

Glutamine-enriched enteral nutrition in very low birthweight infants and allergic and infectious diseases at 6 years of age

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Summary

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In a previous randomised controlled trial, we found that glutamine-enriched enteral nutrition in 102 very low birthweight (VLBW) infants decreased both the incidence of serious infections in the neonatal period and the risk of atopic dermatitis during the first year of life. We hypothesised that glutamine-enriched enteral nutrition in VLBW infants in the neonatal period influences the risk of allergic and infectious disease at 6 years of age. Eighty-eight of the 102 infants were eligible for the follow-up study (13 died, 1 chromosomal abnormality). Doctor-diagnosed allergic and infectious diseases were assessed by means of validated questionnaires. The association between glutamine-enriched enteral nutrition in the neonatal period and allergic and infectious diseases at 6 years of age was based on univariable and multivariable logistic regression analyses. Seventy-six of the 89 (85%) infants participated, 38 in the original glutamine-supplemented group and 38 in the control group. After adjustment, we found a decreased risk of atopic dermatitis in the glutamine-supplemented group: adjusted odds ratio (aOR) 0.23 [95% CI 0.06, 0.95]. No association between glutamine supplementation and hay fever, recurrent wheeze and asthma was found. A decreased risk of gastrointestinal tract infections was found in the glutamine-supplemented group (aOR) 0.10 [95% CI 0.01, 0.93], but there was no association with upper respiratory, lower respiratory or urinary tract infections. We concluded that glutamine-enriched enteral nutrition in the neonatal period in VLBW infants decreased the risk of atopic dermatitis and gastrointestinal tract infections at 6 years of age.

Keywords: neonatal nutrition, RCT, childhood asthma, atopic dermatitis, infection.

Introduction

Allergic diseases form an important health problem worldwide.^{1,2} Many predisposing factors for the development of allergic diseases have been suggested, including low birthweight; however, conflicting results have been found so far in previous studies.^{3–6} The atopic predisposition of preterm infants might differ from term infants.^{4,7–11} This predisposition may vary for different allergic diseases such as atopic dermatitis and asthma. The development of atopic dermatitis is said to be multifactorial, with both genetic and environmental factors playing a role.¹² In a recent study, Kvenshagen *et al.*¹³ found no difference in the

prevalence of atopic dermatitis between preterm and term infants. The association between low birthweight and asthma remains unclear, mainly because of the variety and diversity of factors (e.g. behavioural, environmental, psychological and genetic factors) that may contribute to the development of asthma.¹⁴ In a meta-analysis, Jaakkola *et al.* concluded that preterm infants are at increased risk of developing asthma when compared to term infants.¹⁵

Besides type of feeding, various feeding supplements have been suggested as influencing the development of allergic diseases. One of these supplements is glutamine, an amino acid which might stimulate the

Th1 cytokine response and change the neonatal immune response towards a balanced Th1/Th2 response.¹⁶ In our previous study of 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 hyper-reactivity, as a risk factor for the (future) development of asthma.¹⁷ In the first year of life, bronchial hyper-reactivity is not a very specific condition, and symptoms of respiratory tract infections often resemble symptoms of bronchial hyper-reactivity. However, asthma at 6 years of age is a more specific indicator of 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.^{18,19} In several studies of both enteral²⁰ and parenteral^{21,22} glutamine supplementation, conflicting results have been found regarding the incidence of serious neonatal infections. We hypothesised that glutamine-enriched enteral nutrition in the neonatal period might stimulate the transition of fetal Th2 polarised cytokine responses to adult Th1-polarised responses and might decrease allergic disease later in life. The question arose as to whether this would result in 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 of allergic and infectious diseases at 6 years of age.

Methods

Study population

The initial study, conducted at the VU University Medical Center from September 2001 to July 2003, was a randomised, double-blind placebo-controlled trial of glutamine-enriched enteral nutrition in 102 VLBW infants (gestational age <32 weeks and/or birthweight <1500 g). Infants admitted to the level III neonatal intensive care unit of the VU University Medical Center, Amsterdam, were eligible for participation. In this study, infants received enteral glutamine supplementation (0.3 g/kg/day) or an isonitrogenous placebo supplementation (alanine) between days 3 and 30 of life. Further details of the study design are outlined in the study protocol.¹⁸ 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 6 years of age, parents were not blinded to treatment allocation of their infants any more.

Questionnaire

Questionnaires were sent to the parents of all participating infants, after informed consent was obtained by phone. The questionnaire was adapted from the questionnaire used in the Generation R study.^{23,24} The atopic symptoms were scored using the questionnaire of the 'International Study of Asthma and Allergies in Childhood' (ISAAC).²⁵

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 dyspnoea, wheezing or dry cough at night; 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 media, 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) and potential confounders (type of feeding, breast feeding vs. formula; mode of delivery, vaginal vs. caesarean section; housing characteristics; pets; maternal smoking; siblings; school attendance; maternal educational level and profession).

Outcome measures

Allergic diseases 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 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 when the child was 6 years of age 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 analysed with Student's *t*-test, Mann-Whitney *U*-test and chi-square test or Fisher's exact test for continuous normally distributed data, non-parametric continuous data and dichotomous data, respectively. Univariable logistic regression analysis was performed to assess the association between 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, no. of siblings and school attendance in case of infectious disease) with multivariable logistic regression analyses. All infants received either breast milk (with human milk fortifier) or a standard formula (neonatal star) until they reached the weight of 2000 g, followed by either breast milk without fortifier or regular infant formula. In additional analyses of our study, we found that adjusting for breast feeding and mode of delivery (vaginal delivery vs. 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 as unadjusted odds ratios (OR) and adjusted odds ratios (aOR) with their 95% confidence intervals (CI). A *P*-value below 0.05 was considered statistically significant. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

Results

At 6 years of age, 88/102 (86%) infants were eligible to participate in the follow-up study (13 had died, 1 was excluded because of a chromosomal abnormality). Of these 88 infants, 76 (86%) 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

Table 1. Baseline characteristics

	Glutamine (<i>n</i> = 38)	Control (<i>n</i> = 38)	<i>P</i> -value
Gestational age (weeks)	29.4 (1.7)	28.8 (1.7)	0.07
Birthweight (kg)	1.2 (0.4)	1.2 (0.3)	0.70
≥ 1 serious infection in the neonatal period ^a	23/38 (61%)	32/38 (84%)	0.04
Vaginal delivery	17/38 (45%)	17/38 (45%)	0.93
Breast feeding until 3 months of age	23/38 (61%)	22/38 (58%)	0.69
Age at follow-up (years)	5.9 (0.8)	6.0 (0.7)	0.66
Smoking at home	3/38 (8%)	5/38 (13%)	0.41
Siblings	33/38 (87%)	31/38 (82%)	0.75
Pets at home	10/38 (26%)	11/38 (29%)	0.59
School attendance ^b	37/38 (97%)	37/38 (97%)	1.0
Parental history of atopy	18/38 (47%)	18/38 (47%)	1.0
High maternal education ^c	16/38 (42%)	11/38 (29%)	0.21

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.

Table 2. Neonatal glutamine-enriched enteral nutrition and allergic disease at 6 years of age

	Glutamine (<i>n</i> = 38)	Control (<i>n</i> = 38)	OR [95% CI]	aOR [95% CI]
Atopic dermatitis ^a	3/38 (8%)	11/38 (29%)	0.21 [0.0, 0.83]	0.23 [0.06, 0.95]
Hay fever ^b	2/38 (5%)	4/38 (11%)	0.49 [0.08, 2.83]	0.60 [0.09, 3.94]
Recurrent wheeze ^c	5/38 (13%)	6/38 (16%)	0.81 [0.22, 2.9]	0.72 [0.17, 3.1]
Asthma ^d	5/38 (13%)	7/38 (18%)	0.67 [0.19, 2.3]	0.59 [0.14, 2.4]

Data are number (%).

OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio (adjusted for maternal education, parental history of atopy, smoking and pets at home).

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

^bDoctor-diagnosed.

^c ≥ 2 episodes of wheezing in the last year.

^dDoctor-diagnosed and use of inhalation medication.

study were: difficulties with the Dutch language (*n* = 4), migration to foreign countries (*n* = 3) and family could not be traced (*n* = 5). Baseline neonatal characteristics and characteristics at 6 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 vs. 6.0 ± 0.7 years in glutamine-supplemented and control groups, respectively (*P* = 0.66). Further details of the neonatal period of the participating infants have been previously described.¹⁷

The association between glutamine-enriched enteral nutrition in the neonatal period and doctor-diagnosed allergic disease at 6 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 or asthma at 6 years of age was found (Table 2). At 1 year of age, 10 infants had been diagnosed with atopic dermatitis.¹⁷ In two of them there were no symptoms of atopic dermatitis at 6 years of age. In six infants the skin rash started after 1 year of age (mostly between 2 and 3 years of age).

The association between glutamine-enriched enteral nutrition in the neonatal period and doctor-diagnosed infectious disease at 6 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 6 years of age was found (Table 3).

Table 3. Neonatal glutamine-enriched enteral nutrition and infectious disease at 6 years of age

	Glutamine (<i>n</i> = 38)	Control (<i>n</i> = 38)	OR [95% CI]	aOR [95% CI]
URTI ^a	14/38 (37%)	18/38 (50%)	0.67 [0.19, 2.33]	0.65 [0.24, 1.7]
LRTI ^b	7/38 (18%)	10/38 (26%)	0.63 [0.21, 1.89]	0.69 [0.21, 2.2]
Gastrointestinal tract infection	2/38 (5%)	8/38 (21%)	0.21 [0.04, 1.01]	0.10 [0.01, 0.93]
Urinary tract infection	4/38 (11%)	1/38 (3%)	4.3 [0.46, 40.8]	4.2 [0.36, 49.1]

Data are number (%).

OR, odds ratio; CI, confidence interval. aOR, adjusted odds ratio (adjusted for maternal education, smoking at home, siblings and school attendance).

^aUpper respiratory tract infection: ≥ 1 episode of flu/serious cold, throat infection, otitis media or laryngitis.

^bLower respiratory tract infection: ≥ 1 episode of bronchitis, bronchiolitis or pneumonia.

Discussion

The results of this study indicate that glutamine-enriched enteral nutrition in VLBW infants between 3 and 30 days of life decreased the risk for atopic dermatitis and gastrointestinal tract infections at 6 years of age. However, there was no association between glutamine-enriched enteral nutrition in the neonatal period and other allergic diseases at 6 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 later in life.

Several epidemiological studies have shown that infectious disease early in life leads to decreased allergic disease later in life (the so-called hygiene hypothesis),^{26,27} although other studies could not confirm this finding.^{28,29} However, these studies have considered term and not preterm infants. In our study, the lower incidence of infectious diseases in the 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 Mommers *et al.*^{28,29} The association between infectious disease early in life and later allergic disease is subject to 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 have reported inverse associations between exposure to endotoxin in house-dust and atopy.^{30,31}

Experimental studies have indicated that the presence of glutamine *in vitro* increases the cytokine production of T-lymphocytes.¹⁶ 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,^{32,33} 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 both in the neonatal period and at 1 year of age. In these studies,^{32,33} stimulation with alphaCD3/alphaCD28 or LPS (lipopolysaccharide) resulted in strong cytokine responses with large inter-individual differences, which may explain why we did

not find a stimulating effect of glutamine-enriched enteral nutrition on Th1 cytokine responses.

In a study in rats, glutamine served as a fuel for enterocytes and improved thickness and optical density of the mucus gel.^{34,35} By enteral supplementation of glutamine in neonates, the mucus layer might change, which might lead to altered bacterial adherence and colonisation of the gut. We found both a lower risk of gastrointestinal tract infections and of atopic dermatitis at 6 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 frequently colonised with bifidobacteria compared to infants without allergic disease, at 1 year of age (unpublished data). The question is whether bacterial colonisation of the gut in these infants remains different at 6 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.³⁶ Certain strains of bifidobacteria might have an antagonistic ability against pathogens, as recently reviewed by Servin.³⁷ Even though a difference in gastrointestinal tract infections was found, we are careful in interpreting these data. These results only involved the past 12 months, viral gastrointestinal tract infections are more common than bacterial gastrointestinal tract infections at this age, and the difference only became significant after adjustment for potential confounders.

The development of allergic diseases is multifactorial, in which breast feeding and mode of delivery may play a role. Although exclusive breast feeding was considered to have a protective role, this role has been disputed in recent studies.^{37,38} Delivery by caesarean section might lead to an increased risk of developing asthma.³⁹ In additional analyses of our study, we found that adjusting for breast feeding and mode of delivery (vaginal delivery vs. caesarean section) did not change the results of the primary analysis. Therefore, we only reported the results of the primary analysis.

Only a few studies are available concerning the prevalence of atopic dermatitis in preterm children. Kvenshagen *et al.* studied the prevalence of atopic dermatitis in preterm and term children at 2 years of age.¹³ They found 19.9% of preterm children to be diagnosed with atopic dermatitis at 2 years of age. In our control group, the prevalence of 29% is higher than expected based on that study. However, the prevalence of 8% in

our study group is significantly lower than expected. Based on these results, we conclude that glutamine-enriched enteral nutrition in the neonatal period in VLBW infants reduces the risk of atopic dermatitis at 6 years of age.

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,¹⁸ the sample size of the follow-up study was relatively small. The response rate of 85% contributed to the reliability of the results, but the CIs were wide and the negative findings should be interpreted with particular caution. Secondly, parental report of disease by questionnaire may be subject to reporting bias. Therefore, we analysed doctor-diagnosed symptoms and conditions in validated questionnaires.^{23,24} Thirdly, parents were not blinded to treatment allocation of their children at 6 years of age. 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 which has been validated and applied in several epidemiological studies. In our cohort, no forced expiratory volume in 1 s was measured; however, the diagnosis of asthma included both 'doctor-diagnosed' asthma and the use of inhalation medication.

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References

- 1 Sears MR. Descriptive epidemiology of asthma. *Lancet* 1997; **350**:1–4.
- 2 Gill S. An overview of atopic eczema in children: a significant disease. *British Journal of Nursing* 2006; **15**:494–499.
- 3 Barker DJ, Godfrey KM, Fall C, Osmond C, Winter PD, Shaheen SO. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airway disease. *British Medical Journal* 1991; **303**:671–675.
- 4 Olesen AB, Ellingsen AR, Olesen H, Juul S, Thestrup-Pedersen K. Atopic dermatitis and birth factors: historical follow up by record linkage. *British Medical Journal* 1997; **314**:1003–1008.
- 5 Stańczyk-Przyłuska A, Ligenza I, Wlaziński J, Chlebna-Sokół D. Evaluation of allergic prevalence in prematurely born children. *Polish Pneumology and Allergy* 2005; **73**:36–40.
- 6 Steffensen FH, Sørensen HT, Gillman MW, Rothman KJ, Sabroe S, Fischer P, *et al.* Low birth weight and preterm delivery as risk factors for asthma and atopic dermatitis in young adult males. *Epidemiology* 2000; **11**:185–188.
- 7 Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. *Clinical & Experimental Allergy* 1993; **23**:504–511.
- 8 Kuehr J, Frischer T, Karmaus W, Meinert R, Barth R, Herrmann-Kunz E, *et al.* Early childhood risk factors for sensitization at school age. *The Journal of Allergy and Clinical Immunology* 1992; **90**:358–363.
- 9 Bråbäck L, Hedberg A. Perinatal risk factors for atopic disease in conscripts. *Clinical & Experimental Allergy* 1998; **28**:936–942.
- 10 Savilahti E, Tuomikoski-Jaakkola P, Järvenpää AL, Virtanen M. Early feeding of preterm infants and allergic symptoms during childhood. *Acta Paediatrica* 1993; **82**:340–344.
- 11 Siltanen M, Kajosaari M, Pohjavouri M, Savilahti E. Prematurity at birth reduces the long-term risk of atopy. *The Journal of Allergy and Clinical Immunology* 2001; **107**:229–234.
- 12 Hoffjan S, Epplen JT. The genetics of atopic dermatitis: recent findings and future options. *Journal of Molecular Medicine* 2005; **83**:682–692.
- 13 Kvenshagen B, Jacobsen M, Halvorsen R. Atopic dermatitis in premature and term children. *Archives of Disease in Childhood* 2009; **94**:202–205.
- 14 Wright RJ, Subramanian SV. Advancing a multilevel framework for epidemiologic research on asthma disparities. *Chest* 2007; **132**:757S–769S.
- 15 Jaakkola JJ, Ahmed P, Ieromnimon A, Goepfert P, Laiou E, Quansah R, *et al.* Preterm delivery and asthma: a systemic review and meta-analysis. *The Journal of Allergy and Clinical Immunology* 2006; **118**:823–830.
- 16 Chang WK, Yang KD, Shiao MF. Effect of glutamine on Th1 and Th2 cytokine responses of human peripheral blood mononuclear cells. *Clinical Immunology* 1999; **93**:294–301.
- 17 van den Berg A, van Zwol A, Moll HA, Fetter WP, van Elburg RM. 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. *Archives of Pediatrics & Adolescent Medicine* 2007; **161**:1095–1101.
- 18 van den Berg A, van Elburg RM, Westerbeek EA, Twisk JW, Fetter WP. Glutamine-enriched enteral nutrition in very-low-birth-weight infants and effects on feeding tolerance and infectious morbidity: a randomized controlled trial. *American Journal of Clinical Nutrition* 2005; **81**:1397–1404.
- 19 Neu J, Roig JC, Meetze WH, Veerman M, Carter C, Millsaps M, *et al.* Enteral glutamine supplementation for very low birth weight infants decreases morbidity. *Journal of Pediatrics* 1997; **131**:691–699.
- 20 Vaughn P, Thomas P, Clark R, Neu J. Enteral glutamine supplementation and morbidity in low birth weight infants. *Journal of Pediatrics* 2003; **142**:662–668.

- 21 Lacey JM, Crouch JB, Benfell K, Ringer SA, Wilmore CK, Maguire D, *et al.* The effects of glutamine-supplemented parenteral nutrition in premature infants. *Journal of Parenteral and Enteral Nutrition* 1996; **20**:74–80.
- 22 Poindexter BB, Ehrenkranz RA, Stoll BJ, Wright LL, Poole WK, Oh W, *et al.* Parenteral glutamine supplementation does not reduce the risk of mortality or late-onset sepsis in extremely low birth weight infants. *Pediatrics* 2004; **113**:1209–1215.
- 23 Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, *et al.* The Generation R Study: design and cohort profile. *European Journal of Epidemiology* 2006; **21**:475–484.
- 24 Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, *et al.* The Generation R Study: design and cohort update until the age of 4 years. *European Journal of Epidemiology* 2008; **23**:801–811.
- 25 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, *et al.* International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *European Respiratory Journal* 1995; **8**:483–491.
- 26 Illi S, von Mutius E, Lau S, Bergmann R, Niggemann B, Sommerfeld C, *et al.* Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *British Medical Journal* 2001; **322**:390–395.
- 27 Williams LK, Peterson EL, Ownby DR, Johnson CC. The relationship between early fever and allergic sensitization at age 6 to 7 years. *The Journal of Allergy and Clinical Immunology* 2004; **113**:291–296.
- 28 Benn CS, Melbye M, Wohlfahrt J, Björkstén B, Aaby P. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. *British Medical Journal* 2004; **328**:1223.
- 29 Mommers M, Swaen GM, Weishoff-Houben M, Creemers H, Freund H, Dott W, *et al.* Childhood infections and risk of wheezing and allergic sensitisation at age 7–8 years. *European Journal of Epidemiology* 2004; **19**:945–951.
- 30 Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, *et al.* Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; **355**:1680–1683.
- 31 Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *The New England Journal of Medicine* 2002; **347**:869–877.
- 32 van den Berg A, van Elburg RM, Vermeij L, van Zwol A, van den Brink GR, Twisk JW, *et al.* Cytokine responses in very low birth weight infants receiving glutamine-enriched enteral nutrition. *Journal of Pediatric Gastroenterology and Nutrition* 2009; **48**:94–101.
- 33 van Zwol A, van den Berg A, Nieuwenhuis EE, Twisk JW, Fetter WP, van Elburg RM. Cytokine profiles in one year old very low birth weight infants after enteral glutamine supplementation in the neonatal period. *Pediatric Allergy and Immunology* 2009; **20**:267–270.
- 34 Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate, and aspartate. *The Journal of Biological Chemistry* 1980; **255**:107–112.
- 35 Khan J, Iiboshi Y, Cui L, Wasa M, Sando K, Takagi Y, *et al.* Alanine-glutamine supplemented parenteral nutrition increases luminal mucus gel and decreases permeability in the rat small intestine. *Journal of Parenteral and Enteral Nutrition* 1999; **23**:24–31.
- 36 Falagas ME, Rafailidis PI, Makris GC. Bacterial interference for the prevention and treatment of infections. *International Journal of Antimicrobial Agents* 2008; **31**:518–522.
- 37 Servin AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiology Reviews* 2004; **28**:405–440.
- 38 Kramer MS. Methodological challenges in studying long-term effects of breast-feeding. *Advances in Experimental Medicine and Biology* 2009; **639**:121–133.
- 39 Tollånes MC, Moster D, Daltveit AK, Irgens LM. Cesarean section and risk of severe childhood asthma: a population-based cohort study. *Journal of Pediatrics* 2008; **153**:112–116.