REVIEW ARTICLE

Clinical Effects of Prebiotics in Pediatric Population

ROK OREL AND LEA VODUŠEK REBERŠAK

From University Medical Centre, Ljubljana, University Children's Hospital; Ljubljana, Slovenia.
Correspondence to: Prof Rok Orel, University Children's Hospital, Bohoričeva 20, 1000 Ljubljana, Slovenia. rok.orel@kclj.si

Context: Prebiotics are non-digestible components of food that in a selective manner trigger the expansion of microbes in the gut with valuable effects for the health of the host. In our document, current literature pertaining to the clinical effects of the use of prebiotics for the treatment and prevention of some common pediatric pathology such as infantile colic, constipation, absorption of minerals, weight gain, diarrhea, respiratory infections, and eczema is reviewed.

Evidence: Data was collected through search of the MEDLINE, PubMed, UpToDate, Cochrane Database of Systemic Reviews, and the Cochrane Controlled Trials Register database as well as through references from relevant articles, all until September 2015. However, only the results of publications with adequate methodological quality were included.

Results: Prebiotics seem to be very appealing in treatment of many clinical conditions, explicitly in the fight against constipation, poor weight gain in preterm infants, and eczema in atopic children. In contrast to probiotics, the evidence of true clinical efficacy of prebiotics, supported with exact type and dose information are rather sparse, and there are a limited number of randomized controlled trials concerning prebiotics in children, especially beyond the age of infancy.

Conclusion*:* Large well-designed, controlled, confirmatory clinical trials are required, using commercially available products, to help healthcare providers in making an appropriate decision concerning the appropriate use of prebiotics in different conditions.

Keywords: *Conetipation, Eczema, Nutrition, Prevention*.

Prebiotics are non-digestible components of food that in a selective manner trigger the expansion of microbes in the gut with valuable effects for host health [1]. International Scientific Association for Probiotics and Prebiotics (ISAPP) postulated that prebiotics must fulfil three norms as: (*i*) escape digestion by human digestive enzymes in the upper gastrointestinal tract (GIT); (*ii*) be fermented by intestinal microorganisms; and (*iii*) selectively stimulate the growth and activity of those intestinal microorganisms that are associated with health and well-being of the presenter [2].

All these demands are completed by non-digestible oligosaccharides that consist of three to ten sugar molecules, and are naturally present in fruits, vegetables, cereals, milk, *etc*., or can be industrially produced [1,3,4]. They represent an excellent meal for the resident society of saccharolytic bacteria in our gut. Main end-products of their fermentation are short-chain fatty acids (SCFAs). Different bacteria prefer one energy source over the other [5]. Consequently, diet becomes a powerful tool in directing gut microbial population [6,7]. That is in fact the foundation of prebiotic concept as we have the influence to supply favourite 'fuel' to specific microorganisms recognized as promoters of good health.

At the moment, animal and human studies favor the use of galacto-oligosaccharides (GOS), inulin, and fructo-oligosaccharides (FOS). In addition, to imitate the human milk oligosaccharides (HMO) a mixture of short-chain GOS and long-chain FOS was developed. According to the Commission Directive on infant formulae and follow-on formulae in European Union, fructo-oligosaccharides and galacto-oligosaccharides may be added to infant formulae, nonetheless their proportion must not exceed 0.8 g/100 mL in a combination of 90% oligogalactosyl-lactose and 10% high molecular weight oligofructosyl-saccharose [8].

Nutritional and health benefits of prebiotics are gripping attention among consumers, food and pharmaceutical industry, and health professionals [9,10]. Some experts even established them to be superior to probiotics as they are cheaper to produce, more affordable for mass consumers, trust worthier compared to consuming live bacteria, and less susceptible to environmental stresses; nevertheless, probiotics cannot function in the absence of prebiotics [11].

Special attention should therefore be focused on interpreting studies and conclusions as financial drive is enormous, whereas clinical evidence is often sparse.

For this review, we collected data through search of the MEDLINE, PubMed, UpToDate, Cochrane Database of Systemic Reviews and the Cochrane Controlled Trials Register database as well as through

references from relevant articles. Duplicates were discarded. A search strategy was used based on combination of MeSH terms: 'prebiotics' and 'pediatrics'. The last search was conducted September 5th, 2015. 459 full-text articles were analyzed by two of the authors. A descriptive and explanatory qualitative approach was chosen for the content analysis.

MECHANISMS OF ACTION

Indirect effects of prebiotics arise through stimulation of health-promoting microbial taxa, such as *Bifidobacterium* and *Bacteroides* [12-14], which block intestinal pathogens, improve intestinal barrier function and orchestrate immune pathways. There is even some evidence of gut microbiota influencing brain function over microbiota-gut-brain axis [15].

Direct effects are due to the action of SCFAs. They are largely produced in colon where they act locally; however, some of them reach high concentration in the bloodstream carrying them across the body and allowing them to interact in extra-intestinal reactions [16]. The main SCFAs formed are acetate, propionate and butyrate. Their primary task is the energy supply for intestinal epithelial cells, though they also play a role in gene expression, gut motility and barrier function, metabolite absorption, lipid metabolism, appetite control, insulin resistance, gut-liver axis regulation, and regulation of the immune system, resulting in prevention of infection, diarrhea, constipation and allergies [17].

CLINICAL USES IN CHILDREN

Infantile Colic

In a randomized non-blinded trial, published almost ten years ago, 96 formula-fed infants under 4 months of age with colic received a partially hydrolyzed whey protein formula containing FOS and GOS. They experienced a greater reduction of crying episodes after 7 and 14 days compared with those assigned to a standard formula and simethicone [18]. However, whether the effect is due to partially hydrolysed protein, the prebiotics, or both, is not clear. Pärtty, *et al* [19]. studied preterm infants randomized to receive a mixture of GOS and polydextrose (1:1), probiotics or placebo during first 2 months of life, and followed-up for 1 year. In both pre- and probiotic groups, significantly less frequent crying was observed compared with the placebo group (19% *vs*. 19% *vs*. 47%, respectively; $P = 0.02$) . On the other hand, a systematic review and meta-analysis including 12 prebiotic studies found no impact of prebiotics on the incidence of colic, regurgitation, crying, restlessness or vomiting [20]. Nonetheless, adding prebiotics to infant formula for full-term infants was reviewed. Although, further confirmatory studies are needed, no adverse effects of prebiotics were found during this review.

Constipation

Majority of clinical studies concerning the effects of supplementation of infant formulas with prebiotics confirmed increase in frequency of defecation and/or softer consistency of stools, similar to that of breast-fed infants [21-31]. Current analysis of stool characteristics of infants receiving short-chain GOS (scGOS) and long-chain FOS (lcFOS) in ratio 9:1 showed that effects on stool consistency were more often found to be significant than effects on stool frequency [32]. Bongers, *et al*. [33] published the only therapeutic randomized controlled trial (RCT) using prebiotic formula for functional constipation in 2007. The consumption of a high concentration sn-2 palmitic acid, scGOS/lcFOS 8g/l and partially hydrolyzed whey protein formula resulted in a strong tendency of softer stools in constipated infants, but not in a difference in defecation frequency. In a randomized, double-blind, prospective study [25], it was shown that prebiotics can soften stools and increase stool frequency even in toddlers [25].

A more recent study, [34] indicated a significant rush of motilin following prebiotic supplementation. Motilin being a peptide, produced by endocrine M cells, largely presents especially in duodenum and jejunum. Its essential role is to clean undigested food from the gut by controlling inter-digestive migrating contractions [34]. All together suggesting an association with improved gastric emptying, better tolerance to food and improved digestion in general [35].

Changes in defecation patterns in pediatric population due to prebiotic supplementation mostly result in improvement of abdominal comfort and reduction of prevalence of functional constipation. Since constipation affects one third of children usually before the age of five [36-38] but often persists beyond puberty, these observations are relevant for preventive or curative treatment of this very common functional disorder [39]. Yet, to establish specific doses in avoiding diarrhea, more studies are awaited.

Absorption of minerals

Acidic environment in colon increases solubility of certain minerals [40]. Bioavailability of calcium when consuming prebiotic ingredients has been well-studied. Animal studies verified the positive correlation; efficiency in humans is nevertheless not consistent. Abrams, *et al*. [41] found significantly enhanced calcium absorption and bone mineralization in adolescents after receiving inulin-type fructans daily for a year. On the

contrary, no significant effect of prebiotics was observed on calcium absorption or other markers of bone mineralisation in infants [42]. Recent observations show that prebiotic oligosaccharides enhance iron absorption in deficient rats [43]. Clearly, further human trials are needed, but this seems to be encouraging information, given the prevalence of iron-deficiency in children.

Weight-gain

At the Summer Meeting of the Nutrition Society in 2010, it was announced that an overview of studies investigating effects of oral SCFA on appetite regulation did not reveal a positive connection. The experts concluded that sensory characteristics are those influencing our choice of which food we eat and the quantity of it rather than a physiological effect of SCFA [44]. In children, especially in the first months of life when milk is the basic nutrition, there are some encouraging results. For instance, Mugambi, *et al*. [20] conducted a meta-analysis that summarized positive context of prebiotics in infant formulas and increased weight gain; there was no impact on length or head circumference gain. Whether this is the result of intensified energy harvests by intestinal bacteria and/or increased absorption by enterocytes is not yet clear. It is very likely that the outcome is dose-dependent [14]. Interestingly, these results are to some extent antagonistic with the inverse correlation between fibre intake and obesity known in adults as well as in adolescents [22,45,46]. In fact, dietary fibre reduces the risk of childhood obesity by up to 21% [47]. Furthermore, Dasopoulou, *et al*. [35] found that supplementation of infant formula with scGOS/lcFOS resulted in significantly lower mean cholesterol values compared with preterm neonates fed with standard formula.

Diarrhea

An open-label RCT published six years ago [48], included more than 300 healthy infants, age 1-2 months. The group receiving a GOS/FOS mix had a significantly lower number of gastrointestinal infections and antibiotic use per year [48]. Still, when Duggan, *et al*. [49] studied a group of 282 infants 6-12 months of age, there was no difference in diarrheal prevalence or the mean duration of diarrhea between those receiving an infant cereal enriched with oligofructose with and without prebiotics [49].

Destruction of microbial population in GIT has the power to start the so called antibiotic-associated diarrhea. Preventive intervention by giving prebiotics after or along with antibiotic treatment has so far not been properly evaluated. A RCT published in 2006 by Brunser, *et al*. [50] showed no significant difference in the frequency of antibiotic-induced diarrhea between two groups, aged 1-2 years. The first group received inulin and oligofructose (total of 4.5 g/L) containing milk formula for 3 weeks after they had ended amoxicillin therapy for respiratory infection. The second group received prebiotic-free milk formula [50]. Another trial was organized by the ESPGHAN Working Group on Pro- and Pre-biotics. In this multi-centre trial, children with oral and/or intravenous antibiotic therapy covering common infections were treated with inulin and FOS in age-dependent doses (max 5g/day) for as long as they were taking antimicrobial drugs. These children were below 11 years old and tolerated the mixture well; nonetheless, it had no effect regarding antibiotic-associated diarrhea. The study was stopped before time because of slow recruitment and the working group concluded that overall prevalence of diarrhea was not high and caution must be taken when judging the results. However, there is a need for further research with different prebiotics [51].

Administration of prebiotic compounds *via* oral rehydration solution (ORS) is under investigation. A decade ago, Hoekstra, *et al*. [52] also completed a multi-centre European double-blind randomized placebo controlled study on behalf of the ESPGHAN (European Society for Paediatric Gastroenterology, Hepatology, and Nutrition) Working Group on intestinal infection. The subject was ORS containing a mixture of prebiotics (soy polysaccharides 25%, alpha-cellulose 9%, gum arabic 19%, FOS 18.5%, inulin 21.5%, resistant starch 7%) in the acute diarrhea treatment. Children aged 1 month to 3 years with acute diarrhea resulting in mild-to-moderate dehydration were given either supplemented or non-supplemented ORS [52]. There was no significant difference between participants of the two groups in mean 48-h stool quantity, duration of symptoms and hospitalization [52]. No significant influence on clinical course of acute gastroenteritis was also reported by Israeli analysts. A mixture of 80% lcFOS/scGOS and 20% AOS in a three 2-g sachets per day significantly increased stool consistency (P=0.048) but not total of daily stools number (P=0.66) in 9- to 24-month-old children [53]. A recent randomized controlled trial in Italian children showed significant efficiency when FOS and xylo-oligosaccharides – both 0.35 g/L were consumed along with hypotonic ORS. Children aged 3 months to 3 years in prebiotic group drunk more ORS in the first 24 hours (P<0.001), diarrhea was over after 72 hours in a greater percentage (P=0.01) and their parents missed fewer working days compared to placebo

"team" of parents (P<0.001) [54]. An older double-blind, placebo controlled RCT including boys from Bangladesh enrolled a group of 150 male children, aged 4 to 18 months that looked for medical help because of watery non-cholera diarrhea of less than 48 hours of duration. Adding partially hydrolyzed guar gum to oral rehydration solution resulted in important reduction of total extent of acute gastroenteritis [55].

Respiratory infections

It would be simple, safe and economical if prebiotics would help to prevent respiratory infections. There are some supportive results revealed by Luoto and colleagues in a recent RCT. Ninety-four preterm infants were randomized to receive PDX/GOS 1:1 (600mg/24h day 1 to 30, 1200 mg/24h day 31 to 60), probiotic (*Lactobacillus rhamnosus* GG (LGG); 1×10^9 colony-forming units/24h day 1 to 30, 2×10^9 colony-forming units/24h day 31 to 60) or placebo for 60 days, starting on the third day of life. Follow-up visits were dated five times in the adjacent year. Significantly lower incidence of respiratory tract infections (RTI) was confirmed in the prebiotic group (P<0.001) as well as in the probiotic group (P=0.022). Specifically, Rhinovirus being etiological cause of 80% of contemplated RTIs, occurred less frequently in both non-placebo groups (prebiotic group: P=0.003; probiotic group: P=0.051). There was; however, no difference among participants in severity and duration of RTI [56]. On the contrary, a study published in 2012 found no difference in the incidence of infections of respiratory and gastrointestinal system in preterm neonates supplemented with a prebiotic mixture (80% scGOS/lcFOS + 20% AOS, maximum of 1,5g/kg/day) between days 3 and 30 of life compared to placebo [57].

To summarize, evidence to date is often contradictory but in general does not uphold the role of prebiotics in prevention or treatment of infectious diseases. However, supplementation of prebiotic compounds in infant formulas, as early as preterm period of life, and in ORS seems to be the most useful.

Eczema

Grüber and his team performed an international double-blind placebo-controlled trial in 832 low atopy-risk infants [58]. They were assigned either to the formula containing 6.8 g/L GOS/FOS (9:1) plus AOS 1.2 g/L, or standard formula. Results were compared to 300 breast-fed infants. At one year follow-up, the prebiotic group had almost comparable incidence of eczema to breast-fed babies (5.7 *vs* 7.3%, *vs* controls 9.7%). However, a similarly designed but more recent study found no difference in prevalence of atopic eczema and bronchial hyper-reactivity when GOS/FOS plus acidic oligosaccharides (4:1) mixture was added to milk formula in a lower dose (1.5 g/kg/day) in 92 preterm, low birth weight infants [57]. On the other hand, Ziegler, *et al*. [31] found that unselected term infants fed with chosen blends of prebiotics (polydextrose and GOS: 4 g/L *vs* polydextrose and GOS and lactulose: 8 g/L *vs* control) have a statistically important elevated risk for developing eczema (18% *vs* 4% *vs* 7%) .

When concerning infants with family history of allergic disease, the representative series of studies must be the three completed by Moro and Arslanoglu together with their colleagues. They began in 2006 with a pool of 259 children assigned to extensively hydrolyzed infant formula with or without prebiotics (scGOS/lcFOS 8 g/L) them during the first six month of life. In the intervention group, 9.8% developed atopic dermatitis, and 23.1% in the placebo group (P<0.05) [59]. Blinded, follow-up continued until second birthday, 107 participants dropped out. Cumulative incidences of atopic dermatitis, recurrent wheezing and allergic urticaria were higher in the control group (27.9; 20.6 and 10.3 %, respectively) compared to the prebiotic group (13.6; 7.6, and 1.5%) (P<0.05) [60]. After five years, with 92 children remaining in the study, both the prevalence and the persistence of any allergic symptoms were significantly lower in the supplemented (30.9% and 4.8%, respectively) than in the non-supplemented group (66% and 26%, respectively) (P<0.01). The differences for rhino conjunctivitis (2.4 vs.14%) and atopic dermatitis (19.1 vs. 38%) (P<0.05) were significant but not for persistent wheezing (4.8 vs.14%) [61].

There are still many questions about not only the etiology, immunology and genetics, but also about the role of prebiotic substances in prevention and/or treatment of allergic diseases. Evidence is still very sketchy; however, it seems that early supplementation, at least in predisposed population, might be of benefit.

SUMMARY

Regarding the mechanisms of action, prebiotics seem to be very appealing in prevention and treatment of many clinical conditions as in contrast to probiotics they may have more extensive influence on overall bacterial community in the gut, both regarding its composition and functioning. However, in contrast to probiotics the number of published methodologically adequate clinical trials on the efficacy of prebiotics, especially RCTs, supported with exact type and dose information, is rather sparse, especially beyond the age of infancy. The strongest evidence on beneficial effects of prebiotics in

children exists in relation to the fight against constipation, poor weight-gain in preterm infants and eczema in atopic children. The reasonableness of using prebiotics in some other diseases, including infantile colic, absorption of minerals and infectious diseases is still under investigation. It must be highlighted; however, that the safety profile of prebiotic use is excellent as no adverse effects were found in this review.

In conclusion, due to their strong potential to influence gut microbiota composition and function, as well as the extremely low risk of their use for serious adverse effects, prebiotics seem to be more than a promising tool for supporting health, prevention and treatment of many pathologic conditions in the intestine and beyond it. However, further large clinical studies are required using commercially available products to help health care providers and users in making an appropriate decision concerning the correct use of prebiotics in these conditions.

Contributors: Both authors contributed to review of literature, interpretation of data and manuscript writing.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, *et al*. Prebiotic effects: metabolic and health benefits. Br J Nutr. 2010;104:S1-S63.
2. First ISAPP Annual Meeting Report. Available from: *http://isappscience.org/annual-meeting-reports/* Accessed May 1, 2016.
3. Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: Downstream impacts on host immune function and health, and therapeutic implications. J Gastroenterol. 2014;49:785-98.
4. Weijers CAGM, Franssen MCR, Visser GM. Glycosyltransferase-catalyzed synthesis of bioactive oligosaccharides. Biotechnol Adv. 2008;26:436-56.
5. Morris C, Morris GA. The effect of inulin and fructo-oligosaccharide supplementation on the textural, rheological and sensory properties of bread and their role in weight management: A review. Food Chem. 2012;133:237-48.
6. Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev. 2004;17:259-75.
7. Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. Pharmacol Res. 2013;69:52-60.
8. Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. Available from: *http://eurlex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2006:401:TOC*. Accessed January 24, 2016.
9. Lamsal BP. Production, health aspects and potential food uses of dairy prebiotic galactooligosaccharides. J Sci Food Agric. 2012;92:2020-8.
10. Kothari D, Patel S, Goyal A. Therapeutic spectrum of nondigestible oligosaccharides: overview of current state and prospect. J Food Sci. 2014;79:R1491-8.
11. Homayoni Rad A, Akbarzadeh F, Mehrabany EV. Which are more important: prebiotics or probiotics? Nutrition. 2012;28:1196-7.
12. Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, *et al*. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes. 2011;60:2775-86.
13. Neyrinck AM, Possemiers S, Druart C, Van de Wiele T, De Backer F, Cani PD, *et al*. Prebiotic effect of wheat arabinoxylan related to the increase in bifidobacteria, Roseburia and Bacteroides/Prevotella in diet-induced obese mice. PLoS One. 2011;6:e20944.
14. Parnell JA, Peimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese ICR:LA-cp rats. Br J Nutr. 2012;107:601-13.
15. Wasilewski A, Zieliñska M, Storr M, Fichna J. Beneficial effects of probiotics, prebiotics, synbiotics, and psychobiotics in inflammatory bowel disease. Inflamm Bowel Dis. 2015;21:1674-82.
16. Marcobal A, Sonnenburg JL. Human milk oligosaccharide consumption by intestinal microbiota. Clin Microbiol Infect. 2012;18:12-5.
17. Jakobsdottir G, Nyman M, Fåk F. Designing future prebiotic fiber to target metabolic syndrome. Nutrition. 2014;30:497-502.
18. Savino F, Palumeri E, Castagno E, Cresi F, Dalmasso P, Cavallo F, *et al*. Reduction of crying episodes owing to infantile colic: A randomized controlled study on the efficacy of a new infant formula. Eur J Clin Nutr. 2006;60:1304-10.
19. Pärtty A, Luoto R, Kalliomäki M, Salminen S, Isolauri E. Effects of early prebiotic and probiotic supplementation on development of gut microbiota and fussing and crying in preterm infants: a randomized, double-blind, placebo-controlled trial. J Pediatr. 2013;163:1272-7.e1-2.
20. Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. Nutr J. 2012;11:81.
21. Holscher HD, Faust KL, Czerkies LA, Litov R, Ziegler EE, Lessin H, *et al*. Effects of prebiotic-containing infant formula on gastrointestinal tolerance and fecal microbiota in a randomized controlled trial. J Parenter Enteral Nutr. 2012;36,95S-105S.
22. Williams T, Choe Y, Price P, Katz G, Suarez F, Paule C, *et al*.Tolerance of forumals containing prebiotics in healthy, term infants. J Pediatr Gastroenterol Nutr. 2014;59: 653-8.
23. Scalabrin DM, Mitmesser SH, Welling GW, Harris CL, Marunycz JD, Walker DC, *et al*. New prebiotic blend of polydextrose and galacto-oligosaccharides has a bifidogenic effect in young infants. J Pediatr

Gastroenterol Nutr. 2012;54:343-52.
24. Ashley C, Johnston WH, Harris CL, Stolz SI, Wampler JL, Berseth CL. Growth and tolerance of infants fed formula supplemented with polydextrose (PDX) and/or galactooligosaccharides (GOS): double-blind, randomized, controlled trial. Nutr J. 2012;11:38.
25. Ribeiro TC, Costa-Ribeiro H Jr, Almeida PS, Pontes MV, Leite ME, Filadelfo LR, *et al*. Stool pattern changes in toddlers consuming a follow-on formula supplemented with polydextrose and galactooligosaccharides. J Pediatr Gastroenterol Nutr. 2012;54:288-90.
26. Veereman-Wauters G, Staelens S, Van de Broek H, Plaskie K, Wesling F, Roger LC, *et al*. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. J Pediatr Gastroenterol Nutr. 2011;52:763-71.
27. Westerbeek EA, Hensgens RL, Mihatsch WA, Boehm G, Lafeber HN, van Elburg RM. The effect of neutral and acidic oligosaccharides on stool viscosity, stool frequency and stool pH in preterm infants. Acta Paediatr. 2011;100:1426-31.
28. Vivatvakin B, Mahayosnond A, Theamboonlers A, Steenhout PG, Conus NJ. Effect of a whey-predominant starter formula containing LCPUFAs and oligosaccharides (FOS/GOS) on gastrointestinal comfort in infants. Asia Pac J Clin Nutr. 2010;19:473-80.
29. Bisceglia M, Indrio F, Riezzo G, Poerio V, Corapi U, Raimondi F. The effect of prebiotics in the management of neonatal hyperbilirubinaemia. Acta Paediatr. 2009; 98:1579-81.
30. Rao S, Srinivasjois R, Patole S. Prebiotic supplementation in full-term neonates: a systematic review of randomized controlled trials. Arch Pediatr Adolesc Med. 2009; 163:755-64.
31. Ziegler E, Vanderhoof JA, Petschow B, Mitmesser SH, Stolz SI, Harris CL, *et al*. Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. J Pediatr Gastroenterol Nutr. 2007;44:359-64.
32. Scholtens PA, Goossens DA, Staiano A. Stool characteristics of infants receiving short-chain galacto-oligosaccharides andlong-chain fructo-oligosaccharides: a review. World J Gastroenterol. 2014;20:13446-52.
33. Bongers ME, de Lorijn F, Reitsma JB, Groeneweg M, Taminiau JA, Benninga MA. The clinical effect of a new infant formula in term infants with constipation: a double-blind, randomized cross-over trial. Nutr J. 2007; 6:8.
34. Szurszewski JH. A migrating electric complex of canine small intestine. Am J Physiol. 1969;217:1757-63.
35. Dasopoulou M, Briana DD, Boutsikou T, Karakasidou E, Roma E, Costalos C, *et al*. Motilin and gastrin secretion and lipid profile in preterm neonates following prebiotics supplementation: a double-blind randomized controlled study. J Parenter Enteral Nutr. 2015;39:359-68.
36. Issenman RM, Hewson S, Pirhonen D, Taylor W, Tirosh A. Are chronic digestive complaints the result of abnormal dietary patterns? Diet anddigestive complaints in children at 22 and 40 months of age. Am J Dis Child. 1987; 141:679-82.
37. Ip KS, Lee WT, Chan JS, Young BW. A community-based study of the prevalence of constipation in young children and the role of dietary fibre. Hong Kong Med J. 2005;11:431-6.
38. Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiau J. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology. 2006;130:1519-26.
39. Van Ginkel R, Reitsma JB, Büller HA, van Wijk MP, Taminiau JA, Benninga MA. Childhood constipation: longitudinal follow-up beyond puberty. Gastroenterology. 2003;125:357-363.
40. Sabater-Molina M, Larqué E, Torrella F, Zamora S. Dietary fructooligosaccharides and potential benefits on health. J Physiol Biochem. 2009;65:315-28.
41. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, *et al*. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr. 2005;82:471-6.
42. Hicks PD, Hawthorne KM, Berseth CL, Marunycz JD, Heubi JE, Abrams SA. Total calcium absorption is similar from infant formulas with and without prebiotics and exceeds that in human milk-fed infants. BMC Pediatr. 2012;12:118.
43. Laparra JM, Díez-Municio M, Herrero M, Moreno FJ. Structural differences of prebiotic oligosaccharides influence their capability to enhance iron absorption in deficient rats. Food Funct. 2014;5:2430-7.
44. Darzi J, Frost GS, Robertson MD. Do SCFA have a role in appetite regulation? Proc Nutr Soc. 2011;70:119-28.
45. Carlson JJ, Eisenmann JC, Norman GJ, Ortiz KA, Young PC. Dietary fiber and nutrient density are inversely associated with the metabolic syndrome in US adolescents. J Am Diet Assoc. 2011;111:1688-95.
46. Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. Am J Clin Nutr. 2003;78:920-7.
47. Brauchla M, Juan W, Story J, Kranz S. Sources of Dietary Fiber and the Association of Fiber Intake with Childhood Obesity Risk (in 2-18 Year Olds) and Diabetes Risk of Adolescents 12-18 Year Olds: NHANES 2003-2006. Nutr Metab. 2012;2012:736258.
48. Bruzzese E, Volpicelli M, Squeglia V, Bruzzese D, Salvini F, Bisceglia M, *et al*. A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: An observational study. Clin Nutr. 2009;28:156-61.
49. Duggan C, Penny ME, Hibberd P, Gil A, Huapaya A, Cooper A, *et al*. Oligofructose-supplemented infant cereal: 2 randomized, blinded, community-basedtrials in Peruvian infants. Am J Clin Nutr. 2003;77:937-42.
50. Brunser O, Gotteland M, Cruchet S, Figueroa G, Garrido D, Steenhout P. Effect of a milk formula with prebiotics on the intestinal microbiota of infants after anantibiotic treatment. Pediatr Res. 2006;59:451-6.

51. ESPGHAN Working Group on Probiotics and Prebiotics, Szajewska H, Weizman Z, Abu-Zekry M, Kekez AJ, Braegger CP, Kolacek S, Micetic-Turk D, Ruszczyñski M, Vukavic T. Inulin and fructo-oligosaccharides for the prevention of antibiotic-associated diarrhea in children: report by the ESPGHAN Working Group on Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr. 2012;54: 828-9.
52. Hoekstra JH, Szajewska H, Zikri MA, Micetic-Turk D, Weizman Z, Papadopoulou A, *et al*. Oral rehydration solution containing a mixture of non-digestible carbohydrates in the treatment of acute diarrhea: a multicenter randomized placebo controlled study on behalf of the ESPGHAN working group on intestinal infections. J Pediatr Gastroenterol Nutr. 2004;39:239-45.
53. Vaisman N, Press J, Leibovitz E, Boehm G, Barak V. Short-term effect of prebiotics administration on stool characteristics and serumcytokines dynamics in very young children with acute diarrhea. Nutrients. 2010;2: 683-92.
54. Passariello A, Terrin G, De Marco G, Cecere G, Ruotolo S, Marino A, *et al*. Efficacy of a new hypotonic oral rehydration solution containing zinc and prebioticsin the treatment of childhood acute diarrhea: a randomized controlled trial. J Pediatr. 2011;158:288-92.
55. Alam NH, Meier R, Schneider H, Sarker SA, Bardhan PK, Mahalanabis D, *et al*. Partially hydrolyzed guar gum-supplemented oral rehydration solution in thetreatment of acute diarrhea in children. J Pediatr Gastroenterol Nutr. 2000;31:503-7.
56. Luoto R, Ruuskanen O, Waris M, Kalliomäki M, Salminen S, Isolauri E. Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. J Allergy Clin Immunol. 2014;133:405-13.
57. Niele N, van Zwol A, Westerbeek EA, Lafeber HN, van Elburg RM. Effect of non-human neutral and acidic oligosaccharides on allergic and infectious diseases in preterm infants. Eur J Pediatr. 2013;172:317-23.
58. Grüber C, van Stuijvenberg M, Mosca F, Moro G, Chirico G, Braegger CP, *et al*; MIPS 1 Working Group. Reduced occurrence of early atopic dermatitis because of immunoactive prebioticsamong low-atopy-risk infants. J Allergy Clin Immunol. 2010;126:791-7.
59. 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.
60. 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;138:1091-5.
61. Arslanoglu S, Moro GE, Boehm G, Wienz F, Stahl B, Bertino E. Early neutral prebiotic oligosaccharide supplementation reduces the incidence of some allergic manifestations in the first 5 years of life. J Biol Regul Homeost Agents. 2012;26:49-59.

WEB TABLE I SUMMARY OF RANDOMIZED CONTROLLED TRIAL EVALUATING PREBIOTICS IN INFANTILE CASE AND CONSTIPATION

Prebiotic study	*Inclusion criteria,n*	*Treatment used in study groups*	*Treatment duration*	*Reported outcomes*
Infantile Colic				
Savino, *et al.* [18] 2006	formula-fed infants, aged less than 4 months with infantile colic *n*=199	PG: partially hydrolysed formula with FOS and GOS CG: standard formula and simethicone (6 mg/kg 2×/d)	2 weeks	Reduction of crying episodes in infants with colic after 7 and 14 days in PG.
Pärtty, *et al.* [19] 2013	gestational age 32-36 weeks and birth weight >1500 g *n*=63	PG : mixture of GOS and PDX (ratio 1:1) 600 mg/d day 1- 30 and 1200 mg/d day 31-60 CG: standard formula	first 2 months of life and follow up for 1 year	Significantly less excessive criers in PG.
Constipation				
Holscher, *et al.* [21] 2012	full-term fomula-fed infants *n*=139	PG: a partially hydrolyzed whey formula with GOS&FOS (9:1) 4g/L CG: a partially hydrolyzed whey formula BG	6 weeks	Prebiotic formula was well tolerated. Increased abundance, proportion of bifidobacteria, and reduced fecal pH in PG.
Williams, *et al.* [22] 2014	healthy term infants *n*=180	PG 1: GOS 4 g/L PG 2: GOS 8 g/L CG: standard formula	first 4 months of life	PG1 was well-tolerated in terms of stool consistency. Significantly higher percentage of watery stools in PG2 group. No increase in stool frequency in PGs.
Scalabrin, *et al.* [23] 2012	term infants *n*=230	PG: PDX&GOS (1:1) 4g/L CG: standard formula BG	60 days	PDX&GOS produced soft stools and a bifidogenic effect closer to breast milk.
Ashley, *et al.* [24] 2012	healthy 12- to 16-day old infants *n*=419	PG 1: PDX&GOS (1:1) 4g/L PG 2: GOS 4g/L CG: cow's milk-based infant formula	from 14 to 120 days of age	Softer stooling pattern similar to that reported in breastfed infants in both PGs. No group differences in growth rate.
Ribeiro, *et al.* [25] 2012	healthy 9- to 48-months old children, *n*=129	PG: PDX&GOS 2g/d CG: cow's milk–based follow-on formula	108 days	More frequent and softer stools in PG.
Veereman-Wauters, *et al.* [26] 2011	healthy neonates *n*=110	PG 1: oligofructose& FOS (1:1) 0.8 g/dL PG 2: GOS&FOS (9:1) 0.8 g/dL CG: standard formula BG	first month of life	Stool consistency and bacterial composition of infants in both PGs were closer to the breast-fed pattern.
Westerbeek, *et al.* [27] 2011	preterm infants *n*=113	PG: scGOS&lcFOS and AOS CG: maltodextrin	between days 3 and 30	Prebiotic mixture decreases stool viscosity and stool pH with a trend towards increased stool frequency
Vivatvakin, *et al.* [28] 2010	healthy term infants *n*=144	PG: a whey-predominant formula containing long-chain polyunsaturated fatty acids and GOS&FOS CG: casein-predominant formula BG	from 30 days to 4 months of age	Softer stools, stool microbiota, gastric and intestinal transit times were closer to that of the breast-fed group. No group differences in growth rate.
Bisceglia, *et al.* [29] 2009	healthy newborns *n*=76	PG: scGOS/lcFOS (9:1) 0.8 g/dL CG: standard formula	28 days	A larger number of stools in PG.
Bongers, *et al.* [33] 2007	otherwise healthy constipated term infants aged 3–20 weeks *n*=38	PG: scGOS&lcFOS (9:1) 0.8 g/100 mL, high concentration of *sn*-2palmitic acid and partially hydrolyzed whey protein CG: standard formula	period 1 (3 weeks in PG) and crossed-over to period 2 (CG)	A strong tendency of softer stools but no difference in defecation frequency in PG.

PG - prebiotic group, CG - Control group, BG - breast-fed comparison group , FOS - fructo-oligosaccharides, GOS - galacto-oligosaccharides, , scGOS – short chain GOS, lcFOS – long chain FOS, AOS - acidic oligosaccharides, PDX - polydextrose

WEB TABLE II Summary of RANDOMIZED CONTROLLED TRIALS EVALUATING EFFICACY OF PREBIOTICS IN NUTRIENT ABSORPTION, WEIGHT GAIN AND DIARRHEA

Prebiotic study	*Inclusion criteria n=number of participants*	*Treatment used in study groups*	*Treatment duration*	*Reported outcomes*
Absorption of minerals				
Abrams, *et al*. [41] 2005	9 and 13 years of age and a body mass index between the 5th and 95th percentiles for age and sex *n*= 98	PG: mixed short and long degree of polymerization inulin-type, 8 g/d CG: maltodextrin	8 weeks and 1 year	Calcium absorption was significantly greater in the PG at 8 weeks and at 1 year.
Hicks, *et al*. [42] 2012	term infants to 10 weeks of age with birth weight ≥ 2500 g *n*=74	PG. GOS&PDX (1:1) 4 g/L CG: cow milk-based formula BG	a minimum of 14 days	No significant effect of prebiotics on calcium absorption or other markers of bone mineral metabolism.
Weight gain				
Dasopoulou, *et al*. [35] 2015	healthy formula-fed preterms *n*=167	PG: scGOS&lcFOS, 0.8 g/100 mL CG: preterm formula	16 days	Mean cholesterol and low density lipoprotein (LDL) increased significantly in the CG. Mean weight increased in the CG. Significant surge of motilin in PG.
Diarrhea				
Bruzzese, *et al*. [48] 2009	healthy infants aged 1-2 months *n*=342	PG: GOS&FOS formula CG: standard formula	1 year	The incidence of gastroenteritis lower in the PG.
Duggan, *et al*. [49] 2003	healthy infants aged 6-12 months *n*=251	PG: cereal with oligofructose 0.55 g/15 g cereal CG: non-supplemented cereal	6 months	Prebiotic cereal was not associated with any change in diarrhea prevalence.
Brunser, *et al*. [50] 2006	healthy infants aged 1-2 years *n*=140	PG: inulin and oligofructose, 4.5 g/L CG: standard formula	3 weeks after they had ended amoxicillin therapy for respiratory infects	No significant difference in the frequency of AAD.
ESPGHAN [51] 2012	6 months to 11 years old children with oral and/or intravenous short-term antibiotic therapy covering common infections *n*=105	PG: inulin and FOS age-dependent doses (max 5g/day) CG: maltodextrin	for as long as they were taking antimicrobial drugs	No effect regarding AAD.
Hoekstra, *et al*. [52] 2004	1 month to 3 years old children with acute diarrhea *n*=144	PG: ORS with prebiotic mixture (soy polysaccharides 25%, alpha-cellulose 9%, gum arabic 19%, FOS 18.5%, inulin 21.5%, resistant starch 7%) CG: non-supplemented ORS	during mild to moderate dehydration	No significant difference between participants concerning mean 48-h stool quantity, need for intravenous rehydration, duration of symptoms and hospitalization.
Vaisman, *et al*. [53] 2010	9 to 24 months old children with acute diarrhea *n*=119	PG: 80% lcFOS&scGOS and 20% AOS 2g, 3x/d CG: maltodextrin	12 days	Importantly increased stool consistency but not total of daily stools number .

Contd....

Contd... from previous page

Passariello, *et al.* [54] 2011	3 months to 3 years old children with acute diarrhea *n*=60	PG: ORS with FOS and xilooligosaccharides – both 0.35 g/L CG: non-supplemented ORS	during dehydration	Higher rate of diarrhea resolution at 72 hours, greater ORS intake during first 24 hours and reduced number of missed working days by parents.
Alam, *et al.* [55] 2000	4 to 18 months old infants with watery non-choleric diarrhea of less than 48 hours of duration *n*=150	PG: ORS with partially hydrolyzed guar gum CG: non-supplemented ORS	during dehydration	Important reduction of total duration of acute diarrhea in PG.

PG - prebiotic group, CG - Control group, BG - breast-fed comparison group , FOS - fructo-oligosaccharides, GOS - galacto-oligosaccharides, scGOS – short chain GOS, lcFOS – long chain FOS, AOS - acidic oligosaccharides, PDX – polydextrose, ORS – oral rehidration solution, AAD - antibiotic-associated diarrhea.

WEB TABLE II SUMMARY OF RANDOMIZED CONTROLLED TRIALS EVALUATING EFFICACY OF PREBIOTICS IN RESPIRATORY INFECTIONS AND ALLERGY

Prebiotic study	*Inclusion criteria n=number of participants*	*Treatment used in study groups*	*Treatment duration*	*Reported outcomes*
Respiratory infections				
Luoto, *et al*. [56] 2014	gestational age 32-36 weeks *n*=94	PG: PDX&GOS (1:1), 600 mg/d day 1 to 30, 1200 mg/d day 31 to 60 CG: standard formula	60 days, starting on the third day of life and follow-up for 1 year	Significantly lower incidence of RTI in the PG, no difference among participants in severity and duration of RTI.
Niele, *et al*. [57] 2013	preterm infants (gestational age <32 weeks and/or birth weight <1500 g) *n*=113	PG: prebiotic mixture 80% scGOS&lcFOS + 20% AOS, maximum of 1,5 g/kg/day CG: standard formula	between days 3 and 30 of life	No difference in the incidence of RTI.
Atopic Eczema				
Grüber, *et al*. [58] 2010	healthy term infants with low atopy risk before the age of 8 weeks *n*=1130	PG: formula containing GOS&FOS (9:1) 6.8 g/l plus AOS 1.2 g/l CG: standard formula BG	1 year follow-up	The cumulative incidence of atopic eczema in the PG was in the low range of the breast-fed group.
Niele, *et al*. [57] 2013	preterm infants (gestational age <32 weeks and/or birth weight <1500 g) *n*=113	PG: prebiotic mixture 80% scGOS&lcFOS + 20% AOS, maximum of 1,5g/kg/day CG: standard formula	between days 3 and 30 of life	No difference in prevalence of atopic eczema.
Ziegler, *et al*. [31] 2007	healthy, formula-fed, term infants *n* = 226	PG 1: PDX&GOS 4g/l PG 2: PDX&GOS and lactulose: 8g/l CG: standard formula	up to 120 days of age	PGs had a statistically important risk for developing eczema.
Moro, *et al*. [59] 2006	infants at high risk for atopy *n*=259	PG: extensively hydrolyzed formula with prebiotics scGOS&lcFOS 8 g/l CG: maltodextrine	first six months of life	A beneficial effect of prebiotics on the development of atopic dermatitis.
Arslanoglu, *et al*. [60] 2008	infants at high risk for atopy *n*=152	PG: extensively hydrolyzed formula with prebiotics scGOS/lcFOS 8 g/l CG: maltodextrine	follow-up until second birthday	Cumulative incidence of atopic dermatitis significantly lower in PG.
Arslanoglu, *et al*. [61] 2012	infants at high risk for atopy *n*=92	PG: extensively hydrolyzed formula with prebiotics scGOS&lcFOS 8 g/l CG: maltodextrine	follow-up until fifth birthday	The prevalence and the persistence of any allergic symptoms were significantly lower in the PG.

PG - prebiotic group, CG - Control group, BG - breast-fed comparison group , FOS - fructo-oligosaccharides, GOS - galacto-oligosaccharides, scGOS – short chain GOS, lcFOS – long chain FOS, AOS - acidic oligosaccharides, PDX – polydextrose, RTI - respiratory tract infections.