

# Early Dietary Intervention with a Mixture of Prebiotic Oligosaccharides Reduces the Incidence of Allergic Manifestations and Infections during the First Two Years of Life<sup>1,2</sup>

Sertac Arslanoglu,<sup>3\*</sup> Guido E. Moro,<sup>3</sup> Joachim Schmitt,<sup>4</sup> Laura Tandoi,<sup>3</sup> Silvia Rizzardi,<sup>3</sup> and Gunther Boehm<sup>4,5</sup>

<sup>3</sup>Center for Infant Nutrition, Macedonio Melloni Hospital, University of Milan, Milan 20129, Italy; <sup>4</sup>Numico Research, Friedrichsdorf 61381, Germany; and <sup>5</sup>Sophia Children's Hospital, Erasmus University, Rotterdam 3015 GE, The Netherlands

## Abstract

A mixture of neutral short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS) has been shown to reduce the incidence of atopic dermatitis (AD) and infectious episodes during the first 6 mo of life. This dual protection occurred through the intervention period. The present study evaluated if these protective effects were lasting beyond the intervention period. In a prospective, randomized, double-blind, placebo-controlled design, healthy term infants with a parental history of atopy were fed either a prebiotic-supplemented (8 g/L scGOS/lcFOS) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula during the first 6 mo of life. Following this intervention period, blind follow-up continued until 2 y of life. Primary endpoints were cumulative incidence of allergic manifestations. Secondary endpoints were number of infectious episodes and growth. Of 152 participants, 134 infants (68 in placebo, 66 in intervention group) completed the follow-up. During this period, infants in the scGOS/lcFOS group had significantly lower incidence of allergic manifestations. Cumulative incidences for AD, recurrent wheezing, and allergic urticaria were higher in the placebo group, (27.9, 20.6, and 10.3%, respectively) than in the intervention group (13.6, 7.6, and 1.5%) ( $P < 0.05$ ). Infants in the scGOS/lcFOS group had fewer episodes of physician-diagnosed overall and upper respiratory tract infections ( $P < 0.01$ ), fever episodes ( $P < 0.00001$ ), and fewer antibiotic prescriptions ( $P < 0.05$ ). Growth was normal and similar in both groups. Early dietary intervention with oligosaccharide prebiotics has a protective effect against both allergic manifestations and infections. The observed dual protection lasting beyond the intervention period suggests that an immune modulating effect through the intestinal flora modification may be the principal mechanism of action. *J. Nutr.* 138: 1091–1095, 2008.

## Introduction

Human milk oligosaccharides (HMO),<sup>6</sup> after lactose and lipids, represent the third largest component in human milk (20–23 g/L in colostrum and 12–14 g/L in mature milk). They are important components of the defense system of human milk, having both the prebiotic potential and the direct interaction with the immune cells (1–6). HMO are structurally very complex with a huge diversity and so, identical structures are not available in infant

formulas (6–8). Searching for alternatives to mimic the prebiotic effect of human milk, a prebiotic mixture of 90% short-chain galactooligosaccharides (scGOS) and 10% long-chain fructooligosaccharides (lcFOS) (IMMUNOFORTIS) has been developed (9). Although these oligosaccharides are not identical to HMO, studies in preterm (10) and term infants (11–13) have shown that a formula supplemented with this prebiotic scGOS/lcFOS mixture results in an intestinal microbiota similar to that found in breast-fed infants. Because a balanced intestinal microbiota dominated by bifidobacteria and lactobacilli is crucial for the expansion and education of the immune system early in life (14,15), it could be expected that such a prebiotic mixture might modulate the immune system in bottle-fed infants.

Our hypothesis was that this mixture of prebiotic oligosaccharides could mimic the immune modulatory function of HMO, leading to a reduction in the incidence of allergic manifestations and infections in formula-fed infants. To test this hypothesis, based on a prospective, randomized, double-blind, placebo-controlled nutritional intervention design, we planned a series of prospective trials addressing different outcomes at different time points.

<sup>1</sup> Supported by the European Union EARNEST (Early Nutrition Programming) Project (FOOD-CT-2005-007036). Numico Research provided the scGOS/lcFOS mixture utilized in this study.

<sup>2</sup> Author disclosures: S. Arslanoglu, G. E. Moro, L. Tandoi, and S. Rizzardi, no conflicts of interest; G. Boehm is the Director of Infant Nutrition Research at Numico Research; J. Schmitt is the Head of Research Cooperation at Numico in Germany.

<sup>6</sup> Abbreviations used: AD, atopic dermatitis; HMO, human milk oligosaccharides; lcFOS, long-chain fructooligosaccharides; scGOS, short-chain galactooligosaccharides; URTI, upper respiratory tract infection.

\* To whom correspondence should be addressed. E-mail: asertac@tiscali.it.

The population consisted of healthy term infants with a parental history of atopy and the intervention was early scGOS/lcFOS supplementation (8 g/L of formula) during the first 6 mo of life. The first trials addressed the cumulative incidence of atopic dermatitis (AD) (16) and infections (17) during the intervention period and showed that the mixture led to a significant reduction in the incidence of AD and had a preventive effect against infections during the first 6 mo of life.

We report here the results of a further study aimed at monitoring if these allergy and infection prevention effects are long lasting. This is a 2-y follow-up trial with a double-blind follow-up design evaluating the cumulative incidence of allergic manifestations, episodes of infections, and growth during the first 2 y of life.

## Methods

**Study design.** The study was planned as a randomized, double-blind, placebo-controlled trial. Term infants with a parental history of atopy received either prebiotic-supplemented (8 g/L scGOS/lcFOS) or placebo-supplemented (8 g/L maltodextrin) hypoallergenic formula during the first 6 mo of life (16). The study hypothesis was that the prebiotic-supplemented formula could be protective against allergic manifestations and infections during the first 2 y of life. Infants were enrolled and randomly assigned to 1 of the 2 study groups, scGOS/lcFOS or placebo, according to a preprepared randomization numbers table. For this purpose, the random permuted block method was used with a block size of 4. For blinding, 2 trial formulas were coded by adding the suffix N or O to the product name. Follow-up to the age of 2 y was performed by 2 investigators who were unaware of the formula assignments. Distribution of the formulas to the infants was done by the nurses who were not involved in the first 6 mo of the study.

**Subjects.** Healthy term infants with a parental history of atopic eczema, allergic rhinitis, or asthma in either mother or father were eligible for the study. In all cases, the parental diagnosis was based on a documented physician's certification. Inclusion criteria were: gestational age between 37 and 42 wk, birth weight appropriate for gestational age, and start of formula feeding within the first 2 wk of life. According to the hospital's policy, breast-feeding was recommended to all mothers. The parents were informed about the study at discharge from the maternity ward and were asked to contact the hospital if they started formula feeding. The study protocol was approved by the Ethical Committee of the Macedonio Melloni Hospital, Milan, Italy. Informed written consent was obtained from parents. Two hundred and fifty-nine term infants were enrolled and 206 infants completed the first 6-mo part of the study (16,17). Parents of 152 completers gave consent to participate for the 2-y follow-up trial.

**Nutritional intervention.** Infants whose mothers started formula feeding within the first 2 wk of life were randomly assigned to be fed 1 of the 2 study formulas. The recipe of both formulas was based on a hypoallergenic formula with extensively hydrolyzed cow milk whey protein (Aptamil HA). In the intervention group, this formula was supplemented with 8 g/L scGOS/lcFOS (IMUNOFORTIS) and in the placebo group the same formula was supplemented with 8 g/L maltodextrin (Glucodex 12). Mixed breast- and bottle-feeding was accepted until wk 6 of life. When the mother started formula feeding according to the inclusion criteria but continued breast-feeding for more than 6 wk, the infant was excluded from the study. Duration of feeding with the study formulas was 6 mo. Weaning was started in a standard way for all the infants in the study at 5 mo with fruit followed by weaning purees. Probiotic or prebiotic food supplements were not allowed throughout this period.

**Follow-up protocol.** Data were collected through follow-up visits, diaries written by parents, and telephone calls by trained personnel.

Follow-up visits consisted of a detailed physical examination, evaluation of growth, and structured interviews by the study physicians. These visits were scheduled at 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24 mo of age. Any sign or symptom related to allergy (AD, wheezing episodes, and allergic urticaria) and infection (fever, cough, runny nose, watery stools) was

recorded. Medical documents and reports were noted. Unscheduled visits were conducted when the investigators were contacted by the family regarding the occurrence of symptoms related to allergy or infections.

Diaries and telephone calls were used to collect the data between 2 scheduled visits and to increase the compliance. Parents were instructed to record allergic and infectious symptoms, every episode of fever ( $\geq 38.5^{\circ}\text{C}$ ), clinic visits, tests, physician's diagnosis, prescription of medications, particularly antibiotics, and were asked to contact the investigators and bring these reports and medical documents at each visit.

**Endpoints and definitions.** Primary endpoints were cumulative incidence of allergic manifestations: AD, recurrent wheezing, and allergic urticaria at 2 y of age.

AD was diagnosed according to the criteria described by Harrigan and Rabinowitz (18) and Muraro et al. (19). The diagnosis of AD was confirmed if the following features were detected: pruritus, involvement of the face, skull facial, and/or extensor part of the extremities, and a minimal duration of the symptoms of 4 wk. Severity of AD was scored by using the SCORAD index based on extension and intensity of the skin symptoms, as well as on the subjective symptoms of pruritus and sleep loss as recommended by the European Task Force on AD (20,21). The extent of AD was determined by using the SCORAD figure for infants  $<2$  y.

Recurrent wheezing was defined as 3 or more physician-diagnosed wheezing episodes (19). Allergic urticaria was defined as (22) 2 or more episodes of itching eruptions or swelling with typical appearance provoked by the same allergen.

Secondary endpoints were the number of infectious episodes and growth. To define infectious episodes, 2 different criteria were used: physician-diagnosed infections and episodes of fever ( $\geq 38.5^{\circ}\text{C}$ ) witnessed by the parents. The reason to select a second definition was to have an objective criterion for the diagnosis of infection.

Physician-diagnosed infections included overall, upper respiratory tract infections (URTI), lower respiratory tract infections, otitis media, gastrointestinal infections, and urinary tract infections. Antibiotic prescriptions were recorded separately to determine the infectious episodes requiring antibiotic therapy.

Body weight and length were measured at each clinic visit by experienced nurses and the growth data were assessed at 12, 18, and 24 mo of life. Birth data were derived from the birth records.

**Statistical analysis.** The data were analyzed on a per protocol basis. Time-balanced randomization was performed with the software RANCODE (IDV; seed numbers randomized by reaction time) with a random permuted block size of 4. Sample size was calculated based on analysis of the previous years' incidence of AD in the hospital and assuming an effect size similar to that reported for probiotics at that time (16). Based on this assumption, 108 subjects per group completing the protocol were calculated to provide a power of 80%. The study was completed after a full 2-y enrollment period to exclude seasonal effects.

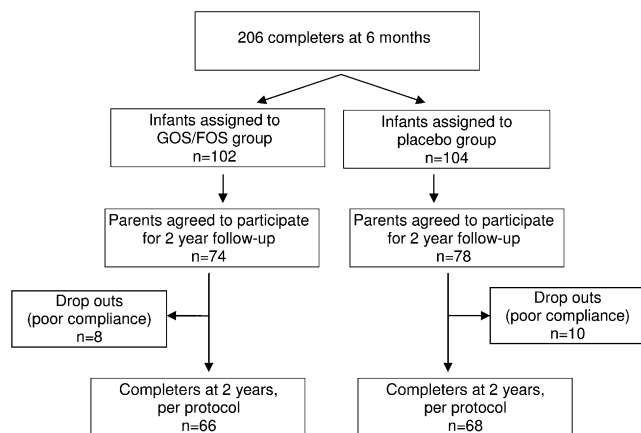
One-way ANOVA and *t* tests were used to compare continuous variables between 2 treatment groups. When equality of variances were not present, we used Mann-Whitney U nonparametric tests. Categorical data were compared by using the chi-square test. Fisher's exact test was performed for the analysis. Differences were considered significant at  $P < 0.05$ . Statistical analyses were performed using the SPSS 10.0 software for Windows.

## Results

Parents of 152 infants agreed to participate in the follow-up study. A total of 134 infants (68 in the placebo, 66 in the scGOS/lcFOS group) completed the 2-y follow-up period (Fig. 1). Baseline characteristics and demographic data of the completers were similar (Table 1).

During the follow-up, the growth expressed as mean body weight and length at 12, 18, and 24 mo was adequate and similar in the placebo and intervention groups (data not shown).

The cumulative incidences of AD, recurrent wheezing, and allergic urticaria were lower in the scGOS/lcFOS group (13.6,



**FIGURE 1** Flow chart showing enrollment and disposition of the subjects throughout the study.

7.6, 1.5%, respectively) than in the placebo group (27.9, 20.6, and 10.3%, respectively;  $P < 0.05$ ) (Fig. 2).

As previously mentioned, 2 different criteria were used to define the infectious episodes for this study: physician-diagnosed infections and fever episodes witnessed by the parents. The number of overall infections was lower in the scGOS/lcFOS group than in the placebo group as assessed by both physician-diagnosed infections ( $P = 0.01$ ) and the number of fever episodes ( $P < 0.0001$ ) (Table 2).

Infants in the scGOS/lcFOS group had fewer episodes of URTI ( $P < 0.01$ ) and tended to have fewer episodes of urinary tract infections ( $P = 0.06$ ). Similarly, infants in the intervention group had fewer episodes of infections requiring antibiotic treatment compared with infants in the placebo group ( $P < 0.05$ ) (Table 2).

In a subgroup of 98 infants with a complete set of stool samples, supplementation with scGOS/lcFOS resulted in a significant increase in the number of bifidobacteria compared with the placebo group at 6 mo (16). The median bifidobacteria count as colony-forming units/g stool at 6 mo of life was 10.3 in the scGOS/lcFOS group and 8.7 in the placebo group ( $P < 0.0001$ ).

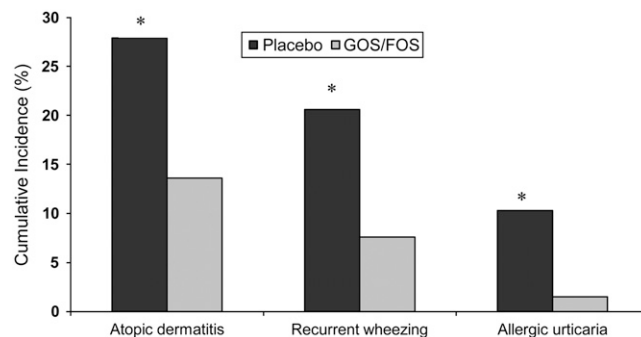
## Discussion

This is the first follow-up study, to our knowledge, showing that an early dietary intervention results in dual prevention for

**TABLE 1** Baseline characteristics and demographic data of subjects who completed the 2-y follow-up<sup>1</sup>

	Placebo	scGOS/lcFOS
<i>n</i>	68	66
Gender ratio (male:female)	1.0	1.1
Birth weight, <i>g</i>	3314 ± 455	3282 ± 505
Mother's age, <i>y</i>	32.6 ± 4.7	33.6 ± 4.9
Atopy in the family, %		
Only maternal	58.8	56.1
Biparental	23.5	25.8
Only paternal	17.6	18.2
Births, <i>n</i>	1.4 ± 0.8	1.4 ± 0.7
Vaginal delivery, %	64.7	66.7
Maternal smoking, %	17.7	16.7
Age at onset of day care, <i>mo</i>	13.1 ± 4.1	11.7 ± 4.2
Furred pets at home, %	11.8	10.6

<sup>1</sup> Values are means ± SD or %.



**FIGURE 2** Cumulative incidence of allergic manifestations at the end of 2-y follow-up period in the scGOS/lcFOS and placebo groups.  $n = 68$  (placebo) or 66 (scGOS/lcFOS). \*Different from placebo,  $P < 0.05$ . Atopic dermatitis was diagnosed according to the criteria described (18,19). Recurrent wheezing was defined as 3 or more physician-diagnosed wheezing episodes (19). Allergic urticaria was defined as 2 or more episodes of itching eruptions or swelling with typical appearance provoked by the same allergen (22).

infection and allergy and that both effects last beyond the intervention period. We have previously shown that prebiotic oligosaccharides significantly reduce the incidence of AD (16) and infections (17) in infants at high-risk for allergy during the 6-mo intervention period. The hypothesis of the present study was that this observed dual preventive effect could last longer, beyond the intervention period through the modulation of the immune system. The rationale for this expectation was that the dietary intervention started very early (within the first 2 wk of life) and went on for 6 mo, a time corresponding to a critical period of life when long-lasting effects can be induced (programming effect).

Confirming this hypothesis, the scGOS/lcFOS mixture led to significant reductions in the cumulative incidence of allergic manifestations (AD, recurrent wheezing episodes, and allergic urticaria) and in the number of infectious episodes (overall, URTI, infections requiring antibiotic therapy, fever episodes) during 18 mo after the termination of oligosaccharide supplementation.

During the last decades, we have become aware of a dramatic increase in allergic diseases throughout the world, a so-called

**TABLE 2** Episodes of infections and fever during the 2-y follow-up period in the scGOS/lcFOS and placebo groups<sup>1</sup>

	Placebo	scGOS/lcFOS
<i>n</i>	68	66
Physician-diagnosed infections		
Overall (any kind of infection)**	5.9 ± 4.1	4.1 ± 3.1
URT <sup>†</sup>	3.2 ± 2.2	2.1 ± 1.8
Lower respiratory tract infections	1.3 ± 0.8	0.9 ± 1.1
Otitis media	0.7 ± 1.2	0.5 ± 1.0
Gastrointestinal infections	0.6 ± 0.9	0.4 ± 0.7
Urinary tract infections	0.1 ± 0.5	0.0 ± 0.0
Infections requiring antibiotic prescriptions*	2.7 ± 2.4	1.8 ± 2.3
Fever episodes recorded by parents <sup>‡</sup>	3.9 ± 2.5	2.2 ± 1.9

<sup>1</sup> Values are means ± SD. \*Different from placebo,  $P < 0.05$ , \*\* $P = 0.01$ , <sup>†</sup> $P < 0.01$ , <sup>‡</sup> $P < 0.0001$ .

“epidemic of allergy.” In fact, recent data suggest that the incidence of atopic disease in children with 1 atopic family member has reached almost 50% by the age of 2 y (23). The cumulative incidence of AD changes between 13–44% in the literature (24). Moreover, Kuehni et al. (25) reported a significant increase in wheezing disorders in preschool children in the UK. The etiology of allergic diseases is multifactorial. The list of lifestyle-related factors that might be associated with this epidemic of the century is long. Although there is no marker that is capable with certainty to predict the development of allergic disease later on, the family history remains the best predictor (24). At this point, primary prevention in high-risk infants becomes crucial as a public health priority. Recently among the primary prevention measures (22,24–32) gut microbiota modifying agents, i.e. probiotics and prebiotics, offer promising horizons (16,33–36).

Evidence shows that differences in the gut microbiota composition during the neonatal period and early infancy precede the development of atopy. Bjorkstein et al. (37) have demonstrated that the presence of fewer bifidobacteria and lactobacilli in the neonatal microbiota precedes the development of atopic diseases. Recently, the KOALA Birth Cohort Study (38) revealed that the presence of *Escherichia coli* was associated with a higher risk of developing eczema, whereas colonization with *Clostridium difficile* was associated with higher risk of developing eczema as well as recurrent wheeze and allergic sensitization.

Human milk is considered the gold standard nutrient in the first 6 mo of life. It promotes a microbiota rich in bifidobacteria and its oligosaccharides play an important role in establishment of this health benefit promoting flora (prebiotic effect). Recently, a prebiotic mixture of scGOS and lcFOS in a 9:1 ratio has been designed for formula-fed infants (9) to provide a prebiotic effect comparable to that of human milk. It has been demonstrated that this mixture has prebiotic activities in infants (10–13) and immunomodulatory effects in animal models (39,40). Also during the 6-mo intervention period of this follow-up study, supplementation with this mixture resulted in a significant increase in the number of bifidobacteria compared with the placebo group (16).

There are only a few published follow-up studies addressing the prevention of allergic disease in infants at high risk by means of intestinal flora modification (33–36,41). In the first prevention trial of Kalliomaki et al. (33), probiotics (*Lactobacillus GG*) were given to the mothers of infants at high risk prenatally and then to the infants for 6 mo. In the next trial conducted by Kukkonen et al. (34), both probiotics (mothers plus infants) and prebiotics (infants) were used as intervention. Both trials showed a reduction in the cumulative incidence of AD by 2 y of age, although the effect sizes were quite different (a reduction of 50% in the first study and 20% in the latter). Our study is the first follow-up trial in which prebiotic oligosaccharide intervention has been applied alone and exclusively to the infants postnatally. In this prevention trial of 134 high-risk infants, the cumulative incidence of AD has been successfully reduced by >50% in 2 y (27.9% in the placebo vs. 13.6% in the scGOS/lcFOS group). This reduction is similar to the reduction obtained by Kalliomaki et al. (46% in the placebo and 23% in the intervention group).

AD is typically the first manifestation of the allergic disorder followed by subsequent respiratory allergic disease (atopic march) (42). In our study, the cumulative incidence of other allergy-associated symptoms, like recurrent wheezing and allergic urticaria, was also significantly lower in the sGOS/lcFOS group compared with the placebo group. We think that the 3-fold reduction in the incidence of recurrent wheezing episodes

(20.6% in the placebo vs. 7.6% in the sGOS/lcFOS groups) might have important clinical implications, because we used very strict definition criteria (3 or more physician-diagnosed episodes in 2 y). Recent literature underlines the importance of recurrent early wheeze in the later development of asthma. Ly et al. (43) showed in a group of 440 children with parental history of allergy that the frequency of recurrent early wheeze was 26.0% and was associated with a 4-fold increase in the odds of asthma at 7 y of age. In this study, recurrent early wheeze was defined as  $\geq 2$  reports of wheezing in the first 3 y of life. Very recently it has been reported by Saglani et al. (44) that the characteristic pathological features of adult asthma have already been developed in preschool children with confirmed wheeze between the ages of 1 and 3 y.

Our data show that the use of this prebiotic oligosaccharide mixture (scGOS/lcFOS) also results in a significant reduction of the total number of infections, respiratory tract infections, fever episodes, and antibiotic prescriptions during the first 2 y of life.

Although we cannot determine the specific mechanism through which this dual prevention occurred, it might be through the modification of the intestinal flora. This interpretation is also supported by the fact that a relationship between allergic diseases and intestinal microbiota early in life has been reported (45,46). As shown in this study, it is possible to have fewer infections and allergic symptoms at the same time through the bifidogenic modification of the intestinal flora soon after birth. However, any potential direct effect of the studied prebiotics on the immune cells and target receptors like lectins (1,47,48) cannot be excluded.

We conclude that early nutritional intervention with prebiotic oligosaccharides seems to be effective in priming the infant's immune system in a balanced way, providing a substantial protection both for allergy and infection. This dual effect can be considered as a typical example of immunological programming. When mother's milk is not available, the supplementation of formulas with prebiotic oligosaccharides early in life may have promising clinical implications.

## Literature Cited

1. Eiwegger T, Stahl B, Schmitt JJ, Boehm G, Gerstmayr M, Pichler J, Dehlinek E, Urbanek R, Sze'p'falusi Z. Human milk derived oligosaccharides and plant derived oligosaccharides stimulate cytokine production of cord blood T-cells in vitro. *Pediatr Res*. 2004;56:536–40.
2. Velupillai P, Harn DA. Oligosaccharide-specific induction of interleukin 10 production by B2201 cells from schistosome-infected mice: a mechanism for regulation of CD41 T-cell subsets. *Proc Natl Acad Sci USA*. 1994;91:18–22.
3. Schumacher G, Bendas G, Stahl B, Beermann C. Human milk oligosaccharides affect P-selectin binding capacities: in vitro investigation. *Nutrition*. 2006;22:620–7.
4. Bode L, Rudloff S, Kunz C, Strobel S, Klein N. Human milk oligosaccharides reduce platelet-neutrophil complex formation leading to a decrease in neutrophil b 2 integrin expression. *J Leukoc Biol*. 2004;76:820–6.
5. Naarding MA, Ludwig IS, Groot F, Berkhout B, Geijtenbeek TB, Pollakis G, Paxton VA. Lewis X-component in human milk binds DCSIGN and inhibits HIV-1 transfer to CD41 lymphocytes. *J Clin Invest*. 2005;115:3256–64.
6. Boehm G, Stahl B. Oligosaccharides from milk. *J Nutr*. 2007;137:S847–9.
7. Boehm G, Stahl B. Oligosaccharides. In: Mattila-Sandholm T, editor. *Functional dairy products*. Cambridge: Woodhead Publishing; 2003. p. 203–43.
8. Bode L. Recent advances on structure, metabolism, and function of human milk oligosaccharides. *J Nutr*. 2006;136:2127–30.

9. Boehm G, Fanaro S, Jelinek J, Stahl B, Marini A. Prebiotic concept for infant nutrition. *Acta Paediatr Suppl.* 2003;91:64–7.
10. Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B, Marini A. Supplementation of an oligosaccharide mixture to a bovine milk formula increases counts of faecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* 2002;86:F178–81.
11. Moro G, Minoli I, Mosca M, Fanaro S, Jelinek J, Stahl B, Boehm G. Dosage related bifidogenic effects of galacto- and fructo oligosaccharides in formula fed term infants. *J Pediatr Gastroenterol Nutr.* 2002;34:291–5.
12. Schmelzle H, Wirth S, Skopnik H, Radke M, Knol J, Böckler HM, Brönstrup A, Wells J, Fusch C. Randomised double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta palmitic acid level, and nondigestible oligosaccharides. *J Pediatr Gastroenterol Nutr.* 2003;36:343–51.
13. Knol J, Scholtens B, Kafka C, Steenbakkers J, Gro S, Helm K, Klarczyk M, Schöpfer H, Böckler HM, et al. Colon microflora in infant fed formula with galacto- and fructo-oligosaccharides: more like breast fed infants. *J Pediatr Gastroenterol Nutr.* 2005;40:36–42.
14. Neu J, Douglas-Escobar M, Lopez M. Microbes and the developing gastrointestinal tract. *Nutr Clin Pract.* 2007;22:174–82.
15. Corthésy B, Gaskins HR, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. *J Nutr.* 2007;137:S781–90.
16. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child.* 2006;91:814–9.
17. Arslanoglu S, Moro G, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J Nutr.* 2007;137:2420–4.
18. Harrigan E, Rabinowitz LG. Atopic dermatitis. *Immunology and Allergy Clinics of North America.* 1999;19:383–96.
19. Muraro A, Dreborg S, Halken S, Host A, Niggemann B, Aalberse R, Arshad SH, von Berg A, Karlens KH, et al. Dietary prevention of allergic diseases in infants and small children. Part II: evaluation of methods in allergy prevention studies and sensitization markers. Definitions and diagnostic criteria for allergic diseases. *Pediatr Allergy Immunol.* 2004;15:196–205.
20. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taïeb A. Clinical validation and guidelines for the SCORAD index: Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology.* 1997;195:10–9.
21. Anonymous. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology.* 1993;186:23–31.
22. von Berg A, Koletzko S, Grühl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, Reinhardt D, Berdel D, German Infant Nutritional Intervention Study Group. The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial. *J Allergy Clin Immunol.* 2003;111:533–40.
23. Rautava S. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol.* 2002;109:119–21.
24. Vandenplas Y. Clinical overview the changing pattern of clinical aspects of allergic diseases. In: Isolauri E, Walker WA, editors. *Allergic diseases and the environment.* Nestlé Nutrition Workshop Series Pediatric Program. Basel (Switzerland): Nestec Ltd Vevey/S. Karger AG; 2004. pp 1–25.
25. Kuehni CE, Davis A, Brooke AM, Silverman M. Are all wheezing disorders in very young (preschool) children increasing in prevalence? *Lancet.* 2001;357:1821–5.
26. Oldaeus G, Anjou K, Björkstén B, Moran JR, Kjellman NI. Extensively and partially hydrolysed infant formulas for allergy prophylaxis. *Arch Dis Child.* 1997;77:4–10.
27. Høst A, Koletzko B, Dreborg S, Muraro A, Wahn U, Aggett P, Bresson JL, Hernell O, Løfeber H, et al. Dietary products used in infants for treatment and prevention of food allergy. Joint Statement of the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) Committee on Hypoallergenic Formulas and the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition. *Arch Dis Child.* 1999;81:80–4.
28. Committee on Nutrition, American Academy of Pediatrics. Hypoallergenic infant formulas. *Pediatrics.* 2000;106:346–9.
29. Halken S, Hansen KS, Jacobsen HP, Estmann A, Faelling AE, Hansen LG, Kier SR, Lassen K, Lintrup M, et al. Comparison of a partially hydrolyzed infant formula with two extensively hydrolyzed formulas for allergy prevention: a prospective, randomized study. *Pediatr Allergy Immunol.* 2000;11:149–61.
30. Arshad SH, Bateman B, Sadeghnejad A, Gant C, Matthews SM. Prevention of allergic disease during childhood by allergen avoidance: the Isle of Wight prevention study. *J Allergy Clin Immunol.* 2007;119:307–13.
31. von Berg A, Koletzko S, Filipiak-Pittroff B, Laubereau B, Grühl A, Wichmann HE, Bauer CP, Reinhardt D, Berdel D, German Infant Nutritional Intervention Study Group. Certain hydrolyzed formulas reduce the incidence of atopic dermatitis but not that of asthma: three-year results of the German Infant Nutritional Intervention Study. *J Allergy Clin Immunol.* 2007;119:718–25.
32. von Berg A. The concept of hypoallergenicity for atopy prevention. *Nestle Nutr Workshop Ser Pediatr Program.* 2007;59:49–57, 57–62.
33. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet.* 2001;357:1076–9.
34. Kalliomäki 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;361:1869–71.
35. Kukkonen K, Savilahti E, Haahela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M. Probiotics and prebiotic galacto-oligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol.* 2007;119:192–8.
36. Abrahamsson TR, Jakobsson T, Böttcher MF, Fredrikson M, Jenmalm MC, Björkstén B, Oldaeus G. Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol.* 2007;119:1174–80.
37. Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and intestinal flora during the first year of life. *J Allergy Clin Immunol.* 2001;108:516–20.
38. Penders J, This C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics.* 2006;118:511–21.
39. Vos AP, Haarman M, Buco A, Govers M, Knol J, Garssen J, Stahl B, Boehm G, M'Rabet L. A specific prebiotic oligosaccharide mixture stimulates delayed-type hypersensitivity in a murine influenza vaccination model. *Int Immunopharmacol.* 2006;6:1277–86.
40. Vos AP, Haarman M, van Ginkel J-WH, Knol J, Garssen J, Stahl B, Boehm G, M'Rabet L. Dietary supplementation of neutral and acidic oligosaccharides enhances Th1-dependent vaccination responses in mice. *Pediatr Allergy Immunol.* 2007;18:304–12.
41. 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;119:184–91.
42. Wahn U, von Mutius E, Lau S, Nickel R. The development of atopic phenotypes: genetic and environmental determinants. *Nestlé Nutr Workshop Ser Pediatr Program.* 2007;59:1–15.
43. Ly NP, Gold DR, Weiss ST, Celedón JC. Recurrent wheeze in early childhood and asthma among children at risk for atopy. *Pediatrics.* 2006;117:e1132–8.
44. Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK. Early detection of airway wall remodelling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med.* 2007;176:858–64.
45. Ouwehand AC, Isolauri E, He F, Hashimoto H, Benoo Y, Salimine S. Differences in Bifidobacterium flora composition in allergic and healthy infants. *J Allergy Clin Immunol.* 2001;108:144–5.
46. Kalliomäki M, Isolauri E. Role of intestinal flora in the development of allergy. *Curr Opin Allergy Clin Immunol.* 2003;3:15–20.
47. Watzl B, Gierbach S, Roller M. Inulin, oligofructose and immunomodulation. *Br J Nutr.* 2005;93 Suppl 1:S49–55.
48. Vos AP, M'Rabet L, Stahl B, Boehm G, Garssen J. Immune modulatory effects and potential working mechanisms of orally applied non-digestible carbohydrates. *Crit Rev Immunol.* 2007;27:97–140.