Probiotics and prebiotics in pediatrics: where are we now?

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In recent years, probiotics and prebiotics have become a hot topic. There are an increasing number of health benefits attributed to them. However, only a few of the benefits have been confirmed in well-conducted randomized controlled trials. This paper was written to evaluate the evidence on the effect of administration of probiotics and prebiotics in children. Electronic databases and the reference lists of publications were searched for randomized controlled trials or their meta-analyses (all up until April 2007). Based on the available evidence, to date, the most extensively studied application and the best documented is the use of some probiotic strains for the treatment of acute infectious diarrhea and prevention of antibiotic-associated diarrhea. Many other benefits, both of probiotics and prebiotics, are largely unproven, but there is a growing body of scientific evidence in support of such benefits. Guidance is needed as to which agent to use, timing, dosage and mode of administration.

Key words: probiotics, prebiotics, children, acute infectious diarrhea, antibiotic-associated diarrhea, necrotizing enterocolitis, nosocomial diarrhea, allergy, acute gastroenteritis, atopic dermatitis, atopic disease.

A growing understanding of the possible role of gut microbiota in health and disease has led to an interest in the development of strategies aimed at manipulating bacterial colonization. These have included the administration of probiotics or prebiotics or a combination of both (synbiotics). This review was prepared following a comprehensive literature search to evaluate the available evidence of their efficacy in children. To identify the published evidence, MEDLINE, EMBASE, the Cochrane Database of Systematic Reviews, and the Cochrane Controlled Trials Register were searched (all up until April 2007) for randomized controlled trials (RCTs) or their systematic reviews or meta-analyses, using relevant keywords.

PROBIOTICS

According to the definition proposed by a group of experts convened by the Food and Agriculture Organization of the United States (FAO), probiotics are currently defined as 'live microorganisms administered in adequate amounts which confer a beneficial health effect on the host'[1]. However, the scientific basis of this definition may be questioned, as animal studies suggest that some probiotic effects can be achieved by nonviable bacteria and even by isolated bacterial DNA[2-4]. Therefore, further modifications of the probiotic definition might be needed.

The criteria that must be fulfilled to classify a microorganism as a probiotic include: (1) human origin; (2) nonpathogenic properties; (3) resistance to technologic processes, i.e., viability in delivery vehicles; (4) stability in acid and bile; (5) adhesion to target epithelial tissue; (6) ability to persist within the gastrointestinal tract; (7) production of antimicrobial substances; (8) ability to modulate the immune system; and (9) ability to influence metabolic activities[5].

In humans, the most commonly used probiotics are bacteria from the genera *Lactobacillus* or *Bifidobacterium*, either as single species or in mixed culture with other bacteria. Other nonpathogenic bacterial genera, including *Escherichia, Enterococcus*, and *Bacillus*, and nonbacterial organisms, such as a nonpathogenic yeast *Saccharomyces boulardii*, have also been used.

There is some debate about whether yogurt starter bacteria cultures, such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp. *bulgaricus*, that are used to ferment milk and turn it into yogurt should be considered probiotics. However, a number of human studies have clearly demonstrated that yogurt containing viable bacteria improves lactose digestion and eliminates symptoms of lactose intolerance; thus, these cultures fulfill the current concept of probiotics[6].

TREATMENT OF ACUTE INFECTIOUS DIARRHEA

The rationale for the use of probiotics to treat and prevent diarrheal diseases is based on the assumption that they modify the composition of the colonic microflora and act against enteric pathogens. However, the exact mechanism by which probiotics might exert their activity against enteropathogens in humans remains unknown. Several possible mechanisms have been proposed, mostly based on results of *in vitro* and animal studies. These include: the synthesis of antimicrobial substances (e.g., *Lactobacillus* GG and *Lactobacillus acidophilus* strain LB have been shown to produce inhibitory substances against some Gram-positive and Gram-negative pathogens)[7-9], competition for nutrients required for growth of pathogens[10,11], competitive inhibition of adhesion of pathogens[12-15], and modification of toxins or toxin receptors[16,17]. Additionally, studies have shown that probiotics stimulate or modify nonspecific and specific immune responses to pathogens: in fact, certain probiotics increase the number of circulating lymphocytes[18] and lymphocyte proliferation[19], stimulate phagocytosis, increase specific antibody responses to rotavirus vaccine strain[20], and increase cytokine secretion, including interferon γ[19,21-23]. Recently, Mack et al.[24] showed that Lactobacillus species (*L. rhamnosus* strain GG, as well as *L. plantarum* strain 299v) inhibit, in a dose-dependent manner, binding of *Enterococcus coli* strains to intestinal-derived epithelial cells grown in a tissue culture by stimulation of synthesis and secretion of mucins (glycoproteins known to have a protective effect in intestinal infections). Furthermore, probiotics have been shown to enhance mucosal immune defenses[25] and protect against structural and functional damage promoted by entero-virulent pathogens in the brush border of enterocytes, probably by interfering with the cross-talk between the pathogen and host cells (i.e., inhibition of pathogen-induced cell signaling)[26]. It is likely that several of the above-described mechanisms operate simultaneously, and they may well differ depending on the properties of an enteric pathogen (e.g., bacterial or viral) and probiotic strain[5].

At least six meta-analyses[27-32] aimed at determining the effect of probiotics in the treatment of acute infectious diarrhea have been carried out (Table I). Based on the results of these meta-analyses, the beneficial effects of probiotics in acute diarrhea in children seem to be: (1) moderate, allowing a reduction of diarrhea duration between 17 to 30 hours; (2) strain-dependent, with *Lactobacillus* GG and *S. boulardii* being amongst the most effective; (3) dose-dependent (greater for doses $>10^{10}$ CFU); (4) significant for watery diarrhea and viral gastroenteritis (well documented for *Lactobacillus* GG), but not for invasive, bacterial diarrhea; (5) more evident when treatment with probiotics is initiated early in the course of disease; and (6) more evident in children in European countries.

PREVENTION OF ANTIBIOTIC-ASSOCIATED DIARRHEA

The rationale for the use of probiotics in antibiotic-associated diarrhea (AAD) is based on the assumption that the key factor in the pathogenesis of AAD is a disturbance in normal intestinal microflora. Indeed, several systematic reviews (with or without meta-analysis) have shown that some probiotic strains are effective in preventing AAD in the general (mainly adult) population (Table II)[33-36].

Evidence from two recent systematic reviews of RCTs in children[37,38] is also encouraging. The first review (search date December 2005) identified six RCTs involving 766 children. The review found that the treatment with probiotics compared with placebo reduced the risk of AAD from 28.5% to 11.9% (relative risk-RR: 0.44, 95% confidence interval-CI: 0.25 to 0.77, random effect model). Preplanned subgroup analysis showed that reduction of the risk of AAD was associated with the use of *Lactobacillus* GG (2 RCTs, 307 participants, RR

Table I. Probiotics in Treatment of Acute Infectious Diarrhea: Results of Meta-Analyses of Randomized Controlled Trials (RCTs)

Outcome measure	Study ID	Number of RCTs (n)	Probiotics	Population	Measure of Effect Size	Effect (95% CI)
Diarrhea lasting ≥3 days	Szajewska et al.[27]	8 (731)	Various	Children	RR	0.4 (0.3-0.5)
	Allen et al.[30]	15 (1341)	Various	Children & adults	RR	0.7 (0.6-0.8)
Duration of diarrhea	Szajewska et al.[27]	8 (731)	Various	Children	WMD	-18 h (-27 to -10)
	Van Niel et al.[28]	9 (675)	Lactobacilli	Children	WMD	-17 h (-29 to -7)
	Huang et al.[29]	18 (1917)	Various	Children	WMD	-19 h (-26 to -14)
	Allen et al.[30]	12 (970)	Various	Children & adults	WMD	-30 h (-42 to -19)
	Szajewska et al.[31]	4 (473)	*S. boulardii*	Children	WMD	-26 h (-31 to -19)
	Szajewska et al.[32]	7 (876)	*Lactobacillus GG*	Children	WMD	-26 h (-46 to -7)

RR: Relative risk. WMD: Weighted mean difference (negative values indicate that duration of diarrhea was shorter in the probiotic than control group). CI: Confidence interval.

Table II. Probiotics in Prevention of Antibiotic-Associated Diarrhea in Children: Results of Meta-Analyses of Randomized Controlled Trials (RCTs)

Study	Number of RCTs (n)	Probiotic(s)	Population	Relative risk (95% CI)	NNT (95% CI)
D'Souza et al.[33]	9 (1214)	Various	Adults + children	0.4 (0.3 to 0.5)	11 (8 to 18)
Cremonini et al.[34]	7 (881)	Various	Adults + children	0.4 (0.3 to 0.6)	9 (7 to 14)
Szajewska et al.[35]	5 (881)	*S. boulardii*	Adults + children	0.4 (0.2 to 0.8)	10 (7 to 16)
Szajewska et al.[37]	6 (766)	Various	Children	0.4 (0.3 to 0.8)	7 (5 to 10)
Johnston et al.[38]	6 (707)	Various	Children	0.4 (0.3 to 0.8) ITT 1.01 (0.6 to 1.6) PP	6 (5-8)

ITT: Intention to treat analysis. NNT: Number needed to treat. PP: Per protocol analysis.

0.3, 95% CI 0.15 to 0.6), S. boulardii (1 RCT, 246 participants, RR 0.2, 95% CI 0.07-0.6), or Bifidobacterium lactis & S. thermophilus (1 RCT, 157 participants, RR 0.5, 95% CI 0.3 to 0.95). It was concluded that probiotics reduce the risk of AAD in children. For every seven patients that would develop diarrhea while being treated with antibiotics, one fewer will develop AAD if also receiving probiotics.

The second review (search date January 2005) of six studies (total n=707 patients) found that the combined results, analyzed with a per-protocol method that reported on the incidence of diarrhea during antibiotic treatment, showed significant benefit of the use of probiotics over placebo (RR 0.43, 95% CI 0.25-0.75). In contrast, the results from intention-to-treat (ITT) analysis were non-significant (RR 1.01, 95% CI 0.64-1.61). However, as indicted by the authors of this review, the validity of ITT analysis in this review can be questioned due to high losses to follow-up.

In conclusion, RCTs in children have provided evidence of a moderate beneficial effect of some probiotic strains in the prevention of AAD. Their use is probably warranted whenever the physician feels that preventing this usually self-limited complication is important.

CLOSTRIDIUM DIFFICILE DIARRHEA

The vast majority of children who have an episode of *C. difficile* diarrhea, whether antibiotic-associated or sporadically acquired, respond to the proper antibiotic treatment with eradication of the infection. However, up to 20% of them experience a recurrence of the infection. The use of probiotics, and especially of *S. boulardii and Lactobacillus* GG, is often advocated to prevent such recurrence.

Two systematic reviews were found. The first review (with no statistical pooling) concluded that available evidence does not support the administration of probiotics with antibiotics to prevent the development of *C. difficile* diarrhea and is inadequate to justify its introduction as a treatment for developed *C. difficile* diarrhea in adults[39]. The second review concluded that only *S. boulardii* was effective in *C. difficile* diarrhea[40]. In spite of some anecdotal evidence of their efficacy, no RCT investigating such possibility in children has been conducted. Well-done RCTs addressing the role of probiotics in *C. difficile*-associated diarrhea both in children and adults are still needed.

PREVENTION OF NOSOCOMIAL DIARRHEA

Nosocomial diarrhea is any diarrhea that a patient contracts in a health-care institution. In children, it is commonly caused by enteric pathogens, especially rotavirus[41]. Depending on the population, type of hospital and standard of care, the reported incidence ranges from 4.5[42] to 22.6[43] episodes per 100 admissions. Nosocomial diarrhea may prolong the hospital stay and increase medical costs. Although hand washing remains an essential infection-control measure, other cost-effective measures to prevent nosocomial diarrhea are being evaluated.

Four RCTs[44-47]examining the use of probiotics to prevent diarrhea in infants and young children admitted to hospitals for reasons other than diarrhea were identified. Two of them evaluated the effect of *Lactobacillus* GG while the other two assessed a combination of *Bifidobacterium bifidum* (recently renamed *B. lactis*) and *S. thermophilus* (Table III). In summary, there is conflicting evidence from two RCTs on the efficacy of *Lactobacillus* GG in the prevention of nosocomial diarrhea. One small RCT suggests a benefit of *B. bifidum* and *S. thermophilus* in sick infants admitted to the hospital, but no such benefit in healthy children in residential care settings. There is currently not enough evidence to recommend the routine use of probiotics to prevent nosocomial diarrhea. However, as this is a field of potentially great benefit, there is a strong need for additional large, well-designed RCTs.

NECROTIZING ENTEROCOLITIS

Preterm neonates in the Intensive Care Unit (NICU) develop a colonic bacterial colonization pattern radically different from that of healthy term infants. Microorganisms that are typical of the breast-fed are only late colonizers;

Table III. Probiotics in Prevention of Nosocomial Diarrhea: Results of Randomized Controlled Trials

Study	Location	N (Age in months)	Probiotic(s) (Dose)	Relative risk (95% CI)	NNT (95% CI)
Szajewska et al.[44]	Pediatric hospitals (Poland)	81 (1-36)	*Lactobacillus* GG ($6x10^9$ CFU)	0.2 (0.06-0.6)	4 (2-10)
Mastretta et al.[45]	Pediatric hospital (Italy)	220 (1-18)	*Lactobacillus GG* (10^{10} CFU)	0.84 (0.6-1.3)	Not significant
Saavedra et al.[46]	Chronic care hospital (USA)	55 (5-24)	*B. lactis Bb12* 10^9 CFU/g + *S. thermophilus* 10^7 CFU/g	0.2 (0.06-0.8)	5 (3-20)
Chouraqui et al.[47]	Residential nurseries or foster care centers (France)	90 (<8)	*B. lactis Bb12* + *S. thermophilus* (min. 10^8 CFU/day)	0.7 (0.4-1.3)	Not significant

NNT: Number needed to treat.

additionally, the reduced exposure to maternal microflora, the utilization of completely sterile feedings and finally the widespread use of antibiotics in the NICU, all greatly diminish the newborn's exposure to commensal bacteria and their chance to effectively colonize the gut. Under these circumstances, the colon actually becomes a reservoir of antibiotic-resistant, potentially harmful germs. Indeed the predominant bacterial flora in preterm babies in the NICU is constituted by staphylococci, enterobacteria such as *Klebsiella*, and enterococci. The most common anaerobes are clostridia. Only a small minority of these neonates are colonized by bacteria that predominate in healthy, breast-fed full-term babies such as bifidobacteria. This abnormal colonization is thought to contribute to the onset of necrotizing enterocolitis (NEC), the most common abdominal emergency of preterm infants in the NICU.

To date, only three RCTs have evaluated the beneficial effect of probiotics in the prevention of NEC (Table IV). The purpose of the first study[48] was to evaluate the effectiveness of *Lactobacillus* GG supplementation in reducing the incidence of NEC, bacterial sepsis, and urinary tract infections in preterm infants. Newborn infants with a gestational age <33 weeks or birth weight <1500 g were randomized to receive standard milk formula supplemented with *Lactobacillus* GG at a dose of 6 x 10^9 CFU once daily until discharge, starting with the first feed, or placebo. Five hundred eighty-five patients were studied. Although NEC (1.4% vs. 2.7%) and urinary tract infections (3.4% vs. 5.8%) were found less frequently in the probiotic group compared to the control group, these differences were not significant. Bacterial sepsis was more frequent in the probiotics group (4.4%) than in the placebo group (3.8%), but this difference was also not significant.

The second well-designed trial[49] compared the outcomes of breast-milk feeds with and without probiotics in preventing NEC and death in very low birth weight (VLBW) infants. Infants with a birth weight <1500 g, who started to feed enterally and survived beyond the seventh day after birth, were randomized in a blind manner to receive breast-milk (from their mother or a milk bank) only (n=187) or breast-milk supplemented with *L. acidophilus* and *Bifidobacterium infantis,* 125 mg/kg, per dose twice daily (n=180), until discharge. The incidence of death or NEC was significantly lower in the probiotic group than the control group (5% vs. 12.8%, P=0.009), as was the incidence of NEC (Bell stage 2 or higher) (1.1% vs. 5.3%, P=0.04). The incidence of NEC or sepsis was lower in the probiotic group than the control group (13.3% vs. 24.6%, P<0.03), as was the incidence of NEC, sepsis, or death (17.2% vs. 32.1%, P<0.009). The incidence of culture-proven sepsis was significantly lower in the probiotic group (P=0.03). None of the blood cultures grew *Bifidobacterium* or *Lactobacillus* species. The number needed to treat (NNT) to prevent one case of NEC was 27. The NNT to prevent one death was 31. Reassuringly, none of the blood cultures found to be positive throughout the duration of the study grew any *Lactobacillus* or *Bifidobacterium*.

Table IV. Probiotics in Prevention of Necrotizing Enterocolitis in Premature Infants: Results of Randomized Controlled Trials

Study	N	Population	Probiotic(s) (Dose)	Relative risk (95% CI)	NNT (95% CI)
Dani et al.[48]	585	Birth weight <1500 g or <33 weeks of gestation	*Lactobacillus* GG (6x10^9 CFU once daily; with milk formula)	0.5 (0.15-1.6)	Not significant
Lin et al.[49]	367	Birth weight <1500 g	*L. acidophilus* + *B. infantis* 125 mg/kg, per dose twice daily; with breast milk)	0.2 (0.05-0.8)	24 (12-142)
Bin-Nun et al.[50]	145	Birth weight ≤1500 g	*B. infantis* + *S. thermophilus* + *B. bifidus* 10^9 CFU/day; with breast milk and/or formula)	0.3 (0.07-0.8)	9 (5-39)

CI: Confidence interval. NNT: Number needed to treat.

In the most recent RCT conducted in a neonatology unit in Israel, a daily feeding supplement of a probiotic mixture (*B. infantis, S. thermophilus, and B. bifidum*) at the dose 10^9 CFU/day reduced both the incidence and severity of NEC in VLBW (≤1500 g) infants. The incidence of NEC was 4% in the probiotic group versus 16.4% in the control group, a relative risk reduction of 75% (95% CI 21-92%), NNT 9 (95% CI 5-39). NEC that did develop was less severe in the probiotic-supplemented neonates (P=0.005). Three of 15 infants who developed NEC died and all deaths related to NEC occurred in the control infants[50].

In summary, these results are promising. However, despite a feasible rationale and promising results, well-conducted trials need to provide more convincing evidence of their efficacy and safety. Until more data is available, it would be wise to avoid prophylactic use of probiotics in premature infants with underlying diseases (e.g. congenital or acquired immunodeficiencies, congenital heart disease, etc.).

ALLERGY PREVENTION

The rationale for using probiotics in prevention and treatment of allergic disorders is based on the concept that appropriate microbial stimuli are vital for an adequate early immunologic development. The intestinal flora of atopic children has been found to differ from that of controls. Atopic subjects have more clostridia and tend to have fewer bifidobacteria than non-atopic subjects[51]. Thus, there is indirect evidence that differences in the neonatal gut microflora may precede or coincide with the early development of atopy. This further suggests a crucial role for a balanced commensal gut microflora in the maturation of the early immune system.

Among three well-conducted RCTs (Table V), *Lactobacillus* GG demonstrated the most significant effects in prevention of allergic disease among high-risk infants. In a randomized, double-blinded placebo controlled trial[52], *Lactobacillus* GG was administered to pregnant and lactating mothers who had at

Table V. Probiotics for Prevention of Atopic Diseases: Results of Randomized Controlled Trials

Author	N	Population	Intervention	Duration of intervention	Outcome	RR (95%CI)	NNT (95%CI)
Kalliomaki 2001 et al.[52]	132/159	Pregnant women with family history of atopic diseases	LGG $1x10^{10}$ (n=77) or placebo (n=82)	2-4 weeks before delivery and for 6 months after birth	AD at 2 years LGG 1/64 (23%) vs. placebo 31/68 (46%)	0.5 (0.3-0.8)	5 (3-16)
Kalliomaki et al.[53]	As above	As above	As above	As above	AD at 4 years of age: LGG 14/53 vs. placebo 25/54	0.6 (0.3-0.97)	6 (3-64)
Kalliomaki et al.[54]	As above	As above	As above	As above	AD at 7 years of age: LGG 43% vs. placebo 66%	0.64 (0.45-0.92)	5 (3-18)
Taylor et al.[55]	188/232	Pregnant women with a family history of atopy	*L. acidophilus* LAVRI-A1 $3x10^{10}$ CFU or placebo	6 months after birth	AD at 6 mo: 26% vs. 23%	1.1 (0.7-1.9)	NS
					AD at 12 mo: 43% vs. 39%	1.1 (0.8-1.6)	NS
					Food allergy at 12 mo: 10% vs. 16%	1.5 (0.7-3.3)	NS
					Positive SPT: 40% vs. 24%	1.6 (1.04-2.6)	NNH 7 (4-70)
Abrahamsson et al.[56]	188/232	Positive family history of atopy	*L. reuteri* $1x10^8$ CFU	From 36th week of gestation until delivery, then for 12 months	Eczema 36% vs 34%	NS	
					IgE-eczema 8% vs 20%	P=0.02	
					Positive SPT 14% vs 31%	P=0.02	

AD: Atopic dermatitis. SPT: Skin prick test. RR: Relative risk. CI: Confidence interval. NNT: Number needed to treat.

least a first-degree relative or partner with atopic eczema, allergic rhinitis, or asthma. One hundred and thirty-two mother-infant pairs were included and treated up to six months postnatally. The frequency of atopic eczema in the probiotic group was half that of the placebo group (15/64 [23%] vs 31/68 [46%]; RR 0.51 [95% CI 0.32-0.84]). The NNT to prevent the onset of atopic eczema was 4.5 (95% CI 2.6-15.6). In direct extension of this study, the four-year and seven-year follow-up examined the prevalence of atopic disease by review of a questionnaire and a clinical examination. Again, children receiving *Lactobacillus* GG had developed less atopic eczema compared with those receiving placebo. However, skin prick test reactivity was the same in both groups. There was no significant difference between the two groups in the percentage of infants with asthma and allergic rhinitis[53,54].

The second RCT (231 newborns with allergy) compared *L. acidophilus* LAVRI-A1 $3x10^9$ CFU or placebo given daily for the first six months of life. A total of 178 infants completed the supplementation period. The trial found that early probiotic supplementation with *L. acidophilus* did not reduce the risk of atopic dermatitis in high-risk infants and was associated with increased allergen sensitization in infants receiving supplements. The authors stated that these findings challenge the role of probiotics in allergy prevention[55].

A very recent double-blind, randomized, placebo-controlled trial in 232 families with allergic disease, of whom 188 completed the study, examined the effect of oral administration of *Lactobacillus reuteri* ATCC 55730 at a dose $1x10^8$ CFU daily from gestational week 36 until delivery, and then from birth until 12 months of age. While there was no effect on infant eczema, the probiotic-treated infants had less IgE-associated eczema at two years of age and therefore possibly run a reduced risk of later developing respiratory allergic disease[56].

In conclusion, some probiotic strains (e.g. *Lactobacillus* GG), but not all, hold promise for a role in the prevention of atopic disorders. Further studies are needed.

PROBIOTICS IN DIETETIC PRODUCTS FOR INFANTS

Probiotics have been added to dietetic products for infants in an attempt to render their gut flora more similar to that of breast-fed babies. The most commonly employed probiotics are bifidobacteria and lactobacilli, in isolated forms or in combination. Several different strains and dosages have been used, but most are in the range of $1x10^6$-$1x10^{11}$ CFU/g of formula powder.

In its position paper[57], the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition concluded that in addition to limited data on safety and clinical effects, there was a lack of published evidence of the long-term clinical benefit of using formulas supplemented with probiotic bacteria. However, short-term benefits have been seen in patients with infectious diarrhea. Interestingly, it has been suggested that bacteria ingested during early infancy are more likely to permanently colonize the intestine than those ingested during later life[58], reinforcing the notion that an earlier intervention in the development of colonic microflora may be beneficial.

PREBIOTICS

The term *prebiotic* was introduced in 1995 by Gibson and Roberfroid[59] as *'nondigestible food components that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thereby improving host health'*. Oligosaccharides, which are contained in human breast milk, are considered the prototype of prebiotics, since they have been shown to facilitate the growth of bifidobacteria and lactobacilli in the colon of breast-fed neonates[60-62]. In the diet, the most common sources are wheat, onions, bananas, garlic, artichokes, and leeks. Prebiotic oligosaccharides can be produced by extraction from plant materials, using microbiological and enzymatic synthesis, and by enzymatic hydrolysis of polysaccharides. In practice, commercial prebiotics are galacto-oligosaccharides (GOS) and inulin-type fructans[63].

MECHANISM OF ACTION

Prebiotics present in human milk, found in food, or supplemented to the diet (e.g., inulin-type fructans, GOS) are not hydrolyzed by small intestinal enzymes; thus, they enter the colon and are fermented, resulting in a more acidic luminal pH and an increased concentration of short-chain fatty acids such as lactic, butyric, propionic and acetic acids.

This in turn results in increased proliferation of certain commensal bacteria, mainly but not exclusively, bifidobacteria and lactobacilli, which function as probiotics to stimulate intestinal host defenses[64]. Thus, prebiotics may be responsible indirectly for some of the beneficial effects of probiotics. In addition, the produced short-chain fatty acids provide an energy source for colonocytes as well as a stimulus for bacterial–epithelial cell 'cross-talk' cellular events, e.g. up-regulation of TLR expression[65]. Several studies have demonstrated the specific effect of prebiotic oligosaccharides in achieving a lower luminal pH and increased concentration of short-chain fatty acids in the colon, as well as an increased concentration of bifidobacteria and lactobacilli; however, long-term studies demonstrating a sustained effect of prebiotics are lacking. In addition, one may deduce that since prebiotics stimulate an increase in bifidobacteria and lactobacilli, the effect of this stimulation on health is similar to that observed with use of probiotics. This assumption, however, needs to be proven in clinical trials. Prebiotics can interact with receptors on immune cells and, thus, provide direct effects that do not require the proliferation of commensal (probiotic) bacteria[66].

Prebiotic carbohydrate properties are not limited to direct and indirect immunomodulation, but also include metabolic functions such as improved mineral absorption and influence on lipid metabolism. Animal studies have shown that inulin-type fructans increase mineral absorption, especially calcium absorption[67] and bone mineralization[68].

PREBIOTIC OLIGOSACCHARIDES IN INFANT FORMULAE

The use of nondigestible carbohydrates (i.e., oligofructosyl-saccharose and oligogalactosyl-lactose) in infant formulae and follow-on formulae has been commented on by the ESPGHAN Committee on Nutrition[69]. To date, there are only limited published data on the evaluation of prebiotic substances in dietetic products for infants. No general recommendation on the use of oligosaccharide supplementation in infancy for preventive or therapeutic purposes can be made. During the time of their administration, prebiotic oligosaccharides in dietetic products have the potential to increase the total number of bifidobacteria in feces and to soften stools. The available data on oligosaccharide mixtures in infant formulae do not demonstrate adverse effects. Validated clinical outcome measures of prebiotic effects in infants should be characterized in future well-designed and carefully conducted RCTs, with relevant inclusion/exclusion criteria and adequate sample sizes. Such trials should also define the optimal quantities, types and intake durations, and safety of different oligosaccharides.

USE OF PREBIOTICS IN SOLID FOODS FOR CHILDREN

One RCT[70] conducted in 56 healthy, term infants aged 4-12 months evaluated the tolerance and gastrointestinal effects of an infant cereal supplemented with either 0.75 fructo-oligosaccharide (FOS) per serving or placebo for 28 days. Compared with the control group, stool consistency was less often described as 'hard' and more likely to be described as 'soft' or 'loose' in the FOS-supplemented group. The mean number of stools was 2.0 ± 0.6 per day in the FOS-supplemented group compared with 1.6 ± 0.7 per day in the control group ($p=0.02$). There was no difference between the groups in crying, spitting-up or colic. No difference in stool pH between the groups was found. There was also no significant difference in growth between the two groups. The authors concluded that FOS supplements added to cereal were well tolerated in doses of up to 3 g/day. FOS consumption led to more frequent and softer stools, without reported diarrhea; it also resulted in less-reported frequency of symptoms associated with constipation such as hard stools or days without a stool. Clinical outcomes were not reported. Limitations of this study included the use of a non-validated tool for parental assessment of stool consistency, a small sample size, and a short follow-up period.

A more recent double-blind RCT[71] involving 35 infants aged 4 to 6 months studied the effect of adding GOS/FOS to solid foods on an increase in bifidobacteria in the intestinal microbiota. ITT analysis revealed no significant difference between the two study groups. Only per-protocol analysis, involving 20 children who complied with the protocol, showed that the percentage of bifidobacteria in feces

increased from 43% to 57% (p=0.03) from week 0 to week 6, but did not significantly change in the control group (36% and 32%, respectively, p=0.4). There were no differences in stool frequency and consistency between the two groups.

USE OF PREBIOTICS FOR PEDIATRIC DISORDERS

Only a few clinical trials have reported health outcomes for children given prebiotic oligosaccharides (for characteristics of studies see Table VI).

Diarrheal Diseases

Prevention is the most important challenge posed by childhood diarrheal diseases, particularly in developing countries. In the past several years, enormous efforts have been made to develop safe and effective vaccines against enteric infections. The most recent data on rotavirus vaccines are encouraging[72,73], but other enteric pathogens still await their turn. Children attending day care centers are also at high risk for developing intestinal and respiratory infections. The successful prevention of these infections would be beneficial to families and society. It can be hypothesized that continuous use of prebiotics might, by providing an immunologic stimulus, prove useful in preventing infectious diseases commonly encountered by young children.

In a large, well-designed study performed in Peruvian infants aged 6 to 12 months (n=282), Duggan et al.[74] compared an infant cereal supplemented with oligofructose (0.55 g/15 g cereal) with a non-supplemented cereal. During a second part of the same trial involving 349 subjects, zinc (1 mg/15 g cereal) was added to both oligofructose-supplemented and control cereals[74]. The authors concluded that the use of cereal supplemented with this type and dose of oligosaccharide (with or without zinc) was not associated with any change in diarrhea prevalence, use of health care resource, or response to *Haemophilus influenzae* type B immunization.

Treatment of Acute Infectious Gastroenteritis

A randomized, double-blind, placebo-controlled multicenter study[75] was conducted to evaluate the efficacy and safety of administering a mixture of nondigestible carbohydrates (NDC), including soy polysaccharide 25%, α-cellulose 9%, gum arabic 19%, FOS 18.5%, inulin 21.5%, and resistant starch 7%, as an adjunct to oral rehydration therapy in the treatment of acute infectious diarrhea in children with mild to moderate dehydration. It was hypothesized that with the incorporation of NDC, some of them (e.g., FOS, GOS and inulin) with prebiotic effects might promote fermentation in the colon, and thus, decrease fecal volume and the duration of the diarrheal illness. One hundred forty-four boys aged 1 to 36 months with diarrhea defined as three or more watery stools per day for >1 day but <5 days with mild or moderate dehydration (World Health Organization criteria) were randomly assigned to receive hypotonic oral rehydration solution (ORS) (Na 60 mmol/L, glucose 111 mmol/L) with or without a mixture of NDC. ITT analysis did not show a significant difference in mean 48-hour stool volumes. The duration of diarrhea after randomization was similar in both groups (82 ± 39 hours vs. 97 ± 76 hours; p= 0.2). There was no significant difference in the duration of hospital stay, and unscheduled intravenous rehydration was comparable in the two groups. No adverse effects were noted. An explanation for the negative results could originate from the type and the amount of NDC added to the ORS. An average dose of 10 to 15 g per episode in relatively mild diarrhea simply may be insufficient to achieve a shorter duration of diarrhea. Furthermore, it is possible that the timing of the intervention was inappropriate, making the addition of NDC to exclusive oral rehydration therapy an insufficient measure.

Prevention of Antibiotic-associated Diarrhea

In contrast to probiotics, there is a paucity of data on the use of prebiotics in the prevention of AAD. The only pediatric double-blind RCT[76] involved 140 children (1 to 2 years of age) who were treated with amoxicillin for acute bronchitis. This study revealed no significant difference in the frequencies of diarrhea in children receiving oligofructose and inulin administered in a milk formula (4.5 g/L) for 21 days after completion of antibiotics compared with placebo (10% *vs.* 6%, RR 0.6, 95% CI 0.2-1.8). However, prebiotics in a milk formula increased fecal bifidobacteria early after amoxicillin treatment.

Table VI. Prebiotics in Children: Summary of Randomized Controlled Trials

Study ID	Clinical condition	Prebiotic(s)	Control	N (age)	Outcome	Effect*
Moore et al.[70]	Tolerance	FOS (0.75 mg/portion of infant cereal)	Placebo (maltodextrin)	56 (4 to 12 mo)	Stool consistency	↓
					Stool frequency	↑
					Crying, spitting-up, colic	NS
					Stool pH	NS
Moro et al.[78]	Prevention of atopic dermatitis in high-risk infants	GOS/FOS (with partial hydrolysate)	Placebo (with partial hydrolysate)	192 (≥1 atopic parent)	Atopic dermatitis	RR 0.4 (0.2-0.85) NNT 8 (5-37)
Passeron et al.[77]	Treatment of atopic dermatitis	Prebiotic (lactose 0.397 g + potato starch 0.759 g) (n=22)	Synbiotic (prebiotic+*L. rhamnosus* Lcr35 $1.2x10^9$ CFU (n=17)	39 (>2 y)	SCORAD (SCORing Atopic Dermatitis) score	NS
Duggan et al.[74]	Prevention of diarrheal disease	Oligofructose (0.55 g/ 15 g cereal)	Non-supplemented cereal	282 (6 to 12 mo)	Episodes of diarrhea	NS
					Episodes of severe diarrhea	NS
					Episodes of dysentery	NS
					Duration of diarrhea	NS
					Postimmunization titers of *H. influenzae*	NS
					Gains in height, visits to clinic, hospitalizations, use of antibiotics	NS
Duggan et al.[74]	Prevention of diarrheal disease	Oligofructose (0.55 g/15 g cereal) + Zn (1 mg/ 15 g cereal)	Non-supplemented cereal	349 (6 to 12 mo)	Episodes of diarrhea	NS
					Episodes of severe diarrhea	NS
					Episodes of dysentery	NS
					Duration of diarrhea	NS
					Postimmunization titers of *H. influenzae*	NS
					Gains in height, visits to clinic, hospitalizations, use of antibiotics	NS
Hoekstra et al.[75]	Treatment of acute gastroenteritis	Mixture (soy polysaccharide 25%, α-cellulose 9%, gum arabic 19%, FOS 18.5%, inulin 21.5%, resistant starch 7%) with oral rehydration therapy	Placebo (cow's milk protein)	144 (1 to 36 mo; boys only)	Stool volume	NS
					Duration of diarrhea	NS
					Duration of hospitalization	NS
					Unscheduled intravenous rehydration	NS
Brunser et al.[76]	Prevention of antibiotic-associated diarrhea	Oligofructose + inulin (Raftilose P95 and Raftiline) 4.5 g/L	Placebo	140 (1-2 y)	Diarrhea	NS
					Fecal bifidobacteria	↑
Griffin et al.[79]	Calcium bioavailability	Oligofructose (8 g/d) + inulin	Placebo (sucrose)	59 (11 to 14 y)	Ca absorption	↑
		Oligofructose (8 g/d)			Ca absorption	NS
Van den Heuvel et al.[80]	Calcium bioavailability	Oligofructose (15 mg/d)	Placebo (sucrose)	12 (14 to 16 y, boys)	Ca absorption	↑
Abrams et al.[81]	Calcium bioavailability	Short- and long-term fructans (8 g/d)	Placebo (maltodextrin)	Young adolescents	Ca absorption	↑ (at 8 wk and 1 y)
					Bone mineral content	↑
					Bone mineral density	↑

*The experimental (prebiotic) group compared with control group.
FOS: Fructo-oligosaccharides. GOS: Galacto-oligosaccharides. Ca: Calcium. NS: Not significant.

Treatment of Atopic Dermatitis

One RCT evaluating the effect of synbiotics against prebiotics and not 'normal' controls in treating atopic dermatitis was found[77]. The trial included 39 children, two years of age or older, who had a minimum score of 15 on the SCORing Atopic Dermatitis (SCORAD) scale. In addition to their usual diet and treatment for atopic dermatitis, children were randomized to receive *L. rhamnosus* in a synbiotic preparation or prebiotics (lactose plus potato starch) alone, 3 times a day for 3 months. Among children receiving synbiotics, the pretreatment SCORAD score was 39.1 versus 20.7 after 3 months of treatment ($p<0.0001$). Among children receiving the prebiotic alone, the pretreatment SCORAD score was 39.3 versus 24.0 after 3 months of treatment ($p<0.0001$). There was no significant difference in SCORAD between the children who received synbiotics versus probiotics. In addition, no difference was found in the number of patients who reached at least 50% and 90% improvement ($p=0.408$ and $p=0.184$, respectively). There was also no difference in the use of topical treatments. Synbiotics and prebiotics were both well tolerated. Although the investigators demonstrated that children with moderate-to-severe atopic dermatitis have a reduction in symptoms following treatment with either prebiotics or synbiotics, these results must be confirmed against placebo and in a larger group of patients.

Prevention of Atopic Disease

One double-blind, randomized, placebo-controlled trial[78] investigated the effect of a prebiotic mixture (90% GOS, 10% long-chain FOS; dosage: 0.8 g/dl) on the intestinal flora and the cumulative incidence of atopic dermatitis during the first six months of life in infants at risk for allergy (with at least one parent with documented allergic disease confirmed by physician). Two hundred and six (79.5%) of 259 infants who were randomly assigned to receive extensively hydrolyzed whey formula supplemented either with 0.8 g GOS/FOS ($n=102$) or maltodextrin as placebo ($n=104$) were included in the per-protocol analysis. The frequency of atopic eczema in the experimental group was significantly reduced compared with placebo group [9.8% vs. 23.1%, RR 0.42 (0.2-0.8), NNT 8 (5-31)]. In a subgroup of 98 infants, parents provided fresh stool samples for microbiological analysis, using plating techniques; the fecal counts of bifidobacteria were higher in the group fed the GOS/FOS formula compared to the placebo group. By contrast, no significant difference was found for lactobacilli counts between groups. This is the first and only observation that prebiotics are able to reduce the incidence of atopic dermatitis, demonstrating the immune-modulating effect of prebiotics during the first months of life. However, these results should not influence practice until confirmed by further studies. ITT analysis was not performed. The drop-out rate of 20% was high. In addition, the prevalence of eczema in the placebo group was relatively high (23.1%), particularly considering the fact that infants in this group received extensively hydrolyzed formula, which is designed to be used as a formula to decrease allergy risk. Collectively, these findings argue for caution in applying the results of this study to current clinical practice.

Bioavailability and Absorption of Calcium

Three double-blind cross-over trials have studied the effects of administering oligosaccharides on the bioavailability and absorption of calcium in adolescents. One RCT[79] ($n=59$) revealed that in girls at or near menarche, calcium absorption was significantly higher in a group receiving an inulin plus oligofructose (8 g/day) mixture than in the placebo group ($38.2 + 9.8\%$ *vs.* $32.3 + 9.8\%$; $p=0.01$); however, no significant difference was seen between the oligofructose without inulin group and those receiving placebo ($31.8 + 9.3\%$ *vs.* $31.8 + 10\%$, p=NS).

Another RCT[80] in 12 healthy male adolescents aged 14-16 years reported that 15 g of oligofructose per day was well tolerated and enhanced fractional calcium absorption (mean difference + SE of difference: $10.8 + 5.6\%$; $p<0.05$, one-sided) compared with individuals receiving sucrose. No information was provided about the overall calcium balance of study subjects in either of these two RCTs. Thus, it is difficult to critically assess the degree of benefit that might be achieved for overall calcium homeostasis.

The most recent RCT[81] in young adolescents assessed the effects on calcium absorption and bone mineral accretion after eight weeks

and one year of supplementation with either 8 g/d of a mixed short- and long-degree of polymerization inulin-type fructan product (fructan group) or maltodextrin (control group). Bone mineral content and bone mineral density were measured before randomization and after one year. Calcium absorption was measured with the use of stable isotopes at baseline and at eight weeks and one year after supplementation. Polymorphisms of the *Fok1* vitamin D receptor gene were also determined. Daily consumption of the combination of prebiotic short- and long-chain inulin-type fructans significantly increased calcium absorption and enhanced bone mineralization during pubertal growth. Effects of dietary factors on calcium absorption may be modulated by genetic factors, including specific vitamin D receptor gene polymorphisms[81]. The importance of this study derives from the demonstration of long-lasting beneficial effects after prolonged use of prebiotics.

CONCLUSIONS

So where are we now? Probiotics and prebiotics have the potential to prevent and treat many disorders in the pediatric population. However, to date, the most extensively studied application and the best-documented area is only the efficacy of probiotics in the treatment of acute infectious diarrhea and prevention of antibiotic-associated diarrhea. Many other benefits of both probiotics and prebiotics are largely unproven, but there is a growing body of scientific evidence in support of such benefits. Guidance is needed as to which agent to use, timing, dosage and mode of administration. As there is still too little evidence, further studies investigating the role of probiotics and prebiotics in clinical practice are required.

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