Chapter 7

General discussion
Long-term effects of glutamine supplementation in VLBW infants

Several trials over the past decade have demonstrated efficacy and safety of glutamine supplementation in critically ill adult patients. In VLBW infants, no evidence of toxicity exits, however glutamine is not used as a routine in this population. In some trials glutamine supplementation in VLBW infants benefits seem clear, however, other trials demonstrate no benefits. Neu et al.\textsuperscript{1} and our group\textsuperscript{2} have demonstrated that glutamine-enriched enteral nutrition between day 3 and 30 of life decreased the incidence of serious infections. Other studies of enteral\textsuperscript{3} and parenteral\textsuperscript{4,5} glutamine supplementation in VLBW infants could not confirm this finding, possibly because of differences in supplementation method, supplementation dose and definition of infections. 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 in their Cochrane review. 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 studies of glutamine supplementation, as well as in the meta-analysis report where the secondary analysis on outcomes in the Vaughn trial and differences in the large versus the small studies were not discussed.\textsuperscript{3} As data from animals and adults as well as some of the smaller studies remains compelling, we think that further research in the area of glutamine supplementation in premature infants is warranted.\textsuperscript{55} Only very few have investigated long-term effects of glutamine supplementation in VLBW infants. In order to understand the pathophysiology and to determine safety and efficacy of glutamine supplementation beyond neonatal period, long-term follow-up is essential.

Neonatal glutamine supplementation and allergic and infectious diseases later in life

In chapter 2 and 3, we describe two studies in which we investigated the relation between glutamine-enriched enteral nutrition in VLBW infants and allergic and infectious diseases at one year (chapter 2) and at six years (chapter 3) of age. We found a decreased incidence of atopic dermatitis, both at one year and at six years of age after neonatal glutamine supplementation. However, we found no effect on the incidence of bronchial hyperreactivity at one year of age and no effect on asthma at six years of age. At one year of age, no effect of glutamine-enriched enteral nutrition in neonatal period on the incidence of infectious diseases was found. At six years of age, a lower rate of gastrointestinal tract infections was found in the glutamine supplemented group.

Allergic diseases form an important health problem worldwide.\textsuperscript{8,9} The background of the development of allergic diseases is suggested to be multifactorial in which both genetic and environmental factors play a role.\textsuperscript{12} Allergic diseases are associated with dysregulation
of Th2 responses. During pregnancy, the maternal immune response is skewed toward Th2 immunity.\textsuperscript{67,68} After birth, the neonatal immune response is shifted towards balanced Th1/Th2 response.\textsuperscript{31} Factors in early life such as mode of delivery\textsuperscript{69,70} and type of feeding\textsuperscript{37} might influence the maturation of the neonatal immune response. Besides type of feeding, various feeding supplements have been suggested to influence the development of allergic diseases. One of these supplements is glutamine, an amino acid which might stimulate the Th1 cytokine response and shift the neonatal immune response towards a balanced Th1/Th2 response.\textsuperscript{25} Experimental studies have indicated that the presence of glutamine in vitro increases the cytokine production of T-lymphocytes.\textsuperscript{25} We hypothesised that neonatal glutamine supplementation therefore might lead to a shift in the neonatal immune response towards a balanced Th1/Th2 response, and thereby resulting in a reduction in allergic diseases.

In the initial study into the effect of glutamine-enriched enteral nutrition in VLBW infants on morbidity and outcome in the neonatal period, we found a lower incidence of serious infections in the glutamine-supplemented group.\textsuperscript{2} Several epidemiological studies have shown that infectious disease early in life lead to decreased allergic disease later in life (the so-called hygiene hypothesis),\textsuperscript{75,76} although other studies could not confirm this finding.\textsuperscript{77,79} These studies\textsuperscript{75-77,79} have been performed in term infants and not in preterm infants. In our study, the lower incidence of infectious diseases in neonatal period in the glutamine-supplemented group was not associated with a higher risk but in fact with a lower risk of allergic diseases later in life, which is in line with the studies of Benn et al. and Monnens et al.\textsuperscript{77,79} The association between infectious disease early in life and allergic disease later in life is subject of ongoing debate. Time and type of the infections, genetic and environmental factors appear to be important determinants of the effect of early infectious disease on the risk of allergic disease later in life. Furthermore, two studies reported an inverse relation between exposure to endotoxin in house-dust and atopy.\textsuperscript{84,85} The development of allergic diseases is multifactorial, in which breastfeeding and mode of delivery may play a role. Although exclusive breastfeeding was considered to have a protective role in the development, this role has been disputed in recent studies.\textsuperscript{89,90} Delivery by caesarean section might lead to an increased risk of developing asthma.\textsuperscript{91} In addition analyses of our studies, we found that adjusting for breastfeeding and mode of delivery (vaginal delivery versus caesarean section) did not change the results of the primary analysis. Another explanation of the lower rate of atopic dermatitis after neonatal glutamine supplementation in VLBW infants might be that changes in the mucus layer of the gut occur due to glutamine supplementation. In a study in rats, glutamine served as fuel for enterocytes and improved thickness and optical density of the mucus gel.\textsuperscript{51,61} By enteral supplementation of glutamine in neonates, thickness and optical density of the mucus gel might improve, which might lead to altered bacterial adherence and colonization of the gut. We found both a lower risk for gastrointestinal tract infections and for atopic dermatitis at six years of age in infants after glutamine-enriched enteral nutrition in the neonatal period, possibly due to changes in the mucus layer of the gut. Microbial colonization of the gut is considered the most important
stimulus for Th1 cytokine responses. Sudo et al. have shown that germ-free mice had prolonged Th2 cytokine responses, whereas Th1 cytokine responses rapidly developed after introduction of commensal intestinal microflora. Furthermore, Björkstén et al. found that coliform bacteria were more prevalent in the commensal microflora of atopic children, while more lactobacilli and bifidobacteria were present in non-atopic children. In our cohort, we found infants with allergic diseases to be less frequent colonized with bifidobacteria compared to infants without allergic disease, at one year of age (chapter 5). The question is whether bacterial colonization of the gut in these infants remains different at six years of age and if so, bifidobacteria may reduce the incidence of gastrointestinal infections.

Bacterial interference which refers to antagonism between bacterial species might also have played a role in our cohort. Certain strains of bifidobacteria might have antagonistic ability against pathogens, as recently reviewed by Servin.

**Neonatal glutamine in VLBW infants and neonatal cytokine responses and cytokine responses later in life**

In previous studies, we found that glutamine-enriched enteral nutrition in the neonatal period decreased both the incidence of serious neonatal infections and atopic dermatitis during the first year of life in very low birth weight (VLBW) infants. We hypothesized that the lower incidence of serious neonatal infections and atopic dermatitis during the first year of life in VLBW infants may result from intervention with glutamine-supplementation and is reflected by enhanced Th1 cytokine responses. However, the results of the studies in chapter 4-I and 4-II in VLBW infants show that glutamine-enriched enteral nutrition between days 3 and 30 of life does not specifically enhance Th1 cytokine responses, as determined following in vitro stimulation of whole blood cells with anti(α)-CD3/α-CD28 or LPS. Stimulation with anti(α)-CD3/α-CD28 represents a polyclonal stimulation of all T cells, while stimulation with LPS may be regarded as exemplary for responsiveness of innate immune cells such as monocytes and macrophages. These cells are implied in the first lines of antimicrobial defense, but appeared unaffected by glutamine-enriched enteral nutrition. Therefore, we concluded that the beneficial effects of glutamine-enriched enteral nutrition on the incidence of serious neonatal infections and atopic dermatitis during the first year of life does not result from major changes in both innate or adaptive immune responses.

During pregnancy, the maternal immune response is skewed towards Th2 immunity, resulting in a neonatal immune response which is dominated by Th2 cytokine responses. After birth, microbial exposure stimulates Th1 cytokine responses and shifts the neonatal immune response toward balanced Th1/Th2 cytokine responses. In addition, other factors early in life, such as mode of delivery and supplementation of probiotics, may influence this maturation process. In the study in the neonatal period (chapter 4-I), we did not find clear neonatal cytokine response patterns. In previous studies, glutamine supplementation increased the Th1
response in septic mice\textsuperscript{96} and adult trauma patients.\textsuperscript{97} In the study in adult trauma patients,\textsuperscript{97} cytokine responses were determined in isolated peripheral mononuclear blood cells following stimulation with phytohemagglutinin. Comparison of all studies is hampered by differences in study populations and methods to determine cytokine responses.

In our studies, stimulation with anti(α)-CD3/α-CD28 or LPS resulted in strong cytokine responses with large interindividual differences, which may explain why we did not find a stimulating effect of glutamine-enriched enteral nutrition on Th1 cytokine responses.

Another explanation of the absence of effects on the Th1 cytokine response after glutamine-enriched enteral nutrition may be that enteral glutamine mainly affects the intestinal immune system and does not reach systemic circulation. This hypothesis is supported by the finding that enterally administered glutamine is almost entirely metabolized by the gut and does not reach the systemic circulation.\textsuperscript{102,103} Recently, van der Schoor et al. showed that enteral supplementation of glutamine in VLBW infants is used to a great extent by the splanchnic tissues.\textsuperscript{52} Enteral glutamine supplementation in VLBW infants may change the mucus layer, which might reduce bacterial translocation and thereby reduce serious infectious morbidity in the neonatal period.\textsuperscript{45}

The development of the T-cell function during childhood has been studied extensively. Atopic diseases seem to be associated with an overt Th2 cytokine profile.\textsuperscript{29} Early exposure to microbes will induce Th1 cytokine responses that shift presumed neonatal default Th2 immune responses towards a balanced Th1/Th2 cytokine profile.\textsuperscript{31} Consequently, delayed transition into adult Th1-polarized cytokine responses may lead to long-term persistent Th2 responses and subsequent allergic disease.\textsuperscript{34}

In high-risk infants, van der Velden et al. found that the first six months of life may represent a critical phase for the induction of atopy associated immune responses in later life. This was reflected by an increased production of Th2 cytokines, possibly due to the absence of counter-regulatory events, e.g. induction of IFN-y producing Th1 cells in the neonatal period.\textsuperscript{38}

In our study described in chapter 4-I, we found no effect of glutamine-enriched enteral nutrition in VLBW infants in the neonatal period on cytokine profiles in neonatal period. In the study described in chapter 4-II, we specifically addressed whether glutamine administration would positively affect the immunological process, characterized by a physiological switch from a Th2 cytokine profile in the neonatal period into a modified Th2 profile later in life. Similar to our findings in the neonatal period, we found ample cytokine responses by peripheral immune-cells with large interindividual differences. Th1 and Th2 cytokine profiles were not different between glutamine-supplemented and control infants. Furthermore, we found no differences in Th1 and Th2 cytokine levels between atopic and non-atopic infants at one year of age. Comparison between our study and the earlier mentioned studies\textsuperscript{37,38} is hampered due to differences in study populations and methods of determination of cytokine profiles.

So far, little information is available on the development of the Th1 and Th2 cytokine profiles during the first year of life of VLBW infants. In our study (chapter 4-II), both Th1 (IFN-y, TNF-α)
and Th2 (IL-10, IL-4) cytokines increased during the first year of life, irrespective of the neonatal treatment. This is in line with the increase of memory cells during the first year of life studies in healthy term infants.28

**Neonatal glutamine in VLBW infants and intestinal microbiota later in life**

In chapter 5, we describe a study in which we found that the beneficial effects of glutamine-enriched enteral nutrition in VLBW infants on atopic dermatitis during the first year of life is not related to changes in intestinal species associated with allergy, such as bifidobacteria, Clostridium histolyticum, Clostridium lituseburense (Chis/lit group) and *Escherichia coli* (*E. coli*) at one year of age.

In a study in rats, glutamine supplementation mediated changes of the mucus layer.51 The mucus layer is an important site for bacterial colonization and its composition may modulate bacterial adherence. Enteral glutamine supplementation in VLBW infants may change the mucus layer, which might reduce bacterial translocation and thereby reduce serious infectious morbidity in the neonatal period. Recently, van der Schoor et al. showed that the majority of dietary glutamine in VLBW infants is utilized in first pass, since dietary glutamine is used to a great extent by the splanchnic tissues.52 Based on the results of the current study, the beneficial effect of glutamine on allergic diseases at one year of age is not related to a direct effect on intestinal species associated with allergy such as bifidobacteria, Chis/lit group and *E. coli*. However, changes of the mucus layer might still play a role in preventing atopic dermatitis during the first year of life. Obtaining intestinal biopsies to study mucus production and composition of these infants would be interesting, however this is not possible due to ethical and technical reasons.

In the study described in chapter 5, we also showed that VLBW infants, who develop allergic diseases during the first year of life, are less frequently colonized with bifidobacteria compared to VLBW infants without allergic diseases. In term infants, this inverse relation between bifidobacteria and allergic diseases was previously described.46 In VLBW infants however, little information is known about the development of intestinal microflora during the first year of life. Since the atopic predisposition of preterm infants might differ from term infants,14,17-21 we think it is interesting to get more insight in potential contributing factors to allergic diseases such as the development of intestinal microbiota during the first year of life in preterm infants.

By studying the development of intestinal microbiota, reflected by bifidobacteria, Chis/lit group and *E. coli* in VLBW infants, we found a significant increase in both the prevalence and number per gram feces of bifidobacteria between the neonatal period and at one year of age. This finding was irrespective of study method and of the presence of allergic or infectious diseases during the first year of life. Bifidobacteria are considered beneficial bacteria as they may enhance the intestinal mucosal barrier, modulate the systemic immune response and inhibit colonization with pathogenic bacteria.47 In breast-fed term infants, bifidobacteria become the
predominant bacteria in the intestinal microflora already after one week of life.\textsuperscript{41,42} In formula-
fed term infants however, the intestinal microflora becomes more diverse during the first week
of life with, apart from bifidobacteria, also clostridia, Enterobacteria, Bacteroides spp. and streptococci.\textsuperscript{43,123,124} Brück et al. showed that the microflora of breast-fed healthy term infants is
stable throughout the first six months of life and clearly dominated by bifidobacteria.\textsuperscript{125} In their
review on intestinal microflora in early infancy, Fanaro et al. showed that the predominance
of bifidobacteria is slightly reduced at three months of age, but remains predominant.\textsuperscript{126} In
our study, the prevalence of bifidobacteria was low in neonatal period, but becomes the pre-
dominant bacteria at age one year in this cohort of VLBW infants. Furthermore, we found that
correction for exclusive breast milk feeding during the first three months of life did not change
the results of the primary analysis.

In the current study, described in chapter 5, we also found that the prevalence of \textit{E. coli}
decreased significantly during the first year of life. This species, which also contains pathogenic
strains, dominated the intestinal microbiota during the neonatal period in our cohort of VLBW
infants. At one year of age, both the prevalence and number per gram feces of \textit{E. coli} were low.
This finding is in line with the low numbers of \textit{E. coli} at 4 and 6 months of age in studies in
healthy term infants.\textsuperscript{126}

Overall, the intestinal microbiota as reflected by bifidobacteria, Chis/lit and \textit{E. coli} of these VLBW
infants gradually becomes similar to the intestinal microbiota of healthy term breastfed infants.

**Neonatal glutamine in VLBW infants and neurodevelopmental outcome later in life**

In chapter six, we described a study that indicated that neonatal glutamine-enriched enteral
nutrition in VLBW infants has no beneficial effects on neurodevelopmental outcome at two
years of age.

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} To develop strategies to improve
long-term outcome, it is important to unravel the pathophysiology of central nervous injury
in VLBW infants. From various studies, it is now clear that early infections such as chorioam-
nionitis, sepsis and necrotizing enterocolitis have an adverse effect on neurodevelopmental
outcome, due to secondary brain damage.\textsuperscript{56,57} In the initial study into the effect of glutamine-
enriched enteral nutrition in VLBW infants on morbidity and outcome in the neonatal period,
we found a lower incidence of serious infections in the glutamine-supplemented group.\textsuperscript{2} We
hypothesized that glutamine-enriched enteral nutrition in VLBW infants may lead to improved
neurodevelopmental outcome in early childhood by reducing the neonatal infectious morbidi-
ty. Although we did not find a direct positive effect of enteral glutamine supplementation
on neurodevelopmental outcome, in additional analysis of our VLBW cohort, infants with a
neonatal infection had a higher risk of neurodevelopmental impairment compared to infants without a neonatal infection. The question remained why the adverse effect of a neonatal infection on neurodevelopmental outcome is not completely counteracted by the anti-infectious effect of glutamine supplementation. A possible explanation could be a difference in severity of infections between glutamine-supplemented and control groups, i.e. more severe infections in the glutamine-supplemented group. In additional analysis of our study, type of pathogen causing the infection, type of infection and short term outcome, were not different in glutamine-supplemented and control infants, therefore this explanation seems unlikely. Another explanation might be that although neonatal glutamine-enriched enteral nutrition decreases the incidence of neonatal infections, it may also have a negative effect on neurodevelopmental outcome. However, in additional analysis of our study, no differences in neurodevelopmental outcome were found in glutamine-supplemented infants with an infection with glutamine-supplemented infants without an infection. Therefore, this explanation also seems unlikely, but we have to remain cautious because the subgroups were small and it was a posthoc analysis.

An important factor in all studies which are performed in neonatal period is the potential disconnect between short term perinatal outcomes and long-term outcomes. This underlines the importance of assessment of long-term outcome and safety of these trials. In the initial study in the neonatal period, safety of glutamine-enriched enteral nutrition was assessed by measuring plasma amino acid concentrations at 4 different time points. It was found that glutamine-enriched enteral nutrition did not alter plasma concentrations of glutamate, a potentially neurotoxic glutamine metabolite, or other amino acids. In addition, no adverse effects of neonatal glutamine-enriched enteral nutrition was observed in any of the infants regarding short-term outcome. Therefore, we concluded that neonatal glutamine-enriched enteral nutrition in a dose of 0.3g/kg/day administered between day 3 and 30 of life is safe in VLBW infants. In our initial study, all cases of severe PIVH (≥ grade III) were randomly assigned in the glutamine-supplemented. However, all cases of PIVH ≥ grade III were diagnosed before the start of glutamine supplementation. As 3 cases with PIVH ≥ grade III died and 1 was lost to follow-up, only 2 were included in the follow-up study. Addition of PIVH to our regression analysis at 2 years of age did not influence our results. In our study described in chapter 6, we found no effects of neonatal glutamine-enriched enteral nutrition in VLBW infants on neurodevelopmental outcome at the corrected age of two years. The absence of adverse neurodevelopmental outcome at the corrected age of two years in VLBW infants who received neonatal glutamine-enriched enteral nutrition supports the previous conclusion of safety of enteral glutamine supplementation in a dose of 0.3 g/kg/d in VLBW infants.
Methodological considerations

The studies in this thesis have several methodological drawbacks. The main methodological consideration to discuss is our sample size. The sample size calculation was based on the primary outcome of the main trial. The sample size of 102 infants in this trial was calculated to be necessary to detect a difference of ≥ 2.5 days to full enteral feeding, assuming a standard deviation of 4.5 days (two-tailed t-test, $\alpha = 0.05$, $\beta = 0.20$). As a result, the sample size at follow-up was relatively small and the follow-up study may be susceptible to a type II error. This type of error is known as a “false negative” error, indicating a test of poor sensitivity.

In all studies our response rate was high, in most studies (chapter 2, 3, 5 and 6) > 80% of the eligible infants participated. Of the 102 infants of the initial study, 13 infants died (12 of them in neonatal period and one at age one year) and one infant was excluded to participate in the follow-up studies due to a serious chromosomal abnormality. Therefore, 89 infants were eligible to participate in our studies at one year of age and 88 infants were eligible to participate at two and six years of age. Due to several reasons (for example migration to foreign country, family could not be traced and in the case of the cytokine responses for which a blood sample was required; refusal to participate in the follow-up trial) we lost a few other infants for our follow-up trials. We compared the baseline characteristics of participating and non-participating infants, and found no differences. Therefore the participating groups are considered to be representative for the whole group of 102 infants.

In our studies at one year of age and at six years of age, similar results where found at both time points. In both studies, a lower rate of atopic dermatitis was found after neonatal glutamine-enriched enteral nutrition. The consistency of our results contributes to the reliability of our studies. Since a difference was detected in this relatively small cohort, it would be interesting to study a much larger cohort. We calculated that approximately 80 subjects in both treatment groups would be required to achieve a statistical significant difference with regard to the incidence of bronchial hyperreactivity. At one year of age, 14% of the infants in the glutamine-supplemented group had bronchial hyperreactivity compared to 33% of the infants in the control group. In our opinion, this might not be a statistical significant difference but might be a clinically relevant difference.

Another methodological consideration is the use of questionnaires in chapter 2 and 3. Parental report of disease by questionnaire may be subject of reporting bias. In particular, the difference between respiratory tract infections and bronchial hyperreactivity may be difficult. Therefore, we analyzed doctor-diagnosed symptoms and conditions in validated questionnaires. In addition, the diagnosis of atopic dermatitis and bronchial hyperreactivity required a combination of doctor-diagnosed symptoms. At six years of age, the diagnosis of asthma is based on the ISAAC questionnaire which is validated and applied in several epidemiological studies. In our
cohort, no forced expiratory volume in 1 second (FEV1) was measured, however the diagnosis included both “doctor diagnosed” asthma and the use of inhalation medication. Furthermore, when the infants were six years of age, their parents were not blinded anymore to treatment allocation. However, all allergic and infectious diseases were diagnosed by independent physicians, who were not involved in this trial.

In the initial study, the rate of neonatal infections was relatively high in both treatment groups. The relatively high rate of infections is in line with results of a surveillance study of nosocomial infections in our neonatal intensive care unit (NICU). We used predefined definitions for nosocomial infections in neonates that were adapted from the current definitions from the Center for Disease Control and Prevention for nosocomial infections in children < 1 year. The relatively high infection rate can be partially explained by our definitions of nosocomial infections and the relatively high device-associated utilization ratios in our NICU.

In chapter 5, we compared intestinal microflora which was determined at two different time points (i.e. in the neonatal period and at the corrected age of one year). However, all samples were analyzed in the same research department (Department of Biomedical Research/ Section Gut Biology and Microbiology, Danone Research BV, Wageningen, The Netherlands) with the use of the same methods and techniques, which contributes to the reliability of our results). FISH is a commonly use method that is well validated and regularly reported. Cells were enumerated by combining in the same hybridization, one group probe with the DAPI-stain for total counts; this will ensure that the ratios obtained are always relative to the total cell count of cells which makes the quantification very accurate. The detection level of FISH is around 10^6 cells per gram, and all samples have been treated in the same way and are therefore comparable. It has been well-demonstrated that relative differences in microflora composition between volunteers can be assessed objectively by the FISH method using similar group-specific probes.

An aspect of the study on neurodevelopmental outcome described in chapter six to discuss here is the predictive value of the Bayley scales for cognitive function. This value has been criticized before. Neurodevelopmental outcome of infants may change, as the infants get older. Therefore, further follow-up study of this cohort may contribute to better understanding whether these effects are transient or permanent.
### Table 1. Long term effects of glutamine supplementation in VLBW infants

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<td>1 year</td>
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VLBW: Very Low Birth Weight  
ELISA: Enzyme-Linked Immuno Sorbent Assay  
FISH: Fluorescent In Situ Hybridization
Conclusions

Table 1 summarizes all results of the studies described in this thesis. In conclusion, glutamine-enriched enteral nutrition in VLBW infants between day 3 and 30 of life decreases the incidence of atopic dermatitis (determined both at one and at six years of age), whereas no effect on the incidence of bronchial hyperreactivity at one year of age and on asthma at six years of age is found. Although glutamine-enriched enteral nutrition leads to a lower rate of serious neonatal infections, there is no effect on the incidence of infectious disease during the first year of life. At six years of age the incidence of gastrointestinal tract infections was lower after neonatal glutamine-enriched enteral nutrition.

Furthermore, glutamine enriched-ental nutrition in VLBW infants does not enhance Th1 cytokine responses, as determined following in vitro whole blood stimulation with anti(α)-CD3/α-CD28 or LPS (determined both in neonatal period and at one year of age). A possible explanation for the lower neonatal infection rate in infants receiving glutamine-enriched enteral nutrition may be that glutamine-enriched enteral nutrition only influences the mucosal and not the systemic immune system. The beneficial effect of glutamine-enriched enteral nutrition on the incidence of serious neonatal infections and atopic dermatitis during the first year of life is not related to changes in Th1 and Th2 cytokine profiles, however, both Th1 and Th2 cytokine profiles increased between the neonatal period and at one year of age in VLBW infants.

The beneficial effect of glutamine-enriched enteral nutrition on the incidence of atopic dermatitis during the first year of life probably is not related to changes in intestinal species associated with allergy. However, in this cohort of VLBW infants, infants with allergic disease were less frequently colonized with bifidobacteria compared to infants without allergic diseases. Between the neonatal period and at one year of age, the prevalence of bifidobacteria increased, of Chis/lit group did not change and of E. coli decreased in this cohort of VLBW infants and gradually became similar to the intestinal microbiota of healthy term breastfed infants.

Our study also indicates that neonatal glutamine-enriched enteral nutrition in VLBW infants does not improve neurodevelopmental outcome at the corrected age of two years. Based on the absence of adverse neurodevelopmental outcome, neonatal glutamine-enriched enteral nutrition in VLBW infants seems to be safe. In line with other studies, we found that neonatal infections in VLBW infants are associated with adverse neurodevelopmental outcome. Further follow-up study of this well-defined cohort of VLBW infants may contribute to a better understanding of long-term effects of neonatal glutamine-enriched enteral nutrition on neurodevelopmental outcome.
Implications for future research

During the neonatal period, glutamine-enriched enteral nutrition might decrease the incidence of serious neonatal infections, but study results are conflicting, probably due to methodological discrepancies. As neonatal infections are associated with poor neurodevelopmental outcome (including cerebral palsy and visual impairment) in early childhood, prospective long-term follow-up studies of neonatal interventional trials are essential.

VLBW infants remain at increased risk of neurodevelopmental sequelae. Well-conducted trials, including neurodevelopmental follow-up might be helpful to identify the mechanisms that contribute to injury and/or recovery of neonatal interventions. Furthermore, there is the potential disconnect between short-term perinatal outcomes and long-term outcomes. A prime example includes the short-term benefit of postnatal steroids in chronic lung disease, but neurodevelopmental delays at long-term.

As recently discussed in a workshop on research issues in neonatology, 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.

Several trials over the past decade have demonstrated efficacy and safety of glutamine supplementation in critically ill adult patients. In VLBW infants, no evidence of toxicity exits, however glutamine is not used as a routine in this population. In some trials, the benefits of glutamine supplementation in VLBW infants seem clear, however in other trials, these benefits were not found. Only very few studies have investigated long-term effects of glutamine supplementation in VLBW infants. In order to understand the pathophysiology and to determine the safety and efficacy of glutamine supplementation beyond neonatal period, long-term follow-up is essential. We think it would be interesting to study long-term effects of other trials of glutamine supplementation in the neonatal period to determine whether positive or negative long-term effects exist. We are not yet convinced that the conclusion of Tubman et al. in their Cochrane review of 2008 is still valid, taking into account important differences in methodology and possible flaws of the included studies. In contrast of their recommendations, we believe further research in the area of glutamine supplementation in premature infants, short and long-term is warranted, as data from animals and adults is highly compelling.

Currently, our follow-up study continues. A comprehensive battery of neurocognitive tasks to infants in the glutamine-enriched enteral nutrition cohort is undertaken and will be compared to a group of term controls. Several domains will be studied; i.e. neurocognitive outcome, academic achievement, behavioral and emotional problems and functional imaging. This will be done in collaboration with the department of Clinical Neuropsychology of the VU University in Amsterdam (prof. dr. J. Oosterlaan, drs J.F. de Kieviet).
Another interesting area of future research is the relation between neonatal sepsis, antibiotic therapy and the later risk of asthma and allergy in VLBW infants. In their recent, large cohort study, Sobko et al. found asthma to be more prevalent after neonatal sepsis and early antibiotic therapy. This study consisted mainly of term infants; we think it would be interesting to study these potential relations in our cohort of VLBW infants, including the relation between antibiotic treatment during the first year of life and asthma and allergy later in life.