for steroidogenesis. Thus, the events leading up to parturition are associated with profound changes in fetal hepatic and placental glutamate and glutamine metabolism that may relate to altered progesterone biosynthesis and raise the question of whether disruption of this pathway might play a role in parturition.

Glutamine as a nutritional supplement

Traditionally, glutamine has not been used as a nutritional supplement for several reasons. Glutamine is considered as a nonessential amino acid because the body has the capability to synthesize this amino acid via the glutamine synthetase reaction. It also has a short half-life in aqueous solutions and the theoretical concern has been raised that its breakdown product, pyroglutamate, might be toxic. With a normal diet and under nonstressed conditions, there seems to be no need for glutamine supplementation in healthy individuals. Whether the same is true for sick or highly stressed individuals not obtaining a normal dietary intake will now be addressed.

Several studies in animals (Fox et al. 1998, Klimberg et al. 1990, O'Dwyer et al. 1989, Suzuki et al. 1993, Van der Hulst et al. 1993, Yoshida et al. 1993) suggested the possibility that glutamine supplementation might prove beneficial in critically ill humans, including very low birth weight neonates. The sudden cessation of glutamine supply to very premature infants who are highly stressed and undergoing rapid growth appeared to be potentially detrimental. There is no glutamine in their total parenteral nutrition and they are frequently not enterally fed for weeks. In the early 1990s we hypothesized that glutamine supplementation in very low birth-weight infants would decrease hospital acquired sepsis. A randomized-masked pilot trial of 68 low birth-weight infants receiving glutamine supplementation during their 1st mo of life demonstrated that 30% of the infants in the control group developed culture-proven sepsis, whereas only 11% in the glutamine supplie-
ment group developed culture proven sepsis (Neu et al. 1997). Along with the drop in sepsis, there was a concurrent blunting of HLA-DR + lymphocytes and CD 16 + T-lymphocytes, which was consistent with decreased stimulation of the immune response secondary to decreased translocation of bacteria or their antigens across mucosal surfaces. Measurement of plasma amino acids showed that the control group had a significant elevation in most of the amino acids during the first two weeks of life. The glutamine-supplemented group demonstrated a blunting of this elevation in several of the amino acids, but most significantly in the gluconeogenic amino acids alanine, glycine, serine and threonine (Roig et al. 1996). A cost analysis demonstrated significant cost reduction for the glutamine-supplemented infants (Dallas et al. 1998).

Another study of glutamine supplementation supplied by the parenteral route (Lacey et al. 1996) demonstrated decreased time required for mechanical ventilation in the glutamine-supplemented infants weighing <800 g.

There have also been several studies of glutamine supplementation in critically ill adults. Ziegler et al. (1992) demonstrated decreased hospital acquired sepsis, improved nitrogen balance and decreased costs of hospitalization (McBurney et al. 1994) in bone marrow transplant recipients. Griffiths et al. (1997) demonstrated decreased mortality and hospital costs in critically ill adults receiving glutamine-supplemented total parenteral nutrition. Another study in the same institution using enteral glutamine supplementation (Jones et al. 1999) showed decreased costs of hospitalization. An intriguing study on adult trauma patients (Houdijk et al. 1998) showed decreased pneumonia and sepsis with enteral glutamine supplementation. Concurrent with the decreased infections was a blunting of the cytokine response to the injury. The authors speculated that the blunted cytokine response was secondary to decreased bacterial translocation through mucosal surfaces, as was speculated in our study in very low birth weight infants.

The beneficial results of glutamine supplementation seen in these preliminary studies in infants and adults have prompted two large multicenter trials in low birth weight infants. The National Institutes of Health Neonatal Network is conducting a study of glutamine supplemented by the intravenous route, whereas the Pediatric Neonatal group is studying the effects of glutamine supplemented by the enteral route. As of this writing, there have been ~1000 infants enrolled in these trials. At the conclusion of these studies, we will know much more about the efficacy and safety of glutamine supplementation by both the enteral and parenteral route. We may know whether one route is more effective than the other in very low birth weight neonates. Although very interesting results might occur from these studies, very little is likely to be learned about the mechanisms of action, the understanding of which will be critical if we are to use this amino acid or its derivatives most effectively.

Mechanisms of glutamine action

Other authors in this volume review several aspects of basic glutamine metabolism. For the sake of brevity, the focus here will be on utilization of the amide nitrogen of glutamine as a precursor to nucleotide and glucosamine biosynthesis.

It is to be remembered that there is a major difference between glutamine and glutamate that might provide for significant differences in activity as either a nutrient or a signaling molecule. This lies primarily in the amide moiety of glutamine. This amide nitrogen is critical in the biosynthesis of purines and pyrimidines (Fig. 3). Glutamine and nucleosides appear to act synergistically in intestinal epithelial proliferation and differentiation. He et al. (1994) demonstrated that when glutamine is in short supply, cell growth is retarded in two different types of intestinal epithelial cells (IEC-6 and Caco-2). These effects could be reversed in both cell types by the addition of nucleosides. The addition of nucleosides to the glutamine poor medium also prevented the depletion of ATP pools. These investigators concluded that nucleoside supplements could enhance the rate of cell proliferation and differentiation as well as spare the need for glutamine during enterocyte growth and development. These studies support the strong interplay between glutamine and nucleosides in the intestinal epithelium.

Glutamine amide nitrogen is also critical for the synthesis of hexosamines, which in turn act as building blocks for other macromolecules (Fig. 4). The biosynthesis of glucosamine 6-P04, the precursor of all hexosamines, requires the transfer of the amide nitrogen of glutamine to fructose 6-P04. Hexosamines, as components of glycoproteins and amino sugars, are very important in maintaining absorptive (via glycosylated membrane digestive hydrolases) and gut barrier functions (via surface mucin and glycoproteins forming intercellular tight junctions). The fuzzy glycocalyx coat of the villi, consisting of a family of glycoproteins and proteoglycans (of which hexosamines are major carbohydrate component), protects intestinal epithelial cells from autodigestion by luminal enzymes and may protect from bacterial invasion. The possibility of glutamine-mediated alteration of the luminal mucus gel is supported by studies of rats nourished with glutamine-free vs.
Glutamine dipeptide supplemented total parenteral nutrition (Khan et al. 1999). Rats supplemented with glutamine demonstrated greater thickness and optical density of mucus gel. A decrease in small intestinal permeability as measured by fluorescein isothiocyanate-dextran was concomitantly noted in the glutamine dipeptide supplemented rats.

Using a Caco-2 cell culture system in transwell plates (Weiss et al. 1999), inhibition of endogenous glutamine synthetase with methionine sulfoximine results in a breakdown of small intestinal interepithelial junctional integrity as determined by electrical impedance and transmission electron microscopy. Preliminary studies also have shown that deprivation of dietary glutamine in an infant rat artificial feeding model increases bacterial translocation and that this effect can be exacerbated by inhibiting intestinal glutamine synthetase by enteral administration of methionine sulfoximine (Neu et al. 2000).

Although most studies point to a nutritional mode of glutamine-mediated effects in the small intestine in terms of proliferation and epithelial protection, some studies suggest that these effects are at least partially mediated via cell signaling by mitogen-activated protein kinases. Rhoads et al. (1997) demonstrated significant synergy between Epidermal Growth Factor and glutamine in the stimulation of these mitogen-activated protein kinases.

Glutamine and the cytokine response

Preliminary studies in humans suggest that in addition to preventing hospital-acquired infections, enterally administered glutamine may modulate the proinflammatory response (Houdijk et al. 1998). With additional validation, we might have an important modality to ameliorate morbidity in low birth weight infants. The intestine is the largest immune organ of the body and is a major organ from which the systemic inflammatory response can be initiated. Over the past several years, it has become more evident that cytokine responses as part of an uncontrolled systemic inflammatory response syndrome play an integral role in premature labor, chronic lung disease, cerebral palsy, necrotizing enterocolitis and sepsis. Whether immunomodulatory nutrients such as glutamine play a role in ameliorating these responses via a mechanism related to the scheme hypothesized in Figure 5 could be an exciting new area of investigation.

Glutamine, glutamate and their closely related metabolites play critical roles in numerous cell processes. Their designation as conditionally essential nutrients downplays their importance in rapidly growing and proliferating cells, such as intestinal epithelium. The rapidly growing fetus and premature infant also seem to depend on an adequate supply of glutamine and its metabolites for growth and normal physiologic functions, such as mucosal integrity and immune responses. Ongoing studies designed to test the efficacy of glutamine supplementation should be conducted with studies designed to understand mechanisms of glutamine as a nutrient and signaling molecule. Studies of the role of the glutamine/glutamate pathways in the maternal-fetal-placental unit could lead to a better understanding of the patchwork of processes involved with premature labor. Additional studies of the relationship of glutamine in fetal-inflammatory response syndrome and systemic inflammatory response should provide keys in the modulation of these responses by nutritional supplements, thus contributing to reduced morbidity.

LITERATURE CITED


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