Glutamine-Enriched Enteral Nutrition in Very Low-Birth-Weight Infants

**Effect on the Incidence of Allergic and Infectious Diseases in the First Year of Life**

Anemone van den Berg, MD, PhD; Annelies van Zwol, MD; Henriëtte A. Moll, MD, PhD; Willem P. F. Fetter, MD, PhD; Ruurd M. van Elburg, MD, PhD

**Objective:** To determine the effect of glutamine-enriched enteral nutrition in very low-birth-weight infants on the incidence of allergic and infectious diseases during the first year of life.

**Design:** Follow-up study.

**Setting:** Tertiary care hospital.

**Participants:** All surviving infants who participated in a trial of glutamine-enriched enteral nutrition in very low-birth-weight infants.

**Intervention:** Enteral glutamine supplementation (l-glutamine, 0.3 g/kg per day) from 3 through 30 days of life.

**Main Outcome Measures:** The incidence of allergic and infectious diseases during the first year of life, as assessed by means of validated questionnaires.

**Results:** Seventy-seven of 90 infants (86%) participated in the follow-up study. Baseline patient, maternal, and environmental characteristics did not differ between the glutamine-supplemented (n=37) and control (n=40) groups, except for the incidence of serious neonatal infections and child care attendance. After adjustment for confounding factors, the risk for atopic dermatitis was lower in the glutamine-supplemented group (odds ratio [OR], 0.13; 95% confidence interval [CI], 0.02-0.97). However, the incidence of bronchial hyperreactivity (OR, 0.34; 95% CI, 0.10-1.21) and infections of the upper respiratory (OR, 0.99; 95% CI, 0.35-2.79), lower respiratory (OR, 0.39; 95% CI, 0.13-1.24), and gastrointestinal (OR 1.25, 95% CI 0.23-6.86) tracts was not different between the treatment groups.

**Conclusions:** Glutamine-enriched enteral nutrition in very low-birth-weight infants decreased the incidence of atopic dermatitis during the first year of life but had no effect on the incidence of bronchial hyperreactivity and infectious diseases during the first year of life.


Several studies in very low-birth-weight (VLBW) infants have investigated the effect of parenteral or enteral glutamine supplementation on morbidity and outcome in the neonatal period. However, little is known about the long-term effects of this nutritional intervention. 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 (eg, allergy and asthma) may contribute to de-liberate choices in neonatal nutritional support in VLBW infants.

Experimental studies have shown that the amino acid glutamine plays an important role in maintaining functional integrity of the gut. In addition, glutamine is used at a high rate by cells of the immune system. Experimental studies have indicated that the presence of glutamine in vitro increases the cytokine production of T lymphocytes after stimulation with various mitogens. A subset of helper T cells denoted as type 1 helper T (T_{H1}) cells is implicated in the cell-mediated resistance to infections, whereas T_{H2} cells are involved in the regulation of antibody responses. The T_{H} cell subsets can be differentiated by the production of a specific panel of cytokines. The T_{H2} cytokine responses dominate the neonatal immune response because the maternal immune response during pregnancy is skewed toward T_{H2} immunity. After birth, microbial exposure stimulates T_{H1} cytokine responses and deviates the neonatal immune response toward balanced T_{H1} and T_{H2} cytokine responses. In addition, other factors early in life such as
mode of delivery and enteral nutrition may influence this maturation process.

Delayed transition from fetal T\(_{H2}\)-polarized cytokine responses to adult T\(_{H1}\)-polarized cytokine responses may lead to long-term dysregulation of T\(_{H2}\) responses and allergic disease. The shift from T\(_{H2}\) toward T\(_{H1}\) cytokine responses can be manipulated in early infancy, as suggested by Martinez and Holt and other groups. We hypothesized that glutamine-enriched enteral nutrition in the neonatal period may enhance the maturation of the immune response and may lead to decreased allergic disease later in life. In addition, our group and others have found that glutamine-enriched enteral nutrition, administered from 3 through 30 days of life, decreased the incidence of serious infections in the neonatal period. The question arose whether this will have consequences for long-term resistance against infectious diseases.

The aim of this follow-up study was to determine the effect of glutamine-enriched enteral nutrition in VLBW infants on the incidence of allergic and infectious diseases during the first year of life by means of validated questionnaires.

The primary end point of the study was time to full enteral feeding, defined as a feeding volume of at least 120 mL/kg per day. Secondary end points included other outcome factors of feeding tolerance, the incidence of serious infections (including sepsis, meningitis, pyelonephritis, pneumonia, and arthritis [clinical signs in combination with a positive culture result]), and other short-term outcome measures (eg, need for ventilator support, age at discharge from the neonatal intensive care unit).

See the study protocol for further details of the study design.

### METHODS

#### ORIGINAL STUDY

#### Study Population

Infants with a gestational age of younger than 32 weeks and/or birth weight of less than 1500 g and admitted to the level III neonatal intensive care unit of the VU University Medical Center were eligible for participation in the study. Exclusion criteria were major congenital or chromosomal anomalies, death within 48 hours after birth, transfer to another hospital within 48 hours after birth, and admission from an extraregional hospital.

#### Randomization, Blinding, and Treatment

After assignment to 1 of 3 birth weight strata (≤799 g, 800-1199 g, and ≥1200 g), infants were randomly allocated within 48 hours after birth to receive enteral glutamine supplementation or isonitrogenous control supplementation. An independent researcher used a computer-generated randomization table based on blocks of 4 (provided by Nutricia Nederland BV, Zoetermeer, the Netherlands) to assign infants to treatment A or B, which corresponded to batch numbers on the nutrition products. Investigators, parents, and medical and nursing staff were unaware of treatment allocation.

Glutamine powder contained 82% l-glutamine and 18% glucose (15.5% [wt/wt] nitrogen; 371 kcal/100 g), whereas the isonitrogenous control powder contained 100% l-alanine (15.7% [wt/wt] nitrogen; 435 kcal/100 g) (provided by Nutricia Nederland BV). The powders were indistinguishable by appearance, color, and smell. From 3 through 30 days of life, l-glutamine supplementation was administered in increasing doses to a maximum of 0.3 g/kg per day in the glutamine-supplemented group. Two members of the nursing staff daily added supplementation to breast milk or preterm formula (Neatal; Nutricia Nederland BV). The preterm formula provides 78 kcal, 2.1 g of protein (casein to whey protein ratio, 40:60), 4.4 g of fat, and 7.5 g of carbohydrate per 100 mL. The preterm formula did not contain free l-glutamine or free l-alanine.

When infants were transferred to other hospitals before the end of the study, the protocol was continued under the supervision of the principal investigator (A.vdB.).

#### THE FOLLOW-UP STUDY

At the corrected age of 1 year, all 90 surviving infants were eligible to participate in the follow-up study. The National Committee on Research Involving Human Subjects and the medical ethical review board of our institution approved the study protocol. Written informed consent was obtained from all parents.

Questionnaires were sent to the parents of all participating infants before the routine outpatient clinic visit of their infant at 1 year. At that time, parents and investigators were still unaware of treatment allocation in the neonatal period. The questionnaire was adapted from questionnaires used in the Prevention and Incidence of Asthma and Mite Allergy Study and the Generation R Study and was designed to collect data on allergic and infectious diseases and environmental, lifestyle, demographic, and socioeconomic characteristics. The questionnaire included the following sections: growth and vaccination status, allergic diseases (including questions about the frequency, severity, and location of atopic skin lesions; the frequency and severity of dyspnea, wheezing, humming/sawing breath sounds, or nightly dry cough; physician visits for these complaints; and family history of atopy), infectious diseases (episodes of severe rhinitis, otitis, pharyngitis, pneumonia, bronchitis or bronchiolitis, meningitis, sepsis, urinary tract infections, gastroenteritis, croup, whooping cough, chickenpox, measles, or skin infections and general practitioner or hospital visit and prescription of medication for infectious diseases), fever episodes (temperature measurements, the frequency of fever episodes, and the presence of other complaints in combination with a fever plus a physician visit), environment (housing characteristics, the presence of pets, smoking in the environment of the child, and child care attendance), and questions about the parents (the education and profession of the parents).

#### OUTCOME MEASURES

Allergic diseases included atopic dermatitis, milk protein allergy, and bronchial hyperreactivity. Atopic dermatitis was defined as a physician-diagnosed rash on at least 1 typical location, such as flexural sites (ankle, knee, and elbow) or around the eyes and ears. Milk protein allergy had to be physician diagnosed. Bronchial hyperreactivity was diagnosed in case of at least 3 of the following physician-diagnosed symptoms: dyspnea, wheezing, humming/sawing breath sounds, or nightly dry cough without rhinitis. Upper respiratory tract infections included at least 1 physician-diagnosed episode of severe rhinitis, pharyngitis, or otitis media. Lower respiratory tract infections included at least 1 physician-diagnosed episode of bronchitis, bronchiolitis, or pneumonia. Gastrointestinal tract infections included at least 1 physician-diagnosed episode of severe rhinitis, pharyngitis, or otitis media. Lower respiratory tract infections included at least 1 physician-diagnosed episode of bronchitis, bronchiolitis, or pneumonia.
infections, urinary tract infections, sepsis, and meningitis included physician-diagnosed episodes.

**STATISTICAL ANALYSIS**

Normally distributed and nonparametric data are presented as mean (SD) and median (range), respectively. Infant, maternal, and environmental characteristics were analyzed with an unpaired t test, Mann-Whitney test, and χ² or Fisher exact test for continuous normally distributed, nonparametric continuous, and dichotomous data, respectively.

Logistic regression was performed to examine whether glutamine-enriched enteral nutrition in the neonatal period influenced the presence of allergic and infectious diseases in the first year of life. In additional analyses, adjustments were made for maternal education, parental history of atopy, exclusive breastfeeding in the neonatal period, smoking, and the presence of pets at home in cases of allergic diseases, and for maternal education, smoking at home, the presence of siblings, and child care attendance in cases of infectious diseases.

A P<.05 (2-tailed) value was considered significant. We used SPSS statistical software, version 11 (SPSS Inc, Chicago, Illinois) for data analysis.

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**RESULTS**

From December 1, 2002, through October 31, 2004, 77 of 90 surviving infants (86%) participated in the follow-up study. Reasons for nonresponse were not understanding Dutch (n=6), failure to return the questionnaire (n=3), refusal of informed consent (n=2), and emigration (n=2). The Figure shows the complete trial profile. Baseline patient, maternal, and environmental characteristics were not different between the glutamine-supplemented and control groups, except for the occurrence of at least 1 serious infection in the neonatal period and child care attendance (Table 1). The mean (SD) age of the infants at follow-up was 1.3 (0.2) vs 1.3 (0.1) years in the glutamine-supplemented and control groups, respectively (P=.65).

The incidence of physician-diagnosed allergic diseases in the first year of life is shown in Table 2. In the glutamine-supplemented group, the risk for atopic dermatitis was lower compared with that of the control group, after adjustment for maternal education, parental history of atopy, exclusive breastfeeding in the neonatal period, and the presence of smoking and pets at home (odds ratio, 0.13; 95% confidence interval, 0.02-0.97; P=.05). No significant relation was found between glutamine-enriched enteral nutrition in the neonatal period and bronchial hyperreactivity in the first year of life. Milk protein allergy occurred in only 2 infants in the control group.

The incidence of physician-diagnosed infectious diseases in the first year of life is shown in Table 3. The risk for upper and lower respiratory and gastrointestinal tract infections was not different in the glutamine-supplemented and control groups, even after adjustment for maternal education, smoking at home, siblings, and child care attendance. Urinary tract infections occurred in 5 infants in the glutamine-supplemented group. All of these infants had congenital malformations of the urogenital tract.

**COMMENT**

The results of this study indicate that glutamine-enriched enteral nutrition in VLBW infants from 3 through 30 days of life decreased the incidence of atopic dermatitis during the first year of life. However, there was no effect on the incidence of bronchial hyperreactivity. In addition, glutamine-enriched enteral nutrition in the neonatal period did not influence the incidence of infectious diseases during the first year of life.

Glutamine-enriched enteral nutrition in VLBW infants may lead to less atopic dermatitis during the first year of life by enhancing maturation of the immune response. Experimental studies showed that optimal glutamine concentrations improve the Th1 cytokine response after mal-
ste´n et al33 reported that coliform bacteria were more prevalent in the commensal intestinal microflora. Furthermore, Bjo¨ rkman responses rapidly developed after the introduction of dichotomous data, respectively.

Portant site for bacterial colonization,30 and its composition may modulate bacterial adherence.31 Microbial colonization of the gut is considered the most important point, data on the composition of the intestinal microflora at the corrected age of 1 year will provide interesting information.

In our study, the difference in incidence of bronchial hyperreactivity did not reach statistical significance, though 14% of the infants in the glutamine-supplemented group had bronchial hyperreactivity in the first year of life, compared with 33% of the infants in the control group. In our opinion, this is a clinically relevant difference, and we suppose that the sample size of the follow-up study was insufficient to detect a statistically significant difference in the frequency of bronchial hyperreactivity between the glutamine-supplemented and control groups. Furthermore, the finding that glutamine-enriched enteral nutrition decreased the risk for atopic dermatitis but not for bronchial hyperreactivity may be explained by the fact that during the first year of life, bronchial hyperreactivity is a less specific condition than atopic dermatitis. Symptoms of a respiratory tract infection often resemble symptoms of bronchial hyperreactivity. Finally, the respiratory status is strongly related to neonatal respiratory problems. The odds ratio for bronchial hyperreactivity in the glutamine-supplemented group was 0.23 (95% confidence interval, 0.06-1.05; \( P = .06 \)) compared with the control group, when additional adjustments were made for the administration of surfactant, the length of ventilator support, and the occurrence of pneumonia in the neonatal period.

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<th>Table 1. Baseline Characteristics</th>
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<td>Infant characteristics</td>
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<td>Vaginal delivery</td>
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<td>≥1 Pneumonia episode</td>
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Abbreviation: NICU, neonatal intensive care unit.

a Unless otherwise indicated, data are expressed as number (percentage) of infants.
b The unpaired t test (2-tailed), Mann-Whitney test, and \( y^2 \) or Fisher exact test was used for continuous normally distributed, nonparametric continuous, and dichotomous data, respectively.
c Indicates during the neonatal study period of 4 weeks.
d Includes sepsis, meningitis, pyelonephritis, pneumonia, or arthritis as diagnosed by a combination of clinical signs and a positive culture result.
e Indicates higher professional or university education.
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. Various epidemiological studies showed that infectious diseases early in life lead to decreased allergic diseases later in life (the “hygiene hypothesis”), although other studies did not confirm this finding. In line with the latter studies, the lower incidence of infectious diseases in the neonatal period in our study was not associated with a higher rate but in fact with a lower rate of allergic diseases later in life.

We hypothesized that the lower incidence of serious neonatal infections in the glutamine-supplemented group could be the result of enhanced T\textsubscript{H1} cytokine responses. Improved T\textsubscript{H1} cytokine responses may also enhance the resistance against infectious diseases later in life. However, the results of this study indicate that glutamine-enriched enteral nutrition in VLBW infants did not decrease the incidence of infectious diseases during the first year of life. Because infectious diseases principally included respiratory tract infections, respiratory status after neonatal respiratory problems and ventilator support may be a dominant determining factor for the frequency of infectious diseases during the first year of life. The smaller number of lower respiratory tract infections during the first year of life in the glutamine-supplemented group approached statistical significance. In addition, in the initial study, 6 infants in the glutamine-supplemented group had 7 episodes of pneumonia, compared with 13 episodes of pneumonia in 9 infants in the control group. In additional analyses, we found that neonatal pneumonia (as diagnosed by clinical symptoms in combination with a positive culture result) indeed increased the risk for a lower respiratory tract infection but did not change the results of the primary analysis (data not shown).

Neither administration of surfactant nor length of ventilator support in the neonatal period influenced the risk of lower respiratory tract infections during the first year of life. The risk for upper respiratory tract infections was not affected by neonatal respiratory status. Although glutamine-enriched enteral nutrition in VLBW infants did not decrease the incidence of infectious diseases during the first year of life. Because infectious diseases principally included respiratory tract infections, respiratory status after neonatal respiratory problems and ventilator support may be a dominant determining factor for the frequency of infectious diseases during the first year of life. The smaller number of lower respiratory tract infections during the first year of life in the glutamine-supplemented group approached statistical significance. In addition, in the initial study, 6 infants in the glutamine-supplemented group had 7 episodes of pneumonia, compared with 13 episodes of pneumonia in 9 infants in the control group. In additional analyses, we found that neonatal pneumonia (as diagnosed by clinical symptoms in combination with a positive culture result) indeed increased the risk for a lower respiratory tract infection but did not change the results of the primary analysis (data not shown).

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Glutamine plays an important role in maintaining intestinal integrity, and glutamine-enriched enteral nutrition in the neonatal period did not particularly affect the risk for gastrointestinal tract infections during the first year of life. Following enteral administration, glutamine is almost entirely metabolized by the gut, and consequently it is unlikely that glutamine-enriched enteral nutrition early in life has a long-term effect on the maintenance of the intestinal integrity.

The response rate of 86% supports the reliability of the results of our study. However, some aspects of our study design need to be addressed. First, because the sample size was based on the sample size calculation for the primary outcome of the initial trial, the sample size of the follow-up study was relatively small. As a consequence, the conclusions of our study are susceptible to a type II error. To achieve a statistically significant difference with regard to the incidence of bronchial hyperreactivity, approximately 80 infants should have been included in both treatment groups. Second, parental report of disease by questionnaire may be subject to reporting bias. In particular, it might be difficult for parents to tell the difference between respiratory tract infections and bronchial hyperreactivity. Therefore, we analyzed physician-diagnosed symptoms and conditions in validated questionnaires. In addition, for the diagnosis of atopic dermatitis and bronchial hyperreactivity, a combination of physician-diagnosed symptoms was required. Maternal health interviews are an important tool to estimate the prevalence of childhood morbidity in developing countries. A study of the validity of morbidity assessment questionnaires showed that retrospective interviews can achieve the sensitivity and specificity needed to identify common childhood diseases. Nevertheless, physician-diagnosed events as reported by the parents may decrease standardization in the outcome measures and lead to information bias. Furthermore, the corrected age of 1 year may be too early to investigate the incidence of allergies and infections because these diseases can manifest themselves at an older age. Long-term follow-up may show further differences between the glutamine-supplemented and control groups. Finally, our study provided epidemiological data and did not provide data on underlying pathophysiological processes. Studies of cytokine responses and intestinal microbiota following glutamine-enriched enteral nutrition in VLBW infants may elucidate these processes.

In conclusion, this is, to our knowledge, the first study into the long-term effects of glutamine supplementation in VLBW infants. Our results indicate that glutamine-enriched enteral nutrition in VLBW infants from 3 through 30 days of life decreased the incidence of atopic dermatitis during the first year of life, whereas no effect on the incidence of bronchial hyperreactivity was found. Thus, glutamine supplementation in VLBW infants may lead to long-term health benefits. Although glutamine-enriched enteral nutrition led to a lower rate of serious neonatal infections, there was no effect on the incidence of infectious diseases during the first year of life. Further follow-up of this well-defined cohort of VLBW infants with respect to the incidence of allergic and infectious diseases may contribute to a better understanding of the maturation of the immune response and the role of glutamine supplementation in this process.

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Correspondence: Anemone van den Berg, MD, PhD, Department of Pediatrics, VU University Medical Center, De Boelelaan 1117, 1081 HV, Amsterdam, the Netherlands (a.vandenberg@vumc.nl).

Author Contributions: All investigators had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs van den Berg and van Zwol contributed equally to the manuscript. Study concept and design: van den Berg, Moll, Fetter, and van Elburg. Acquisition of data: van den Berg, van Zwol, and van Elburg. Analysis and interpretation of data: van den Berg, van Zwol, Moll, and van Elburg. Drafting of the manuscript: van den Berg, van Zwol, and van Elburg. Critical revision of the manuscript for important intellectual content: Moll, Fetter, and van Elburg. Statistical analysis: van den Berg. Obtained funding: van Elburg. Administrative, technical, and material support: van den Berg, van Zwol, and van Elburg. Study supervision: van den Berg, Moll, Fetter, and van Elburg.

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