Chapter 1

Introduction
Adapted from: Long-term effects of neonatal glutamine-enriched nutrition in very low birth weight infants

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Submitted

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Glutamine supplementation in very low birth weight infants

Several studies in very low birth weight (VLBW) infants have investigated the effect of parenteral or enteral glutamine supplementation on morbidity, mortality and outcome in the neonatal period.\textsuperscript{1-5} None of these studies detected a statistically significant effect of glutamine supplementation on mortality in VLBW infants. In two studies\textsuperscript{1,2} in VLBW infants, glutamine-enriched enteral nutrition decreased incidence of neonatal infections. Other studies on enteral\textsuperscript{3} and parenteral\textsuperscript{4,5} glutamine supplementation in VLBW infants could not confirm this finding, possibly because of differences in supplementation methods, supplementation dose and definition of infections. Vaughn et al. found an improvement in intestinal function and a decrease in grades 3-4 intraventricular hemorrhages or periventricular leukomalacia after enteral glutamine supplementation in the neonatal period.\textsuperscript{3} With regard to other short term outcome measures (incidence of necrotising enterocolitis, growth during trial period, time to establish full enteral feeding and time till discharge) no differences were found between infants who received glutamine supplementation and controls.\textsuperscript{1-5} So far, little is known about the long-term effects of this nutritional intervention. VLBW infants remain at increased risk of neurodevelopmental sequelae. Well-conducted trials, including neurodevelopmental follow-up might be helpful to identify the mechanism that contribute to injury and recovery of neonatal interventions.\textsuperscript{6} Furthermore, there is the potential disconnect between short term perinatal outcomes and long-term outcomes. A prime example includes the benefit of postnatal steroids in chronic lung disease, but long-term neurodevelopmental delays.\textsuperscript{6}

As recently discussed in a workshop on research issues in neonatology,\textsuperscript{7} knowledge about the effect of nutritional interventions on long-term outcome and disease later in life (e.g. allergy and asthma) may contribute to deliberate choices in neonatal nutritional support in VLBW infants.

This thesis concerns the long-term effects of glutamine-enriched enteral nutrition in very low birth weight infants, including effects on a) allergic and infectious diseases, b) cytokine response c) intestinal microbiota and d) neurodevelopmental outcome later in life.
Neonatal glutamine in VLBW infants and allergic and infectious diseases later in life

Allergic diseases form an important health problem worldwide, with a high disease burden and high annual healthcare costs. The background of the development of allergic diseases is suggested to be multifactorial in which both genetic and environmental factors play a role. Preterm birth might also play a role in the development of allergic disease, however conflicting results have been found so far in previous studies. The atopic predisposition of preterm infants might differ from term infants and this predisposition may vary for different allergic diseases such as atopic dermatitis and asthma. In a recent study, Kvenshagen et al. found no difference in prevalence of atopic dermatitis between preterm and term infants. The association between low birth weight and asthma remains unclear, mainly due to the variety and diversity of factors (for example behavioural, environmental, psychological and genetic factors) that might contribute to the development of asthma. In a meta-analysis, Jaakkola et al. concluded that preterm infants have an increased risk to develop asthma when compared to term infants.

Besides type of feeding, various feeding supplements have been suggested to influence the development of allergic diseases. One of these supplements is glutamine, a non-essential amino acid which might stimulate the Th1 cytokine response and shift the neonatal immune response towards a balanced Th1/Th2 response. In a murine asthma model, Ko et al. found that systemic glutamine administration suppressed the key features of asthma, such as airway eosinophilia, mucus formation, and airway type 2 cytokine production, as well as late airway hyperresponsiveness, by suppression of the production of Th2 cytokines (IL-4, IL-5 and IL-13). By stimulating the Th1 response, glutamine supplementation in neonatal period in VLBW infants, might reduce the risk for allergic diseases later in life. In chapter 2 and chapter 3, we describe the effects of neonatal enteral glutamine supplementation on allergic and infectious diseases at one- and at six years of age in VLBW infants.

Neonatal glutamine in VLBW infants and cytokine response later in life

During pregnancy, the maternal immune response is skewed towards Th2-type immunity, to avoid the risk of abortion. As a consequence, the neonatal immune response is dominated by a Th2-type immune response. Th2 type cells produce IL-4, IL-5, IL-10, and IL-13 and promote humoral immunity. Exposure to microbes stimulates Th1 cytokine responses and shifts the neonatal immune response towards balanced Th1/Th2 cytokine responses. Delayed transition from fetal Th2-polarized cytokine responses to adult Th1-polarized cytokine responses may lead to long-term dysregulation of Th2 responses and allergic disease. The shift from Th2 cytokine towards Th1 cytokine responses can be manipulated in early infancy, as suggested by Martinez et al.
and other groups. In their study on high-risk infants, van der Velden et al. found that the first six months of life represent a critical time window for the initiation of immunological changes resulting in the development of atopy.

The amino acid glutamine is used at a high rate by cells of the immune system. It has been indicated that, by increasing the availability of glutamine in vitro, the proliferation of T-lymphocytes in response to T-cell mitogens, phagocytosis and antigen presentation by monocytes and Th1 cytokine response is stimulated. In chapter 4-I and 4-II, we describe the effects of neonatal enteral glutamine supplementation on cytokine profiles, both in the neonatal period and at one year of age in VLBW infants.

Neonatal glutamine in VLBW infants and intestinal microbiota later in life

Bacterial colonization of the gastrointestinal tract starts at birth during passage of the maternal vaginal canal. Various factors might influence this process of colonization, for example mode of delivery, environmental and nutritional factors.

In their studies on the ‘microbiota hypothesis’, Shreiner et al. demonstrate that endogenous microbiota play a significant role in shaping the development of the immune system. This hypothesis is supported by the fact that mice can develop allergic airway responses to allergens if their endogenous microbiota is altered at the time of first allergen exposure. Over a decade ago, it was already shown that the intestinal microbiota between allergic and non-allergic infants differs, with a higher prevalence of aerobic micro-organisms allergic infants, compared to non-allergic infants.

Bifidobacteria and lactobacilli are considered beneficial bacteria, as their presence may enhance the intestinal mucosal barrier, modulate the systemic immune response and inhibit colonization with potentially harmful bacteria. In VLBW infants, colonization with bifidobacteria and lactobacilli is often delayed compared to healthy term breastfed infants. Furthermore, the numbers of potentially harmful bacteria in VLBW infants is generally higher during the neonatal period compared to term infants. With the advent of the Human Microbiome Roadmap project, additional information may be garnered about the effects of nutrient supplementation on the wide variety of intestinal microbiota and their development with these nutrients in the growing infant.

Glutamine serves as fuel for enterocytes. In a study in rats, glutamine supplementation improved thickness and optical density of the mucus gel of the intestine. By enteral supplementation of glutamine in neonates, the mucus layer might change and therefore lead to altered bacterial adherence and colonization of the gut. In a recent study by van der Schoor et al., enteral supplementation of glutamine in VLBW infants was used to a great extend by the splanchnic tissues. In our previous study, glutamine-enriched enteral nutrition in the neonatal period did not change intestinal microbiota of the gut in VLBW infants. In their multicenter trial in 649 VLBW infants, Vaughn et al. found that infants receiving enteral glutamine supplementation
had less often gastrointestinal dysfunction, reflected by clinical signs of feeding intolerance.\textsuperscript{3} In their review on glutamine supplementation, Tubman et al. found no relation between glutamine supplementation in VLBW infants and the incidence of necrotizing enterocolitis (NEC) or time to establish full enteral feedings.\textsuperscript{54} Therefore, Tubman et al. suggest to focus further research on the effect of glutamine supplementation in case of severe gastrointestinal disease in preterm infants.\textsuperscript{54} However, we think that this absence of evidence may be due to inherent flaws in the designs of some of the original studies of glutamine supplementation. As data from animals and adults is highly compelling, we think that further research in the area of glutamine supplementation in premature infants is warranted.\textsuperscript{55} In \textbf{chapter 5}, we present a study that investigates the effect of neonatal glutamine supplementation in allergic and non-allergic VLBW infants on intestinal microbiota at one year of age.

\textbf{Neonatal glutamine in VLBW infants and neurodevelopmental and growth outcome later in life}

Neonatal infections in VLBW infants are associated with poor neurodevelopmental outcome (including cerebral palsy and visual impairment) in early childhood.\textsuperscript{56} To develop strategies to improve long-term outcome, understanding the pathophysiology of central nervous injury in VLBW infants is important. From various studies, it is now clear that infections such as chorioamnionitis, sepsis and necrotizing enterocolitis have an adverse effect on neurodevelopmental outcome, due to secondary brain damage.\textsuperscript{57} Neonatal infections are a frequent problem in VLBW infants.\textsuperscript{58} In studies in VLBW infants, our groups have found that glutamine-enriched enteral decreased the incidence of neonatal infections.\textsuperscript{1,2} Other studies on enteral and parenteral glutamine supplementation in VLBW infants could not confirm this finding, possibly because of differences in supplementation method, supplementation dose and definition of infections.\textsuperscript{3-5} For example, in the study of Poindexter et al. the extra parenteral glutamine replaced other, in part essential, amino acids in order to have an isonitrogenous amino acid solution.\textsuperscript{5} In a secondary analysis of the multicenter trial of parenteral glutamine supplementation, Poindexter et al. found that early administration of parenteral amino acids improved growth at 36 weeks postmenstrual age and decreased the incidence of suboptimal head growth at 18 months of age, which is associated with poor neurodevelopmental outcome.\textsuperscript{59} However, the authors did not provide data about neurodevelopmental outcome in the glutamine-supplemented versus the control group.\textsuperscript{59} In a study in 69 VLBW infants, Korkmaz et al. found that long-term enteral glutamine supplementation may lead to significant improvements of growth in all body measures.\textsuperscript{60} We hypothesized that glutamine-enriched enteral nutrition in VLBW infants may lead to improved neurodevelopmental outcome in early childhood by reducing the neonatal infectious morbidity. Our study on neurodevelopmental outcome of infants receiving neonatal glutamine supplementation is described in \textbf{chapter 6}.
In chapter 7, we discuss the results of our studies and implications for future research. Finally, a summary of the thesis is provided in chapter 8.