Chapter 3

Glutamine-enriched enteral nutrition in very low birth weight infants and allergic and infectious diseases at six years of age

A. van Zwol\textsuperscript{a}, H.A. Moll\textsuperscript{b}, W.P.F. Fetter\textsuperscript{a}, R.M. van Elburg\textsuperscript{a}.

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\textsuperscript{a}Department of Pediatrics, VU University Medical Center Amsterdam; \textsuperscript{b}Department of Pediatrics, Sophia Children’s Hospital, Erasmus Medical Center Rotterdam, the Netherlands.
Abstract

Objective(s): Previously, we found that glutamine-enriched enteral nutrition in 102 very low birth weight (VLBW) infants decreased both the incidence of serious neonatal infections and the incidence of atopic dermatitis during the first year of life. We hypothesized that neonatal glutamine-enriched enteral nutrition in VLBW infants might reduce the risk for allergic and infectious disease at age six years.

Design: Follow-up study. Setting: Tertiary care hospital. Participants: All surviving infants, who participated in a trial of glutamine-enriched enteral nutrition in VLBW infants. Intervention: Enteral glutamine supplementation between days 3 and 30 of life. Outcome measures: The incidence of allergic and infectious disease at six years of age, as assessed by means of validated questionnaires. Results: 76/89 (85%) infants participated (38 glutamine-supplemented, 38 controls). After adjustment, the risk for atopic dermatitis was lower in the glutamine-supplemented group (aOR 0.23, 95% CI 0.06-0.95). No association with glutamine supplementation and other allergic diseases was found. The risk for gastrointestinal tract infections was lower in the glutamine-supplemented group (aOR 0.10, 95% CI 0.01-0.93). No association with glutamine supplementation and other infections was found. Conclusions: Neonatal glutamine-enriched enteral nutrition in VLBW infants decreased the risk for atopic dermatitis and gastrointestinal tract infections at age six years.
Introduction

Allergic diseases form an important health problem worldwide. Many predisposing factors have been suggested including low birth weight, however conflicting results have been found so far in previous studies. The atopic predisposition of preterm infants might differ from term infants. This predisposition may vary for different allergic diseases such as atopic dermatitis and asthma. The background of the development of atopic dermatitis is suggested to be multifactorial in which both genetic and environmental factors play a role. 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, an amino acid which might stimulate the Th1 cytokine response and shift the neonatal immune response towards a balanced Th1/Th2 response. In our previous study in very low birth weight (VLBW) infants, neonatal glutamine supplementation decreased the incidence of atopic dermatitis during the first year of life. In the same study, we found a trend towards a lower incidence of bronchial hyperreactivity, as a risk factor for the (future) development of asthma. In the first year of life, bronchial hyperreactivity is not a very specific condition, symptoms of respiratory tract infections often resemble symptoms of bronchial hyperreactivity. On the contrary, asthma at six years of age is a more specific indicator for allergic disease.

Beside an effect on the development of allergic diseases, glutamine supplementation in the neonatal period might reduce the incidence of serious infections in the neonatal period. In several studies of both enteral and parenteral glutamine supplementation, conflicting results were found regarding the incidence of serious neonatal infections. We hypothesized that glutamine-enriched enteral nutrition in the neonatal period might stimulate the transition of fetal Th2 polarized cytokine responses to adult Th1-polarized responses and might decrease allergic disease later in life. The question arose whether this would have consequences for long-term resistance against infectious disease. The aim of this follow-up study was to determine the effect of glutamine-enriched enteral nutrition in VLBW infants on the risk for allergic and infectious disease at six years of age.
Patients and methods

Study population

The initial study, conducted at the VU University Medical Center from September 2001 to July 2003, was a randomized, double-blind placebo-controlled trial of glutamine-enriched enteral nutrition in 102 VLBW infants (gestational age <32 weeks and/or birth weight <1500 g). Infants, admitted to the level III neonatal intensive care unit (NICU) of the VU University Medical Center, Amsterdam, were eligible for participation in the study. In this study, infants received enteral glutamine supplementation (0.3 g/kg/day) or an isonitrogenous placebo supplementation (alanine) between day 3 and 30 of life. For further details of the study design, we refer to the study protocol. Baseline characteristics of the follow-up cohort were recorded in the initial study. The national central committee on research involving human subjects and the medical ethical review board of our institute approved the study protocol. All parents gave written informed consent. At six years of age, parents were not blinded to treatment allocation of their infants anymore.

Questionnaire

Questionnaires were sent to the parents of all participating infants, after obtaining informed consent by phone. The questionnaire was adapted from the questionnaire used in the Generation R study. The atopic symptoms were scored using the questionnaire of the “International Study of Asthma and Allergies in Childhood” (ISAAC). The questionnaire was designed to collect data on allergic and infectious disease and environmental, lifestyle, demographic and socio-economic characteristics. The questionnaire included the following sections: allergic diseases (including questions about the frequency, severity, and location of itchy skin lesions; the frequency and severity of dyspnea, wheezing or nightly dry cough; doctor visits for these complaints; doctor diagnosed asthma; use of inhalation medication; doctor diagnosed hay fever and family history of atopy), doctor diagnosed infectious diseases (including episodes of rhinitis, otitis, pharyngitis, croup, pneumonia, bronchitis or bronchiolitis, urinary tract infections, gastroenteritis, skin infections with general practitioner or hospital visits with or without prescription of medication for infectious diseases), potential confounders (type of feeding; breastfeeding versus formula, mode of delivery; vaginal versus caesarean section, housing characteristics, pets, maternal smoking, siblings, school attendance, maternal educational level and profession).
Outcome measures

Allergic disease included atopic dermatitis, hay fever, recurrent wheeze and asthma. Atopic dermatitis was defined as a doctor-diagnosed skin rash on ≥1 typical location: flexural sites (ankle, knee and elbow) or around eyes and ears. Recurrent wheeze was defined as ≥2 episodes of wheezing in the last year. Hay fever had to be doctor-diagnosed. Asthma had to be both doctor-diagnosed and children had to use inhalation medication.

Upper respiratory tract infections included ≥1 doctor-diagnosed episode of severe cold, throat infection, otitis media or laryngitis. Lower respiratory tract infections included ≥1 doctor-diagnosed episode of bronchitis, bronchiolitis or pneumonia. Gastrointestinal infections included ≥1 doctor-diagnosed episode of vomiting or diarrhea with fever. Urinary tract infections included ≥1 doctor-diagnosed episode of fever and a positive urine culture. Questionnaires were sent to the parents at the infant age of six years and all questions involved the past 12 months.

Statistical analysis

Normally distributed and non-parametric data are presented as mean (SD) and median (range), respectively. Infant, maternal and environmental characteristics were analyzed with Student’s t-test, Mann-Whitney U test and chi-square test or Fisher’s exact test for continuous normally distributed data, nonparametric continuous data and dichotomous data, respectively. Univariate logistic regression analysis was performed to report on the association of glutamine-enriched enteral nutrition in the neonatal period and allergic and infectious diseases at 6 years of age. Subsequently, we adjusted for potentially confounding variables which have been found to be common risk factors for allergic and/or infectious disease (maternal education, parental history of atopy, smoking and pets at home in case of allergic disease, and for maternal education, smoking at home, siblings and school attendance in case of infectious disease) with multivariate logistic regression analysis. All infants received either breast milk (with human milk fortifier) or a standard formula (Nenatal Start) until they reached the weight of 2000 grams, followed by either breast milk without fortifier or regular infant formula. In addition analyses of our study, we found that adjusting for breastfeeding and mode of delivery (vaginal delivery versus caesarean section) did not change the results of the primary analysis. Therefore, we only reported the results of the primary analysis. Measures of association are presented by crude odds ratios (OR) and adjusted odds ratios (aOR) with their 95% confidence interval (CI). A p-value below 0.05 was considered significant.

SPSS 16.0 (SPSS Inc., Chicago, IL) was used for data analysis.
Results

At six years of age, 89/102 (87%) infants were eligible to participate in the follow-up study. Of these 89 infants, 76 (85%) finally participated in the study: 38 infants received enteral glutamine supplementation and 38 received placebo supplementation during the neonatal period. Reasons for not participating in the study were: difficulties with the Dutch language (n=4), migration to foreign country (n=4) family could not be traced (n=5). Baseline neonatal characteristics and characteristics at six years of age were not different between glutamine-supplemented and control groups, except for the occurrence of ≥1 serious infection in the neonatal period (Table 1).

The age of the infants at follow-up was 5.9 ± 0.8 versus 6.0 ± 0.7 years in glutamine-supplemented and control group, respectively (p=0.66). Further details of the neonatal period of the participating infants have been previously described. The association between glutamine-enriched enteral nutrition in the neonatal period and doctor-diagnosed allergic disease at six years of age is shown in Table 2. The risk for atopic dermatitis was decreased in the glutamine-supplemented group compared to the control group, after adjustment for maternal education, parental history of atopy, smoking and pets at home (aOR 0.23, 95% CI 0.06-0.95). No association between glutamine-enriched enteral nutrition in the neonatal period and hay fever, recurrent wheeze and asthma at six years of age was found (Table 2).

At the age of one year, ten infants were diagnosed with atopic dermatitis. In two of them there were no symptoms of atopic dermatitis at the age of six years. In six infants the skin-rash started after the age of one year (mostly between two and three years of age).

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Glutamine (n=38)</th>
<th>Control (n=38)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wks)</td>
<td>29.4 (1.7)</td>
<td>28.8 (1.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.2 (0.4)</td>
<td>1.2 (0.3)</td>
<td>0.70</td>
</tr>
<tr>
<td>≥1 serious infection in the neonatal perioda</td>
<td>23/38 (61%)</td>
<td>32/38 (84%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>17/38 (45%)</td>
<td>17/38 (45%)</td>
<td>0.93</td>
</tr>
<tr>
<td>Breastfeeding until three months of age</td>
<td>23/38 (61%)</td>
<td>22/38 (58%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Age at follow-up (years)</td>
<td>5.9 (0.8)</td>
<td>6.0 (0.7)</td>
<td>0.66</td>
</tr>
<tr>
<td>Smoking at home</td>
<td>3/38 (8%)</td>
<td>5/38 (13%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Siblings</td>
<td>33/38 (87%)</td>
<td>31/38 (82%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Pets at home</td>
<td>10/38 (26%)</td>
<td>11/38 (29%)</td>
<td>0.59</td>
</tr>
<tr>
<td>School attendanceb</td>
<td>37/38 (97%)</td>
<td>37/38 (97%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Parental history of atopy</td>
<td>18/38 (47%)</td>
<td>18/38 (47%)</td>
<td>1.0</td>
</tr>
<tr>
<td>High maternal educationc</td>
<td>16/38 (42%)</td>
<td>11/38 (29%)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Data are mean (SD), or number (%). aSepsis, meningitis, pyelonephritis, pneumonia or arthritis as diagnosed by a combination of clinical signs and a positive culture. b> 1 day/week

cHigher professional or university education.
The association between glutamine-enriched enteral nutrition in the neonatal period and doctor-diagnosed infectious disease at six years of age is shown in Table 3. The risk for gastrointestinal tract infections was lower in the glutamine-supplemented group compared to the control group, after adjustment for maternal education, smoking at home, siblings and school attendance (aOR 0.10, 95% CI 0.01-0.93). No association between glutamine-enriched enteral nutrition in the neonatal period and upper respiratory tract, lower respiratory tract, or urinary tract infections at six years of age was found (Table 3).

**Discussion**

The results of this study indicate that glutamine-enriched enteral nutrition in VLBW infants between days 3 and 30 of life decreased the risk for atopic dermatitis and gastrointestinal tract infections at six years of age. However, there was no association between glutamine-enriched enteral nutrition in neonatal period and other allergic and infectious diseases at six years of age. Glutamine-enriched enteral nutrition in the neonatal period may have enhanced the
maturation of the immune response and, thereby, have led to a decreased incidence of atopic dermatitis and/or gastrointestinal tract infections later in life.

Several epidemiological studies have shown that infectious disease early in life lead to decreased allergic disease later in life (the so-called hygiene hypothesis), although other studies could not confirm this finding. However, these studies 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. 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, several studies reported inverse associations between exposure to endotoxin in house-dust and atopy.

Experimental studies have indicated that the presence of glutamine in vitro increases the cytokine production of T-lymphocytes. We hypothesised that neonatal glutamine supplementation therefore might lead to a deviation in the neonatal immune response towards a balanced Th1/Th2 response. Th subsets can be differentiated based on the production of a specific panel of cytokines. In our previous studies, neonatal glutamine supplementation was not associated with alterations in cytokine profiles (interferon-gamma, tumor necrosis factor-alpha, interleukin [IL]-2, IL-4, IL-5, and IL-10) of peripheral blood cells upon stimulation with either anti-alphaCD3/alphaCD28 or lipopolysaccharide in both neonatal period and at one year of age. In addition, cytokine profiles were not different between atopic and non-atopic infants, at one year of age.

In a study in rats, glutamine served as fuel for enterocytes and improved thickness and optical density of the mucus gel. By enteral supplementation of glutamine in neonates, the mucus layer might change, 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. In this same 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 have played a role in our cohort. Certain strains of bifidobacteria might have antagonistic ability against pathogens, as recently reviewed by Servin. Even though a difference in gastrointestinal tract infections was found, we are careful interpreting these data since this only involved the past 12 months.

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. Delivery by caesarean section might lead to an increased risk of developing asthma. In addition analyses of our study, we found that adjusting for breastfeeding and mode of delivery (vaginal delivery versus caesarean section) did not change the results of the primary analysis. Therefore, we only reported the results of the primary analysis.

Some aspects of our study design need to be addressed. Firstly, as the sample size calculation of the study was based on the primary outcome of the main trial, the sample size of the follow-up study was relatively small. The response rate of 85% contributed to the reliability of the results. Secondly, 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. Thirdly, parents were not blinded to treatment allocation at the age of six year of their children. However, all allergic and infectious diseases were diagnosed by independent physicians, who were not involved in this trial. Finally, the diagnosis of asthma is based on the ISAAC questionnaire validated and applied in several epidemiological studies. In our cohort, no forced expiratory volume in 1 second (FEV1) was measured, however the diagnose included both “doctor diagnosed” asthma and the use of inhalation medication.