

Effect of non-human neutral and acidic oligosaccharides on allergic and infectious diseases in preterm infants

Nicky Niele · Annelies van Zwol ·
Elisabeth AM Westerbeek · Harrie N Lafeber ·
Ruurd M van Elburg

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Abstract Short-term supplementation of non-human neutral and acidic oligosaccharides during the first postnatal weeks may enhance the maturation of the immune response in preterm infants and may lead to less allergic and infectious diseases during the first year of life. In a randomized controlled trial, 113 preterm infants (gestational age <32 weeks and/or birth weight <1500 g) were allocated to receive enteral neutral and acidic oligosaccharide supplementation or placebo between days 3 and 30 of life. The median age at follow-up was not different in both groups: 12 months corrected age (interquartile range [IQR], 11–15) in the prebiotic mixture group and 12 months corrected age in the placebo group (IQR, 10–19), respectively. In addition, baseline patient, maternal, and environmental characteristics were not different between the prebiotic mixture ($n=48$) and placebo ($n=46$) group. Incidence of allergic and infectious diseases was assessed by validated questionnaires. In total, 94/98 (96 %) of the eligible, surviving infants participated in this follow-up study. The incidence of atopic dermatitis (odds ratio [OR], 0.80; 95 % confidence interval [CI], 0.24–2.67), bronchial hyper-reactivity (OR, 1.04; 95 % CI, 0.38–2.87) and infections of the upper respiratory (OR, 0.95; 95 % CI, 0.37–2.44), lower respiratory (OR, 1.03; 95 % CI, 0.37–2.88), and gastrointestinal (OR, 1.77; 95 % CI, 0.55–5.73) tract was not different between the groups. Adjustment for potential confounding factors did not change the results of the primary analysis. **Conclusion:** Short-term enteral supplementation of

non-human neutral and acidic oligosaccharides during the neonatal period in preterm infants does not decrease the incidence of allergic and infectious diseases during the first year of life.

Keywords Allergic diseases · Infectious diseases · Neutral and acidic oligosaccharides · Preterm infants

Introduction

As survival of preterm infants has increased over the past decades, reduction of morbidity of these infants is an important issue. Preterm birth may play a role in the development of allergic disease; however, conflicting results have been found so far in previous studies [7, 13, 18]. The first postnatal weeks of extra-uterine life represent a critical time window for the initiation of immunological changes resulting in the development of atopy [36].

The intestinal microbiota plays an important role in the maturation of the innate and adaptive immune system [12]. The intestinal microbiota communicates extensively with the intestinal immune system, and this interaction leads to metabolic and immunologic reactions by the epithelial cells and the underlying lymphoid cells and is called the bacterial-epithelial “cross talk” [19]. The intestinal bacterial colonization with bifidobacteria and lactobacilli is delayed in preterm infants, whereas the number of pathogenic bacteria is higher [40]. Several authors have found that especially a balanced intestinal microbiota with a dominance of bifidobacteria and lactobacilli may decrease the risk to develop allergic diseases [14, 33]. Human milk increases the growth of bifidobacteria and lactobacilli in preterm infants [29]. As human milk is not always sufficiently available, attempts have been made to develop a formula similar to human milk.

N. Niele · A. van Zwol · E. A. Westerbeek · H. N. Lafeber ·
R. M. van Elburg (✉)
Department of Pediatrics, Division of Neonatology,
VU University Medical Center Amsterdam,
De Boelelaan 1117,
1081 HV Amsterdam, The Netherlands
e-mail: rm.vanelburg@vumc.nl

R. M. van Elburg
Danone Research, Centre for Specialised Nutrition,
Wageningen, The Netherlands

An important component of human milk is non-digestible human milk oligosaccharides. Over 200 human milk oligosaccharides have been identified with significant variability between individuals over time [24]. Of these oligosaccharides, approximately 80 % are neutral and 20 % are acidic oligosaccharides [4]. Non-human milk oligosaccharides, such as neutral short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) and pectin-derived acidic oligosaccharides (pAOS), have been produced to come closer to the effects of these human milk oligosaccharides. Neutral oligosaccharides have been studied in term and preterm infants [1–3, 6, 10, 17, 21–23]. Supplementation of neutral oligosaccharides stimulates a bifidogenic flora with a decrease of pathogens [3, 17, 23], attenuates the Th2 allergy response [30], reduces the incidence of intestinal and respiratory infections in term infants [1, 2, 6], and certain clinical allergic diseases (atopic dermatitis, recurrent wheezing, and allergic urticaria) in term infants [2, 10, 22]. However, most studies using supplementation with neutral oligosaccharides have been performed in term infants, limiting the evidence of the possible benefits of supplementation in preterm infants.

In vitro, acidic oligosaccharides from aqueous carrot extracts have an anti-adhesive and antimicrobial effect [15], human milk-derived acidic oligosaccharides have an immuno-modulatory effect by directly stimulating the immune system [9], and a mixture with pAOS better attenuates a proallergic Th2-type immune response than neutral oligosaccharides alone [38]. Few clinical studies have been performed with acidic oligosaccharides in addition to neutral oligosaccharides [10, 41]. In a randomized controlled trial with neutral and acidic oligosaccharides, the incidence of endogenous infections and ≥ 2 serious infectious episodes was lower in infants who received a mean supplementation dose of ≥ 50 % of the maximum supplementation during the study period [39]. We hypothesized that short-term supplementation of neutral and acidic oligosaccharides during the first postnatal weeks may enhance the maturation of the immune response in preterm infants and may lead to less allergic and infectious diseases during the first year of life.

Therefore, the aim of this follow-up study was to determine the effect of short-term enteral supplementation of non-human neutral and acidic oligosaccharides (scGOS/lcFOS/pAOS) during the neonatal period in preterm infants on the incidence of allergic and infectious diseases during the first year of life.

Materials and methods

Trial design and patients

In a double-blind, placebo controlled randomized controlled trial (trial registration: ISRCTN 16211826), 113 infants with a gestational age < 32 weeks and/or birth weight < 1500 g,

admitted to the level III neonatal intensive care unit (NICU) of the VU University Medical Center, Amsterdam [39, 41], were included. The endpoint of the present follow-up study was the incidence of allergic and infectious diseases during the first year of life. Further details of the study design and the primary outcome have been previously published [39, 41].

Randomization, blinding, and treatment

After assignment to one of three birth weight groups (≤ 799 , 800–1199, and ≥ 1200 g), infants were randomly allocated < 48 h after birth to receive either enteral supplementation of a prebiotic mixture of 80 % scGOS/lcFOS and 20 % pAOS or a placebo mixture of maltodextrin. An independent researcher used a computer-generated randomization table (provided by Danone Research, Friedrichsdorf, Germany) to assign infants to treatment with the prebiotic mixture or placebo. Investigators, parents, and medical and nursing staff were unaware of treatment allocation. The randomization code was broken after complete data analysis was performed.

The prebiotic mixture and the placebo were prepared and packed sterile (Danone Research, Friedrichsdorf, Germany). The two powders were indistinguishable by appearance, color, and smell. During the study period, both mixtures were monitored for stability and possible microbiological contamination.

The supplementation of the prebiotic mixture or placebo was administered in increasing doses between days 3 and 30 of life to a maximum of 1.5 g/kg/day to human milk or preterm formula (Nenatal Start®). Due to osmolarity reasons, each infant had an individual feeding scheme, depending on birth weight and daily amount of feeding. If an infant received ≤ 100 mL/kg/day enteral feeding, 1 g scGOS/lcFOS/pAOS or placebo was added per 60 mL enteral feeding. If an infant received > 100 mL/kg/day, 1 g scGOS/lcFOS/pAOS or placebo was added per 100 mL enteral feeding. Two members of the nursing staff added the daily supplementation to human milk or to preterm formula according to the parents' choice.

After discharge, all infants received human milk or Nenatal Start (without oligosaccharides or probiotics) until term and Nenatal 1 (without oligosaccharides or probiotics) until the corrected age of 6 months.

Outcome measures

Validated questionnaires were sent to the parents of all participating infants before the outpatient clinic visit of their infant at the corrected age of 1 year. The questionnaire was adapted from questionnaires used in the Prevention and Incidence of Asthma and Mite Allergy Study [5] and the Generation R Study [11] and was designed to collect data on allergic and infectious diseases and environmental, lifestyle, demographic, and socio-economic characteristics. The questionnaire included the

following sections: growth status, allergic diseases, infectious diseases, fever episodes, environment, and questions about the parents. Further details of the questionnaire have been previously published [35].

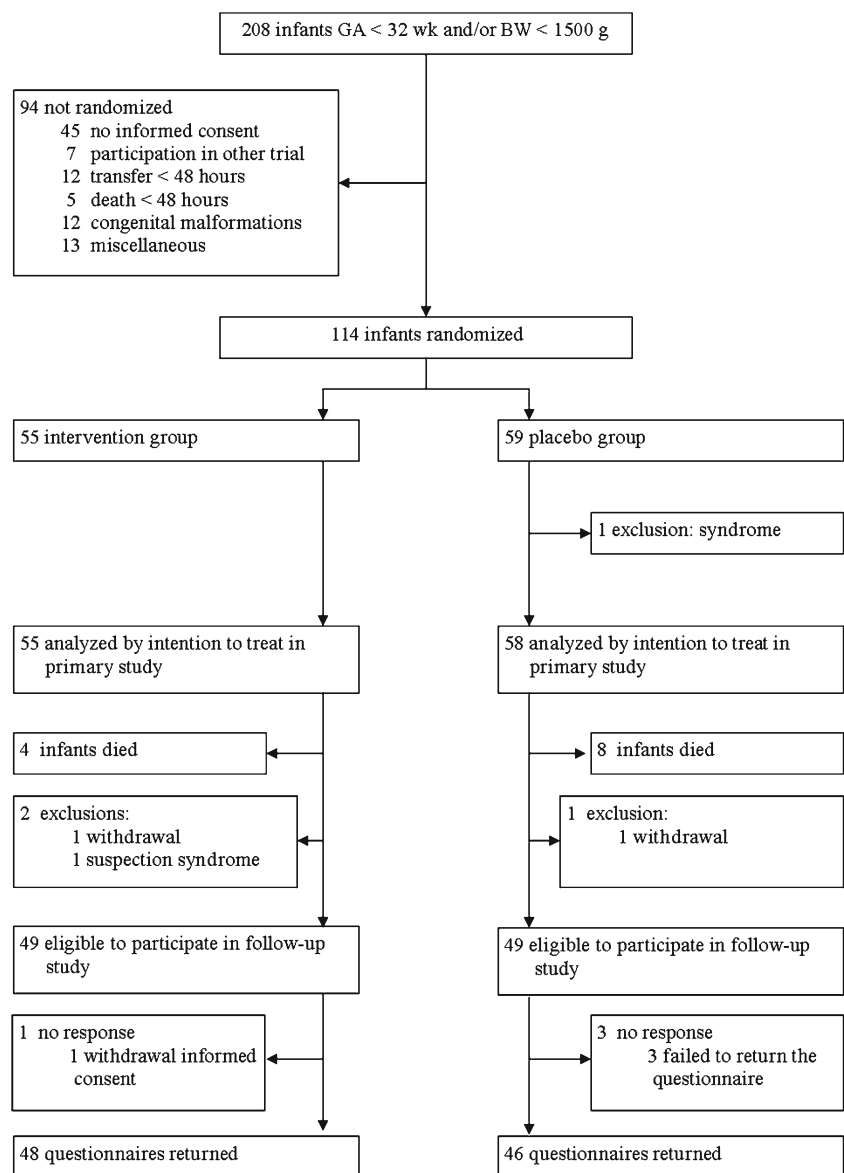
Allergic diseases were defined as previously described [35, 37]. Allergic diseases consisted of atopic dermatitis and bronchial hyper-reactivity. Atopic dermatitis was defined as a physician-diagnosed rash on at least one typical location, such as flexural sites (ankle, knee, and elbow) or around the eyes and ears. Bronchial hyper-reactivity was diagnosed in case of at least three 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 one physician-diagnosed episode of severe rhinitis, pharyngitis, or otitis media. Lower respiratory tract infections included at least

one physician-diagnosed episode of bronchitis, bronchiolitis, or pneumonia. Gastrointestinal tract infections, urinary tract infections, sepsis, and meningitis included only physician-diagnosed episodes [35].

Statistical analysis

Infant, maternal, and environmental characteristics were analyzed by unpaired *t* test, Mann–Whitney, and χ^2 or Fisher's exact test for continuous normally distributed, nonparametric continuous, and dichotomous data, respectively. Logistic regression analysis was performed to determine whether supplementation with the prebiotic mixture during the neonatal period influenced the incidence of allergic and infectious diseases during the first year of life. In additional analyses, adjustments were made for maternal education,

Fig 1 Profile of the initial and follow-up study



family history of atopy, smoking, the presence of pets at home, and exclusive breastfeeding in the neonatal period 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* value <0.05 (two-tailed) was considered significant. SPSS 19.0 (SPSS Inc, Chicago, IL) was used for data analysis.

Ethical approval

The study protocol is approved by the medical ethical review board of VU University Medical Center Amsterdam. Written informed consent was obtained from all parents.

Results

In total, 98/113 infants from the initial study were eligible at the corrected age of 1 year (12 infants died: four in the prebiotic mixture group and eight in the placebo group. The most common causes of death were respiratory and/or circulatory insufficiency or multiorgan failure as a result of necrotizing enterocolitis or infection; three were excluded: two on request of the parents and one because of suspicion of a metabolic syndrome). Of the eligible patients, 94/98 (96 %) infants participated in the follow-up study. Reasons for not participating in the follow-up study were: failure to return the questionnaire (*n*=3) and withdrawal from the

study (*n*=1) (Fig. 1). Baseline infant, maternal, environmental, and nutritional characteristics were not different in the prebiotic mixture and the placebo group. Median age at follow-up was not different in both groups: 12 months corrected age (interquartile range [IQR], 11–15) in the prebiotic mixture group and 12 months corrected age in the placebo group (IQR, 10–19), respectively (Table 1).

In total, 94 questionnaires were returned of which 91 questionnaires could be analyzed for allergic diseases. Three questionnaires could not be analyzed because of unknown family history of atopy. For infectious diseases, 92 questionnaires could be analyzed as parents of two infants did not record data on infections. The incidence of physician-diagnosed allergic diseases was not different in the prebiotic mixture and the placebo group, also after adjustment for maternal education, family history of atopy, smoking, pets at home, and exclusive breastfeeding during neonatal period (Table 2). The incidence of physician-diagnosed upper and lower respiratory and gastrointestinal tract infections was not different in the prebiotic mixture and the placebo group, also after adjustment for maternal education, smoking at home, siblings, and child care attendance (Table 3). Statistical analysis could not be performed for urinary tract infections (*n*=1, prebiotic mixture group), meningitis (*n*=0), and sepsis (*n*=0). In additional analyses, adjustment for human milk feeding and the use of antibiotics did not affect the results of the primary analyses.

Table 1 Baseline characteristics

	Prebiotic mixture (<i>n</i> =48)	Placebo (<i>n</i> =46)
Gestational age (weeks)	30.1 (1.6)	29.5 (2.0)
Birth weight (kg)	1.4 (0.4)	1.3 (0.3)
Vaginal delivery	27/48 (56 %)	26/46 (57 %)
≥1 serious infection in the neonatal period ^a	18/48 (38 %)	21/46 (46 %)
≥2 serious infections in the neonatal period	4/48 (8 %)	7/46 (15 %)
≥1 serious endogenous infection in the neonatal period ^b	6/48 (13 %)	10/46 (22 %)
Antibiotic use during the neonatal period	35/48 (73 %)	35/46 (76 %)
Mechanical ventilation requirement during the neonatal period	25/48 (52 %)	24/46 (52 %)
Corrected age at follow-up (months)	12 (11–15)	12 (10–19)
Family history of atopy	31/48 (65 %)	21/43 (49 %)
Maternal history of atopy	26/48 (54 %)	18/43 (42 %)
Smoking	2/48 (4 %)	6/46 (13 %)
Siblings	20/48 (42 %)	23/46 (50 %)
Pets at home	22/48 (46 %)	18/46 (39 %)
Child care attendance ^c	35/48 (73 %)	32/46 (70 %)
High maternal education ^d	28/48 (58 %)	20/46 (44 %)
Exclusive breastfeeding during the neonatal period	31/48 (65 %)	28/46 (61 %)
Breastfeeding ≥3 months	12/48 (25 %)	12/46 (26 %)
Breastfeeding ≥6 months	6/48 (13 %)	6/46 (13 %)

Data are mean (SD), median (interquartile range), or number (percent)

^aA new positive culture after adequate antibiotic treatment of the previous infectious episode with clinical recovery

^bNon-coagulase-negative staphylococci infections (non-CoNS infections)

^c>1 day/week

^dHigher professional or university education

Table 2 Prebiotic mixture and allergic diseases at 1 year of age

	Prebiotic mixture (<i>n</i> =48)	Placebo (<i>n</i> =43)	OR (95 % CI)	aOR (95 % CI)
Atopic dermatitis ^a	7/48 (15 %)	8/43 (19 %)	0.75 (0.25–2.27)	0.80 (0.24–2.67)
Bronchial hyper-reactivity ^b	14/48 (29 %)	10/43 (23 %)	1.36 (0.53–3.49)	1.04 (0.38–2.87)

Data are number (percent)

OR odds ratio, CI confidence interval, aOR adjusted odds ratio (adjusted for maternal education, family history of atopy, smoking, pets at home, and exclusive breastfeeding during neonatal period)

^a Skin rash on ≥ 1 typical location

^b Indicates ≥ 3 of the following symptoms: dyspnea, wheezing, humming/sawing breath sounds, or nightly dry cough without rhinitis

Discussion

Short-term supplementation of a prebiotic mixture of non-human neutral and acidic oligosaccharides during days 3–30 of life in preterm infants had no effect on the incidence of allergic and infectious diseases during the first year of life. Previous clinical studies in both term and preterm infants with oligosaccharides showed varying results emphasizing the role of composition and duration of supplementation and study population [1, 2, 6, 8, 10, 22].

In term infants with a high risk of allergy, supplementation with neutral oligosaccharides during the first 6 months of life had a protective effect on both atopic dermatitis [22] and infections [1]. At 2 years of age, the protective effect persisted for atopic dermatitis, recurrent wheeze, allergic urticaria, and infections [2]. In an observational study in unselected healthy term infants, a formula with neutral oligosaccharides during 12 months reduced intestinal infections during the first year of life [6]. On the contrary, shorter supplementation of neutral oligosaccharides during 12 weeks in healthy infants did not reduce the incidence of allergic diseases [8]. In term infants at low risk of atopy, a formula with prebiotic mixture with neutral and acidic oligosaccharides (pAOS) during 6 months had positive effect on primary prevention of atopic dermatitis

[10]. In summary, the preventive effect of supplementation of oligosaccharides on allergic and infectious diseases seems to be dependent on the dosage and duration of supplementation [23] and the risk of allergy of the study population. In the present study, we supplemented the prebiotic mixture for 1 month because the first month of life represents a critical time window for the initiation of immunological changes [36]. Reasons that in the present study short-term supplementation with neutral and acidic oligosaccharides had no positive effect on the development of atopy and allergy were found, may be first of all because both preterm infants at high and at low risk for atopy were included. Secondly, 63 % of the infants in our study were exclusively fed with human milk during the neonatal period. However, in an additional post hoc analysis, we did not find a difference in outcome after adjustment for human milk feeding during the immediate neonatal period, a period of ≥ 3 months, and a period of ≥ 6 months. Thirdly, in preterm infants, there are many confounding factors related to the type of treatment that may influence the development of allergic diseases [16]. Therefore, a larger cohort is required to evaluate the effect in preterm infants at high or low risk for atopy. In preterm infants, the intestinal colonization with beneficial bacteria is already delayed [40] which could further be delayed by the frequent use of antibiotics [31]. In some

Table 3 Prebiotic mixture and infectious diseases at 1 year of age

	Prebiotic mixture (<i>n</i> =48)	Placebo (<i>n</i> =44)	OR (95 % CI)	aOR (95 % CI)
URTI ^a	34/48 (71 %)	30/44 (68 %)	1.13 (0.47–2.76)	0.95 (0.37–2.44)
LRTI ^b	12/48 (25 %)	9/44 (21 %)	1.30 (0.49–3.46)	1.03 (0.37–2.88)
Gastrointestinal tract infection	10/48 (21 %)	6/44 (14 %)	1.67 (0.55–5.05)	1.77 (0.55–5.73)
Urinary tract infection	1/48 (2 %)	0/44 (0 %)	— ^c	— ^c
Sepsis/meningitis	0/48 (0 %)	0/44 (0 %)	— ^c	— ^c

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

^a Upper respiratory tract infection: ≥ 1 episode of serious rhinitis, pharyngitis, or otitis media

^b Lower respiratory tract infection: ≥ 1 episode of bronchitis, bronchiolitis, or pneumonia

^c Incidence did not allow statistical analysis

studies, an association between early use of antibiotics and the later development of allergic diseases was found [20], although this association is weak in other studies [28, 42]. In this framework, two types of hypothesis may apply: the so-called hygiene hypothesis [43] and the microbiota hypothesis [32]. These hypotheses have been postulated to explain the increase in the incidence of allergic diseases during the past decades in developed countries [25, 26]. The “hygiene hypothesis” implies that early childhood infections inhibit the tendency to develop allergic diseases later in life [43]. The “microbiota hypothesis” implies that perturbations in the intestinal microbiota in developed countries (due to antibiotic use, dietary changes, and other lifestyle differences) have disrupted the normal microbiota-mediated mechanisms of immunological tolerance [32]. Preterm infants are frequently exposed to high doses of broad-spectrum antibiotics early in life which could further delay the development of the intestinal colonization [31]. We hypothesize that the frequent use of broad-spectrum antibiotics in our NICU could have reduced the effect of the prebiotic mixture on the intestinal microbiota, leading to a less pronounced effect on the immune system and consequently on the development of allergic diseases. However, adjustment for use of antibiotics in our study did not change the results of the primary analyses. Interestingly, we did find a relation between early antibiotics and the development of allergic diseases later in life. However, as our study was not designed to determine whether this relation is causal, no firm conclusions can be drawn from this analysis.

To limit the effect of reporting bias, we only analyzed physician-diagnosed symptoms and diseases with a validated questionnaire [5, 11, 35]. One of the difficult differential diagnoses during infancy is to distinguish between viral-induced wheezing and multifactor-induced wheezing. Viral infections have been implicated in the pathogenesis of asthma and have been shown to account for 50 % of the exacerbations of asthma [27]. Furthermore, the expression of allergic diseases varies with age. Atopic dermatitis, starting in early childhood, is a major risk factor for asthma and allergic rhinitis later in life [34]. It may have been too early to diagnose some manifestations of allergic diseases, such as asthma and allergic rhinitis.

In conclusion, short-term enteral supplementation of a prebiotic mixture of non-human neutral and acidic oligosaccharides between days 3 and 30 of life in preterm infants does not decrease the incidence of allergic and infectious diseases during the first year of life. Long-term follow-up of this cohort regarding health and disease is important.

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Conflict of interest RvE is also an employee of Danone Research. None of the authors had a financial relationship with the funding source.

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