Clinical Nutrition 28 (2009) 156–161

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://intl.elsevierhealth.com/journals/clnu

Original Article

A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: An observational study

Eugenia Bruzzese [a], Monica Volpicelli [a], Veronica Squeglia [a], Dario Bruzzese [b], Filippo Salvini [c], Massimo Bisceglia [d], Paolo Lionetti [e], Mario Cinquetti [f], Giuseppe Iacono [g], Sergio Amarri [h], Alfredo Guarino [a,*]

[a] Dipartimento di Pediatria, Università di Napoli "Federico II", Via S. Pansini 5, 80131 Napoli, Italy
[b] Dipartimento di Statistica medica, Università di Napoli "Federico II", Napoli, Italy
[c] Dipartimento di Pediatria, Università di Milano, Ospedale S. Paolo, Milano, Italy
[d] Divisione di Terapia Intensiva Neonatale, Ospedale di Crotone, Italy
[e] Divisione di Pediatria, Ospedale Meyer, Firenze, Italy
[f] Dipartimento di Pediatria, Università di Verona, Ospedale Civile Maggiore, Verona, Italy
[g] Divisione di Pediatria, Ospedale Di Cristina, Palermo, Italy
[h] Dipartimento di Pediatria AUSL, Ravenna, Italy

ARTICLE INFO

Article history:
Received 21 July 2008
Accepted 16 January 2009

Keywords:
Prebiotics
Intestinal microflora
Bifidobacteria
Immune system

SUMMARY

Background & aim: The addition of prebiotics to infant formula modifies the composition of intestinal microflora. Aim of the study was to test the hypothesis that prebiotics reduce the incidence of intestinal and respiratory infections in healthy infants.

Methods: A prospective, randomized, placebo-controlled, open trial was performed. Healthy infants were enrolled and randomized to a formula additioned with a mixture of galacto- and fructo-oligosaccharides or to a control formula. The incidence of intestinal and respiratory tract infections and the anthropometric measures were monitored for 12 months.

Results: Three hundred and forty two infants (mean age 53.7 ± 32.1 days) were enrolled. The incidence of gastroenteritis was lower in the supplemented group than in the controls (0.12 ± 0.04 vs. 0.29 ± 0.05 episodes/child/12 months; $p = 0.015$). The number of children with more than 3 episodes tended to be lower in prebiotic group (17/60 vs. 29/65; $p = 0.06$). The number of children with multiple antibiotic courses/year was lower in children receiving prebiotics (24/60 vs. 43/65; $p = 0.004$). A transient increase in body weight was observed in children on prebiotics compared to controls during the first 6 months of follow-up.

Conclusions: Prebiotic administration reduce intestinal and, possibly, respiratory infections in healthy infants during the first year of age.

1. Introduction

Infectious diseases are generally less frequent in breast-fed infants than in formula-fed infants[1,2] and this may be due in part to the peculiar pattern of microbial colonization typical of mother's milk.[3,4] Prebiotics are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of a limited number of bacterial species in the colon".[5] Their addition to infant formulas is expected to exert beneficial effects on health. However, although prebiotics increase the load of lactobacilli and bifidobacteria and make intestinal microflora of formula-fed infants similar to that observed in breast-fed infants,[6–9] it is not clear whether this translates into clinically relevant effects.

The intestinal microbiota of breast-fed infants is generally dominated by bifidobacteria and lactic acid bacteria, whereas the intestinal microecology of formula-fed infants is more similar to that of adults in that it contains heavier loads of Bacteroides, Clostridia and Enterobacteriaceae.[3,4,10] It is now becoming clear that the intestinal microflora is in a symbiotic relationship with the host and may drive immune imprinting in the early life.[11–13] Therefore dietary components that are capable of modifying the composition of intestinal microbiota may affect the postnatal

* Corresponding author. Tel.: +39 081 7464232; fax: +39 081 5451278.
E-mail address: alfguari@unina.it (A. Guarino).

0261-5614/$ – see front matter © 2009 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2009.01.008

development of the immune system, leading to clinical effects. A link between changes in intestinal microflora and prevention of diseases has been shown for probiotics. Administration of selected probiotics has been associated with the prevention of gastrointestinal infections[14] and with a reduced incidence of respiratory tract infections.[15] We recently reported that administration of *Lactobacillus* GG reduced the incidence of pulmonary exacerbations and of hospital admissions in cystic fibrosis patients.[16] For prebiotics there is virtually no evidence of clinical effects.

Despite the wealth of evidence that a galacto/fructo-oligosaccharide (GOS/FOS) mixture positively affects the intestinal microflora, it is not yet clear whether this results in clinically relevant effects. Recently it was shown that term infants fed with a formula supplemented with a prebiotic mixture containing polydextrose, oligosaccharides and lactulose had growth and stool characteristics more similar to those of breast-fed infants compared with infants fed with a standard formula.[17] Very recently, a formula supplemented with the GOS/FOS mixture significantly reduced the development of atopic dermatitis in high risk infants.[18] The same prebiotic mixture reduced episodes of all types of infections in infants at risk of atopy.[19] We designed this prospective, case-controlled, randomized study to test the hypothesis that prebiotics may have clinically relevant effects in a normal infant population. To this aim, we investigated whether early administration of a GOS/FOS-containing formula affected the risk of intestinal and/or respiratory infections in infants.

2. Patients and methods

2.1. Study design

A multicenter, prospective, randomized, placebo-controlled open trial with two intervention groups was carried out with the collaboration of family pediatricians. Due to the pilot nature of the study, and in line with the proposal by participating family pediatricians, an open-study design was applied. Healthy infants aged between 15 and 120 days were enrolled by 38 pediatric practitioners in 7 Italian regions, after informed consent was obtained from the parents. The inclusion criteria were gestational age between 37 and 42 weeks, birth weight greater than 2500 g, and introduction of formula feeding after at least 15 days of exclusive feeding with maternal milk. Exclusion criteria were twins, clinically significant illness of the mother, congenital immunodeficiency, any chronic or progressive diseases, proven or suspected allergy, and previous intake of pro- or prebiotics. Infants were enrolled when formula milk was introduced and this was always done independently from the study.

Eligible infants were randomly assigned to receive either a standard infant formula or a prebiotic-supplemented formula for 12 months. The two formulas were identical except for the addition of a mixture of GOS/FOS in a ratio of 9:1. The concentration of GOS/FOS was 0.4 g/100 ml and the two formulas showed a similar osmolality (280 mOsm/l and 290 mOsm/l, respectively). Randomization was conducted using a random numbers table with a block design for groups of 10 infants.

Informed consent was obtained from the parents of all enrolled children. The study was approved by the local ethics committee.

2.2. Study end points

Primary outcome measures were incidence of acute diarrhea, incidence of upper and lower respiratory tract infections, and number of antibiotic courses prescribed for respiratory infections. Acute diarrhea was defined as a stool pattern with 3 or more loose or watery stools/day lasting at least for 3 days. Otitis, pharyngitis, laryngitis, tracheitis and bronchitis were considered as upper respiratory tract infections (URTIs). The diagnosis was made by the family pediatrician based on specific symptoms, with or without fever. All episodes of respiratory infections were recorded during a follow-up of 12 months. Antibiotic courses were also recorded. Secondary outcome measures, used to assess nutritional adequacy, were weight, length and head circumference gain. All the parameters were recorded at enrolment and were monitored at 3, 6, 9 and 12 months after enrolment.

2.3. Study protocol

Each infant underwent the routine evaluation by pediatrician and was monitored for 12 months after enrolment. Every 3 months, the routine visit included a medical examination and measurements of weight, length and head circumference by standard methods.[20] Further clinical assessment was performed in case of gastrointestinal or respiratory symptoms. Primary and secondary outcome measures were recorded on a specific case-report form.

2.4. Statistical analysis

The sample sizes required to detect a reduction of 30% in the incidence of acute diarrhea and of URTI, at a 5% significance level and with 85% of power, included 150 infants for acute diarrhea and 200 infants for URTI per group. These numbers were based on the expected incidence of 0.5 episodes/year of acute diarrhea in children up to 3 years of age and of 5 episodes/year of URTI in children up to 4 years of age. A total of 342 children were enrolled in the study (169 in GOS/FOS group and 173 in the control group). A total of 201 children completed the study (96 in GOS/FOS group and 105 in the control group). The drop out rate at the different follow-up times was compared using the Kaplan–Meier method. Log-Rank test showed no significant differences ($p = 0.212$). In order to test a *Missing Completely At Random* (MCAR) mechanism, at each time (3, 6, 9, 12 months), the growth parameters of the lost to follow-up children, taken at the previous follow-up times (respectively, at the enrolment time and at 3,6 and 9 months), were compared with that of the infants who continued the study, using the *t*-test for independent samples and no significant differences were detected ($p \gg 0.05$, data not shown). The lack of any differences in the drop out pattern of the two groups allowed us to consider in the statistical analysis of the outcome variables, only those children who effectively complete the study, thus we perform a per protocol analysis. However, in order to measure the effective power of the statistical procedures (based on the reduced sample size), a post-experimental power analysis was performed. The power curves were evaluated by using the upper bound of the confidence intervals (with a confidence level of 95%) for the standard deviation of each outcome variable, assuming that the data were normally distributed.

The results of the two formula groups were evaluated with the *t*-test for anthropometric measurements (results expressed as means $\pm$ SD) and for the incidence of acute diarrhea and URTI (results expressed as means $\pm$ SEM). We used the χ^2 analysis to compare the proportion of children with URTI and with acute diarrhea, as well as to compare the rates of antibiotic use in the two groups. Value of $p < 0.05$ was considered significant.

3. Results

3.1. Baseline features of infants enrolled

Fig. 1 shows the flow chart of enrolled children throughout the study. A total of 349 infants were assessed for eligibility, 7 were

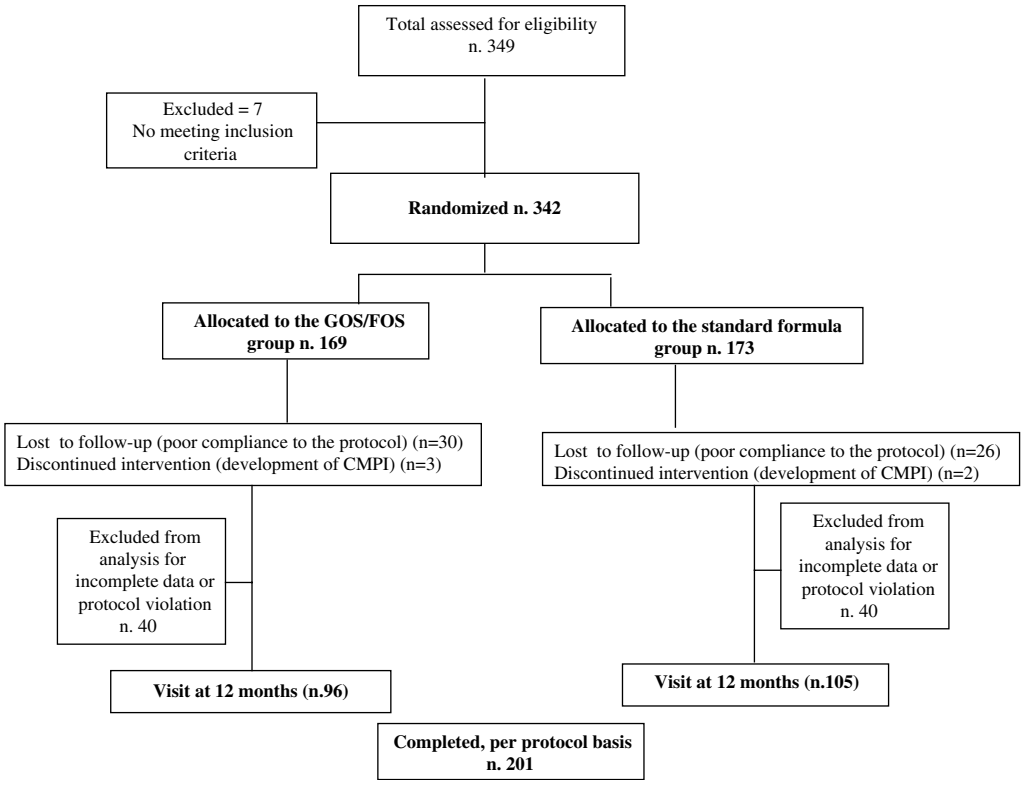

Fig. 1. Flow chart of children enrolled in the study. A total of 349 infants were assessed for eligibility; 342 infants were assigned randomly to the GOS/FOS formula ($n = 169$ infants) or to the control formula ($n = 173$ infants).

excluded (2 were born at 36 weeks of gestation, 2 were twins, and 3 were small for gestational age). Thus, the study population consisted of 342 infants who were randomly assigned to the GOS/FOS formula ($n = 169$ infants) or to the control formula ($n = 173$ infants). At enrolment, there were no differences between the two groups in terms of gender, gestational age, mean weight at birth, mean duration of breastfeeding, mean age, weight, length, head circumference (Table 1). The relative number of children lost to follow-up was similar in the two groups. Seventy-three infants in the GOS/FOS group and 68 in the control group failed to complete the study because of non-compliance and protocol violation (such as early introduction of weaning or probiotic assumption) or because of incomplete data, and a total of 201 infants completed the study. During the first 3 months of the study, 3 infants in the GOS/FOS group and 2 in the standard formula group developed cow's milk protein intolerance (CMPI). There was no evidence of a cause–effect relationship between the introduction of enriched formula and cow's milk protein intolerance.

3.2. Safety and tolerance

There was no report of major side effects. The stool pattern of children receiving prebiotics was generally characterized by softer but not diarrheic stools and in no case was the prebiotic formula withdrawn.

3.3. Primary outcome measures

As shown in Fig. 2, the rate of diarrheal episode/child was significantly lower in children receiving the GOS/FOS formula than in controls (0.12 ± 0.04 vs. 0.29 ± 0.05 episodes/child/12 months; C.I. 95% mean difference – 0.3–0.03; $p = 0.015$) (Fig. 2A). In addition, the number of children with at least 1 episode of acute diarrhea was significantly lower in the GOS/FOS group 10/96 (10.4%) vs. 26/109 (23.8%); $p = 0.01$, RR: 0.44; C.I. 95% RR: 0.22–0.86 (Fig. 2B).

The number of episodes of URTI was lower in the GOS/FOS group, but the difference was not significant ($p = 0.4$). The number of children with at least 1 episode of URTI was similar in the two groups. However, among the children with at least 1 episode of URTI, the number of children with recurrent URTI, defined as more than 3 episodes of URTI in 12 months, was lower in children fed with the GOS/FOS formula (17/60 vs. 29/65) and the difference was close to significance ($p = 0.06$; Fig. 3A).

Administration of prebiotics was associated with a lower number of antibiotic prescription. The mean rate of antibiotic courses prescribed for children fed with GOS/FOS was significantly lower compared to controls (1.03 ± 0.15 vs. 1.48 ± 0.16; $p = 0.038$; C.I. 95% mean difference – 0.88–0.02). Moreover, the percent of children receiving 2 or more antibiotic courses/year was

Table 1
Baseline characteristics of the children enrolled in the study.

	GOS/FOS group ($n = 169$)	Standard formula group ($n = 173$)
Gender (M/F)	80/89	83/90
Gestational age (weeks)	39.14 ± 1.35	39.83 ± 1.38
Mean weight at birth (g)	3168.7 ± 479.6	$32{,}386 \pm 507.5$
Mean duration of breastfeeding (day)	60.7 ± 48.5	60.2 ± 48.8
Enrolment parameters		
Mean age (days)	54 ± 32.6	53.9 ± 34.1
Mean weight (g)	4645.6 ± 1191	4643.7 ± 1183
Mean length (cm)	55.3 ± 4.3	55.3 ± 4.4
Mean head circumference (cm)	37.7 ± 3.8	37.4 ± 3.8

Data reported as means ± SD.

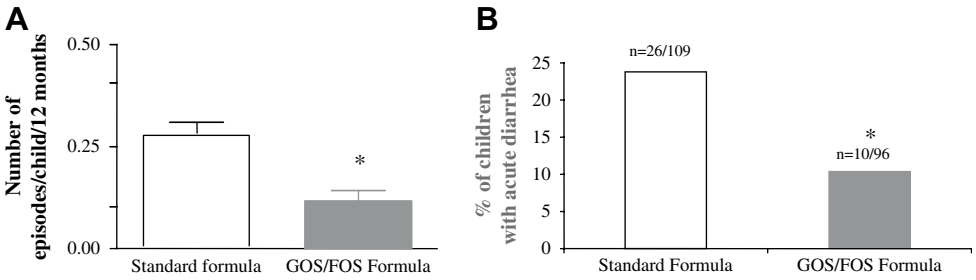

Fig. 2. Acute gastroenteritis in children receiving GOS/FOS enriched formula and in controls. Mean number of episodes of gastroenteritis was significantly lower in the GOS/FOS group than in controls ($p = 0.01$) (A). A lower proportion of children with at least 1 episode of gastroenteritis was observed in the GOS/FOS group ($p = 0.01$) (B).

significantly lower in children receiving prebiotics (24/60 vs. 43/65; $p = 0.004$; RR: 0.6; C.I. 95% RR: 0.42–0.82 (Fig. 3B)). The latter finding is consistent with the observed reduced number of children with recurrent URTI in the GOS/FOS group.

The number of lower respiratory tract infections was very low in both controls and GOS/FOS group (4 vs. 6 episodes, respectively) and no further evaluation was made.

3.4. Secondary outcome measures

Secondary outcome measures are shown in Fig. 4. The average growth parameters (i.e., weight, length and head circumference) were within the normal ranges in both groups. However, the mean body weight was significantly increased at 3 and 6 months of follow-up, in the GOS/FOS formula group compared to the control group ($p < 0.01$), whereas it was similar in the two groups at 9 and 12 months of follow-up (Fig. 4A). Mean body length was significantly greater in the GOS/FOS group at 3, 6, 9 and 12 months of follow-up than in controls ($p < 0.05$) (Fig. 4B). Mean head circumference was similar in the two groups at 3, 6, 9 and 12 months of follow-up (data not shown).

4. Discussion

Prebiotic addition to infant formula is associated with increased costs compared with standard infant formula and it is therefore important to establish whether this intervention is associated with clinical effects.

The results of this study provide evidence of an association between the administration of prebiotics and clinical effects in healthy infants. The most convincing finding is the preventive effect exerted against intestinal infections. This was supported by the reduction of the total number of diarrheal episodes and of the number of children with at least 1 gastroenteritis episode in those receiving the enriched formula. In this context, it is noteworthy that probiotics have been found to give protection against acute gastroenteritis.[14]

A less robust but even more interesting observation is the reduction of respiratory infections. Although the direct parameter was not significantly different between the two groups, all outcome measures enclosed in the evaluation pointed toward a decreased incidence of respiratory infections in the GOS/FOS group. The protective effect by prebiotics was further supported by the significant reduction of antibiotic courses in infants receiving the enriched formula.

A preventive effect by modified intestinal microflora against non-intestinal infections is not entirely new. We and others have observed a lower incidence of respiratory infections after the administration of probiotics in children at increased risk of respiratory infections,[15,16] such as those with cystic fibrosis. Recently it has been published that the administration of GOS/FOS enriched formula protects formula-fed infant, at high risk of atopy, against infections during the first 6 months of life.[19]

Infections of the intestinal or respiratory tract, although of mild degree in the vast majority of infants, are very frequent and are associated with substantial costs. Preventive strategies are based on vaccination against the most frequent agents, i.e., influenza virus and rotavirus,[21,22] whereas specific therapies are lacking. The results of our study may open new perspectives in terms of prevention of frequent infections in infancy through functional nutrition in early infancy.

However, the preventive effects on infections was not the only clinical effect we observed. The growth pattern differed between controls and children of the GOS/FOS group, with the latter showing a significant transient increase in weight and height at 3 and 6 months. Growth parameters of the two groups of children

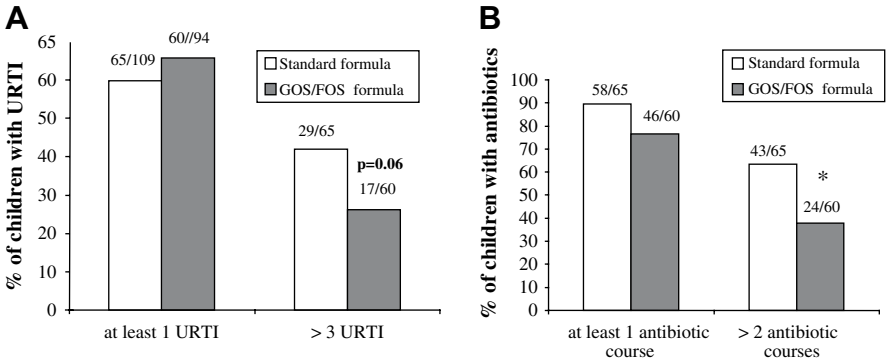

Fig. 3. Upper respiratory tract infections and antibiotic use in children receiving GOS/FOS enriched formula and in controls. A lower, but not significant, proportion of children with more than 3 episodes of URTI was observed in the GOS/FOS (A). The percent of children receiving ≥2 antibiotic courses/year was significantly lower in children receiving prebiotics ($p = 0.02$) (B).

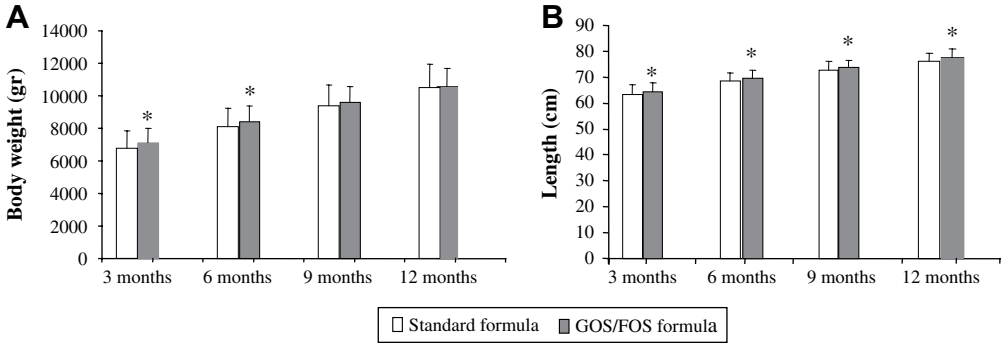

Fig. 4. Growth parameters in children receiving GOS/FOS enriched formula and in controls. Children in GOS/FOS group showed a significantly higher mean body weight compared to controls during the first 6 months of follow-up ($p < 0.01$) (A). This difference was not observed at 9 and 12 months of follow-up. Children in GOS/FOS group showed a mean length significantly higher compared to controls at 3, 6, 9 and 12 months of follow-up ($p < 0.05$) (B).

were similar at subsequent controls and at the end of the study. In contrast, the significant difference in length persisted, although it was of little absolute magnitude. It seems unlikely that the changes in growth parameters result from fewer infections, rather, a metabolic mechanism could be involved given the capacity of oligosaccharides to increase intestinal calcium absorption.[23] Whether prebiotics may induce a transient modification of a growth parameter in healthy children remains to be confirmed and does not beg substantial clinical implications.

Our study has several limitations, the main of which being its open label design. This was discussed with the pediatricians involved in the study and it was concluded that a double blind design was not easily feasible. Both formulas were already commercially available and the introduction of blinding may have contributed in reducing the population size. It was eventually decided to adopt an observational protocol for this study. The relatively high dropouts, due to a long term observation, did not allow an intention to treat analysis, but the lack of difference in dropouts between the two groups and the post-experimental power analysis confirmed the statistical significance of our results.

Although many infant formulas enriched with prebiotics are commercially available, there is little proof of clinical advantages. The available data are largely of microbiological nature and based on the concept of making intestinal microecology of formula-fed infants similar to that of breast-fed infants. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition has made a call for clinical trails on prebiotics in order to produce recommendations on their inclusion in infant formula.[24] Prebiotics are already included in infant formula, which stresses the need for clinical trials. When investigating the effects of functional nutrition in healthy subjects, the transfer from animal or experimental results to clinical application may be hampered by the high number of variables and confounding factors. Clinical research, may provide, through field studies, answers to the obvious question of whether investing money to improve infant formula is justified by clinically measurable benefits.

Conflict of interest

The study was partially supported by an unrestricted grant from Numico Research, Friedrichsdorf, Germany. None of the authors have a conflict of interest and received any personal gain from this study.

Acknowledgments

The study was partially supported by an unrestricted grant from Numico Research, Friedrichsdorf, Germany. The sponsor has no role in the study design, in the collection analysis and interpretation of data, in the writing the manuscript and in the decision to submit the manuscript for publication. None of the authors received any personal gain from this study.

All authors contributed significantly to the study and take public responsibility for the content of the article, including the conception, design, and conduct of the study and for data interpretation. All the authors have read and approved the final manuscript.

- Alfredo Guarino and Eugenia Bruzzese carried out the study and data analysis and drafted the manuscript.
- Monica Volpicelli contributed to study design, to collection of the data and to writing the manuscript.
- Veronica Squeglia collected the data and contributed to the data analysis.
- Dario Bruzzese performed the statistical analysis.
- Filippo Salvini participated in the study design and coordinated family pediatricians in the enrolment of infants.
- Massimo Bisceglia participated in the study design and coordinated family pediatricians in the enrolment of infants.
- Paolo Lionetti participated in the study design and coordinated family pediatricians in the enrolment of infants.
- Mario Cinguetti participated in the study design and coordinated family pediatricians in the enrolment of infants.
- Giuseppe Iacono participated in the study design and coordinated family pediatricians in the enrolment of infants.
- Sergio Amarri participated in the study design and coordinated family pediatricians in the enrolment of infants.

The authors thank Jean Ann Gilder for editing of text.

Appendix

Pediatricians who participated in the study: Mannocci M (Florence), Belfiori M (Florence), Generoso M (Florence), La Rosa S (Florence), Vitali Rosati G (Florence), Pasquini A (Florence), Pomo A (Florence), Cecere G (Naples), Castaldo C (Naples), Marino A (Naples), De Franchis R (Naples), La Torraca AF (Modena), Martone P (Modena), Renne P (Modena), Lalinga G (Modena), Sulla AM (Crotone), Aloisio A (Crotone), Senatore S (Crotone), Leopardi F (Crotone), Cofano D (Milano), Ravelli M (Milan), Palla F (Milan), Torre R (Milan), Villa AL (Milan), Carrara D (Milan), Venturi MC (Milan), Guasti E (Verona), Polidoro E (Verona), Ariamone L (Verona), Bondovalli S (Verona), Muscarella A (Palermo), Mancuso G (Palermo), Alfano G (Palermo), Zingone A (Palermo), Mancuso C (Palermo), Gentile M (Palermo), Rizzarri R (Palermo), Antonino G (Palermo).

References

1. Howie PW, Forsyth JS, Ogston SA, Clark A, Florey CD. Protective effect of breast feeding against infection. *Br Med J* 1990;**300**:11–6.
2. Kunz C, Rudloff S, Baier W, Klein N, Strobel S. Oligosaccharides in human milk: structural, functional and metabolic aspects. *Annu Rev Nutr* 2000;**20**: 699–722.
3. Newburg DS. Oligosaccharides in human milk and bacterial colonisation. *J Pediatr Gastroenterol Nutr* 2000;**30**:S8–17.
4. Orrhage K, Nord CE. Factors controlling the bacterial colonization of the intestine in breast fed infants. *Acta Paediatr* 1999;**88**(Suppl. 430):47–57.
5. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995;**125**:1401–12.
6. Boehm G, Stahl B. Oligosaccharides. In: Mattila-Sandholm T, editor. *Functional dairy products*. Cambridge: Woodhead Publ; 2003. p. 203–43.
7. 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**:291–5.
8. Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B, et al. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of fecal bifidobacteria in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2002;**86**:F178–81.
9. Knol J, Scholtens P, Kafka C, Steenbakkers J, Gro S, Klarczyk M, et al. Colon microflora in infants fed formula with galacto- and fructo-oligosaccharides: more like breast-fed infants. *J Pediatr Gastroenterol Nutr* 2005;**40**: 36–42.
10. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 2000;**30**:61–7.
11. Ouwehand A, Isolauri E, Salminen S. The role of the intestinal microflora for the development of the immune system in early childhood. *Eur J Nutr* 2002;**41**(Suppl. 1):132–7.
12. Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001;**108**:516–20.
13. Köhler H, McCormick BA, Walker WA. Bacterial-enterocyte crosstalk: cellular mechanisms in health and disease. *J Pediatr Gastroenterol Nutr* 2003;**36**: 175–85.
14. Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhoea in infants and children: a systematic review of published randomized, double-blind, placebo-controlled trials. *J Pediatr Gastroenterol Nutr* 2001;**33**:S17–25.
15. Hatakka K, Savilahti E, Ponka A, Meurman JH, Poussa T, Nase L, et al. Effect of long term consumption of probiotic milk on infection in children attending day care centres: double blind, randomised trial. *Br Med J* 2001;**322**:1327.
16. Bruzzese E, Raia V, Spagnuolo MI, Volpicelli M, De Marco G, Maiuri L, et al. Effect of *Lactobacillus* GG supplementation on pulmonary exacerbations in patients with cystic fibrosis: a pilot study. *Clin Nutr* 2007;**26**:322–8.
17. Ziegler E, Vanderhoof JA, Petschow B, Mitmesser SH, Stolz SI, Harris CL, et al. Term infants fed formula supplemented with selected blends of prebiotic grow normally and have soft stools similar to reported for breast-fed infants. *J Pediatr Gastroenterol Nutr* 2007;**44**:359–64.
18. 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.
19. Arslanoglu S, Moro GE, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first six months of life. *J Nutr* 2007;**137**:2420–4.
20. Johnson TS, Engstrom JL, Gelhar DK. Intra and interexaminer reliability of anthropometric measurements in term infants. *J Pediatr Gastroenterol Nutr* 1997;**24**:497–505.
21. Vesikari T, Van Damme P, Giaquinto C, Gray J, Mrukowicz J, Dagan R, et al, European Society for Paediatric Gastroenterology, Hepatology, and Nutrition/ European Society for Paediatric Infectious Diseases. Evidence-based recommendations for rotavirus vaccination in Europe. *J Pediatr Gastroenterol Nutr* 2008;**46**(S2):S38–48.
22. American Academy of Paediatrics Committee on Infectious Diseases. Prevention of influenza: recommendations for influenza immunization of children 2006–2007. *Pediatrics* 2007;**119**:846–51.
23. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, et al. A combination of prebiotic short and long-chain insulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. *Am J Clin Nutr* 2005;**82**:471–6.
24. Agostoni C, Axelsson I, Goulet O, Koletzko B, Michaelsen KF, Puntis JWL, et al, ESPGHAN Committee on Nutrition. Prebiotic oligosaccharides in dietetic products: a commentary by ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2005;**39**:465–73.