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Prebiotic Oligosaccharides in Dietetic Products for Infants: A Commentary by the ESPGHAN Committee on Nutrition

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ABSTRACT
This article by the ESPGHAN Committee on Nutrition summarizes available information on the effects of adding prebiotic oligosaccharides to infant and follow-on formulae. Currently there are only limited studies evaluating prebiotic substances in dietetic products for infants. Although administration of prebiotic oligosaccharides has the potential to increase the total number of *bifidobacteria* in feces and may also soften stools, there is no published evidence of clinical benefits of adding prebiotic oligosaccharides to dietetic products for infants. Data on oligosaccharide mixtures in infant formulae do not demonstrate adverse effects, but further evaluation is recommended. Combinations and dosages in addition to those so far studied need to be fully evaluated with respect to both safety and efficacy before their use in commercial infant food products. Well-designed and carefully conducted randomized controlled trials with relevant inclusion/exclusion criteria, adequate sample sizes and validated clinical outcome measures are needed both in preterm and term infants. Future trials should define optimal quantity and types of oligosaccharides with prebiotic function, optimal dosages and duration of intake, short and long term benefits and safety. At the present time, therefore, the Committee takes the view that no general recommendation on the use of oligosaccharide supplementation in infancy as a prophylactic or therapeutic measure can be made. ***JPGN 39:465–473, 2004.***
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PREFACE

The potential benefits of adding probiotics, prebiotics or both (synbiotics) to dietetic products for children and infants are of considerable interest. Some infant formulae or follow-on formulae with added probiotics or prebiotics are already marketed in many countries. The ESPGHAN Committee on Nutrition has recently commented on probiotics in dietetic products for infants (1) and on non-digestible carbohydrates in the diet of infants and young children (2). Here the Committee summarizes available information on the effects of adding oligosaccharides considered to be of a prebiotic nature to infant and follow-on formulae. In preparing this comment the Committee reviewed documents of other expert groups on the use of prebiotic oligosaccharides in dietetic products for infants and young children. The Committee also reviewed randomized clinical trials (RCTs) on infant and follow-on formulae with prebiotics and clinical trials that examined the effects of different prebiotic oligosaccharides used for infants and children.

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Establishment of the Gut Microflora in Infancy

The development of a normal intestinal microflora is a gradual process. At birth, the human gastrointestinal tract is sterile, but within hours it is colonized by different types of bacteria, mainly environmental and maternal (vaginal, fecal and oral flora) coliforms and streptococci (3–5). Vaginally delivered full-term infants are colonized very early by anaerobic bacteria, predominantly *Bacteroides* (6). In contrast, infants delivered by caesarian delivery exhibit delayed colonization by anaerobes and by *Enterobacteria* and gram-negative organisms (3). Although some recent studies found no significant differences between the intestinal microflora of breast-fed and formula-fed infants (7), several other studies

suggested that the intestinal microflora differs between these two populations. Breastfeeding induces predominance of lactobacilli and bifidobacteria (8–10), whereas formula-fed infants develop an intestinal microflora that is rich in *Enterobacteria* and gram-negative organisms (11). The introduction of complementary foods during weaning is a further critical time point in the colonization process when the composition of the microflora shifts to a more adult pattern, with fewer *Escherichia coli* and *Clostridium* spp and more *Bacteroides* and gram-positive anaerobic cocci (12,13). In the older child and adult, more than 400 bacterial species are found in the colon.

Potential Effects of a Bifidobacteria-Dominant Microflora in Breast-Fed Infants

Breast-fed infants are less susceptible to infectious diseases. The lower incidence of gastrointestinal and other infections found in breast-fed infants (14–16) may be related in part to the early pattern of microbial colonization. The colonizing bifidobacteria and lactobacilli may inhibit the growth of pathogenic microorganisms through the production of lactic, acetic and other organic acids, with a consequent decrease of intraluminal pH that inhibits the growth of some bacterial pathogens. In contrast, formula feeding tends to favor a flora associated with a near neutral pH of the feces. Moreover, bifidobacteria and lactobacilli compete with potentially pathogenic bacteria for nutrients and epithelial adhesion sites. The gut flora also modulates the recovery of substrates through fermentation of nondigestible carbohydrates and nitrogen salvage, and affects mucosal growth and the absorption of water and nutrients (17). Accumulating evidence also indicates that the gut flora modulates mucosal physiology, barrier function and systemic immunologic and inflammatory responses (18).

Factors Relevant to a Bifidobacteria-Dominant Microflora

The colonic microflora depends on a constant supply of nutrients and growth factors. Thus, various components in breast milk or formula either stimulate or inhibit the growth of different types of bacteria. However, such effects may not be based on single growth promoting substances but rather on a complex set of interacting factors. Those implicated in the bifidogenic effects of human milk include the specific composition and relatively low concentration of protein (19), the low phosphorus content (20), the presence of lactoferrin (an iron-binding protein that has been shown in vitro to inhibit the growth of several pathogens) (21) and the presence of nucleotides (22) and oligosaccharides. Below we briefly summarize current knowledge on human oligosaccharides, the third largest component in human milk.

Human Oligosaccharides

Human milk contains more than 130 different oligosaccharides at a concentration of 15–23 g/L in colostrum and 8–12 g/L in transitional and mature milk (23,24). The carbohydrate chains of almost all oligosaccharides in human milk so far isolated contain lactose at the reducing terminal. Other monosaccharides are glucose, galactose, fucose, N-acetylglucosamine and sialic acid. Synthesis of oligosaccharides starts mainly from lactose moieties by transglycosyltransferases. Recently, human milk oligosaccharides were shown to be resistant to enzymatic digestion in the upper gastrointestinal tract (25). Human milk oligosaccharides appear to bind to specific carbohydrate receptors on mucosal cell surfaces and to act as receptor analogues. Complex oligosaccharides act as competitive receptors on the host cell surface, thereby preventing adhesion of a number of bacterial and viral pathogens. Part of the undigested oligosaccharides in human milk may serve as substrates for colonic fermentation and contribute to stimulation of the growth of *Bifidobacteria* in the colon. The promotion of a *Bifidobacteria*-dominant flora might have beneficial effects in infants, such as some protection against enteric infections. Human milk oligosaccharides are often regarded as a model for the addition of oligosaccharides of a prebiotic nature to infant formulae or follow-on formulae even though the biologic role of human milk oligosaccharides appears to be far more complex than the roles of the simple oligosaccharides presently added to formulae. Because of the variety, variability, complexity and polymorphism of their structure, it is currently not feasible to replicate the oligosaccharide component of human milk in infant and follow-on formulae (26).

Modulation of Intestinal Microflora

The growing understanding of the possible role of a bifidobacteria-dominant gut flora has led to the development of different strategies aimed at manipulating bacterial colonization in formula-fed infants. These include administration of probiotics or prebiotics or the combination of both (synbiotics) (27).

Probiotics have been defined as live bacteria that colonize the gut and provide a health benefit to the host (28,29). Bacterial genera most often used to induce probiotic effects are lactobacilli and bifidobacteria that form part of the normal intestinal microflora of humans. However, because these organisms are indigenous to the colon, a second strategy is to enhance their growth and metabolic activity with a selective carbon and energy source, such as oligosaccharides, providing these organisms with a competitive advantage over other resident bacteria (i.e., the prebiotic approach).

Prebiotics are 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

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the colon and thereby improving host health (29,30). To be effective, prebiotics should largely escape digestion and absorption in the upper gastrointestinal tract, reach the large bowel and be used selectively by microorganisms that have been identified to have health promoting properties (31). The usual candidates are bifidobacteria and lactobacilli. In light of ongoing research, it is important to stress that the spectra of target strains may be wider than expected. Prebiotics may be fermented by many strains (32) according to their metabolic characteristics, and several bacterial communities have been identified in the intestinal tract of infants (33,34). Indeed, it is known that inulin-type fructans increase the production of acetate and butyrate, indicating that populations other than bifidobacteria may benefit, as bifidobacteria do not produce butyrate. Therefore, other, as yet still undefined, strains could be selectively promoted by prebiotics (35).

Although undigested nitrogen- and lipid-containing compounds may also have prebiotic effects (22,23), the most commonly studied and used components of infant feeds with proposed prebiotic nature are nondigestible carbohydrates. These are a heterogeneous group of dietary substances that are derived primarily from plants (2). The principal groups of dietary nondigestible carbohydrates comprise fiber and nonstarch polysaccharides, a proportion of dietary starches that are not digested and absorbed in the small intestine of healthy individuals. Furthermore, a proportion of disaccharides such as lactose (35) that may be incompletely digested in the small intestine especially in young infants, oligosaccharides and polysaccharides, and synthetic and modified complex carbohydrates such as thickening agents.

In Europe and the USA, the main prebiotics used in food manufacturing are fructans, i.e., inulin and oligofructosyl-saccharose (oligofructose; fructooligosaccharides, FOS) derived from inulin. In addition, oligogalactosyl-lactose (oligogalactose; galactooligosaccharides) is used. In Japan, prebiotics in use also include isomaltooligosaccharides, soybean oligosaccharides, gentiooligosaccharides and xylooligosaccharides. Generally, these oligosaccharides are extracted from plants or synthesized from lactose or sucrose by enzymatic methods (36). It should be noted that there are considerable differences in utilization patterns between bacterial strains and species. This has implication for the development and use of oligosaccharides with expected prebiotic effects and requires further evaluation (31,37). Substances with more selective prebiotic and enhanced anti-pathogen effects and those with more desirable storage, processing and organoleptic properties are under development (38).

It has been suggested that the use of prebiotics might lead to increased resistance to pathogens (mainly gastrointestinal tract pathogens), modulation of the systematic immune response and of allergic risk, improved bowel function and laxative effects, reduced risk of colon cancer, reduction in cholesterol and blood lipids and enhanced calcium bioavailability and bone mineralization (31,39). However, for demonstration of any clinical benefits in infants and young children, well-designed clinical trials in these age groups that should follow current scientific standards (preferably controlled, randomized, and double-blind trials) are needed (40,41).

Expert Committee Reports on the Use of Oligosaccharides with Claimed Prebiotic Effects in Infant Formulae

The Scientific Committee on Food (SCF) of the European Commission recently commented on the use of nondigestible carbohydrates (i.e., oligofructosyl-saccharose and oligogalactosyl-lactose) in infant formulae and follow-on formulae (42). The report stated that the inclusion of up to 0.8 g/100 ml of a combination of 90% oligogalactosyl-lactose and 10% high molecular weight oligofructosyl-saccharose to infant formulae and follow-on formulae, for which data were available, raised no major concerns. However, further information should be gathered on safety and benefits of this combination as well as other forms of oligosaccharides in infant formulae and follow-on formulae. The SCF noted that the available trials comprise only a limited number of infants and some compare formula whereas other components, not just short chain carbohydrate content, had been modified. The SCF recommended, therefore, that further information should be collected for this combination of short chain carbohydrates (or any others) with regards to suitability and safety in infant formulae and follow-on formulae. The SCF also concluded that based on the data available, fructans other than oligofructosyl-saccharose should not be included in infant formulae and follow-on formulae. The SCF noted that particular attention should be given to the effects on growth and body composition and nutrient bioavailability in young infants, particularly with respect to protein and amino acid utilization, and on water balance, urine output and urine osmolarity in infants and in neonates.

The French Agency for Food Safety (AFFSA) Working Group on Infant Food and Modification of the Intestinal Flora concluded that prebiotics used in infant formulae should be fully characterized (i.e., origin, identification, concentration). Safety of prebiotics should be assessed according to the criteria used for authorized ingredients. Conditions of storage (temperature, humidity) should be mentioned on the product labeling. Although allergic reactions to prebiotics have not been described in infants and young children, AFSSA recommended follow-up of infants at risk for allergy fed prebiotic containing formulae in view of one reported anaphylactic reaction to inulin and oligofructose in an adult (43). AFSSA also raised concerns on the use of prebiotics in infants with congenital or acquired immunodeficiency and in the premature newborns. No beneficial clinical effects of prebiotics added to infant formulae other than an effect on stool

frequency and consistency have been demonstrated. Careful assessment of both safety and efficiency of formulae containing prebiotics should be made before commercialization, according to published criteria including at least two randomized control studies. Special attention should be given to water and mineral balances (44).

Systematic Review of Clinical Trials on Dietetic Products with Prebiotic Oligosaccharides in Infant Formula

Three databases (MEDLINE, EMBASE and Cochrane Controlled Trials Register) were searched until January, 2004 for randomized and quasirandomized (i.e., allocating participants according to date of birth, hospital number) controlled trials of infant or follow-on formulae supplemented with oligosaccharides with presumed prebiotic effects. Furthermore, all references to review articles and in the identified trials were examined. A separate search was made using names of individual authors known to be experts in this field. No limit was imposed as to the language of publication. Letters to the editor, abstracts and proceedings from scientific meetings were excluded. All health related outcomes (end points) reported by authors were considered. After the exclusion criteria were applied, the search strategies yielded only three papers assessing the clinical effects of feeding infant formula supplemented with prebiotic oligosaccharides (for characteristics of included studies and outcome measures, see Table 1) (45–47). Two trials were excluded (one trial described the same study group as in included trial (48), and one was an open and nonrandomized study (49). One potentially relevant report could not be not located (50). The three included studies recruited a total of 286 participants divided into the experimental (n = 148) and control (n = 138) groups. Participants were preterm (one RCT) and term (two RCTs) infants. Only one type of prebiotic oligosaccharide mixture (90% galactooligosaccharides + 10% FOS) produced by the same manufacturer was tested. In one of the trials, infant formula with further modifications (partially hydrolyzed whey protein, modified vegetable oil with a high ß-palmitic acid) was used (47).

Main Results

All three RCTs found that the supplementation of preterm or term infant formulae with the addition of 0.4 g/dL, 0.8 g/dL or 1 g/dL of a mixture of 90% galactooligosaccharides and 10% FOS compared with standard formula resulted in a significant increase of fecal *Bifidobacteria*. According to the results of one study this effect was dose dependent, with a concentration of the galactooligosaccharides/FOS mixture of 0.8 g/dL of formula inducing a higher number of *Bifidobacteria* than the concentration of 0.4 g/dL (45). Two RCTs evaluated the effect of oligosaccharide supplementation on fecal *Lactobacilli*, and an increase in the total number was demonstrated in one (45). Two RCTs (in term and preterm infants) found no effect of oligosaccharide supplementation on the number of infants with positive fecal culture for potentially pathogenic bacterial species (*Bacteroides, E. coli, Clostridium, Enterobacter, Citrobacter, Proteus, Klebsiella* and *Candida)* (45,46). All three RCTs found that oligosaccharides softened stool consistency and increased stool frequency, with the induction of more watery stools. According to one RCT this effect was dose dependent (45). One RCT found a dose-dependent effect of a mixture of prebiotic oligosaccharides on lowering fecal pH (45).

Three studies reported growth characteristics. Two of them examined growth from birth to the age of 1 month in preterm (46) and term (45) infants and found no significant group differences. In the third study (47), weight gain was higher in the study formula but only in girls and only during the first 6 weeks of the 12-week study period. Head circumference was greater after 12 weeks but, again, only in girls. Furthermore, the sum of skinfold measurements was higher but only in boys.

One RCT evaluated biochemical values of protein status (i.e., urea nitrogen, albumin, prealbumin, and amino acids) and reported no significant differences between the study groups (47). No adverse effects other than the occurrence of loose stools were reported.

In summary, the systematic review showed that published data on the efficacy and safety of prebiotic supplemented term and preterm infant formulae are scarce. Only one type of prebiotic oligosaccharide mixture used in products from the same manufacturer was tested in controlled trials.

Although short-term effects on increasing the total number of bifidobacteria in stools were demonstrated, there are no data on major clinical or long-term benefits. The total number of bifidobacteria was reported but not the effects on different bifidobacteria strains or on different pathogenic bacteria. It is unclear whether the total number of bifidobacteria in stools is related to any functional outcome (e.g., immune or inflammatory modulation). In addition, preterm infants, especially those treated under intensive care conditions, often have an abnormal pattern of gut colonization (51,52), and their gut flora may contain only small numbers of lactobacilli and bifidobacteria considered as target microorganisms for oligosaccharide supplementations. Clearly, the specific benefits and risks of oligosaccharide supplementation in this group of infants need careful evaluation in future trials.

None of the RCTs addressed clinically important outcome (e.g., gastrointestinal infection, allergic disease). Thus it is not possible to support or refute prebiotic oligosaccharide supplementation in infant formulae as a preventive or therapeutic measure for any childhood disease.

The induction of more watery stools may provide a relevant benefit in infants suffering from constipation but has the potential to increase the risk of dehydration in some infants. There is a concern (discussed in details by

the SCF) (42) that a particular risk may exist for infants during the first months of life with renal immaturity and a poor ability to concentrate urine if an additional stress on water balance is induced, for example, by fever, respiratory distress, infectious diarrhea, high dietary renal solute loads or refusal of the infant to accept appropriate quantities of fluids. Furthermore, an increase of stool frequency and change in stool consistency might theoretically interfere with the bioavailability of nutrients, although available data do not show this to be a major problem.

The impact on growth is an important part of the safety evaluation of breast milk substitutes (41,53). Two of the three RCTs examined growth from birth to the age of 1 month in preterm and term infants and found no significant difference. However, in the study of preterm infants there were only 15 infants in each group (46), which gives a power to detect a difference of 1 standard deviation (SD). In the study of term infants there were about 30 in each group (45), which gives a power to show a significant difference of about 0.7 SD between the groups. On the other hand, in both studies the growth data were almost the same in the groups studied with no tendency for a systematic difference. The number of subjects included in the third trial (47) is approximately sufficient to detect a meaningful growth difference (0.5 SD) according to currently acceptable standards (42,53). It is also similar to recommendations of the American Academy of Pediatrics (54), although the duration of these growth studies is shorter than desirable (53). Nevertheless, the growth differences observed in this largest study might also be explained by other variations in composition between the control and the study formula. Not only were oligosaccharides added to the study formula, but it also had a higher protein content with a different composition (hydrolyzed whey protein), and there was a higher percentage of palmitic acid in the ß-position with a probable benefit for fat and energy absorption, as compared with the control formula.

Other Randomized Controlled Clinical Trials on the Use of Oligosaccharides with Presumed Prebiotic Effects in Pediatric Populations

Few clinical trials have reported health outcomes for children given oligosaccharides.

In a large well-designed study performed in infants aged 6 to 12 months (n = 282), Duggan et al. (55) compared an infant cereal supplemented with oligofructose (0.55 g/15 g cereal) with a non-supplemented cereal. No significant difference was found in the mean duration of diarrhea (10.3 ± 9.6 vs. 9.8 ± 11.0 days, P = NS). During a second part of the same trial in 349 subjects, zinc (1 mg/15 g cereal) was added to both oligofructose-supplemented and control cereals (56). Again, no significant difference was found in mean duration of diarrhea (10.3 ± 8.9 vs. 9.5 ± 8.9 days, P = NS). In both trials, postimmunization titers of antibody to *Haemophilus influenzae* type B were similar in all groups, as were gains in height (no data on weight), number of visits to clinic, hospitalizations and use of antibiotics. It was concluded that the use of cereal supplemented with this type and dose of oligosaccharide was not associated with any change in diarrhea prevalence, use of health care resource or response to *Haemophilus influenzae* type B immunization.

Another RCT in 56 healthy term infants aged 4–12 months evaluated the tolerance and gastrointestinal effects of an infant cereal supplemented with either 0.75 FOS per serving or placebo for 28 days. Stool consistency was less often described as ''hard,'' and more likely to be described as ''soft'' or ''loose,'' in the FOS than in control 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 was found for stool pH. There was 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/d. FOS consumption led to more frequent and softer stools without reported diarrhea and less-reported frequency of symptoms associated with constipation such as hard stools or days without stool (56). Clinical outcome was not reported.

A recent randomized double blind placebo controlled multicenter study performed by the ESPGHAN Working Group on Intestinal Infections in 144 boys aged 1 to 36 months found that a mixture of nondigestible carbohydrates (soy polysaccharide 25%, α-cellulose 9%, gum arabic 19%, fructo-oligosaccharides 18.5%, inulin 21.5%, resistant starch 7%) was ineffective as an adjunct to oral rehydration therapy in the treatment of acute infectious diarrhea in children with mild to moderate dehydration. Intention-to-treat analysis did not show significant differences in mean 48 hour stool weight in the experimental group compared with the placebo group (140 ± 124 g/kg vs. 143 ± 114 g/kg, P = 0.4). The duration of diarrhea after randomization was similar in both groups (82 ± 39 hours vs. 97 ± 76 hours, P = 0.2). There were no significant differences in the duration of hospital stay (111 ± 44 hours vs. 126 ± 78 hours; P = 0.3), and unscheduled intravenous rehydration was similar in both groups (21.4% vs. 16.2%, P = 0.4). No significant adverse effects were noted (57).

Two double blind crossover trials studied the effects of oligosaccharides on bioavailability and absorption of calcium in adolescents. One RCT (n = 59) found that in girls at or near menarche calcium absorption was significantly higher in the 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), but no significant difference was seen between the oligofructose group and the placebo group (31.8 ± 9.3% vs. 31.8 ± 10%, P = NS) (58). Another RCT in 12 healthy male adolescents aged 14–16 years demonstrated that 15 g of oligofructose per

TABLE 1. *Characteristics of included studies*

Study	Randomization	Allocation concealment	Blinding	Dropouts	Participants	Experimental group	Control group	Duration of Intervention	Effect on stool *Bifidobacterium*
Boehm, 2002	No details given	Unclear	Not mentioned	Not described	N = 42 Preterm infants; <32 weeks gestation (Italy)	N = 15 PF + GOS/FOS (1 g/dL)	N = 15 PF + placebo (malto-dextrin) N = 12 Breast-fed infants (reference group)	28 days	28 days *vs* day 0 ↑ (in general) ($p < 0,05$); 1 g/dL IF *vs* placebo, $p < 0.008$
Moro, 2002	No details given	Unclear	Not mentioned	Not described	N = 90 Full term infants; AGA (Macedonia, Italy)	N = 30 IF+ GOS/FOS 0.4 g/dl N = 27 IF + GOS/ FOS 0.8 g/dl	N = 33 IF+ placebo (malto-dextrins)	28 days	↑number: 1 day *vs*. 28 days (IQR; cfu/g ± SD) 0.4 g/dL IF, 8.5 ± 1.9 *vs* 9.3 ± 1.6 0.8 g/dL IF, 7.7 ± 6.1 *vs* 9.7 ± 0.8 placebo, 8.8 ± 6.1 *vs* 7.2 ± 4.9 0.4g/dL; 0.8g/dL IF *vs* placebo $p < 0.01$; 0.4g/dL *vs* 0.8 g/dL IF, $p < 0.01$
Schmelzle, 2003	Sealed envelopes; random number table with a block design for groups of four; stratified by sex and by study Analysis per protocol; intention to treat analysis performed, but not shown	Yes	DB, method not described	N = 52 (34%) No significant difference between the groups (25 *vs* 27)	N = 154; 37-42 weeks gestation; BW 10-90pc; exclusive IF feeding by age 14 days (Germany)	N = 76 IF+GOS/FOS 0.8 g/dL + partially hydrolysed whey protein; high beta-palmitic acid level, starch;	N = 78 Standard IF	12 wks	Per gram of wet feces Entry *vs* 6 wk; 0.8 g/dL IF, 3.87×10^9 *vs* 1.03×10^{10} ($p < 0.005$) Control group: 3.5×10^9 *vs* 5.6×10^9 (NS) Number of bifidobacteria/ total number of bacteria (%) Entry *vs* 6 wk: $p = 0.002$; Control group *vs* 0.8 g/dL IF at 6 wk, $p = 0.003$

IF = infant formula; PF = preterm formula; BM = breast milk; NA = not assessed; NS = not significant.

day were well tolerated and enhanced fractional calcium absorption (mean difference ± SE of difference: 10.8 ± 5.6%; $P < 0.05$, one-sided) (59). No information was given on the overall calcium balance of the study subjects in these two RCTs and thus it is difficult to assess the degree of benefit that might be achieved for calcium balance.

In summary, a very limited number of controlled trials have addressed health outcomes of oligosaccharide addition to the diet of older infants and children. Two RCTs in infants aged 4 to 12 months did not report adverse effects. One study reported softer stools. One large RCT reported no effect of oligofructose with respect to reduction of infectious diarrhea and other infections. Current data do not support the use of oral rehydration solution with added fructooligosaccharides, inulin and other nondigestible carbohydrates in children with mild to moderate diarrhea. Prebiotics may have an effect on calcium absorption in adolescents, and there was no

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TABLE 1. *(Continued) Characteristics of included studies*

Effect on stool *Lactobacillus*	Effect on potentially pathogenic microflora	Stool frequency	Stool consistency	Stool pH	Anthropometric parameters	Biochemical parameters	Tolerance/adverse effects
Significant increase in all groups with no significant effect of the oligosaccharide supplement	NS	BM placebo; $p < 0.0001$; BM *vs* 1 g/dL IF, $p = 0.2$	Placebo vs 1 g/dL IF, $p < 0.01$; placebo *vs* BM, $p < 0.0003$	NA	Weight gain (g/day, 1-28 days): 1 g/dL IF 29.8 ± 4; placebo 29.8 ± 4.1; BM 29.7 ± 3.3 (NS between the 3 groups) Length gain (cm/wk, 1-28 days): 1 g/dl IF 0.99 ± 0.05; placebo 0.98 ± 0.05; BM 1.01 ± 0.04 (NS between the 3 groups)	NA	No effect on incidence of crying, regurgitation, and vomiting
↑number: 1 day *vs.* 28 day (IQR ± SD) 0.4 g/dL IF, 3.3 ± 0.2 *vs* 5.9 ± 1.5 0.8 g/dl IF, 3.4 ± 0.2 *vs* 5.6 ± 2.1 placebo, 3.4 ± 0.2 *vs* 3.4 ± 1.8 0.4g/dl IF & 0.8g/dL IF > placebo ($p < 0.001$) 0.4 *vs* 0.8 g/dL IF, NS	NS	Placebo *vs* 0.8 g/dL $p < 0.01$	0.8 g/dL IF *vs* placebo, $p < 0.0001$; 0.8 g/dL *vs* 0.4 g/dL IF, $p < 0.01$	0.4 g/dL IF, pH 5.48-5.44; 0.8 g/dL IF, pH 5.54-5.19; placebo, pH 5.5-6.1	Weight gain (g/day), 1-28 days: 0.4 g/dL IF: 35.1 ± 6.7; 0.8 g/dL IF: 35.9 ± 6.5; placebo: 36.8 ± 8.3 (NS) Length gain (cm/wk, 1-28 day): 0.4 g/dL IF: 0.88 ± 0.23; 0.8 g/dL 0.87 ± 0.17; placebo 0.87 ± 0.16 (NS)	NA	No effect on incidence of crying, regurgitation, vomiting
NA	NA			NA	Weight gain (g/day), entry-6 wk, 0.8 g/dL IF ♀ > control group ♀ ($p < 0.05$), entry -12 wk, NS Length (mm/day), entry-6 wk, NS; entry-12 wk, NS HC, entry-12 week 0.8 g/dL IF ♀ > control ♀ ($p < 0.05$) Sum of skinfolds, entry-12 week, 0.8 g/dL IF ♂ > control ♂ ($p < 0.05$); 0.8 g/dL IF ♀ > control ♀ (Ns)	(All at 6 weeks). Total protein, albumin, urea NS; prealbum-in 0.8 g/dL IF < control ($p < 0.002$); TYR 0.8 g/dL < control ($p < 0.05$); THR 0.8 g/dL IF > control ($p = 0.001$). ILEU 0.8 g/dL IF > control ($p < 0.05$). LYS 0.8 g/dL > control ($p = 0.007$)	No adverse effects

evidence of adverse effects. However, no firm conclusion on the effects of prebiotics in children or infants can be drawn on the basis of the available data. Further large studies with clinically important outcomes are needed.

Reports on the Use of Oligosaccharides in Adults

Several reviews of the literature are available (60,61). In brief, a relatively small number of oligosaccharides considered as prebiotics has been evaluated. At present, there is strong evidence of an increase of the total number of bifidobacteria in stools. Insufficient evidence is available to state that inulin or oligofructose might have a cholesterol-lowering effect. Effects on plasma triacylglycerol concentrations remains to be elucidated. Inulin and fructooligosaccharides seem to have dose-related laxative effects indicating potential use of these products in the treatment of constipation. No effect on symptoms of irritable bowel syndrome was reported with oligofructose

or fructooligosaccharides. The result of one RCT suggests no efficacy in the prevention of traveller's diarrhea. There is some evidence that prebiotic substances may enhance intestinal calcium absorption in postmenopausal women, but no information is available on calcium balance and bone mineral content. Also, the effect on other minerals (Mg^{2+}, Fe^{2+}, and Zn^{2+}) is unclear.

In summary, knowledge on the clinical effects of prebiotic oligosaccharide administration in adults is very limited. Many claims for the potential health benefits of prebiotics remain unsubstantiated. There is no scientific basis for generalizing from data obtained in adults to infants and children without further consideration of age-related physiological differences. Prebiotics can be generally regarded as safe, although it was reported that in adults fructans may cause dose-dependent gastrointestinal side effects (62). Furthermore, the occurrence of repeated anaphylactic reaction to inulin and oligofructose was reported in one adult (43). A recent study in rats showed that dietary fructooligosaccharides and lactulose improve the colonization resistance to *Salmonella enteritidis*, but—in contrast to most expectations—concomitantly impair the intestinal resistance of rats to translocation (i.e., the passage of viable bacteria from the gastrointestinal tract through the epithelial mucosa) (63) of this invasive pathogen. No such effects were observed with resistant starch, wheat fiber or cellulose supplementation of the diet (64).

CONCLUSIONS AND RECOMMENDATIONS

- Currently there are only limited published data on the evaluation of prebiotic substances in dietetic products for infants. Therefore, 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.
- There is no published evidence of other clinical benefits of adding prebiotic oligosaccharides to dietetic products for infants.
- The available data on the oligosaccharide mixtures in infant formulae do not demonstrate adverse effects.
- Validated clinical outcome measures of prebiotic effects in infants should be characterized in further well-designed and carefully conducted randomized controlled trials with relevant inclusion/exclusion criteria and adequate sample size. Such trials should also define the optimal quantities, types and intake durations and safety of different oligosaccharides.
- Further evaluation is required before the general use of prebiotics in premature infants and/or infants with special conditions (e.g., immune deficiency).

REFERENCES

1. ESPGHAN Committee on Nutrition. Probiotic bacteria in dietetic products for infants: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2004;38:365–74.
2. ESPGHAN Committee on Nutrition. Nondigestible carbohydrates in the diets of infants and young children: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2003;36:329–37.
3. Rotimi VO, Duerden B. The development of the bacterial flora in normal neonates. *J Med Microbiol* 1981;14:51–62.
4. Brook I, Barrett CT, Brinkman CR 3rd, et al. Aerobic and anaerobic bacterial flora of the maternal cervix and newborn gastric fluid and conjunctiva: a prospective study. *Pediatrics* 1979;63:451–5.
5. Tannock GW, Fuller R, Smith SL, et al. Plasmid profiling of members of the family *Enterobacteriaceae*, lactobacilli, and *bifidobacteria* to study the transmission of bacteria from mother to infant. *J Clin Microbiol* 1990;28:1225–8.
6. Long SS, Swenson RM. Development of anaerobic fecal flora in healthy newborn infants. *J Pediatr* 1977;91:298–301.
7. Satokari RM, Vaughan EE, Favier CF, et al. Diversity of *Bifidobacterium* and *Lactobacillus* spp. in breast-fed and formula-fed infants as assessed by 16S rDNA sequence differences. *Microb Health Dis* 2002;14:97–105.
8. Orrhage K, Nord CE. Factors controlling the bacterial colonization of the intestine in breast-fed infants. *Acta Paediatr Suppl* 1999;430: 47–57.
9. Benno Y, Sawada K, Mitsuoka T. The intestinal microflora of infants: composition of fecal flora in breast-fed and bottle-fed infants. *Microbiol Immunol* 1984;28:975–86.
10. Lunderquist B, Nord CE, Winberg J. The composition of the faecal microflora in breast-fed and bottle-fed infants from birth to eight weeks. *Acta Paediatr Scand* 1985;74:45–51.
11. Mevissen-Verhage EA, Marcelis JH, de Vos MN, et al. *Bifidobacterium*, *Bacteroides*, and *Clostridium* spp. in fecal samples from breast-fed and bottle-fed infants with and without iron supplementation. *J Clin Microbiol* 1987;25:285–9.
12. Mata LV, Urrutia JJ. Intestinal colonization of breast-fed children in a rural area of low socio-economic level. *Ann N Y Acad Sci* 1971; 176:93–109.
13. Stark PL, Lee A. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J Med Microbiol* 1982;15:189–203.
14. Howie PW. Protective effect of breastfeeding against infection in the first and second six months of life. *Adv Exp Med Biol* 2002;503: 141–7.
15. Howie PW, Forsyth JS, Ogston SA. Protective effect of breast feeding against infection. *Br Med J* 1990;300:11–6.
16. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding (Cochrane Review). *The Cochrane Library* 2003, Issue 3.
17. Kelleher SL, Casas I, Carbajal N, et al. Supplementation of infant formula with the probiotic *Lactobacillus reuteri* and zinc: impact on enteric infection and nutrition in infant rhesus monkeys. *J Pediatr Gastroenterol Nutr* 2002;35:162–8.
18. Sudo N, Sawamura S, Tanaka K, et al. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997;159: 1739–45.
19. Heine WE, Mohr C, Wutzke KD. Host-microflora correlations in infant nutrition. *Prog Food Nutr Sci* 1992;16:181–97.
20. Radke M, Mohr C, Wutzke KD, Heine W. Phosphate concentration: does reduction in infant formula feeding modify the micro-ecology of the intestine? [in German]. *Monatsschr Kinderheilkd* 1992; 140(Suppl 1):S40–4.
21. Bullen JJ, Rogers HJ, Leigh L. Iron-binding proteins in milk and resistance to *Escherichia coli* infections in infants. *Br Med J* 1972; 1:69–75
22. Gil A, Corral E, Martinez A, et al. Effects of the addition of nucleotides to an adapted milk formula on the microbial pattern of

faeces in term infants. *J Clin Nutr Gastroenterol* 1986;1: 127–32.
23. Kunz C, Rodriguez M, Koletzko B, et al. Nutritional and biochemical properties of human milk, part I. General aspects, proteins and carbohydrates. *Clin Perinatol* 1999;26:307–33.
24. Kunz C, Rudloff S, Baier W, et al. Oligosaccharides in human milk: structural, functional, and metabolic aspects. *Ann Rev Nutr* 2000; 20:699–722.
25. Engfer MB, Stahl B, Finke B, et al. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *Am J Clin Nutr* 2000;71:1589–96.
26. Erney RM, Malone WT, Skelding MB, et al. Variability of human milk neutral oligosaccharides in a diverse population. *J Pediatr Gastroenterol Nutr* 2000;30:131–3.
27. Crittenden RG. Prebiotics. In: Tannock GW, ed. *Probiotics: A Critical Review*. Wymondham: Horizon Scientific Press, 1999:141–156.
28. Fuller R. Probiotics in human medicine. *Gut* 1991;32:489–92.
29. Bellisle R, Diplock AT, Hornstra G, et al. Functional food science in Europe. *Br J Nutr* 1998;80(Suppl):S3–193.
30. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microflora: introducing the concept of prebiotics. *J Nutr* 1995;125:1401–12.
31. Macfarlane GT, Cummings JH. Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? *Br Med J* 1999;318:999–1003.
32. Favier CF, Vaughan EE, De Vos WM, et al. Molecular monitoring of succession of bacterial communities in human neonates. *Appl Environm Microbiol* 2002;68:219–26.
33. Favier CF, De Vos WM, Akkermans ADL. Development of bacterial and bifidobacterial communities in feces of newborn infants. *Anaerobe* 2003;9:219–29.
34. Van Loo J, Cummings J, et al. Functional food properties on non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII-AIRII-CT94–1095). *Br J Nutr* 1999;81:121–32.
35. Szilagyi A. Review article: lactose—a potential prebiotic. *Aliment Pharmacol Ther* 2002;16:1591–602.
36. Playne MJ, Crittenden R. Commercially available oligosaccharides. *Bulletin of the International Dairy Federation* 1996;313:10–22.
37. Hopkins MJ, Cummings JH, Macfarlane GT. Interspecies differences in maximum specific growth rates and cell yields of *bifidobacteria* cultured on oligosaccharides and other simple carbon sources. *J Appl Microbiol* 1998;85:381–6.
38. Rastall RA, Gibson GR. Prebiotic oligosaccharides: evaluation of biologic activities and potential future developments. In: Tannock GW, ed. *Probiotics and Prebiotics: Where Are We Going?* Wymondham: Caister Academic Press, 2002:108–48.
39. Van Loo J, Cummings J, Delzenne N, et al. Functional food properties on non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). *Br J Nutr* 1999;81:121–32.
40. ESPGHAN Committee on Nutrition. Core data for nutrition trials in infants: a discussion document—a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2003;36: 338–42.
41. ESPGHAN Committee on Nutrition. The nutritional and safety assessment of breast milk substitutes and other dietary products for infants: a commentary by the ESPGHAN Committee on Nutrition. *J Pediatr Gastroenterol Nutr* 2001;32:256–8.
42. Scientific Committee on Food. Report of the Scientific Committee on Food on the revision of essential requirements of infant formula and follow-up formula (adopted on 4 April 2003). SCF/CS/NUT/IF/65 Final 18 May 2003.
43. Gay-Crosier F, Schreiber G, Hauser C. Anaphylaxis from inulin in vegetables and processed food. *N Engl J Med* 2000;18:1372–3.
44. AFSSA (Agence Française de Sécurité Sanitaire des Aliments—French Food Safety Agency). Infant food and modification of intestinal flora (working document) [in French]. 2003. Available at: http://www.afssa.fr/ftp/afssa/basedoc/Floreintestinale.pdf.
45. Moro G, Minoli I, Mosca M, et al. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. *J Pediatr Gastroenterol Nutr* 2002;34:291–5.
46. Boehm G, Lidestri M, Casetta P, et al. Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of fecal *bifidobacteria* in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2002;86:F178–81.
47. Schmelzle H, Wirth S, Skopnik H, et al. Randomized double-blind study of the nutritional efficacy and bifidogenicity of a new infant formula containing partially hydrolyzed protein, a high beta-palmitic acid level, and nondigestible oligosaccharides. *J Pediatr Gastroenterol Nutr* 2003;36:343–51.
48. Fusch C, Skopnik H, Wirth S, et al. Double-blind, randomized, placebo-controlled study to evaluate the nutritional efficacy of Omneo 1. Final study results. *Pediatrika* 2001;21:49–54.
49. Alarcon PA, Tressler RL, Mulvaney A, et al. Gastrointestinal tolerance of a new infant milk formula in healthy babies: an international study conducted in 17 countries. *Nutrition* 2002;18:484–9.
50. Yamamoto Y, Yonekubo A. A survey of physical growth, nutritional intake, fecal properties and morbidity of infants related to feeding methods. *Shoni Hoken Kenkya* 1993;52:465–71.
51. Blakey JL, Lutitz L, Barnes GL, et al. Development of gut colonisation in preterm neonates. *J Med Microbiol* 1982;15:519–29.
52. Gewold IH, Schwalbe RS, Taciak VL, et al. Stool microflora in extremely low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 1999;80:F167–73.
53. Koletzko B, Ashwell M, Beck B, et al. Characterisation of infant food modifications in the European Union. *Ann Nutr Metab* 2002; 46:231–42.
54. American Academy of Pediatrics. *Clinical Testing of Infant Formulae With Respect to Nutritional Suitability for Term Infants.* Report of AAP Task Force. Rosemont, IL: American Academy of Pediatrics; 1989.
55. Duggan C, Penny ME, Hibberd P, et al. Oligofructose-supplemented infant cereal: two randomized, blinded, community-based trials in Peruvian infants. *Am J Clin Nutr* 2003;77:937–42.
56. Moore N, Chao C, Yang LP, et al. Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. *BR J Nutr* 2003;90:581–7.
57. Hoekstra JH, Szajewska H, Abu Zikri M, et a. Oral rehydration solution containing a mixture of nondigestible 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.
58. Griffin IJ, Davila PM, Abrams SA. Non-digestible oligosaccharides and calcium absorption in girls with adequate calcium intakes. *Br J Nutr* 2002;87:S187–91.
59. Van den Heuvel EG, Muys T, van Dokkum W, et al. Oligofructose stimulates calcium absorption in adolescents. *Am J Clin Nutr* 1999; 69:544–8.
60. Conway PL. Prebiotics and human health: the state-of-the-art and future perspectives. *Scand J Nutr* 2001;45:13–21.
61. Andersson H, Asp N-H, Bruce Å, et al. Health effects of probiotics and prebiotics: a literature review on human studies. *Scand J Nutr* 2001;45:58–75.
62. Safety evaluation of fructans. Nordic Council of Ministers, Copenhagen 2000. *TemaNord* 2000;523:115.
63. Van Leeuwen PA, Boermeester MA, Houdijk AP, et al. Clinical significance of translocation. *Gut* 1994;35(Suppl):S28–34.
64. Bovee-Oudenhoven IMJ, ten Bruggencate SJM, Lettink-Wissink MLG, et al. Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonisation but stimulate translocation of *Salmonella* in rats. *Gut* 2003;52:1572–8.