ORIGINAL ARTICLE AIRWAY DISEASES

Synbiotics prevent asthma-like symptoms in infants with atopic dermatitis

L. B. van der Aa[1], W. M. C. van Aalderen[1], H. S. A. Heymans[2], J. Henk Sillevis Smitt[3], A. J. Nauta[4], L. M. J. Knippels[4], K. Ben Amor[4], A. B. Sprikkelman[1] & the Synbad Study Group*

[1]Department of Pediatric Respiratory Medicine and Allergy, Emma Children's Hospital, Academic Medical Center; [2]Department of Pediatrics, Emma Children's Hospital, Academic Medical Center; [3]Department of Dermatology, Academic Medical Center, Amsterdam; [4]Danone Research – Centre of Specialised Nutrition, Wageningen, the Netherlands

To cite this article: van der Aa LB, van Aalderen WMC, Heymans HSA, Henk Sillevis Smitt J, Nauta AJ, Knippels LMJ, Ben Amor K, Sprikkelman AB, the Synbad Study Group. Synbiotics prevent asthma-like symptoms in infants with atopic dermatitis. *Allergy* 2011; **66**: 170–177.

Keywords
asthma; prebiotics; prevention; probiotics; synbiotics.

Correspondence
Leontien B. van der Aa, Department of Pediatric Respiratory Medicine and Allergy, Emma Children's Hospital, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands.
Tel.: +31205662968
Fax: +31206917735
E-mail: l.b.vanderaa@amc.nl

*E. K. George, MD, PhD, Department of Pediatrics, Medical Center Alkmaar, Alkmaar, the Netherlands.
N. J. van den Berg, MD, Department of Pediatrics, FlevoHospital, Almere, the Netherlands.
D. Bosman, MD, PhD, Department of Pediatrics, Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands.
G. A. M. Tytgat, MD, PhD, Department of Pediatrics, Zaans Medical Center, Zaandam, the Netherlands.
E. Ree, MD, Department of Pediatrics, Sint Lucas Andreas Hospital, Amsterdam, the Netherlands.
M. A. van Houten, MD, PhD, Department of Pediatrics, Spaarne Hospital, Hoofddorp, the Netherlands.

Accepted for publication 15 April 2010

DOI:10.1111/j.1398-9995.2010.02416.x

Edited by: Hans-Uwe Simon

Abstract

Background: Infants with atopic dermatitis (AD) have a high risk of developing asthma. We investigated the effect of early intervention with synbiotics, a combination of probiotics and prebiotics, on the prevalence of asthma-like symptoms in infants with AD.

Methods: In a double-blind, placebo-controlled multicentre trial, ninety infants with AD, age <7 months, were randomized to receive an extensively hydrolyzed formula with *Bifidobacterium breve* M-16V and a galacto/fructooligosaccharide mixture (Immunofortis®), or the same formula without synbiotics during 12 weeks. After 1 year, the prevalence of respiratory symptoms and asthma medication use was evaluated, using a validated questionnaire. Also, total serum IgE and specific IgE against aeroallergens were determined.

Findings: Seventy-five children (70.7% male, mean age 17.3 months) completed the 1-year follow-up evaluation. The prevalence of 'frequent wheezing' and 'wheezing and/or noisy breathing apart from colds' was significantly lower in the synbiotic than in the placebo group (13.9% *vs* 34.2%, absolute risk reduction (ARR) −20.3%, 95% CI −39.2% to −1.5%, and 2.8% *vs* 30.8%, ARR −28.0%, 95% CI −43.3% to −12.5%, respectively). Significantly less children in the synbiotic than in the placebo group had started to use asthma medication after baseline (5.6% *vs* 25.6%, ARR −20.1%, 95% CI −35.7% to −4.5%). Total IgE levels did not differ between the two groups. No children in the synbiotic and five children (15.2%) in the placebo group developed elevated IgE levels against cat (ARR −15.2%, 95% CI −27.4% to −2.9%).

Conclusion: These results suggest that this synbiotic mixture prevents asthma-like symptoms in infants with AD.

Atopic dermatitis (AD) is a chronic, itching, inflammatory skin disease that often presents in infancy (1). The disease can be the first manifestation of the so-called atopic march, the natural progression of allergic disorders, with subsequent development of asthma and allergic rhinitis. Approximately 40% of the children with AD will develop asthma later in childhood (2).

Over the past decades, the prevalence of allergic disease has risen in Western countries. This increase is hypothesized, among other things, to result from diminished microbial exposure, leading to an altered composition of the intestinal microbiota (3). It has been shown that intestinal microbiota composition differs between children with and without atopy. This difference precedes sensitization and clinical symptoms, suggesting a causal relationship (4, 5).

Modulation of the intestinal microbiota with probiotics, living micro-organisms with immunomodulatory effects, or prebiotics, nondigestible food ingredients that stimulate the growth and/or activity of one or a limited number of beneficial gut bacteria (6), could possibly offer a new way of prevention or treatment of allergic disease. Clinical trials in this area have focused mainly on AD; however, in view of the high prevalence of asthma in children with AD, another important aspect is to explore whether probiotics and prebiotics can bring the atopic march to a halt and prevent subsequent development of allergic airway disease in these children.

Animal studies show that probiotics can inhibit the allergic airway response and allergic sensitization by induction of regulatory T cells (7, 8). However, in several clinical trials investigating the prevention of allergic disease with probiotics in high-risk children (children with allergic parents or siblings), no effect on the prevalence of asthma or asthma-like symptoms was found (9–13). In most of these trials, *Lactobacillus* species were used. Because probiotic effects are strain specific (14), other probiotic strains might be more efficacious. A specific prebiotic oligosaccharide mixture that increases the amount of bifidobacteria in the colon has been shown to significantly reduce the incidence of recurrent wheezing in 2-year-old children with atopic parents (15), suggesting that bifidobacteria might be good candidates for prevention of allergic airway disease. Up to now, no trials have been conducted to explore whether probiotics, prebiotics or a combination of both, i.e. synbiotics, can prevent the development of asthma or asthma-like symptoms in children with AD.

We recently performed a randomized controlled trial of 12 weeks to study the effect of a synbiotic combination of *Bifidobacterium breve* M-16V and a specific mixture of 90% short-chain galactooligosaccharides (scGOS) and 10% long-chain fructooligosaccharides (lcFOS) (Immunofortis®, Nutricia Cuijk B. V, Cuijk, the Netherlands) on AD severity in infants (16). This specific synbiotic mixture has been shown to inhibit the allergic airway response in mice (17) and to reduce allergen-specific Th2-responses and improve peak expiratory flow in allergic asthmatic adults (van de Pol et al., submitted for publication). Participants of our trial were followed up 1 year after the intervention to evaluate the prevalence of asthma-like symptoms and asthma medication use.

Methods

Participants

Ninety full-term infants, aged <7 months, fulfilling Hanifin and Rajka criteria for AD (18), were recruited between September 2005 and February 2007 from Pediatric and Dermatologic outpatient clinics of seven participating hospitals, regional Baby Health Clinics, and through advertisements in magazines. Inclusion criteria included a SCORing Atopic Dermatitis (SCORAD) (19) score >15, exclusive formula feeding at time of enrolment, no other major medical problems, and no use of probiotics or immunomodulatory medication during the 4 weeks before enrolment. Written informed consent was obtained from both parents of all participants.

Study design

Participants were randomized, using computer-generated four-block design lists, drawn up by a statistician, with stratification according to recruiting hospital and current use of topical steroids, to receive either an extensively hydrolyzed whey-based formula (Nutrilon Pepti®; Nutricia, Zoetermeer, the Netherlands) with additional synbiotics or the same formula without synbiotics for a period of 12 weeks. Patients were enrolled by the investigator (LvdA) and sequentially assigned a patient number connected to a formula code. Formulas were prepared and coded by Danone Research and dispensed by the pharmacy of the Academic Medical Center. Both formulas were identical with respect to smell, taste, texture, color and packaging. The investigator (LvdA), participant's own physicians and parents were all blind to the treatment groups. One year after the start of the intervention period, participants returned for a follow-up visit, performed by the same investigator, who was still blinded to the treatment groups. During this visit, parents were asked about respiratory symptoms (cough, shortness of breath, noisy/rattly breathing, wheezing) and medication use of their child, using a validated questionnaire (20, 21). The protocol was approved by the Medical Ethics Committees of all participating centers. The trial is registered in the ISRCTN register: ISRCTN69085979.

Synbiotics

Synbiotics consisted of *B. breve* M-16V (Morinaga Milk Industry Co, Ltd., Tokyo, Japan) at a dose of 1.3×10^9 cfu/100 ml and a mixture of 90% scGOS and 10% lcFOS (Immunofortis®), 0.8 g/100 ml (22). Formula was given on demand. The product was stable for at least 18 months when stored at room temperature (20–25°C).

Outcome measures

The primary outcome measure of this randomized controlled trial, change in severity of AD after 12 weeks of intervention, is published elsewhere (16). Respiratory outcome measures at follow-up were as follows: (i) prevalence of respiratory symptoms predictive of asthma: frequent wheezing, defined

as ≥3 episodes after the intervention period, and wheezing apart from colds (23), (ii) current use of asthma medication (beta-2 agonists, anticholinergics, inhaled corticosteroids, prescribed by patient's own physicians) and (iii) total serum IgE levels and the presence of elevated specific IgE (≥0.35 kU/l) against aeroallergens.

Blood samples

At baseline and 1-year follow-up, total serum IgE and specific IgE levels against house dust mite (HDM) (d1), cat (e1) and dog (e2) were determined using the CAP FEIA system (Phadia, Uppsala, Sweden). Specific IgE was considered elevated if ≥0.35 kU/l. A subgroup of patients with IgE-negative AD was defined as patients with AD without elevated total and/or specific IgE levels at baseline (total IgE was considered elevated if ≥5 kU/l in infants aged <3 months and ≥15 kU/l in infants aged >3 months, reference values of the Academic Medical Center, Amsterdam).

Statistics

Sample size was determined for the primary outcome of the study, AD severity (16). To detect a clinically relevant 25% difference in SCORAD score reduction between the synbiotic and placebo group at a 5% significance level with 80% power, 35 children per group were required. To allow for a 20% drop out rate, 90 children were included in the original study. Data analysis was carried out according to a pre-established statistical analysis plan. All analyses were carried out on intention-to-treat basis. Parametric data were analyzed with unpaired *t*-tests. Nonparametric data were analyzed with the Mann–Whitney *U*-test. Binary data were analyzed using the χ^2-test, or Fisher's exact test when appropriate, and results are represented as absolute risk reduction (ARR) with 95% confidence intervals (CI). Bivariate logistic regression analyses were performed to investigate potential confounders. For each of the respiratory outcome measures (frequent wheezing, wheezing apart from colds, wheezing and/or noisy/rattly breathing apart from colds and asthma medication use), a univariate model was made with treatment as the determinant and nine bivariate models were made in which treatment was combined with the following variables as second covariate: gender, SCORAD score at baseline, elevated total or specific IgE at baseline (yes/no), breastfed before the intervention (yes/no), asthma medication use at baseline, parental smoking, parental asthma, furry pets in the home and early day care attendance. If the regression parameter (beta) of treatment was changed >10% by entering another covariate in the model, this covariate was considered to be a confounder, and the adjusted effect of treatment was reported. SPSS software (15.0, Chicago, IL, USA) was used for all analyses.

Role of the study sponsor

The study was designed by LvdA, WvA, HH, JSS, AS and the Synbad Study Group in collaboration with the study sponsor. The study sponsor had no role in the collection, analysis and interpretation of the data. LvdA wrote the first draft of the manuscript and had full access to all the data.

Results

Baseline characteristics

Eighty-two infants completed the intervention. Of these children, 75 (91%) completed the 1-year follow-up evaluation (Fig. 1). Baseline characteristics of all randomized children and of the children who completed the follow-up visit are shown in Table 1. There were no significant differences between the two groups. Mean age at 1-year follow-up was 17.5 months (SD 1.6) in the synbiotic and 17.2 (SD 1.8) in the placebo group.

Asthma-like symptoms and asthma medication use

The prevalence of asthma-like symptoms and use of asthma medication in the synbiotic and the placebo group at 1-year follow-up are shown in Table 2. Frequent wheezing (≥3 episodes after the intervention period), and wheezing and/or noisy/rattly breathing apart from colds were significantly less prevalent in the synbiotic than in the placebo group. Wheezing apart from colds did not differ significantly between the two groups ($P = 0.056$). Significantly fewer children in the

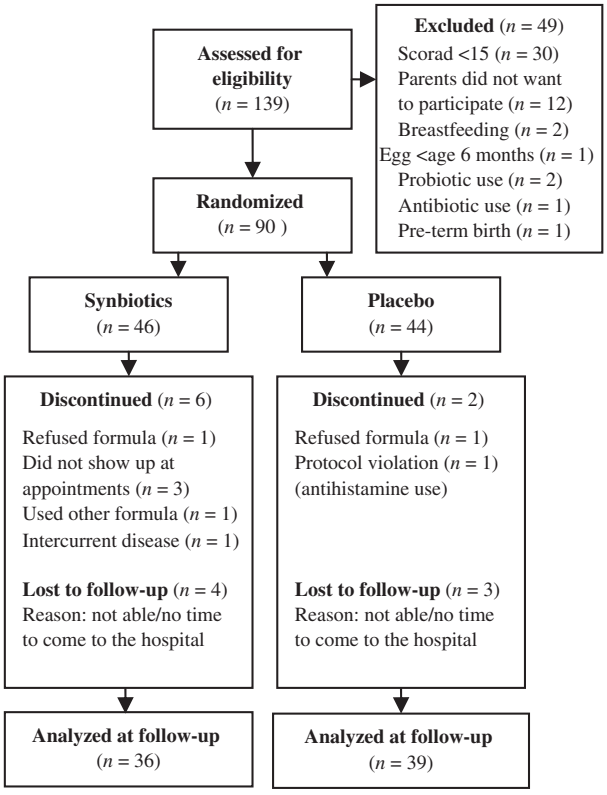

Figure 1 Flowchart of the participants.

Table 1 Baseline characteristics of all randomized children and those who completed the 1-year follow-up visit

	All randomized children		Children seen at follow-up	
	Synbiotics (n = 46)	Placebo (n = 44)	Synbiotics (n = 36)	Placebo (n = 39)
Male, *n* (%)	31 (67.4)	28 (63.6)	27 (75.0)	26 (66.7)
Age at baseline (months), mean (SD)	5.0 (1.4)	4.8 (1.5)	4.9 (1.4)	4.8 (1.6)
SCORAD index, mean (SD)	35.6 (10.6)	34.7 (12.6)	35.4 (10.8)	33.9 (10.6)
Breastfed before intervention, *n* (%)	34 (73.9)	32 (72.7)	26 (72.2)	27 (69.2)
Duration of breastfeeding (weeks), median (range)	8.0 (1–24)	8.0 (2–22)	9 (1–24)	9 (2–22)
Parental asthma, *n* (%)	17 (37.8)	20 (45.5)	13 (37.1)	18 (46.2)
Parental smoking, *n* (%)	16 (35.6)	12 (27.3)	10 (28.6)	11 (28.2)
Day care, *n* (%)	14 (30.4)	13 (29.5)	11 (30.6)	13 (33.3)
Pets, *n* (%)	14 (30.4)	15 (34.1)	9 (25.0)	13 (33.3)
Cats	6 (13.0)	7 (15.9)	3 (8.3)	7 (17.9)
Dogs	5 (10.9)	6 (13.6)	4 (11.1)	6 (15.4)
Older siblings, *n* (%)	26 (56.5)	21 (47.7)	21 (58.3)	18 (46.2)
Probiotic use after study, *n* (%)			1 (3.0)	2 (5.9)
Asthma medication, *n* (%)	5 (10.9)	5 (11.4)	5 (13.9)	5 (12.8)
Cough*, *n* (%)	30 (65.2)	31 (70.5)	25 (69.4)	28 (71.8)
Wheezing*, *n* (%)	10 (21.7)	11 (25.0)	7 (19.4)	8 (20.5)
Noisy/rattly breathing*, *n* (%)	19 (41.3)	26 (59.1)	17 (47.2)	23 (59.0)

SCORAD, SCORing Atopic Dermatitis.
*During the 2 weeks before baseline, parent reported.

Table 2 Prevalence of asthma-like symptoms and asthma medication use at 1-year follow-up

	Synbiotics (n = 36) *n* (%)	Placebo (n = 39) *n* (%)	Difference (ARR) (95% CI) %	*P*-value*
Frequent wheezing†	5 (13.9)	13 (34.2) [n = 38]	−20.3 (−39.2 to −1.5)	0.04
Wheezing apart from colds	1 (2.8)	7 (17.9)	−15.2 (−28.4 to −2.0)	0.056
Wheezing and/or noisy breathing apart from colds	1 (2.8)	12 (30.8)	−28.0 (−43.4 to −12.5)	0.001
Asthma medication	5 (13.9)	13 (33.3)	−19.4 (−38.1 to −0.8)	0.049
Asthma medication at follow-up and not at baseline (new users)	2 (5.6)	10 (25.6)	−20.1 (−35.7 to −4.5)	0.02

ARR, absolute risk reduction.
*χ^2-test.
†≥3 episodes after intervention period.

synbiotic group than in the placebo group used asthma medication at time of follow-up. There were also significantly less new users of asthma medication (children who were using asthma medication at follow-up, but not at baseline) in the synbiotic than in the placebo group. None of the potential confounders changed the beta of treatment >10%, and therefore none of these variables were considered to be confounders.

IgE sensitization

Total IgE

Median total serum IgE concentrations at baseline and 1-year follow-up are shown in Fig. 2. At baseline, total IgE was similar in the synbiotic and the placebo group (8.7 kU/l, range 2.3–191, *vs* 13.8 kU/l, range 2.8–230, respectively, $P = 0.22$). At follow-up, total IgE was lower in the synbiotic group than in the placebo group; however, this difference was not statistically significant (20.4 kU/l, range 2.9–628, *vs* 47.7 kU/l, range 3.7–1529, respectively, $P = 0.15$). In the subgroup of children who were IgE negative at baseline ($n = 25$), total IgE at follow-up was significantly lower in the synbiotic group than in the placebo group (7.8 kU/l, range 5.0–68.9, *vs* 18.8 kU/l, range 6.8–168, respectively, $P = 0.008$); however, there was also a small, but statistically significant, difference between the two groups at baseline (5.8 kU/l, range 2.3–8.7, *vs* 8.0 kU/l, range 2.8–14.3, respectively, $P = 0.047$). To correct for this, the change in total serum IgE concentration between 1-year follow-up and baseline (ΔIgE) in the synbiotic and the placebo group was also

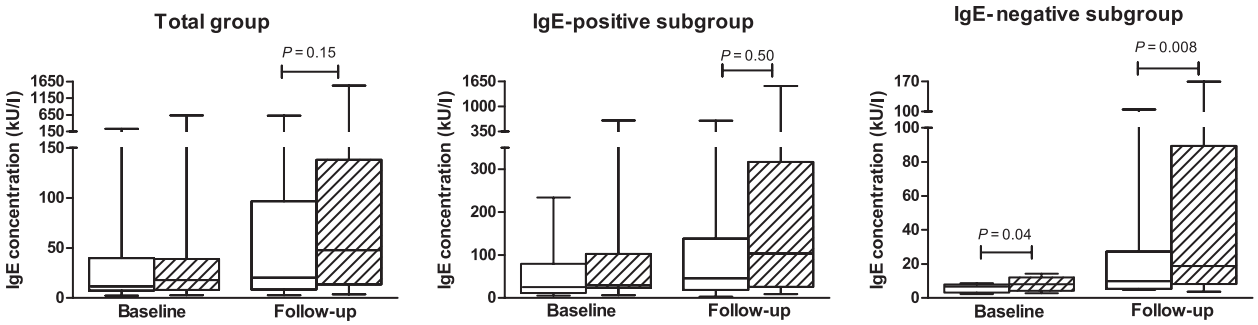

Figure 2 Median total serum IgE concentration (kU/l) in the synbiotic (white bars) and the placebo group (striped bars) at baseline and 1-year follow-up. Results given for total study population (baseline: synbiotics $n = 31$, placebo $n = 35$, follow-up: synbiotics $n = 29$, placebo $n = 34$), IgE-positive subgroup (baseline: synbiotics $n = 18$, placebo $n = 20$, follow-up: synbiotics $n = 15$, placebo $n = 18$) and IgE-negative subgroup (baseline: synbiotics $n = 13$, placebo $n = 15$, follow-up: synbiotics $n = 11$, placebo $n = 14$).

Table 3 Change in total IgE concentration between baseline and 1-year follow-up

	Synbiotics median (range)	Placebo median (range)	*P*-value
Total study population			
Total IgE (kU/l) follow-up-baseline [*n*]	5.0 (−14.5 to 437) [26]	10.6 (−118 to 1521) [32]	0.77
IgE-positive subgroup			
Total IgE (kU/l) follow-up-baseline [*n*]	26.7 (−14.5 to 437) [15]	13.1 (−118 to 1521) [18]	0.70
IgE-negative subgroup			
Total IgE (kU/l) follow-up-baseline [*n*]	3.0 (−0.75 to 60.9) [11]	10.6 (−6.84 to 155) [14]	0.04

calculated (Table 3). In the total study population and the IgE-positive subgroup, there was no significant difference in ΔIgE between the synbiotic and the placebo group. However, in the IgE-negative subgroup, ΔIgE was significantly lower in the synbiotic than in the placebo group.

Specific IgE against aeroallergens

The percentage of children with elevated specific IgE levels against cat, dog or HDM at baseline and 1-year follow-up is presented in Fig. 3. At follow-up, the percentage of children with elevated specific IgE against cat was significantly lower in the synbiotic group than in the placebo group (6.9% *vs* 30.3%, ARR −23.4%, 95% CI: −41.6% to −5.2%, $P = 0.03$). The number of children with elevated specific IgE against cat at follow-up that did not yet have elevated cat-IgE at baseline, was 0 out of 29 (0%) in the synbiotic group and 5 out of 33 (15.2%, none of these five children had cats in the home) in the placebo group (ARR −15.2%, 95% CI −27.4% to −2.9%, $P = 0.053$). The percentage of children with elevated specific IgE against HDM was also lower in the synbiotic group, but this difference was not statistically significant (10.3% *vs* 15.2%, $P = 0.71$). No significant difference was observed in the percentage of children with elevated specific IgE against dog.

Discussion

We showed that the prevalence of asthma-like symptoms and the prevalence of asthma medication use at 1-year follow-up were significantly lower in infants with AD who had received

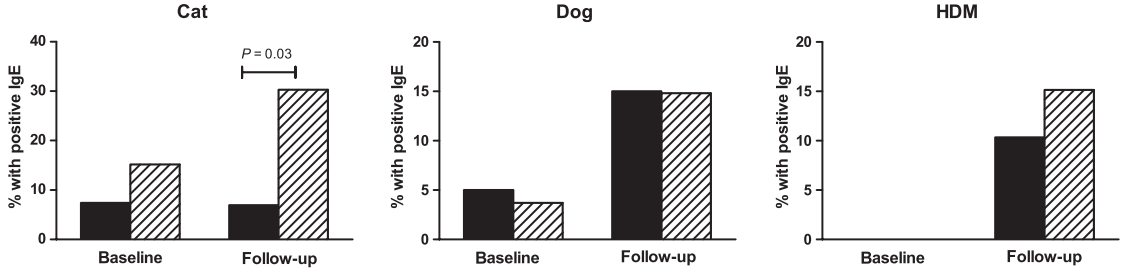

Figure 3 The percentage of children with elevated specific IgE (≥0.35 kU/l) against cat (e1), dog (e2) and house dust mite (HDM) (d1) in the synbiotic (black bars) and the placebo group (striped bars) at baseline and follow-up (cat and HDM: synbiotics $n = 29$, placebo $n = 33$, dog: synbiotics $n = 20$, placebo $n = 27$).

an infant formula with added synbiotics, *B. breve* M-16V and a scGOS/lcFOS mixture (Immunofortis®), than in those who had received the placebo.

To our knowledge, no previous studies on the efficacy of probiotics, prebiotics or synbiotics in children with AD have also explored a possible preventive effect on asthma-like symptoms in these children. Our results are consistent with a prevention study in healthy infants with a parental history of atopic disease that showed that administration of the specific prebiotic mixture of scGOS/lcFOS during 6 months significantly reduced the incidence of recurrent wheezing (≥3 physician-diagnosed episodes/2 years) compared to placebo (7.6% *vs* 20.6%) (15).

In contrast to our results, two prevention studies investigating the solitary effect of probiotics in healthy infants at high risk for allergic disease did not show an effect on prevalence of recurrent wheeze at age 1/2 years (10, 11), and one study even showed an increased prevalence of recurrent (≥5) episodes of wheezing bronchitis in the probiotic group (12). These studies did not describe other important variables such as wheeze apart from colds and asthma medication use. In two other prevention studies in high-risk infants, one with pro- and one with synbiotics, asthma prevalence was determined at age 4/5 years, and no difference between the treatment and placebo groups was found (9, 13).

The discrepancy between these studies and our study can possibly be explained by the solitary use of probiotics (instead of synbiotics). Another factor might be the probiotic strains that were used, because probiotic effects are strain specific. In an animal study, for example, it was shown that one probiotic organism, *Lactobacillus reuteri*, but not another, *Lactobacillus salivarius*, was able to attenuate antigen-induced airway eosinophil influx, local cytokine responses and hyper-responsiveness to methacholine (8). Therefore, strain-selection experiments should precede clinical trials to adequately identify the probiotic strain that is most efficacious for a specific clinical problem. The strain that we used, *B. breve* M-16V, has been shown to suppress airway hyper-responsiveness and pulmonary inflammation in a murine model for asthma, in combination with the prebiotic scGOS/lcFOS mixture and was more effective than several other *Bifidobacterium* and *Lactobacillus* strains (17, 24).

Children with AD have a chance of approximately 40% to develop asthma later in childhood (2), compared to a chance of 5–10% in the general population (25). In this group of high-risk infants, we demonstrated a statistically significant and clinically relevant benefit of synbiotics on the prevalence of asthma-like symptoms and asthma medication use. However, as AD severity was the primary outcome measure, this study was not powered to detect an effect on these, secondary, outcome measures, and the number of children with asthma-like symptoms was small. Also, there were a few small, not statistically significant, baseline differences between the two groups, such as a slightly higher percentage of parental asthma and pets in the home in the placebo group. To assess a possible confounding effect of these and several other variables, we performed multivariate logistic regression analyses, which showed that these variables were no confounders.

To diagnose asthma-like symptoms, we used a questionnaire and did not confirm the reported wheezing. It has been shown that parents sometimes misunderstand the term wheeze, which could theoretically lead to both over- and underestimation of the real prevalence (26). However, although this problem is of great importance in epidemiological studies, misunderstanding of the term wheeze in randomized controlled trials is likely to occur in both groups, and therefore probably does not substantially influence results regarding group differences. Moreover, misunderstanding is known to have less impact on prevalence estimates in more severe categories of wheeze (26), so our results regarding frequent wheezing and wheezing apart from colds were probably not greatly affected by this problem.

The underlying mechanism of the lower prevalence of asthma-like symptoms in the synbiotic group is not yet fully understood. It is known that asthma-like symptoms are often related to respiratory infections. For example, infants with recurrent asthma-like symptoms often have human rhinovirus in their bronchial epithelium (27). Synbiotics have been shown to decrease the number of respiratory infections (28). Therefore, a hypothesis could be that, in our study, synbiotics reduced the prevalence of asthma-like symptoms by lowering the respiratory infection rate. We did not record respiratory infections during the 1-year follow-up period; however, the number of respiratory infections (lower and upper) during the intervention period did not differ between the synbiotic and the placebo group (data not shown). Moreover, we showed a significant difference in wheezing/noisy breathing without concurrent respiratory infections between the two groups, indicating that another mechanism has to be involved.

Animal studies show that intervention or treatment with probiotics abrogate Th2-responses and inhibit allergic airway disease by inducing regulatory T cells, with associated increase in IL-10 and/or TGF-β production (7, 8, 29, 30). This induction of regulatory T cells does not only occur locally, in the intestine, but also systemically, and up regulation has been demonstrated in the lung compartment (7). It has been suggested that probiotic administration during the sensitization phase is essential for adequate immune modulation and prevention of allergic disease (7). In our trial, the administration of synbiotics in early infancy could have coincided with the aeroallergen sensitization phase in several of our participants, resulting in modulation of the immune response against these allergens and prevention of asthma-like symptoms, while the effect on already established allergic disease, AD, was limited. In line with this hypothesis, the increase in total serum IgE between baseline and follow-up in the IgE-negative subgroup was significantly greater in the placebo than in the synbiotic group, indicating that this synbiotic mixture might prevent an increase in IgE in those children who are not yet sensitized. Although significantly less children in the synbiotic group were sensitized to cat, this difference was not significant ($P = 0.053$), when only those children with sensitization after baseline were included in the analysis.

Ultimately, one would not only want to prevent asthma-like symptoms in infants with AD but also the actual devel-

opment of asthma. In general, most wheezing in preschool children is transient and does not predispose to asthma in later life (31). Although it is hard to predict which wheezing infants will develop asthma, several predictive factors have been identified. These include having AD, frequent wheezing and wheezing apart from colds (23, 32). As all children included in our study had AD and we found significant group differences in these specific predictive variables, it seems plausible that there will also be group differences in asthma prevalence.

In conclusion, we demonstrated that infants with AD who have received a specific synbiotic mixture, *B. breve* M-16V and a scGOS/lcFOS mixture (Immunofortis®), for a period of 3 months, have a lower prevalence of asthma-like symptoms and asthma medication use at 1-year follow-up than those who have received placebo. These results suggest that this synbiotic mixture prevents asthma-like symptoms. The infants included in our study will be followed up to age 5–6, when they are old enough for lung function tests and bronchial hyper-responsiveness measurements, to determine whether this synbiotic mixture also prevents the development of asthma. Our results have to be confirmed in further, larger clinical studies using the same synbiotic mixture.

Acknowledgments

We thank J. H. van der Lee, MD, PhD, Department of Pediatric Clinical Epidemiology, Emma Children's Hospital, Academic Medical Center, Amsterdam, the Netherlands for advice regarding the statistical analyses.

Funding

This study was financially supported by Danone Research – Centre for Specialised Nutrition.

References

1. Kay J, Gawkrodger DJ, Mortimer MJ, Jaron AG. The prevalence of childhood atopic eczema in a general population. *J Am Acad Dermatol* 1994;**30**:35–39.
2. Gustafsson D, Sjoberg O, Foucard T. Development of allergies and asthma in infants and young children with atopic dermatitis–a prospective follow-up to 7 years of age. *Allergy* 2000;**55**:240–245.
3. Noverr MC, Huffnagle GB. The 'microflora hypothesis' of allergic diseases. *Clin Exp Allergy* 2005;**35**:1511–1520.
4. Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; **107**:129–134.
5. Penders J, Thijs C, van den Brandt PA, Kummeling I, Snijders B, Stelma F et al. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut* 2007;**56**: 661–667.
6. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995;**125**:1401–1412.
7. Feleszko W, Jaworska J, Rha RD, Steinhausen S, Avagyan A, Jaudszus A et al. Probiotic-induced suppression of allergic sensitization and airway inflammation is associated with an increase of T regulatory-dependent mechanisms in a murine model of asthma. *Clin Exp Allergy* 2007;**37**:498–505.
8. Forsythe P, Inman MD, Bienenstock J. Oral treatment with live Lactobacillus reuteri inhibits the allergic airway response in mice. *Am J Respir Crit Care Med* 2007;**175**:561–569.
9. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003;**361**:1869–1871.
10. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2007;**119**:184–191.
11. Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, Bjorksten B et al. Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007;**119**:1174–1180.
12. Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. *Pediatrics* 2008;**121**:e850–e856.
13. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *J Allergy Clin Immunol* 2009;**123**:335–341.
14. Wickens K, Black PN, Stanley TV, Mitchell E, Fitzharris P, Tannock GW et al. A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2008;**122**:788–794.
15. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *J Nutr* 2008;**138**:1091–1095.
16. van der Aa LB, Heymans HS, van Aalderen WM, Sillevis Smitt JH, Knol J, Ben Amor K et al. Effect of a new synbiotic mixture on atopic dermatitis in infants: a randomized controlled trial. *Clin Exp Allergy* 2010; **40**(5):795–804.
17. Hougee S, Knippels LMJ, Nauta AJ, Folkerts G, Nijkamp FP, Knol J et al. Oral treatment with a specific synbiotic mixture inhibits the allergic airway response in mice. *Allergy* 2008;**63**(Suppl. 88):558.
18. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980;**92**:44–47.
19. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology* 1993;**186**:23–31.
20. Sprikkelman AB, Heymans HS, van Aalderen WM. Development of allergic disorders in children with cow's milk protein allergy or intolerance in infancy. *Clin Exp Allergy* 2000;**30**:1358–1363.
21. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;**7**:954–960.
22. Moro G, Minoli I, Mosca M, Fanaro S, Jelinek J, Stahl B et al. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *J Pediatr Gastroenterol Nutr* 2002;**34**:291–295.
23. Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000;**162**(4 Pt 1):1403–1406.

24. Hougee S, Vriesema AJ, Wijering SC, Knippels LM, Folkerts G, Nijkamp FP et al. Oral treatment with probiotics reduces allergic symptoms in ovalbumin-sensitized mice: a bacterial strain comparative study. *Int Arch Allergy Immunol* 2009;**151**:107–117.
25. Pearce N, it-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* 2007;**62**:758–766.
26. Michel G, Silverman M, Strippoli MP, Zwahlen M, Brooke AM, Grigg J et al. Parental understanding of wheeze and its impact on asthma prevalence estimates. *Eur Respir J* 2006;**28**:1124–1130.
27. Malmstrom K, Pitkaranta A, Carpen O, Pelkonen A, Malmberg LP, Turpeinen M et al. Human rhinovirus in bronchial epithelium of infants with recurrent respiratory symptoms. *J Allergy Clin Immunol* 2006;**118**:591–596.
28. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T et al. Long-term safety and impact on infection rates of postnatal probiotic and prebiotic (synbiotic) treatment: randomized, double-blind, placebo-controlled trial. *Pediatrics* 2008;**122**:8–12.
29. Repa A, Grangette C, Daniel C, Hochreiter R, Hoffmann-Sommergruber K, Thalhamer J et al. Mucosal co-application of lactic acid bacteria and allergen induces counter-regulatory immune responses in a murine model of birch pollen allergy. *Vaccine* 2003;**22**:87–95.
30. Zuany-Amorim C, Sawicka E, Manlius C, Le MA, Brunet LR, Kemeny DM et al. Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells. *Nat Med* 2002;**8**:625–629.
31. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;**332**:133–138.
32. Ly NP, Gold DR, Weiss ST, Celedon JC. Recurrent wheeze in early childhood and asthma among children at risk for atopy. *Pediatrics* 2006;**117**:e1132–e1138.