Potential Roles and Clinical Utility of Prebiotics in Newborns, Infants, and Children: Proceedings from a Global Prebiotic Summit Meeting, New York City, June 27-28, 2008

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Initial bacterial colonization, including colonization with health-positive bacteria, such as bifidobacteria and lactobacilli, is necessary for the normal development of intestinal innate and adaptive immune defenses. The predominance of beneficial bacteria in the gut microflora of breast-fed infants is thought to be, at least in part, supported by the metabolism of the complex mixture of oligosaccharides present in human breast milk, and a more adult-type intestinal microbiota is found in formula-fed infants. Inadequate gut colonization, dysbiosis, may lead to an increased risk of infectious, allergic, and autoimmune disorders later in life. The addition of appropriate amounts of selected prebiotics to infant formulas can enhance the growth of bifidobacteria or lactobacilli in the colonic microbiota and, thereby, might produce beneficial effects. Among the substrates considered as prebiotics are the oligosaccharides inulin, fructo-oligosaccharides, galacto-oligosaccharides, and lactulose. There are some reports that such prebiotics have beneficial effects on various markers of health. For example, primary prevention trials in infants have provided promising data on prevention of infections and atopic dermatitis. Additional well-designed prospective clinical trials and mechanistic studies are needed to advance knowledge further in this promising field. *(J Pediatr 2009;155:S61-70)*.

The intestine of the human fetus *in utero* is thin and immature, with a slow turnover of mucosal cells and a paucity of lymphoid elements. In contrast, the intestine of the newborn, after the bacterial colonization process has begun, contains an active and mature epithelium with all forms of enterocytes expressed and an abundance of lymphoid tissue. The maturation process is followed closely by marked changes in the immune protective function of the intestinal tract. These changes indicate that active bacterial colonization exerts profound effects on gut function and structure.

Establishment of Bacterial Colonization

The process of bacterial colonization of the intestine begins at the time of delivery (phase 1 of gut colonization), when the fetus leaves the germ-free intrauterine environment and enters the extra-uterine setting. Term infants have evidence of intestinal colonization by the day after vaginal delivery, and the process continues through breast feeding (phase 2 of gut colonization) and weaning (phase 3 of gut colonization) in a stepwise manner.

By approximately the age of 18 months, the colonic bacterial microbiota is complete.[1] The normal adult gut flora includes roughly 500 species of bacteria.[2] The diverse species present as the gut microflora exist in a symbiotic relationship with the host, providing, for example, energy to colonocytes in the form of short-chain fatty acids produced by bacterial fermentation and the production of vitamin K that is made available to eukaryotic cells of the host.[3] The resident microflora also provide a balanced ecosystem that serves to protect against long-term colonization by potentially pathogenic bacteria—a feature referred to as colonization resistance.

Microorganisms present in large numbers in the normal microflora of the breast-fed infant are also present, albeit in lower absolute numbers, after weaning. Some of these genera are considered potentially health-promoting bacteria

FISH	Fluorescent in situ hybridization
FOS	Fructo-oligosaccharide
GOS	Transgalacto-oligosaccharide (galacto-oligosaccharide)
sIgA	Secretory immunoglobulin A
Th	T-helper

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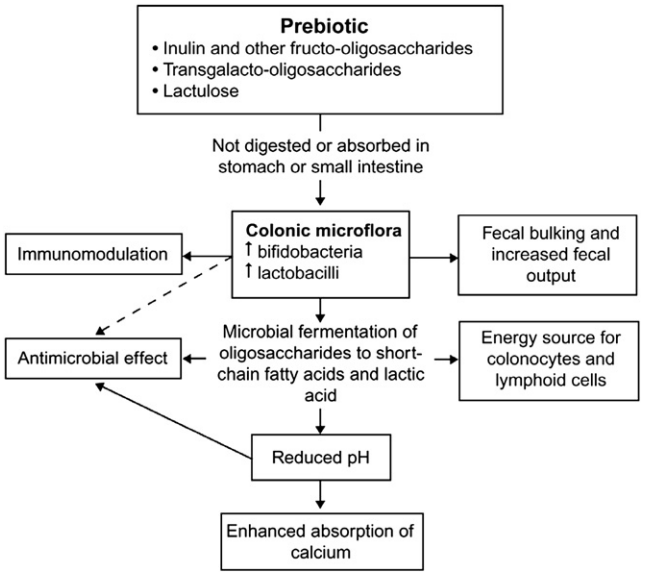

Figure. Representation of potential or proven effects of prebiotics in the intestinal tract.

(for instance, *Bifidobacterium* and *Lactobacillus*). The composition of the intestinal microflora is influenced by the type of initial oral feeding, as determined with standard culture techniques. In breast-fed infants, the gram-positive nonsporulating bacilli bifidobacteria and lactic acid-producing organisms (lactobacilli) predominate, whereas a more adult-type flora in which *Enterobacteriaceae*, *Clostridium*, and *Bacteroides* predominate is seen in formula-fed infants.[4] Studies with molecular identification and genetic detection methodologies have confirmed these findings.[4]

The predominance of beneficial bacteria in the gut microbiota of breast-fed infants is thought to result from the fermentation of oligosaccharides—non-digestible carbohydrates consisting of several linked monosaccharides (typically 3-10 simple sugars)—in breast milk. Human milk contains approximately 8% of total carbohydrates as oligosaccharides (roughly between 5 and 13 g/L, with levels as high as 24 g/L in colostrum).[5] There are >130 different oligosaccharides with ≥3 monosaccharides present in mature human milk. The molecular structure of these oligosaccharides is highly variable,[6] based in large part on genetic differences. The composition and concentration of human milk oligosaccharides change during lactation: 90% are neutral, and 10% are negatively charged and acidic.

Oligosaccharides pass unabsorbed through the small intestine into the colon, where they are fermented by resident bifidobacteria to short-chain fatty acids and lactic acid, creating a milieu with a pH of approximately 5.7 (**Figure**).[7] In contrast, the gut microflora of formula-fed infants produces a different profile of short-chain fatty acids and a pH in the local microenvironment of approximately 7.0.[7] Oligosaccharides in breast milk provide protection against enteric infections, likely because of their prevention of pathogen binding to the intestinal epithelium.[8] Oligosaccharides in human milk also protect the nursing infant by acting as glycoconjugate receptors, which can inhibit the binding of enteropathogens to host cell surface receptors.[9]

Impaired Bacterial Colonization

Premature birth or cesarean delivery may result in inadequate phase 1 gut colonization, with a restricted and sparse intestinal bacterial microbiota. Despite the stimulus of phases 2 and 3 of gut colonization, final colonization may be delayed, which may contribute to making these children more susceptible to gut pathogens. Infants with an inadequate phase 1 of colonization also may be more prone to the development of immune-mediated diseases. For instance, one study noted an increased risk of food allergy in the offspring of mothers with a history of food allergy who were delivered via cesarean method, compared with babies delivered vaginally by mothers with or without a history of food allergy.[10] A large, prospective, birth-cohort study found that infants delivered via cesarean method had an increased risk of diarrhea during the first year of life and allergic sensitization at the age of one year.[11]

The widespread use of broad-spectrum antibiotics during the perinatal period, particularly in the vulnerable preterm baby, also may result in inadequate phase 1 gut colonization. A study in 3-week-old mice showed that when the aminoglycoside antibiotic kanamycin was administered orally for 7 days, a shift in the T-helper (Th)1/Th2 balance toward a Th2-predominant response was observed, manifested by a striking increase in immunoglobulin E responses.[12] Oral introduction of intestinal bacteria (specifically, *Enterococcus faecalis* or *Lactobacillus acidophilus*, but not *Bacteroides vulgaris*) after oral antibiotic administration in 3-week-old mice prevented development of this Th2-shifted immunity.[13] Thus, both quantitative and qualitative disturbances of the intestinal microflora during infancy might prevent postnatal Th1-cell maturation, resulting in a Th2-polarized immune predominance. This shift to increased Th2 activities, with up-regulation of immunoglobulin E responses to environmental antigens, serves as a model for the development of atopy.

Consequences of Altered Bacterial Colonization

The establishment of bacterial colonization during infancy has important clinical implications, including protection against infection through competition with enteric pathogens, positive metabolic effects, and augmentation of both innate and adaptive host immune responses. Epidemiologic data indicate that decreases in numbers of infectious diseases in developed countries between the years 1950 and 2000—in part, as the result of improved sanitation, widespread immunization, and antibiotic therapies—are associated with a shift in disease burden, characterized by an increase in the prevalence of immune-mediated disorders such as allergic and autoimmune diseases.[14] This phenomenon has been referred to as the "hygiene hypothesis," which asserts that the intestinal mucosal immune system fails to develop properly when exposure to colonizing environmental microorganisms is decreased during infancy. The net result is an increased burden of allergic and autoimmune diseases.[14] This

Table I. Definitions of Prebiotics

- Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth, activity, or both of one or a limited number of bacteria in the colon and thus improve host health[20]
- Non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth of one or a limited number of bacterial species in the colon, such as bifidobacteria and lactobacilli, which have the potential to improve host health[21]
- A non-viable food ingredient selectively metabolized by beneficial intestinal bacteria[22]
- A selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastrointestinal microflora, that confers benefits on host well-being and health[23]
- A selectively fermented ingredient that results in specific changes in the composition, activity, or both of the gastrointestinal microbiota, thus conferring benefit(s) on host health[24]
- Substrates that arrive undigested in the colon, where they serve to stimulate the growth or metabolic activity of one or a few bacterial species with demonstrable benefit to the host organism[5]

hypothesis is supported by a study in which the frequency of allergic symptoms in children living in non-farm environments was greater than that in farm-dwelling children.[15] Initial bacterial colonization and exposure to antigens in farm settings may well enhance the development of intestinal mucosal defense mechanisms.

Development of Gut Defenses

The development of normal mucosal defenses is complex and multifactorial. Increasingly, the critical importance of bacterial-epithelial cell cross talk in the development of normal host defenses is recognized.[16] Host defense functions are already developed at birth, but are not operational until colonizing bacteria stimulate either epithelial cells or lymphoid elements in the intestine. In the absence of appropriate gut colonization, a Th2 immune response predominates,[17] which could be an important factor in the observed increases in allergic and autoimmune disorders in the last half century.

Modulation of Colonization in Formula-fed Infants

A variety of options are available for modifying microbial gut colonization in infants fed formula. These options include modification of the nutrient composition (eg, protein quantity and quality); micronutrients such as iron that are required as essential growth factors by some bacteria; the amount of lactose, some of which reaches the colon undigested in infants, hence acting as a prebiotic; buffering capacity and other factors affecting colonic pH; and other compositional aspects, all which may have a considerable impact on gut colonization. Live bacteria, such as *Bifidobacteria* and *Lactobacilli*, can be added to infant formula and considered as probiotics when benefits to host health are documented after their provision in adequate amounts.[18] Alternatively, prebiotics can be added as substrates that arrive undigested into the colon, where they serve to stimulate the growth, metabolic activity, or both of one or a few bacterial species with demonstrable benefit to the host.[5] Combinations of probiotics and prebiotics are called synbiotics, whereas nonviable bacteria are called postbiotics, which are used in fermented and pasteurized infant formula.[19,20] This manuscript focuses on the potential usefulness of prebiotics in infant formulas.

Overview of Prebiotics

Prebiotics have been defined in a number of ways (Table I).[5,21-25] Criteria that must be met for classification as a prebiotic include resistance to gastric acid and hydrolysis by mammalian enzymes, lack of significant gastrointestinal absorption, fermentation by the intestinal microflora, and selective stimulation of either the growth or the activity of intestinal bacteria with demonstrable benefit for host health and well-being in studies meeting accepted scientific standards. There continues to be debate about whether the affected bacteria should be restricted to bifidobacteria and lactobacilli. The gut microbiota is extraordinarily complex. It is certain to contain other positive genera and, as knowledge of the composition and functionality of the intestinal microflora expands, other bacterial targets for prebiotic effects likely will emerge. Currently, only certain non-digestible oligosaccharides—saccharide polymers containing simple sugars—fulfill all the criteria for classification as a prebiotic.[26]

Among the substances currently considered to be prebiotics are the oligosaccharides inulin (long-chain fructo-oligosaccharides; lcFOS), short-chain fructo-oligosaccharides (scFOS), transgalacto-oligosaccharides (also called galacto-oligosaccharides; GOS), and lactulose.[27] Inulin is a fructan (a polymer of fructose molecules) typically consisting of 10 to 60 fructose units; scFOS has the same structure as inulin, but the fructose chains are shorter, typically consisting of 2 to 7 fructose units. Lactulose consists of one unit of fructose and one unit of galactose. It should be noted that the structural composition of these carbohydrates is very different from the complex mixture of human milk oligosaccharides, which comprise >100 different molecular structures[8,28] and currently cannot be added to infant formula. However, the bifidogenicity of inulin/FOS,[29,30] GOS,[31] lactulose,[32,33] and lcFOS/scGOS has been reported in

a number of publications.[34-36] These findings also suggest that the degree of prebiotic response is dependent on starting levels of bifidobacteria, because lower populations elicit a more marked effect. Furthermore, the ability of bifidobacteria to ferment prebiotics varies with the substrate tested and with the specific *Bifidobacterium* species and strain under evaluation.[37] The longer the chain length, the more sustained the fermentation pattern becomes.[38] A concurrent increase in lactobacilli also has been noted in some studies.[34]

Prebiotics can be incorporated into a wide range of food products, including cereals, biscuits, drinks, baked products, and infant formulas. Inulin-type fructans and particularly lcFOS/scGOS have been used in infants and children, and FOS has been used in weaning foods consumed by toddlers with the goal of increasing fecal bifidobacteria numbers and decreasing fecal clostridia numbers during consumption.[39] However, the long-term effects of prebiotics on the composition of the gut microbiota and on intestinal function remain to be defined.

Testing of prebiotics has been conducted by using pure or mixed bacterial cultures and a variety of cell lines *in vitro*, in animal models, and in human feeding studies. DNA-based methodologies, such as 16S ribosomal RNA sequencing, and traditional microbiologic cultures and biochemical techniques to identify colonic bacteria have been used in such evaluations. Potential prebiotics for use in infant formulas must be tested in controlled trials in infants to document their safety, effects on the gut microflora, and potential clinical benefits. An actual biologic effect cannot be presumed *a priori*, because potential interactions between food components and the added prebiotic(s) may negate a beneficial effect on health.

Clinical Evidence by Age Group

There is some evidence that prebiotics can increase the numbers of bifidobacteria, lactobacilli, or both present in the gut lumen.[40] Prebiotics, however, also may provide other important functions, including, for example, anti-adhesive effects that reduce the binding of pathogenic bacteria to colonocytes[23] and modulating effects on immunologic processes at the level of gut-associated lymphoid tissue.[41] Additional purported benefits of prebiotics are listed in Table II.

Use of Probiotics and Prebiotics in Premature Infants

Premature infants with a gestational age ≥34 weeks generally are breast- or formula-fed and receive full feeding after only a few days. By contrast, infants with a gestational age <34 weeks often receive most of their early nutrition intravenously, with a slow progression to enteral feedings via nasogastric tube. Very premature infants also have a slow acquisition of an intestinal microflora, particularly bifidobacteria,[42] while being exposed to environmental microorganisms from the neonatal intensive care unit and to multiple antibiotics.[43]

Randomized controlled studies in premature infants generally with a gestational age of at least 34 weeks indicate that selected probiotics may reduce the risk of necrotizing enterocolitis,[42,44,45] but the populations are highly heterogeneous, and the studies lack the power to draw definitive conclusions. Accordingly, it is currently not known whether prebiotics might reduce the frequency of necrotizing enterocolitis in at-risk premature babies. Additional studies, including a larger number of subjects from multiple centers, must be conducted to confirm efficacy and to establish safety before probiotics or prebiotics become a standard of clinical practice in the neonatal intensive care unit.

Table II. Potential Effects of Prebiotics

- Confirmed
  - Selective proliferation of beneficial bacteria, such as bifidobacteria
  - Softer stools
- Possible and promising effects
  - Fecal bulking and regular bowel motions
  - Increased resistance to colonization by enteric pathogens
  - Immune modulation, including innate, adaptive, and regulatory functions
  - Stimulation of beneficial microbial activities, through selective fermentation
    - Production of volatile short-chain fatty acids
      - Trophic and anti-neoplastic effects
      - Induction of peristalsis and improved laxation
      - *De novo* lipogenesis, with reduced serum cholesterol levels
      - Reduced pH
        - Increased absorption of calcium and magnesium
        - Hostile environment for pathogens

Boehm et al[35] compared the effect of a cow milk formula supplemented with a mixture of GOS and inulin (10 g/L in a 9:1 ratio) with that of an unsupplemented control formula taken for 28 days on the fecal flora and stool characteristics of 30 preterm infants with a maximum gestational age of 32 weeks. A third study group was fed fortified human breast milk. Bifidobacteria counts in the prebiotic-treated group increased significantly compared with the control formula ($P = .0008$), reaching the upper range of counts found in the breast milk group. Stool frequency was lower in the control group, compared with both the prebiotic ($P = .0079$) and the breast milk groups ($P < .0001$). Stool consistency was harder in the control group compared with babies receiving either prebiotic-supplemented formula ($P = .0102$) or fortified breast milk ($P = .0003$). Whether such prebiotic mixtures will reduce intolerance to enteral feeds in preterm newborns needs to be evaluated in rigorous clinical trials with clear and reproducible primary outcome measures.

Mihatsch et al[46] studied the effects of a GOS/FOS prebiotic-supplemented formula (10 g/L) compared with a control formula taken for 14 days on feeding tolerance in 20 preterm infants with a mean gestational age of 27 weeks who were receiving full enteral formula feedings. Prebiotic supplementation altered both stool consistency and intestinal transit time, compared with the control formula. Additional studies are necessary to determine whether prebiotics in preterm formula will facilitate the advancement of enteral feedings and enterocyte maturation, which could reduce the duration of intravenous nutrition, its adverse associated effects, and associated risk of catheter-related infections.

Kapiki et al[47] compared the effect of formula supplemented only with FOS (4 g/L) with a control formula containing maltodextrin for 14 days on the fecal flora of 56 preterm infants with a maximum gestational age of 36 weeks. In the prebiotic-treated group, numbers of bifidobacteria in stool and the proportion of infants colonized with bifidobacteria were higher after 7 days compared with the control group ($P = .032$ and $P = .030$, respectively); there was also a significantly higher number of *Bacteroides* species in the FOS group ($P = .029$). Prebiotic supplementation also increased daily stool frequency ($P = .008$ versus control group). Weight gain was significantly greater in the control group ($P < .05$), which could have been caused by the added maltodextrin.

There are still unanswered questions specific to the preterm infant, but which also may apply to the term newborn and developing infant. For instance, it is not clear that there is sufficient rationale and enough confirming evidence to provide prebiotics to a premature infant who may not have established a gut microbiota that can appropriately use oligosaccharides for promoting health. In this instance, there is a potential concern that the substrate may be used to promote the growth of either potential pathogens or commensal luminal organisms that are capable of translocating the immature gut epithelial barrier and causing systemic disease as opportunistic pathogens. A recent study provided evidence of increased bacterial translocation in the intestine of immature rats fed a milk formula containing GOS and inulin.[48] Clearly, further study in this area is necessary. The potential for long-term effects from manipulation of the gut microbiome early in life, including manipulation with prebiotics, is currently not known.[49]

Role of Prebiotics for Term Infants

Whether prebiotics should be added to infant formulas has been considered previously by experts in the field.[5] Studies show that prebiotic oligosaccharides may increase the levels of bifidobacteria present in the gut and soften stools. However, it must be noted that there are still relatively few published clinical trials evaluating the efficacy and safety of prebiotics in food products targeted for infants.

Prebiotic Effects on Stool Composition

A number of studies have assessed the effects of prebiotic-supplemented infant formulas on stool consistency and frequency. For instance, Moro et al[34] compared the effects of formulas containing mixtures of short-chain GOS and inulin in a 9:1 ratio (total of 4 g/L and 8 g/L, respectively) with those of a maltodextrin-containing control formula taken for 28 days in 90 term newborns. Stool frequency increased only in the supplemented group given the "dose" of 8 g/L prebiotic ($P < .01$ versus both 4 g/L and control groups). Stool consistency also softened only in the group receiving 8 g/L, without a significant change in the prebiotic-supplemented study group receiving 4 g/L. Stool pH increased during the study period in the control group, but did not change in the 4 g/L group and decreased in the 8 g/L group. At the end of the study period, stool pH was significantly lower in both prebiotic-supplemented formula groups compared with the control group ($P < .05$) and was within the range typical for the stools of breast-fed infants.

By using the fluorescent in situ hybridization (FISH) approach to assess the fecal microbiota at enrollment and after 6 weeks, Knol et al[40] compared a standard formula with a formula containing both short-chain GOS and inulin (total: 8 g/L in a 9:1 ratio) in healthy bottle-fed term newborns. Expressed as a percentage of total bacteria, the proportion of bifidobacteria was higher in the prebiotic-treated group compared with the control formula group (69% versus 34%, $P < .05$). In addition, the proportion of *Escherichia coli* and *Clostridium* species was lower in the prebiotic-treated group.

Moore et al[50] evaluated the effect of infant cereal supplemented with either 0.75 g FOS per serving or placebo for 28 days on gastrointestinal tolerance in 56 healthy infants. Stool consistency was more likely to be described as either soft or loose in the FOS group compared with the placebo group. The mean number of stools per infant per day was 1.99 in the FOS group, compared with 1.58 in the placebo group ($P = .02$). These results indicate that FOS consumption leads to softer and more regular stools, without diarrhea, and lower frequency of signs of constipation, such as hard stools or days without stooling.

More recently, Scholtens et al[51] compared the effect of breast milk, a formula containing inulin and short-chain GOS (6 g/L in a 9:1 ratio) and formula without prebiotics on the composition of the intestinal microflora, stool pH, and development of the fecal secretory immunoglobulin A (sIgA) response in 187 healthy infants during the first 26 weeks of life. As determined by using the FISH approach, the percentage of bifidobacteria was higher in the prebiotic group (60.4%) compared with the control group (52.6%, $P = .04$). The percentages of *Clostridium* were 0.0% and 32.7%, respectively, in the prebiotic and control groups ($P = .006$). Stool pH also was decreased significantly in the prebiotic-treated group. As measured with immunoassay, fecal sIgA was higher in the prebiotic group (719 μg/g) compared with the control group (263 μg/g, $P < .001$). These results indicate an effect of prebiotic supplementation of infant formula on mucosal immune response.

Infant follow-up formulas that are fermented with lactic acid-producing bacteria during the production process, with or without pasteurization, contain no viable bacteria and have been used widely in Europe[19] also appear to reduce diarrhea episodes during infancy. It should be mentioned, however, that 2 randomized, blinded, controlled studies of prebiotics (infant cereal supplemented with 0.55 g of oligofructose per 15 g cereal with or without zinc) in 282 infants living in a community with a high burden of intestinal infections failed to show an association with change in diarrhea prevalence.[52] The investigators speculated that the lack of effect might be caused by the relatively low dose of prebiotic used in the study, the inadequate statistical power of the study, or most infants continuing to be breast fed during the study.

Prebiotics and Atopy

Osborn and Sinn[53] reviewed published randomized controlled trials assessing the effectiveness of prebiotics given to infants for the prevention of allergic diseases including atopic dermatitis and food hypersensitivity. Of 7 eligible studies, only 2 reported an allergic disease outcome, in a total of 432 infants. Eczema was significantly decreased in 1 of these studies, but not in the other. The inconsistent outcomes are potentially attributable to different risk for allergic diseases in enrolled infants, the prebiotic formulation, or the manner in which eczema was measured. Analysis of the other 5 studies, which assessed infant growth as the endpoint, found no anthropometric differences or consistent adverse effects. These reviewers concluded that, currently, there is insufficient evidence to determine the role of prebiotic supplementation of infant formulas for prevention of allergic disease and food hypersensitivity. One small trial of prebiotic oligosaccharides with excess losses reported a reduction in eczema in high-risk, formula-fed infants (see below). Further trials are needed to determine whether this finding persists in a longer period, applies to other manifestations of allergic disease, is associated with reductions in allergen sensitization, and is reproducible.

One published study reported that eczema is decreased with the use of prebiotics. Moro et al[54] conducted a double-blind, randomized trial to determine whether a partial hydrolysate formula containing inulin and short-chain GOS (8 g/L in a 9:1 ratio) for 6 months had an effect on the incidence of atopic dermatitis compared with a maltodextrin-containing formula. Subjects were term infants with a parental history of atopic eczema, allergic rhinitis, or asthma. After 6 months, atopic dermatitis developed in fewer infants in the prebiotic-treated group than in the control group (10% versus 23%, $P = .014$) on the basis of a validated clinical quantitative index. However, there was a relatively high dropout rate of 20.5%. Arslanoglu et al[55] described the results of a follow-up of this study until 2 years of life. The cumulative incidence of atopic dermatitis, recurrent wheezing, and allergic urticaria were higher in the maltodextrin group (27.9%, 20.6%, and 10.3%, respectively) than in the prebiotic group (13.6%, 7.6%, and 1.5%, respectively; $P < .05$).

Kalliomäki et al[56] showed that infants with evidence of atopy (defined as positive results on at least 1 skin prick test) at 1 year of age had significantly more *Clostridium* species ($P = .04$) and tended to have fewer bifidobacteria ($P = .11$) in stool examined by using the FISH approach at 3 weeks of age, compared with non-atopic infants. These findings suggest that there could be an association between the intestinal microflora and maturation of immune function with a non-atopic state.

Prebiotics and Infection

The frequency of infections has been assessed in a study that compared a partial hydrolysate formula containing an inulin and a short-chain GOS (8 g/L in a 9:1 ratio) with a maltodextrin formula taken for 6 months.[57] The recipe for both formulas was based on a hypoallergenic formula with extensively hydrolyzed cow milk protein. During the study period, in a *post hoc* analysis, infants in the prebiotic group experienced fewer episodes of infections ($P = .01$ versus control group). The cumulative incidence of recurring respiratory infections also was lower in the prebiotic group (10%) compared with those receiving formula supplemented with maltodextrin alone (14%, $P < .05$ versus control). In the unblinded portion of this study, at 2 years after ingestion, prebiotic-supplemented formula was associated with fewer episodes of any kind of infection ($P = .01$ versus control), including upper respiratory infections ($P < .01$ versus control), infections necessitating treatment with an antibiotic ($P < .05$ versus control), or fever episodes recorded by parents ($P < .0001$ versus control).[55] Bruzzese et al[36] conducted a clinical study in which healthy infants between 15 and 120 days of age were randomized to receive either a standard infant formula with prebiotics (inulin/GOS mixture in a 9:1 ratio) or an infant formula without prebiotics. After 3, 6, 9, and 12 months, data on episodes of intestinal and respiratory tract infections were collected. Infants receiving the prebiotic mixture for 12 months had significantly fewer episodes of intestinal and respiratory tract infections.

Prebiotics in Neonates

The evolution of infant formula has been driven by a desire to make formula composition closer to that of human breast milk. The qualitative protein content of current commercially available formulas is less different from that of human milk than in previously used types of formula, although protein composition continues to differ markedly. However, deviation from the composition of human milk may also be desirable. For example, in infant formulas, hydrolyzed proteins, which are not found in human milk, might reduce allergenicity of such formulas compared with formulas containing intact cow milk-derived protein. Most recently, prebiotics have been considered for addition to infant formula, although the complex and variable oligosaccharide content of human breast milk cannot be reproduced precisely. Ideally, prebiotic ingredients should replicate the effects of oligosaccharides present in human milk on the colonic microflora. A number of studies have reported that infants who are fed formulas supplemented with prebiotics have a stool consistency and pattern similar to that of breast-fed infants.[58-60]

Ziegler et al[61] conducted a randomized, double-blind study to evaluate the tolerability and effect on infant growth of oligosaccharides added to formula for 120 days in 226 healthy term newborns. Babies were assigned to ingest control formula alone, the same formula supplemented with a blend of polydextrose and GOS (50:50 ratio, 4 g/L), or formula supplemented with a blend of polydextrose, GOS, and lactulose in a 50:33:17 ratio (8 g/L). Anthropometric measurements taken at 14, 30, 60, 90, and 120 days did not show any differences in the 3 study groups in either body weight or length growth rates. Stools were looser in the supplemented groups compared with the control group at 30, 60, and 90 days. Stool frequency was higher in the polydextrose/

GOS/lactulose group at 30 days compared with both the polydextrose/GOS ($P = .017$) and the control groups ($P = .021$). The frequency of diarrhea was higher in the polydextrose/GOS group ($P = .008$), the frequency of eczema was significantly higher in the polydextrose/GOS group ($P = .008$), and irritability was significantly higher in the polydextrose/GOS/lactulose group ($P = .027$). The most frequent reason given for withdrawal from the study was gas, particularly in the polydextrose/GOS/lactulose group.

Prebiotics Beyond Infancy

To date, there have been several randomized, double-blind, placebo-controlled trials assessing the potential health benefits of prebiotics in children 1 to 18 years of age.[62-69] These studies include a broad range of outcomes, which are considered below.

Prebiotics and the Gut Microflora

Waligora-Dupriet et al[62] gave FOS mixed in either cereal or drinks for 21 days, compared with a maltodextrin control. These investigators assessed the effect on the cultured intestinal microflora of 20 healthy children between 7 and 19 months of age. The prebiotic supplement was well tolerated, with significantly less flatulence, diarrhea, and vomiting (all $P < .05$) and fever ($P < .05$) than in the control group. However, there was no significant increase in the number of bifidobacteria after prebiotic supplementation, compared with maltodextrin.

Brunser et al[63] studied the effect of a cow milk formula supplemented with FOS and inulin versus a prebiotic-free control, both taken for 3 weeks, on the intestinal microflora of 140 children 1 to 2 years of age after treatment of acute bronchitis for 1 week with 50 mg/kg/day of amoxicillin. The FISH approach on stool samples obtained at the beginning and end of antibiotic therapy showed that amoxicillin decreased total fecal bacteria and increased levels of *E coli*. Feces obtained on days 7 and 21 of prebiotic supplementation showed that the prebiotic-supplemented formula significantly increased levels of bifidobacteria ($P < .05$) compared with control formula. Other intestinal bacteria were unaffected, and adverse clinical effects were not observed. These results indicate that prebiotics might help reestablish a normal balance of intestinal bacteria after a course of antibiotic therapy.

Prevention of Diarrhea

Binns et al[64] assessed the effect of a milk product containing a probiotic (*Bifidobacterium lactis*) plus a prebiotic blend of FOS plus acacia gum versus a control milk product taken twice daily for 5 months on the incidence of diarrhea (defined as ≥4 stools per day). This study included 496 healthy children 1 to 3 years of age who attended day care. After controlling for age, milk consumption rate and concurrent family illnesses, children consuming the synbiotic-containing product for at least 10 days (n = 315) experienced a 20% reduction in episodes of acute diarrhea (adjusted risk ratio, 0.80; 95% CI, 0.70-0.91).

Adjunct to Oral Rehydration Therapy

Hoekstra et al[65] evaluated the efficacy and safety of a hypotonic oral rehydration solution with or without a mixture of FOS and inulin for use in the treatment of acute infectious diarrhea (defined as ≥3 watery stools daily for >1 day but <5 days) with mild or moderate dehydration in 136 boys between 1 and 36 months of age. The intention-to-treat analysis did not show significant differences in groups in the mean 48-hour stool volume, duration of diarrhea, duration of hospital stay, or the need for unscheduled intravenous rehydration.

Atopic Dermatitis

Passeron et al[66] evaluated the efficacy of prebiotics alone versus a synbiotic (with the probiotic *Lactobacillus rhamnosus*) administered 3 times daily for 3 months on the severity of atopic dermatitis in 39 children 2 to 13 years of age. The prebiotic preparation was derived from the fermentation broth for *L rhamnosus* and contained skimmed milk powder (0.344 g), potato starch (0.759 g), and lactose (0.397 g). The atopic dermatitis severity-scoring index decreased from 39 to 24 in the prebiotic-treated group and from 39 to 21 in the group receiving the synbiotic ($P < .0001$ for each versus baseline). However, there was no decrease in the number of applications of topical therapy for atopic dermatitis in either group. Inclusion of an untreated group should be considered in future comparable trials.

Calcium Absorption and Bone Mineralization

Griffin et al[67] performed a study with a crossover design to assess the effects of FOS alone, FOS plus inulin, and placebo provided in orange juice 3 times daily for 3 weeks on calcium absorption in 59 healthy girls between 11 and 14 years of age. Each 3-week treatment period was separated by a 2-week washout period, and all subjects consumed roughly 1500 mg of calcium daily throughout the study. Calcium absorption was measured from the cumulative fractional excretion of oral and intravenous tracers over 48 hours with a dual stable isotope technique. Calcium absorption was significantly higher in the teenagers allocated to taking FOS plus inulin ($P = .01$ compared with the other 2 groups), but no significant difference was seen between the group receiving FOS alone and the placebo group. A similar study of 12 male adolescents reported that FOS alone stimulated fractional calcium absorption.[70]

A longer-term study evaluated the effect of a prebiotic consisting of mixed short- and long-chain inulin-type fructans versus a maltodextrin control taken for 12 months on calcium absorption and bone mineralization in 100 healthy, non-obese, early pubertal (Tanner stage 2 or 3) adolescents.[68] Calcium absorption was measured at baseline, at 8 weeks, and after 1 year. Bone mineral content and bone mineral density were measured before randomization and after 1 year. Compared with the control group, calcium absorption was greater in the prebiotic-treated group at 8 weeks ($P < .001$ versus control) and at 1 year ($P = .04$ versus control). The prebiotic group also had a greater increment in whole-body bone mineral content ($P = .03$ versus control) and bone mineral

density ($P = .01$ versus control). These observations may have particular importance because bone accretion is maximal during this stage of life.[71]

Obesity

Abrams et al[69] assessed the effects on body mass index of mixed short- and long-chain inulin-type fructans, compared with maltodextrin as placebo, taken for 12 months in 97 adolescents. Body fat was measured with dual-energy x-ray absorptiometry. Compared with the control group, subjects who received the prebiotic supplement had a smaller increase in body mass index ($P = .016$) and total fat mass ($P = .022$). Differences between groups were significant even in subjects with a calcium intake ≥700 mg/day. These differences were maintained 1 year after supplementation was discontinued.

Adverse Effects of Prebiotics

Despite theoretical concerns aforementioned, a search of the biomedical literature does not provide consistent evidence of adverse effects after the use of prebiotics in human subjects at a variety of ages. This includes an absence of compelling data indicating that there is the potential to enhance the growth of pathogens, at least with the present formulations and at the concentrations currently used.

As aforementioned, Ziegler et al[61] described a higher frequency of diarrhea in the group receiving formula supplemented with 4 g/L of a prebiotic blend of polydextrose and GOS (50:50 blend) or 8 g/L of a prebiotic blend of polydextrose, GOS, and lactulose (50:33:17 ratio) compared with the group receiving control formula without prebiotics (18% versus 4 %, $P = .008$). These authors also reported a higher incidence of eczema in the 4 g/L prebiotic-treated group compared with the control group (18% versus 7%, $P = .046$) and in the 4 g/L group compared with the 8 g/L group (18% versus 4%, $P = .008$). Additional studies in other research settings are required to confirm or refute these observations, and to establish the reasons for these effects.

d-lactic acidosis is a disorder with neurologic symptoms that has been described in patients with short bowel syndrome or after jejuno-ileal bypass surgery. d-lactic acidosis occurs when large amounts of carbohydrate (eg, because of malabsorption) are available for fermentation by lactobacilli dominating in the gut, leading to production of excessive d-lactate, which is then absorbed.[72,73] It is currently not known whether prebiotics predispose patients to d-lactic acidosis, but this possibility should be considered in the appropriate clinical setting (unsteady gait and altered level of consciousness postprandially) and, when observed, reported in the peer-reviewed medical literature.

Conclusions

Most studies indicate that, when taken in sufficient amounts, prebiotics soften stools, increase stool frequency (without episodes of diarrhea), and increase the ratio of bifidobacteria to total fecal bacteria. In infants receiving prebiotic-supplemented formula, water balance remains normal and, in most studies, the infants continue to grow appropriately. The combination of inulin-type fructans and GOS in a 9:1 ratio at concentrations as high as 8 g/L has been added to infant formulas in Europe for >6 years, after having been declared suitable for use in foods by the European Commission Scientific Committee on Food in December 2001,[74] and added to the accepted components for use in infant formulas.[75] The committee observed that the addition of the prebiotic mixture of inulin-type fructans and GOS in a 9:1 ratio at a concentration of 8 g/L to infant formula is considered safe, although additional data on growth, body composition, nutrient availability, and water balance still must be obtained.

The relevance of reported outcome measures, however, still is not clear, at least for decreasing disease and promoting health. In addition, stool culture-based methods are suboptimal and should be replaced with DNA-based molecular detection techniques. In addition, a number of questions remain about the mechanisms underlying the potential benefits of prebiotics. Clearly, additional research in each of these areas, including the use of appropriate animal models, is desirable. ■

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