

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

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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.

Arch Pediatr Adolesc Med. 2007;161(11):1095-1101

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.¹⁻⁵ However, little is known about the long-term effects of this nutritional intervention. As recently discussed in a workshop on research issues in neonatology,⁶ knowledge about the effect of nutritional interventions on long-term outcome and disease later in life (eg, allergy and asthma) may contribute to deliberate 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.⁷⁻¹⁰ In addition, glutamine is used at a high rate by cells of the im-

mune system.¹¹ Experimental studies have indicated that the presence of glutamine in vitro increases the cytokine production of T lymphocytes after stimulation with various mitogens.¹²⁻¹⁴ 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 response¹⁵ because the maternal immune response during pregnancy is skewed toward T_H2 immunity.^{16,17} After birth, microbial exposure stimulates T_H1 cytokine responses and deviates the neonatal immune response toward balanced T_H1 and T_H2 cytokine responses.¹⁵ In addition, other factors early in life such as

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mode of delivery^{18,19} and enteral nutrition²⁰ 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 responses²¹ and allergic disease.^{22,23} The shift from T_H2 toward T_H1 cytokine responses can be manipulated in early infancy, as suggested by Martinez and Holt²⁴ and other groups.^{20,25} 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 group⁵ and others³ 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.

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 (Nenatal; 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.).

Study End Points

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.²⁶

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 Central 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 Study²⁷ and the Generation R Study²⁸ 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 itchy 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, 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 χ^2 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.

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.

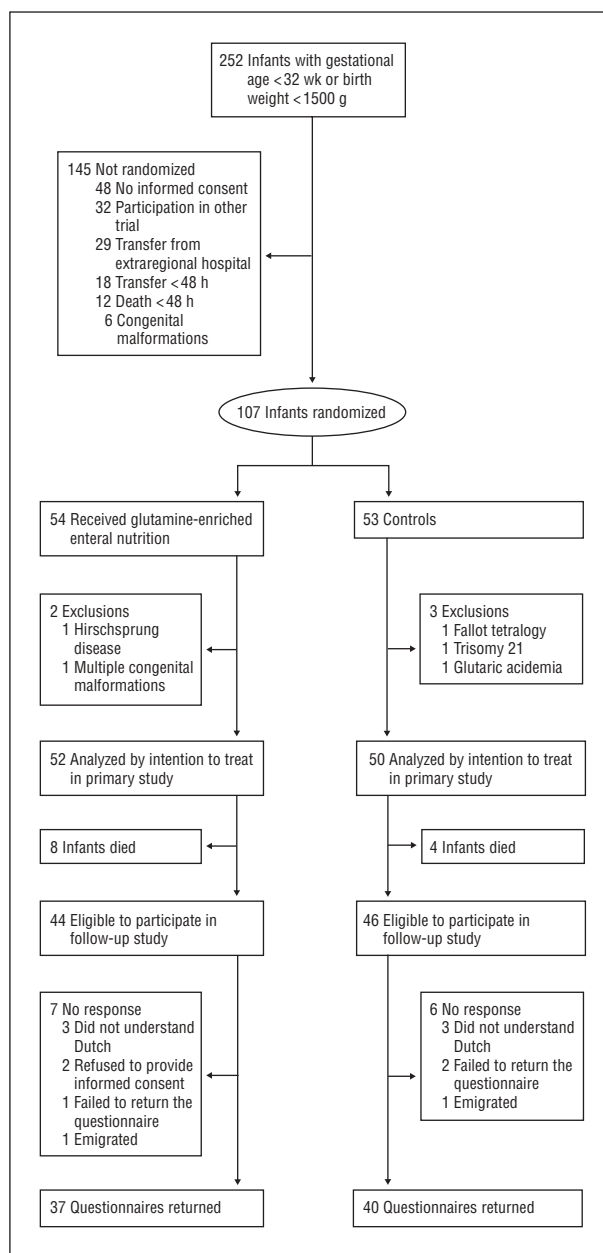


Figure. Profile of original and follow-up studies.

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 T_H1 cytokine response after mal-

Table 1. Baseline Characteristics

Characteristic	Glutamine-Supplemented Group (n=37) ^a	Control Group (n=40) ^a	P Value ^b
Infant characteristics			
Vaginal delivery	16 (43)	18 (45)	.88
Gestational age, mean (SD), wk	29.4 (1.6)	29.1 (1.7)	.44
Birth weight, mean (SD), kg	1.20 (0.37)	1.20 (0.32)	.99
Male sex	18 (49)	22 (55)	.58
Exclusive breast milk feeding ^c	24 (65)	20 (50)	.19
Administration of surfactant	18 (49)	18 (45)	.75
Ventilator support, median (range), d	2 (0 to 53)	4.5 (0 to 61)	.10
≥1 Serious infection in the neonatal period ^d	23 (62)	33 (83)	.05
≥1 Pneumonia episode	4 (11)	6 (15)	.74
Age at discharge from NICU, median (range), d	21 (2 to 121)	27 (3 to 109)	.23
Age at discharge from hospital, median (range), d	60 (35 to 180)	69 (35 to 162)	.32
Age at follow-up, mean (SD), y	1.3 (0.2)	1.3 (0.1)	.65
Age at follow-up to 1 y of corrected age, median (range), wk	0.9 (−5.3 to 12.1)	0.9 (−4.6 to −22.1)	.66
Maternal characteristics			
Age at birth, mean (SD), y	30.3 (4.7)	28.3 (5.5)	.09
White race	32 (86)	36 (90)	.45
High maternal education ^e	19 (51)	12 (30)	.06
Environmental characteristics			
Smoking at home	7 (19)	7 (18)	.87
Siblings	16 (43)	20 (50)	.55
Parental history of atopy	19 (51)	19 (48)	.74
Pets at home	18 (49)	23 (58)	.44
Child care attendance > 1 d/wk	25 (68)	15 (38)	.008

Abbreviation: NICU, neonatal intensive care unit.

^aUnless otherwise indicated, data are expressed as number (percentage) of infants.

^bThe unpaired *t* test (2-tailed), Mann-Whitney test, and χ^2 or Fisher exact test was used for continuous normally distributed, nonparametric continuous, and dichotomous data, respectively.

^cIndicates during the neonatal study period of 4 weeks.

^dIncludes sepsis, meningitis, pyelonephritis, pneumonia, or arthritis as diagnosed by a combination of clinical signs and a positive culture result.

^eIndicates higher professional or university education.

nutrition²⁹ or stimulation with phytohemagglutinin, bacillus Calmette-Guérin, and the measles virus.¹⁴ Furthermore, glutamine supplementation improved the thickness and optical density of the intestinal mucus gel in rats receiving parenteral nutrition.⁹ The mucus layer is an important site for bacterial colonization,³⁰ and its composition may modulate bacterial adherence.³¹ Microbial colonization of the gut is considered the most important stimulus for the development of T_H1 cytokine responses.^{15,25,32} Sudo et al²¹ showed that germ-free mice had prolonged T_H2 cytokine responses, whereas T_H1 cytokine responses rapidly developed after the introduction of commensal intestinal microflora. Furthermore, Björkstén et al³³ reported that coliform bacteria were more prevalent in the commensal microflora of atopic children, whereas more lactobacilli and bifidobacteria were present in nonatopic children. In a study of probiotic supplementation in full-term infants at high risk for developing atopy (*Lactobacillus GG* was given prenatally to mothers with a familial history of atopy and postnatally for 6 months to their infants), the frequency of atopic dermatitis in the probiotic group was half that of the placebo group.³⁴ Unfortunately, the authors did not provide data on the composition of the intestinal microflora in these infants. Although our group found that glutamine-enriched enteral nutrition in the neonatal period decreased the incidence of atopic dermatitis in the first year of life, we did not find increased numbers of lactobacilli or bifidobacteria in the

intestinal microflora in the first 4 weeks of life.³⁵ At this 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 0.25 (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.

Table 2. Physician-Diagnosed Allergic Diseases

Allergic Disease	No. (%) of Infants		Analysis	OR (95% CI)	P Value ^a
	Glutamine-Supplemented Group (n=37)	Control Group (n=40)			
Atopic dermatitis ^b	2 (5)	8 (20)	Crude	0.23 (0.05-1.16)	.08
			Adjusted ^c	0.13 (0.02-0.97)	.05
Milk protein allergy ^d	0	2 (5)	...	...	...
Bronchial hyperreactivity ^e	5 (14)	13 (33)	Crude	0.33 (0.1-1.03)	.06
			Adjusted ^c	0.34 (0.10-1.21)	.10

Abbreviations: CI, confidence interval; OR, odds ratio; ellipses, not applicable.

^aLogistic regression analysis.

^bIndicates rash on at least 1 typical location: flexural sites (ankle, knee, and elbow) or around eyes and ears.

^cAdjusted for maternal education, parental history of atopy, exclusive breastfeeding in the neonatal period, and the presence of smoking and pets at home.

^dIndicates diagnosed by a physician. The incidence did not allow statistical analysis.

^eIndicates ≥ 3 of the following physician-diagnosed symptoms: dyspnea, wheezing, humming/sawing breath sounds, or nightly dry cough without rhinitis.

Table 3. Physician-Diagnosed Infectious Diseases

Infectious Disease	No. (%) of Infants		Analysis	OR (95% CI)	P Value ^a
	Glutamine-Supplemented Group (n=37)	Control Group (n=40)			
Upper respiratory tract infection ^b	24 (65)	23 (58)	Crude	1.36 (0.54-3.43)	.51
			Adjusted ^c	0.99 (0.35-2.79)	.99
Lower respiratory tract infection ^d	7 (19)	14 (35)	Crude	0.43 (0.15-1.24)	.12
			Adjusted ^c	0.39 (0.13-1.24)	.11
Gastrointestinal tract infection	4 (11)	3 (8)	Crude	1.50 (0.31-7.18)	.62
			Adjusted ^c	1.25 (0.23-6.86)	.80
Urinary tract infection ^e	5 (14)	0	...	...	...
Sepsis/meningitis ^e	0	0	...	...	...

Abbreviations: CI, confidence interval; OR, odds ratio; ellipses, not applicable.

^aDetermined by means of logistic regression analysis.

^bIndicates at least 1 episode of serious rhinitis, pharyngitis, or otitis media.

^cAdjusted for maternal education, smoking at home, siblings, and child care attendance.

^dIndicates at least 1 episode of bronchitis, bronchiolitis, or pneumonia.

^eThe incidence of urinary tract infections and sepsis/meningitis did not allow statistical analysis.

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.⁵ Several epidemiological studies showed that infectious diseases early in life lead to decreased allergic diseases later in life (the "hygiene hypothesis"),^{36,37} although other studies did not confirm this finding.³⁸⁻⁴⁰ 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_H1 cytokine responses. Improved T_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 in-

cluded 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.⁵ 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 glu-

tamine plays an important role in maintaining intestinal integrity,⁷⁻¹⁰ 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,⁴¹ 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,⁵ 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.^{27,28} In addition, for the diagnosis of atopic dermatitis and bronchial hyperreactivity, a combination of physician-diagnosed symptoms was required.^{27,28} 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.^{42,43} 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 microflora 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 understand-

ing of the maturation of the immune response and the role of glutamine supplementation in this process.

Accepted for Publication: May 10, 2007.

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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.

Financial Disclosure: None reported.

Funding/Support: For the initial study, Nutricia Nederland BV provided Neonatal, glutamine, and placebo supplementation.

Role of the Sponsor: Nutricia Nederland BV was not involved in the design and conduct of the study, the collection and interpretation of the data, or the preparation of the manuscript.

Additional Contribution: Jos W. R. Twisk, PhD, provided statistical assistance.

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Scurvy: Right Treatment, Wrong Reason. Citric acid, used with great advantage, though inferior to lemon juice or lime juice, of the utmost value, both as preventive and a curative agent. Its efficacy believed to be due to its potassium salts. Oranges are highly useful.
—From *Materia Medica Pharmacy and Therapeutics*, 1906