Review Article

Fructans in the first 1000 days of life and beyond, and for pregnancy

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Inulin-based prebiotics are non-digestible polysaccharides that influence the composition of the gut microbiota in infants and children, notably eliciting a bifidogenic effect with high short chain fatty acid levels. Inulin, a generic term that comprises β-(2,1)-linked linear fructans, is typically isolated from the chicory plant root, and derivatives such as oligofructose and long chain inulin appear to have different physiological properties. The first 1000 days of a child's life are increasingly recognized as a critical timeframe for health also into adulthood, whereby nutrition plays a key role. There is an ever increasing association between nutrition and gut microbiota composition and development, with life health status of an individual. This review summarizes the latest knowledge in the infant gut microbiota from preterms to healthy newborns, as well as in malnourished children in developing countries. The impact of inulin or mixtures thereof on infants, toddlers and young children with respect to intestinal function and immunity in general, is reviewed. Possible benefits of prebiotics to support the gut microbiome of malnourished infants and children, especially those with infections in the developing world, are considered, as well as for the pregnant mothers health. Importantly, novel insights in metabolic programming are covered, which are being increasing recognized for remarkable impact on long term offspring health, and eventual potential beneficial role of prebiotic inulins. Overall increasing findings prompt the potential for gut microbiota-based therapy to support health or prevent the development of certain diseases from conception to adulthood where inulin prebiotics may play a role.

Key Words: review, inulin, infant, pregnancy, gut microbiota

INTRODUCTION

Inulin, an energy storage polymer in plants, is a generic term that covers all β-(2,1)-linked linear fructans, with a variable degree of polymerisation (DP) and mostly one terminal glucose-unit (Figure 1). Due to the β-(2,1) bonds, inulin is scarcely or indigestible by host digestive enzymes in the small intestine; it reaches the colon intact where it is fermented to short-chain fatty acids (SCFA)

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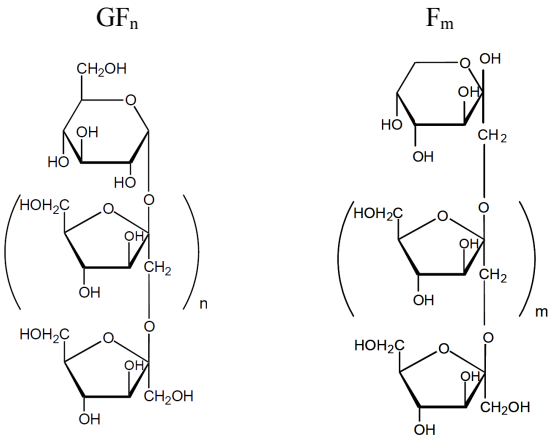

Figure 1. Chemical structure of inulin and oligofructose. G: glucose; F: fructose; n or m: number of fructose moieties; DP (Degree of Polymerization) = n+1, or m+2.

and gases by colonic bacteria; the SCFA are taken up by the host and subsequently metabolised. Thereby inulin is a dietary fibre, as well as its derivative oligofructose. Well-known inulin types of fructans and prebiotics applied in infant foods relevant to this review are listed in Table 1. Although inulin-type fructans occur in a large variety of plants chicory (*Cichorium intybus*, L.) is the preferred source for industrial production.[1,2] Native inulin is usually purified from the chicory plant, and its partial enzymatic hydrolysis product oligofructose (OF) and long-chain inulin are produced from native inulin. Fructo-oligosaccharides (FOS) can also be produced from sucrose with fructosyltransferases from *Aspergillus* spp.[3]

Inulin and oligofructose may be termed prebiotics. Prebiotic activity may be defined as the selective stimulation of growth and/or activity of one or a limited number of microbial genus(era)/species in the gut microbiota that confer health benefits to the host.[4,5] The initial focus of prebiotic research was on the bifidogenic effect of fructans as this was the most prominent measureable effect as first noted in Japan with sc-FOS.[6] Bifidobacteria are saccharolytic bacteria and considered positive for the intestinal tract especially in infants stemming from Henry Tissier's research in the early 1900's.[7] Numerous interventions have shown the bifidogenic effects of inulin and oligofructose in infants, and children,[4,8] and they are widely applied in infant formula today for their prebiotic properties and more recently in growing-up milks for toddlers.

Based on relevant recent research, various aspects connected with the use of fructan prebiotics with special attention for inulin and oligofructose in food for infants, toddlers and young children were discussed with an international group of nutrition experts in Jakarta, Indonesia on October 13-14, 2014. Additionally the use of prebiotics during pregnancy, the effects for the unborn child and the pregnant mother were addressed. Taken together this period of 9 months pregnancy and the first two years of life constitute the first 1000 days of life. Researchers have identified the first 1000 days of a child's life as a critical window of time for a person's lifelong health and proper nutrition is key. The emphasis was on human rather than animal model studies. This review is an outcome of the discussion meeting and the focus is on research that may be more relevant to the Asian region. It covers novel insights in the gut microbiota in both healthy and malnourished infants and children, the impact of fructans on the microbiota and health aspects such as bowel habit and immunity, and the long term health of the infant and young child via metabolic foetal programming and epigenetic regulation, which are being increasing recognized and researched.

INFANT GUT MICROBIOTA AND LONG TERM IMPLICATIONS

Colonization of the infant gut contributes to the intestinal homeostasis and mucosal barrier function, that both are essential for our health, at the start of life and apparently also in adulthood.

Developing infant gut microbiota

An overview of factors influencing the gut microbiota and the growing infant is illustrated in Figure 2. The infant receives its first microbes from the mother. Increasing studies show that the baby's meconium, the first stool passed after birth, already contains bacteria closely resembling microbes in the mother's oral cavity.[9,10] These remarkable findings indicate that colonization of the infant commences *in utero,* in accordance with growing evidence for microbial maternal transmission being widespread across the animal kingdom.[11] This universal phenomenon supports the essential nature of gut microbiota speculated to have consequences for the health of the offspring.

The development of the gut microbiota in the newborn has been extensively researched. Increasingly eloquent molecular technologies are applied to study the infant microbiota since their introduction. Briefly, facultative anaerobes create an anaerobic environment in which subsequently obligate anaerobic species can thrive. Human milk provides a continuous inoculum of lactic acid bacteria and *Bifidobacterium* spp., and the transfer of bacterial types via breast milk is effective regardless of preterm and term delivery, caesarean or vaginal delivery.[12] The microbiota of breast-fed infants is typically dominated by bifidobacteria, whereas the microbiota of formula-fed infants is more diverse.[13,14] In a study of Chinese infants during the first six months using 16S rRNA sequencing, the main groups present were Enterobacteriaceae, Veillonellaceae, Bacteroidaceae and Bifidobacteriaceae, and bifidobacteria were also significantly higher in human milk-fed infants.[15]

Numerous studies have also demonstrated that the mode of delivery affects the composition of the newborn's microbiota. Caesarean section was associated with a lower total microbial diversity and delayed colonisation of the *Bacteroidetes* phylum.[16-18] Other factors influencing this composition include infant hospitalization and antibiotic use, antibiotic use in the pregnant mother, solid-feeding practices and day care attendance.[19,20]

The microbiota of pre-term infants is different to that of full term new-borns. The microbiota of pre-term infants harbours higher levels of facultative anaerobes and in addition has generally less diversity, lower levels of SCFA and a delayed acquisition of the normal anaerobic gut microbiota including bifidobacteria.[21] The bacterial colonization of the premature infant gut proceeds through

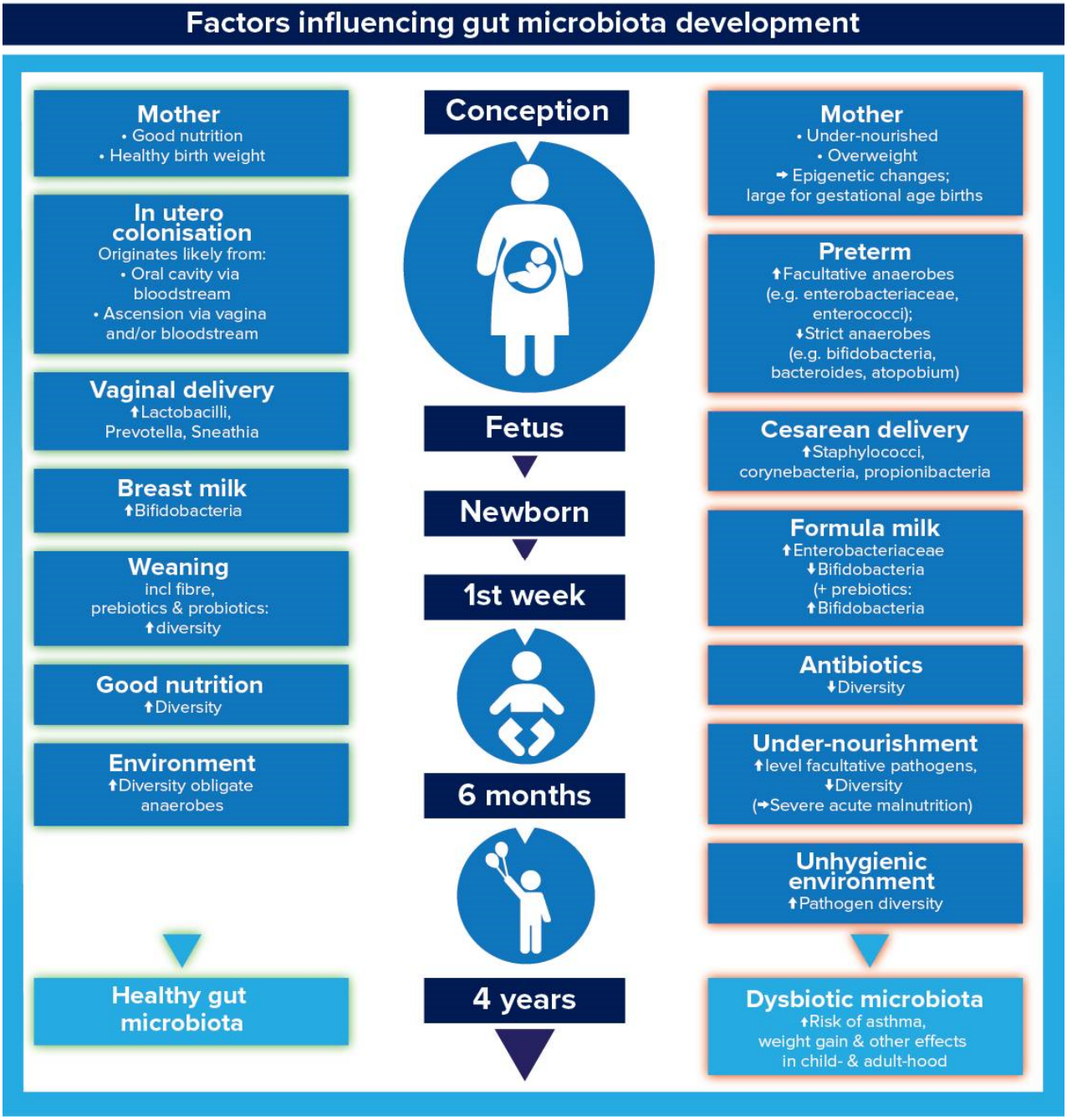

Figure 2. Infographic illustrating the impact of factors on the development of gut microbiota in the infant to small child. The left and right boxes indicate potentially positive and negative factors respectively for healthy development of the gut microbiota and the growing infant.

a patterned progression from bacilli to gammaproteobacteria to clostridia. The rate of progression, but not the sequence, is influenced by antibiotics, vaginal or Caesarean delivery, diet and age.[22] Pre-term infants are more prone to diseases such as necrotising enterocolitis (NEC) and late onset sepsis (LOS), both significant causes of mortality. Recently published data suggest that both diseases might be associated with abnormal patterns of the faecal microbiota without a clear involvement of known potential pathogens, such as *Staphylococcus aureus*, although a novel pathogen may be involved in the onset of NEC.[23,24]

Bifidobacteria deserve a special mention as they generally dominate the infant gut microbiota, being stimulated by oligosaccharides in the milk. Members of this genus are rarely pathogenic, and are primarily carbohydrate-fermenting bacteria producing principally short chain fatty acids (SCFA) which are known to be beneficial to host health. Furthermore, lactic acid-producing bacteria such as bifidobacteria and lactobacilli have been implicated in the maintenance of colonisation resistance, through a variety of mechanisms.[25]

Introduction of solid food at weaning modifies and increases the diversity of the infant gut microbiota, the bifidobacteria decrease, and apparently the so-called enterotypes observed in adults are established at this stage.[26,27] Several studies indicate that after about 3 years the children's microbiota has developed into an adult-type although not as diverse.[28,29] It has been suggested that the development of the intestinal microbiota in infants is influenced by so many parameters, including medical, cultural factors, as well as mode of delivery, diet, familial

environment, diseases, and therapies, that it is nearly impossible to define a universal standard for intestinal colonization and development of the intestinal microbiota.[30]

Study of the gut microbiota of a large cohort of Asian children (7-11 yr olds) showed the microbiota highly reflected their country of residence, with the majority of children in China, Japan and Taiwan harbouring a *Bifidobacterium*/*Bacteroides* cluster, whereas those from Indonesia and Khon Kaen in Thailand mainly harboured a *Prevotella*-type.[31] Predictive metagenomics suggested that different dietary habits and life style played a role in this.

Infant's microbiota and health later in life

It is intriguing to consider that events early in life may determine the activity of our gut microbiota for the rest of our life. Currently the consequences of these changes in the gut microbiota on host physiology and health is the subject of substantial investigations.[32,33] The impact of the infant gut microbiome on obesity risk has recently been reviewed which implicate several gut microbiota mechanisms.[34] Studies suggested that a higher level of *Bifidobacterium* and *Collinsella* and lower *S. aureus* during the first months of life was associated with maintenance of a normal weight in growing infants and children.[17,35]

The development of the microbiota and its relationship with the immune system, notably with allergy, has been recently comprehensively reviewed.[36] Several studies provide support that atopic disease might be associated with the composition of the gut microbiota. One of the first reports showed that there is a difference in *Bifidobacterium* microbiota in allergic and healthy breast-fed infants (aged 2-7 months).[37] Allergic infants harboured adult-like *Bifidobacterium* species with a predominance of *B. adolescentis*, whereas healthy infants had a more typical infant microbiota with mainly *B. bifidum* and to a lesser extent *B. infantis* and *B. breve*. Moreover, differences in the neonatal gut microbiota in infants with and without development of atopy was described.[38] In infants at high risk for atopic disease they found that atopic subjects had more faecal clostridia and (a trend for) less faecal bifidobacteria than non-atopic subjects. Additionally, the presence of *E. coli* was linked to a higher risk of developing eczema, whereas colonization with *C. difficile* was associated with a higher risk of eczema, recurrent wheeze and allergic sensitisation.[39] A detailed investigation of *Bifidobacterium* spp. was not carried out in this latter investigation. The early microbial diversity might be associated with atopic eczema as this parameter was lower in both Swedish and British infants with atopic eczema than in infants without eczema.[40] Moreover, infants receiving a formula with a mixture of GOS/lcFOS and pectin-derived acidic oligosaccharides (pAOS) showed a bifidogenic shift and concomitantly experienced a lower risk for atopic eczema and upper respiratory tract infections which seemed to persist for 5 years for the risk of atopy[41] and for 2 years for the risk of infections.[42] A recent analysis of a large cohort of infants in the Canadian Healthy Infant Longitudinal Development (CHILD) Study, showed that infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life, and this was supported by microbiota transfer experiments to germfree mice.[43]

Thus, ever increasing data points to associations between the infant's gut microbiota composition and health later in life are published. It is too early to make any general statements about these associations, i.e. whether they are also causal relationships. Nonetheless, the findings enhance the potential for gut microbe-based therapies, potentially with prebiotics and probiotics, to prevent the development of certain diseases from childhood.

FRUCTANS, BIFIDOGENIC EFFECTS AND BOWEL HABIT IN CHILDREN

The bifidogenic effect of human milk was recognised in the early 1900s. Human milk contains about 10 g/L of human milk oligosaccharides (HMOs) which are the third most prevalent component. HMOs have a complex molecular structure with several different sugars namely galactose, glucose, fucose, N-acetylglucosamine and sialic acid.[44] A variety of functions is attributed to them as well as the bifidogenic effect. Obviously the structures of the HMOs and inulin are different, nevertheless, some of the properties of the HMO can be approached by inulin ingredients, especially the bifidogenic effect, and possibly also some of the associated health benefits.

Inulin is a normal constituent in a regular Western diet with wheat and onions as the main sources and daily intakes may range from 2 to 8 g/day (average 5 g/day) in the US.[1,45] Data specific for the South East Asian region on inulin consumption are hardly available. The high banana consumption in many Asian countries may contribute to the daily inulin intake more than in a typical Western diet as banana contains about 0.2% inulin.

There is a significant body of literature showing that inulin is well-tolerated by infants and children based on numerous interventions some of which are described in Table 2. This is logical as fructans and other fermentable fibers likely comprised an important part of the diet of humans tens of thousands of years ago.[46] Infant formulae supplemented with inulin, especially infant and follow-on formulae, as well as growing up milk for children aged 6 months and older, are used on a large scale in Asia much more so than in Europe or the USA.

Tolerance

A significant body of literature confirms that inulin is well-tolerated, also by very young children and infants.[47,48] In adults the only reported effects of consuming up to 20 g doses per day are (minor increases in) bloating and flatulence.[49] There is less data on tolerance for children, but daily doses of inulin of about 1.5 g seem to be well tolerated in infants[47,48] and 5 g/d in children aged 7-8 years.[50] Moreover, oligofructose in dosages of 6-12 g/d apparently does not lead to too many unwanted side effects in children aged 6-12 years,[51] and there were no adverse effects with 1 - 5 g/d of native inulin in children aged 6 m - 10 y.[52] Also higher dosages of oligofructose, 8 g/d in children aged 7-11 years or 15 g/d in children aged 12-18 years, did not lead to unwanted side effects.[53]

In this context it should also be mentioned that in the generally lactase non-persistent Asian population a mod-

Table 1. Description of different types of fructans and GOS

Type	Description
Native inulin	Inulin as extracted from chicory with †DP 2-60 and average DP 9-12
OF	Oligofructose with DP 2 to 8 (average DP 4), is produced from native inulin by partial enzymatic hydrolysis
Lc-inulin	Long chain inulin with DP 10-60 (average DP above 21), is produced from native inulin from chicory; also known as lcFOS or lc-oligofructose, in combination with scGOS
(sc)FOS	(short chain) Fructo-oligosaccharides with DP 2 to 5 (hence short-chain), are produced enzymatically from sucrose (average DP 4)
(sc)GOS	(short chain) Galacto-oligosaccharides with DP 2 to 5, are produced enzymatically from lactose (average DP 5)

†DP: degree of polymerization.
The structure of the inulins from chicory root is presented in Figure 1.

est dairy consumption being an important vehicle for formulae should be encouraged.[54,55]

Bifidogenic effect

An overview of the effects of inulin-type fructans alone, or in combinations with GOS, on the composition of the microbiota in pre-term, new-born infants, and toddlers with an age of about 4 months and older, is presented in Table 2. Most of the studies are randomised controlled trials (RCT), either parallel (P) or cross-over (CO). Bifidogenic effects have been consistently shown, sometimes accompanied with increases in lactobacilli, initially with plate counts and later with molecular techniques such as fluorescent in situ hybridization (FISH) and quantitative PCR (qPCR). GOS alone also shows bifidogenic effects in infants and toddlers.[56,57] A bifidogenic effect in preterm infants for oligofructose or a scGOS/lcFOS mixture has also been observed.[58,59]

The prebiotics are effective in solid foods,[60] follow-on formulae,[48,61] other milk-based drinks[62] or in enteral formula.[63] Bifidogenic effects in these infants and children occur with a few grams per day. It is not known which combination may give the highest bifidogenic effect; different daily dosages were used and likely the magnitude of the effect is more dependent on the starting value of faecal bifidobacteria,[47,48] an effect that has also been found in adults.[8] In most of the studies only the concentration of prebiotic in the final formula is given, and since the intake is unknown, the daily intake of prebiotics cannot be accurately derived; for example, with a level of 0.8 g/100 mL of formula, the intake may range from 2 to 5 g/d in newborns to 6 month infants, respectively.

New prebiotic mixtures are being developed for use in infant food to develop the best mixture in physiological terms besides generating patented mixtures for a unique commercial position. Many of the studies presented in Tables 2 and 3 were supported by industry. In a recent systematic review of the association between funding source and outcomes in trials of prebiotics added to infant formula, it was concluded that the source of funding did not effect the majority of outcomes in favour of the sponsors' products.[64] Most of the studies were carried out in Europe and there is little data available for infants from South East Asia. It is noteworthy that the addition of non-digestible oligosaccharides to infant or follow-on formulae has been deemed as non-essential by the European Food Safety Authority (EFSA) in its opinion on the essential composition of infant and follow-on formulae.[65] This is largely because there is currently no conclusive evidence that bifidobacteria in general, nor specific members of the bifidobacteria, have a certain quantifiable impact on infant health. However, paediatric experts support the application of prebiotics in infant formula in those circumstances where the mother cannot breast-feed.[66]

Bowel habit

Fructans alone and in combination with GOS improve bowel habit in infants by promoting softer stools and/or increasing stool frequency (Tables 2 and 3). Bifidogenic effects appear to be associated with the improved bowel habit, for example, consumption of native inulin in formula-fed infants gives both a bifidogenic effect as well as an increase in stool weight.[47] GOS on its own also positively effects bowel habit in formula-fed infants by increasing stool frequency in combination with a bifidogenic effect, an increase in faecal acetate and a decreased faecal pH.[56,57,67-69] A GOS/lcFOS/pAOS mixture was shown to soften stool consistency without affecting stool frequency in healthy infants.[70] In preterm infants this mixture led to a decreased stool viscosity and a trend for increased stool frequency.[71]

The numerous studies strongly suggest that fructans inulin, oligofructose and GOS show positive effects on bowel habit in infants. The mechanism for these effects may reside in the increased fermentation giving more biomass, more SCFA and gas that may stimulate peristalsis and softer stools. The addition of prebiotics makes the formula more comparable to breast milk not only in terms of the effect on microbiota composition but also for bowel habit. In a systematic review on treatments for chronic childhood constipation it was concluded that there is some evidence that fibre supplements are more effective than placebo;[72] however there were no well-designed randomised trials yet for evaluation of fructan prebiotics. It should also be mentioned that most of the studies were carried out in Europe (Table 1) and that little data are available for infants from South East Asia.

FRUCTANS: INFECTION AND IMMUNITY

The effect of prebiotics on the performance of the immune system can be investigated by various approaches such as impact on immune parameters[73] and response to vaccinations, as well as intestinal or systemic infections and atopy, as described below. The immunological properties of inulin-type fructans has been comprehensively reviewed.[74] The focus below is on human infant studies.

Table 2. Effect of fructans on gut microbiota and bowel habit from preterm to about 4 months infants

Reference	Population	Age	Dosage	Study design, duration, subject number	Bifidobacteria	Other gut microbes	Bowel habit and other markers
Studies with native inulin							
47	Korea	13 wk	1.5 g/d	RCT, B, CO; 21d; n=14	plate counts ↑*	Lactobacilli ↑*	Stools softer, faeces amount increased. Frequency of defecation not affected
170	Serbia	8-9 wk	4 g/L†	B, P; 28d; n=21	plate counts ↑*	Lactobacilli ↑	Stool frequency, consistency & side effects were similar to breast-fed
Studies lc-inulin and OF mix from chicory roots							
171	Belgium	Term infants	4-8 g/L	RCT, DB, P; 28d; n=110	FISH ↑* (for 8 g/L)	ND	Stool frequency decreased but less than for breast-fed, softer stools
172	Spain	40 d	8 g/L	RCT, DB, P; 4 mo; n=128	plate counts of frozen -80 samples ↑ (trend)	With I/OF more like breast-fed	Softer stools; higher frequency compared to control (minus prebiotic)
Studies with OF							
58	Greece	Preterm, 0-2 wk	4 g/L	RCT, DB, P; 14d; n=56	plate counts ↑*	*Bacteroides* ↑, *E.coli* & enterococci ↓*	Stool frequency increased; mean weight gain higher in placebo
173	US	Healthy term	1.5 or 3 g/L	RCT, B, P; 5 wk; n=72	plate counts ↑ after 7d with 1.5 g/L	Bacteroides, enterococci and clostridia ↑*	Softer stools, especially with 3 g/L
174	US	Healthy term	1.5 or 3 g/L	RCT, B, P; 12 wk; n=212	NS	NS	Anthropometric measurements - normal growth; 3 g/L less constipation than control & 1.5 g/L
175	Philippines	Healthy term 7-14 d	3 or 5 g/L	RCT, DB, P; 8 wk; n=300	FISH ↑* at 3 & 5 g/L	Bifidobacteria level more like breast-fed	5 g/L OF more like HM-fed infant stool consistency
176	US	Healthy term	3 g/L	RCT, DB, P; 8 wk; n=95	FISH ↑*		Softer stools versus formula without OF, no effect on frequency
Studies with scFOS							
177	French	Healthy term 4d	4 g/L	RCT, DB, P; 4 mo; n=61	qPCR ↑*	ND	Specific IgA higher with scFOS (4 g/L) than placebo (maltodextrin) formula; Somatic growth similar between groups
Studies with scGOS/lc-inulin (9:1)							
178	Italy	Preterm	10 g/L	RCT, DB, P; 4 wk; n=15	plate counts ↑*	ND	Stool frequency lower in control than in GOS/FOS or human milk groups. Stool consistency harder in control, but fairly stable in GOS/FOS & human milk groups

RCT: randomised controlled trial; (D)B: (double) blind; CO: cross over; P: parallel groups; ND: no data; NS: non-significant; FISH-FCM: fluorescent in situ hydridisation-flow cytometry; qPCR: quantitative polymerase chain reaction; pAOS: pectin derived acidic oligosaccharides.

†Mixture of native inulin and short chain inulin (Lugonja, pers. comm.).

*↑/↓= significant increase/decrease (p<0.05).

Table 2. Effect of fructans on gut microbiota and bowel habit from preterm to about 4 months infants (cont.)

Reference	Population	Age	Dosage	Study design, duration, subject number	Bifidobacteria	Other gut microbes	Bowel habit and other markers
Studies with scGOS/lc-inulin (9:1)							
179; 180	Italy	Healthy term	4 or 8 g/L	RCT, DB, P; 1 mo; n=90	↑*	Lactobacilli ↑*	Softer stool with increasing dose from 0.4 to 0.8 g/L; stool frequency different for 0.8 g/L
181	Germany	Healthy term	8 g/L	RCT, DB, P; 3 mo; n=102	FISH ↑*		GOS: FOS formula stools were softer
59; 182; 183	Germany	Healthy term	8 g/L	RCT, DB, P; 6 wk; n=68	FISH; ↑* 5 nuclease assays for bifidobacteria	Lactobacilli ↑*	ND
184	Germany	Preterm	1 g/ 100 mL	RCT, DP, P; 14 d; n=20	ND	ND	Reduced stool viscosity, increased intestinal transit
185	Algeria	Healthy term 7w at start	6 g/L + pAOS	RCT, DB, P; variable length; n=82	FISH-FCM ↑*	Bacteroides ↓*	No statistically significant differences between groups stool characteristics. Child growth similar.
186	Greece	Healthy term	8 g/L	RCT, DB, P; 6 wk; n=140	FISH ↑ (NS)	Clostridia ↓*	Increased stool frequency, softer consistency with prebiotics. No effect on growth

RCT: randomised controlled trial; (D)B: (double) blind; CO: cross over; P: parallel groups; ND: no data; NS: non-significant; FISH-FCM: fluorescent in situ hydridisation-flow cytometry; qPCR: quantitative polymerase chain reaction; pAOS: pectin derived acidic oligosaccharides.

†Mixture of native inulin and short chain inulin (Lugonja, pers. comm.).

*↑/↓= significant increase/decrease (p<0.05).

Table 3. Effect of inulin fructans on gut microbiota and bowel habit in infants from 5 months of age to children

Reference	Population	Age	Dosage	Study design, duration, subject no.	*Bifidobacterium*	Other gut bacteria	Bowel habit and other markers
Studies with native inulin							
48; 61	Malaysia	5-12 mo	0.75-1.25 g/d	B, P; 35 d; n=36	Plate counts ↑* at 1.25 g/d	Clostridia ↓* at all doses	Lower faecal pH, no effect on frequency or consistency
50	Vietnam	7-8 y	5 g/d	RCT, DB, P; 3 mo; n=454	qPCR ↑‡		Bowel habit†
Studies with 70:30 inulin:OF mix from chicory roots							
62	Chile	1-2 y (after antibiotic treatment)	2.3 g/d‡	RCT, DB, P; 21d; n=140	FISH ↑*	No effects on Bacteroides, some clostridia, *E. coli*	No effect on bowel habit (frequency, consistency)
63	China	1-12 y (cancer patients)	0.8 g/d‡	RCT, DB, P; 30 d; n=67	Plate counts ↑ NS	Lactobacilli ↑*, no effect on Enterobacteria	No effect on stool consistency
Studies with OF							
187	France	7-19 mo	2 g/d	RCT, DB, P; 21d; n=20	Plate counts ↑NS	Clostridia ↓*	Less flatulence with OF
Studies with scGOS/lc-inulin (9:1)							
60	Netherlands	4-6 mo	4.5 g/d	RCT, DB, P; 42d; n=15	FISH↑*	Lactobacilli ↑*	No significant changes in bowel habit
Study with mix lc-inulin/GOS/soy fibre/resistant starch (1:1:0.5:0.1)							
188; 189	Netherlands	1-13 y (constipated children)	8 g/d	RCT, DB, P; 8wk; n=97	FISH↑*	Lactobacilli ↑*	No effect on bowel habit (frequency) compared to lactulose. Stool consistency softer with lactulose

RCT: randomised controlled trial; (D)B: (double) blind; P: parallel groups; NS: non-significant; FISH: fluorescent in situ hydridisation; qPCR: quantitative polymerase chain reaction.
†No data
‡Bifidobacteria increase higher with inulin fortified milk than control milk
*↑/↓= significant increase/decrease ($p<0.05$)

Table 4. Effects of different fructans on immune parameters in infants

Reference	Fructans used	Study design, duration, subject number	Study group (age)	Outcome
Inulin or OF				
75	OF/inulin (7:3, 0.2 g/kg BW/d)	RCT, DB, 10 wk, n=55	Infants (7-9 mo)	Higher blood IgG levels after measles vaccination
76	OF (0.7 g/d)	RCT, B, 6 mo, n=282	Infants (6-12 mo)	No effect on antibody response after vaccination with *H. influenzae* type B vaccine
scFOS				
177	scFOS (4 g/L)	RCT, DB, P, 4 mo n=61	Infants (4d)	Trend for higher antipolio IgA with sc FOS
Mixtures with other prebiotics				
78	scGOS/lc-FOS (9:1, 0.6 g/dL formula)	RCT, DB, parallel, 32 wk, n=57	Term Infants (3 d)	Higher faecal IgA (no effect of probiotic *B. animalis*)
79	scGOS/lc-FOS (9:1, 0.6 g/dL formula)	RCT, DB, parallel, 26 wk, n=187	Term infants	Higher faecal sIgA levels
190	scGOS/lc-FOS (9:1, 0.6 g/dL formula)	RCT, DB, parallel, 26 wk, n=187 (same study as 5)	Term infants	No changes compared to breast-fed infants in serum IgG, lymphocyte sub-populations and cytokines
77	scGOS/lc-FOS (9:1; 6.8 g/L) + pAOS (1.2 g/L)	RCT, DB, parallel, n=1130	Healthy term infants	No effect on vaccination response to *H. influenza* and tetanus immunization
191	scGOS/lc-FOS (9:1; 6.8 g/L) + pAOS (1.2 g/L)	RCT, DB, P, 2 wk, n=104	Acute diarrhea patients (6-24 m)	Decrease in TNF-α with prebiotic, decrease in stool numbers and increase in stool consistency in both groups

BW: body weight; RCT: randomised controlled trial; (D)B: (double) blind; P: parallel groups; pAOS: pectin derived acidic oligosaccharides.

Effects on immune parameters

The effect of prebiotics on the performance of the immune system has been investigated by measuring the antibody response to vaccination, as well as the response of certain immune markers in infants with atopic inflammatory conditions, which has resulted in promising but inconsistent outcomes (Table 4). For example, an increased antibody response after measles vaccination was observed in healthy infants for an inulin-oligofructose mix,[75] while another study with oligofructose revealed no effect after an influenza vaccination.[76] Another series of studies with GOS/lcFOS in a ratio of 9:1 (Table 4) also showed no effect on vaccination response to *H. influenza* and tetanus immunization,[77] while two studies showed higher faecal secretory IgA (sIgA) levels, a high score marker of gastrointestinal defence; compared with standard formula-fed infants.[78,79]

Effects on infections

An overview of studies on the effect of inulin or oligofructose alone or in combination with other fibres on the outcome of infections or allergy in preterm and term infants and young children is presented in Table 5.

Preterm infants are prone to NEC and LOS, both diseases whereby the immature intestinal tract and gut microbiota are believed to play a role. Meta-analyses showed that supplementation with prebiotics (GOS, oligofructose, GOS/lcFOS) are safe for use in preterm infants, and resulted in significantly higher bifidobacteria or beneficial microbes.[80,81] There was no decrease in the incidence of NEC, although the number of trials and children were too low to draw a clear conclusion on the effects of the prebiotics on the risk of developing NEC or LOS. In the recent ProPre-Save study, the efficacy of probiotic *B. lactis*, inulin and the synbiotic of both was tested on the prevention of NEC in very low birth weight infants.[82] The prebiotic alone was not effective, whereas both the probiotic and the synbiotic decreased NEC; the intake of inulin was very low in this study (0.9 g/d). Prebiotic, probiotic and synbiotic treatments all led to a lower mortality rate and to shorter stays in intensive care.

In term infants, while there were some promising outcomes for infections such as diarrhoeal, gastroenteritis and respiratory infections, studies with oligofructose or inulin alone on diarrhoea were inconsistent in Western infants and children (Table 5). Studies performed in developing countries such as Indonesia, India, as presented in Table 6 below suggested the prebiotic FOS had positive effects on diarrhoea. Regrettably it is not known whether FOS or oligofructose was used in one of these studies.[83] In a study with native inulin at dosages adapted to body weight (BW) in children aged 6 months to 12 years, no effect was found on the incidence of antibiotic associated diarrhoea (AAD), but the study was in fact underpowered due to the too low (and very slow) inclusion rate.[52] Several studies using a 9:1 scGOS/lcFOS mixture showed significantly (or a trend towards) less infectious periods, especially for respiratory infections and this effect seemed to persist over years (Table 6).[41,42,84-86]

Effects on atopy and allergy

There are so far no publications with an inulin-based prebiotic alone for atopy. Consumption of 1-2 g/d kestose (scFOS with DP3) significantly reduced the severity of atopic dermatitis although surprisingly, there was no simultaneous bifidogenic effect.[87] The prebiotic 9:1

Table 5. Effects of fructans and/or other prebiotics on infections and atopy in infants and children

Reference	Fructans used	Study design, duration, subject number	Target group/ condition	Outcome
Inulin or OF on infections				
75	OF (1.1 g/d)	RCT, DB, n=123	Infants 4-24 mo/ diarrhoea prevalence	No effect on diarrhoea incidence, less occurrence of fever
187	OF (2 g/d)	RCT, DB, n=20; 21d	Infants 7-19 mo/ diarrhoea prevalence	Less episodes with fever or diarrhoea, lower number of infectious disease requiring antibiotic treatment
52	Inulin (1-5 g/d, depending on BW)	RCT, DB, P, n=100	Children aged 6 m -10 y treated with antibiotic	No effect on incidence of AAD
82	Inulin (0.9 g/d)	RCT, DB, P	Very low birth weight infants	No effect on NEC of inulin, lower mortality and shorter stay in IC with inulin
Mixtures of fructans and other prebiotics on infections				
120	Mix in ORS†	RCT, DB, P, n=144; treatment until diarrhoea stopped	Boys aged 3-36 mo with oral hydration therapy/ acute diarrhoea	No effect on duration of diarrhoea or of hospital stay
84	scGOS/lcFOS (9:1, 8 g/L formula)	RCT, DB, 6 m, parallel, n=206	Infants at risk for atopy (>2 wk)/ Infections	Less incidence of respiratory infections, reduction in number of infectious periods
41	Not applicable	Follow up from previous study after 2 yrs, n=152	Follow up from previous study/ Infections	Effects described above (ref. 84) last beyond intervention period
42	Not applicable	Follow up from previous study after 5 yrs, n=89	Follow up from previous study/ Infections	Effects described above (ref. 84) last beyond intervention period for atopic dermatitis, not for recurrent wheezing
85	scGOS/lcFOS (9:1, 4 g/L)	Randomised open trial, parallel, 12 mo, n=342	Infants (average age 53 d)/ Infections	Lower incidence of acute gastro-enteritis, less multiple antibiotic treatments per year, trend for less respiratory tract infections
89	scGOS/lcFOS (9:1, 6.8 g/L) + pAOS (1.2 g/L)	RCT, DB, 1 y, parallel, n=830	Healthy term infants	No effect on number of fever episodes
86	scGOS/lcFOS + pAOS enteral	RCT, n=113, 4 wk	Preterm infants	Trend towards lower incidence of serious infectious morbidity
93	scGOS/lcFOS (9:1, 6.8 g/L) + pAOS (1.2 g/L)	RCT, DB, n=113, 27 d	Preterm infants	No effect on incidence of allergic and infectious diseases
Mixtures of fructans and other prebiotics on atopy and inflammation				
192	scGOS/lcFOS (9:1, 0.8 g/100 mL formula)	RCT, DB, parallel, 6 mo, n=259	Infants at risk for atopy	Less development of dermatitis; no change in severity
87	Kestose (DP 3 sc-FOS) 2 g/d	RCT, DB, parallel,12 w, n=29	Infants with atopic dermatitis	Lower severity score in kestose group
91	scGOS/lcFOS (9:1, 0.8 g/dL formula)	RCT, DB, parallel, 6 mo, n=84 (same study as with 1)	Infants at risk for allergy	Reduction of total IgE, cow's milk allergen specific IgG1, no effect on vaccination specific Ig levels
90	scGOS/lcFOS (9:1, 0.8 g/100 mL formula)	RCT, DB, parallel, 6 mo, n=74 (same study as with 1)	Infants at risk for allergy	Reduction of Ig free light chain levels (relevant for acute allergic skin response)
92	scGOS/lcFOS (9:1) + pAOS	RCT, DB, parallel, 1 yr, n =1130	Healthy term infants with low risk for atopy	Rate of atopic dermatitis lower in prebiotic group

RCT: randomised controlled trial; (D)B: (double) blind; P: parallel groups; BW: body weight; AAD: antibiotic associated diarrhoea; pAOS:pectin derived acidic oligosaccharides.
†Mix of soy polysaccharide, cellulose, gum arabic, OF, inulin, resistant starch (25:9:19:18.5:21.5:7).

scGOS/lcFOS mixture lead to an improvement in atopy.[88,89] The recent finding that administration of the scGOS/lcFOS mixture can reduce Ig free light chain levels in infants at risk for allergy deserves further study.[90] Although GOS had a bifidogenic effect in infants at a consumption of 4 g/L in formula, there was no effect on infections or allergic manifestations.[57] In addition, in infants at risk for allergy who had the scGOS/lcFOS mixture, a reduction of total IgE and cow milk allergen specific IgG was found.[91] The results of a European multicentre study involving 1,130 infants and using a specific mixture of scGOS, lc-inulin and pAOS showed a lower

incidence of atopic dermatitis in children with a low risk for atopy,[92] but no effect on the occurrence of fever periods.[89] So far just one study reported on the effect of scGOS/lc-inulin/pAOS in preterm infants on the incidence of atopic dermatitis, bronchial hyper-reactivity and infections of the lower or upper respiratory tract or on gastrointestinal infections and this mixture appeared to have no effect.[93] A Cochrane review concluded that there is some evidence that a prebiotic supplement added to infant foods may prevent eczema but it was unclear whether it may have an effect on other allergic diseases including asthma.[94] Thus further research is required before routine application of prebiotics can be recommended for this purpose.

FRUCTANS IN MALNOURISHED INFANTS AND CHILDREN

Malnutrition includes both under- and over-nutrition, but this section concerns under-nutrition, which includes delayed growth of children as well as signs and symptoms of vitamin-, mineral-, essential fatty acid-, and protein-deficiencies. Childhood malnutrition is a global health problem.[95]

Microbiome in malnourished children

There are increasing indications that the gut microbiota may contribute to this malnutrition disorder.[96] Malnourishment affected the composition of the faecal microbiota negatively as demonstrated in a metagenomic study of malnourished and healthy children from Bangladesh.[97] In a large cohort of Malawian twin pairs (0-3 yr) with severe acute malnutrition (SAM), metagenomics of the gut microbiomes showed a transient improvement upon treatment with therapeutic food but this regressed upon termination.[98] Transplantation of faecal communities from several discordant (malnourished and healthy) twin pairs into gnotobiotic mice demonstrated that the Malawian diet plus SAM microbiome combination culminated in marked weight loss in the mice, accompanied by other metabolic perturbations, again, only transiently improved with therapeutic food. Notably there were prominent increases in certain bifidobacteria, lactobacilli, ruminococci and *Faecalibacterium prausnitzii*. These findings implicate the gut microbiome as a causal factor in this form of SAM. Furthermore, the effects of two widely applied nutritional interventions for malnutrition was investigated on the gut microbiota in Bangladeshi children (2 yr).[99] Despite improvement of the nutritional status, the gut microbiota was only partially and transiently restored by both of these therapeutic treatments. Thus the gut microbiota failed to develop into a representative mature microbiota as in healthy young children. Therapeutic food interventions have reduced mortality in children with SAM, but incomplete restoration of healthy growth remains a problem. The authors proposed that more prolonged nutritional interventions with existing/ new foods may be needed to ensure a mature gut microbiota and improve clinical outcomes. More recently, new analysis of the Malawi and Bangladesh twin cohorts microbiota linked reduced microbiota diversity with stunting and especially increased relative abundance of *Acidaminococcus* sp. was associated with future linear growth deficits.[100] The faecal microbiota of 992 children (0-59 months) with diarrhoea and diarrhoea-free controls from low-income countries in West and East Africa and Southeast Asia were analysed using high throughput 16S rRNA sequencing.[101] Known pathogens and others such as *Escherichia / Shigella, Granulicatella* species and *Streptococcus mitis/S. pneumoniae*, were associated with the diarrhoea while a great diversity of obligate anaerobic lineages correlated with healthy status children. The children with severe illness tended to have a poorer diversity of microbiota. Other studies showed that multiple pathogens are found in asymptomatic children and there appears to be synergistic effects between pathogens.[102,103] Quantitative microbial detection will be essential to understand the microbiota and diarrhoeal pathogens in developing countries settings.

Impact of prebiotics on mineral absorption

Inulin-type fructans have been demonstrated to stimulate mineral absorption; especially calcium and magnesium absorption from the colon may be enhanced in adolescents and postmenopausal women.[104] A significant increase in apparent absorption, apparent retention and net retention of iron was seen in 7-8 month old infants supplemented with 1 g/d native inulin.[61] Infants supplemented with 0.75, 1 and 1.25 g/day inulin showed a significant increase in magnesium retention, whereas a significant increase in absorption and retention of zinc was seen in infants supplemented with only 0.75 g/day inulin. No significant improvement in calcium absorption or retention was observed with all dosages of inulin studied. The data do indicate a lower faecal pH and a trend for a raised faecal SCFA level at all inulin intake rates. Both are markers for improved fermentation in the colon.[48] An improvement of calcium absorption in formula-fed infants with a polydextrose/GOS (1:1) mixture was not detected.[105] Thus the type of fibre seems critical.

An *in vitro* study of dairy infant formulas supplemented with a variety of soluble dietary fibres or modified starches showed that the type of fibre can affect calcium, iron and zinc availability.[106] Notably calcium availability was increased by 11% with inulin supplementation of the formula with the in vitro technique which predicts the release of micronutrients from meals. Finally studies with young rats offer supportive evidence for improved calcium absorption; inulin, oligofructose, GOS or GOS/lcFOS increased calcium absorption and/or bone mineralization in growing rats.[107,108] A mixture of GOS/lcFOS in growing rats increased bone mineralization due to an increased calcium absorption,[109] and similar data were reported for GOS alone in growing rats.[108] The latter results were further substantiated in a clinical study, showing increased calcium absorption in young girls (10-13 years) upon supplementation with GOS.[110] There have been studies in growing anaemic rodents, using prebiotics long chain inulin and oligofructose which appear to differentially affect the expression of intestinal proteins involved in intestinal iron uptake, but outcomes for improved iron status are inconclusive.[111] Iron status may be measured with haemoglobin concentration, and serum iron and ferritin/ transferrin in children.

Table 6. Impact of ORS with prebiotics and select carbohydrates on diarrhoeal outcomes

Reference	Prebiotic/ carbohydrate	Population, design, subject number, age duration	outcome
Fructans			
193	scFOS (probably) 2.5-5 g/d	Indonesia; n=119; 1-14 yr	Duration of diarrhoea shorter
76	OF 0.55 g/d	Peru; n=282; 6 mo	No effect on diarrhoea prevalence
83	FOS (type not known) 3.2 or 4%	Indonesia: n=192; 6 mo old; 6 mo	3.2%, fewer episodes of diarrhoea; 3.2 & 4% reduced for diarrhoeal episodes of <2 days
Synbiotics			
113	Synbiotic 2000 Forte: *Pediococcus pentoseceus, Lc. mesenteroides, L. paracasei* and *L. plantarum*, inulin, oat bran, pectin, resistant starch (2.5 g each)	Malawi; n=795; 5-168 mo; 33 days	No improvement in pre-specified nutritional and clinical outcomes
114	Synbiotic: *B. longum, L. acidophilus*, inulin (30%), GOS (70%), long chain polyunsaturated fatty acids	Indonesia; n=393 at 12 mo; 1 yr	Increased weight gain; no differences in vaccines response, stool characteristics and neurodevelopmental measures
115	Synbiotic: GOS (2.4 g/d), *B. lactis*	India; n=312; 1-3 yr; 1 yr	Significant reduction of dysentery, respiratory morbidity and febrile illness; no impact on diarrhoea
ORS			
120	ORS NDP mix: soy polysaccharide, cellulose, gum arabic, OF, inulin, resistant starch (25:9:19:18.5:21.5:7)	Egypt, Greece, Israel, Italy, Poland, Portugal, Slovenia, Holland; n=144; 1-36 mo males	No significant difference between duration of mild diarrhea, stool output, or of hospital stay
118	Amylase resistant starch-ORS	India; n=178; 6 mo-3 yr	Significantly shortened duration of diarrhea
121	FOS 0.35 g/L; xylo-oligos 0.35 gL; zinc 1 mmol/L in ORS	Italy; SB, C; n=59; 3-36 mo	Reduced diarrhea duration in children; fewer drugs needed

ORS: oral rehydration solution; NDP: non-digestible polysaccharide.

It is noteworthy that measurements of mineral status can be challenging in children, especially in developing countries due to lack of adequate facilities (such as for stable isotopes or atomic absorption spectrometry for zinc). For example, the long term effect of calcium is typically measured as changes in BMD (bone mineral density) by DXA (Dual Energy X-ray absorptiometry) but this is not possible in young children due to repeated exposure to radiation. Zinc status is difficult to measure and only long term effect/status can be measured in hair. To conclude, data from a limited number of human studies and supporting data from animal and in vitro trials suggest that inulin and oligofructose as well as other prebiotics may improve absorption of minerals such as calcium, magnesium, zinc or iron in infants. Nevertheless, quality interventions are required to substantiate these effects.

Potential for fructans in recovery from malnourishment in developing countries

So far there is a paucity of studies on prebiotics, probiotic and synbiotics in treatment of malnourishment to date and there are no comparative studies with prebiotics alone so the effect cannot be established.[112] Table 6 presents the studies for synbiotics as functional food or in milk for infants and children in developing countries. In the PRONUT study (pro- and prebiotics for severe acute malnutrition), the Synbiotic2000 Forte functional food gave no improvement in the treatment of SAM over the control therapeutic food for malnutrition in a HIV-prevalent setting after 33 days.[113] The authors recommended to further study SAM outpatients and moderately malnourished children as well as perform gut metagenomics to gain understanding of the outcomes of such studies. The other two synbiotic studies of a year long, showed beneficial effects such as a clear weight gain for Indonesian children which was the primary parameter,[114] or reduction in episodes of dysentery and respiratory tract infections, though no impact on diarrhoea in Indian children.[115]

Acute diarrhoea is one of the principal causes of morbidity and mortality among children in low-income countries and the second leading cause of death among children less 5 years old globally. Oral Rehydration Solution (ORS) developed in the 1970s has had a massive impact in reducing mortality caused by diarrhoea by helping replace fluids and prevent further dehydration. There are limited studies on the effectiveness of prebiotics in ORS (Table 6). Most ORS is in the form of a sugar (glucose)-salt solution but more recently other glucose-polymer ingredients have been investigated and the limited evidence suggests an improved effect.[116] Other observations suggest that increasing SCFAs like butyrate in the colon may function similarly to glucose in the small intestine to enhance fluid and sodium absorption during acute diarrheal illness.[117] Interventions performed with high amylase maize starch-ORS gave promising results supporting this hypothesis.[118] The inclusion of resistant starch in ORS of severely malnourished children with cholera in Bangladesh led to an increase in diversity as compared to

glucose rehydration alone, supporting that the inclusion of fermentable fibres or prebiotics might be advantageous.[119] However, ORS comprising a mixture of nondigestible carbohydrates including inulin and oligofructose amongst others was ineffective in treating boys with mild non-cholera diarrhoea.[120] A hypotonic ORS containing zinc with prebiotics improved resolution of acute diarrhoea in Italian young children, and fewer adjunctive drugs were required for the treatment of diarrhoea.[121] Zinc alone was moderately efficacious in reducing the severity of acute diarrhoea (not hospitalized) in 6-35 month-old urban Indian children[122] and apparently zinc enhances the absorption of water and electrolytes across the intestinal mucosa.[117] Thus, the individual benefit of prebiotic fructans alone in ORS remains to be studied but its ability to produce SCFAs suggest that it might play a role in recovery from diarrhoeal disease.

In conclusion, quantitative metagenomic studies of gut microbiota especially in the developing world may lead to a better understanding of the role of the microbiota in pathogenesis and treatment of malnutrition as well as under-nourished children with diarrhoea. So far, there is no data available on the use of specifically prebiotics in malnutrition, but prebiotics as part of synbiotics may improve health status in children living in conditions unfavourable for proper growth. A workshop held by the International Scientific Association for Probiotics and Prebiotics (ISAPP), has provided useful recommendations when considering interventions with pre- or probiotics in malnourished subjects.[123] In addition, a comprehensive systematic review of the evidence assessing interventions, programmes and/or guidelines to treat infants and children under 5 years of age who have SAM also provides valuable recommendations.[124]

FRUCTANS DURING PREGNANCY AND METABOLIC PROGRAMMING

The central role of nutrition in pregnancy for health and well being of the pregnant mother, as well as that of the offspring is generally established. Increasing knowledge suggests that the maternal health and diet can have profound effect on the offspring's health; foetal metabolic programming will be discussed below.

A comprehensive analysis of the gut microbiome in pregnant women revealed a dramatic remodelling over the course of pregnancy.[125] Remarkably, by the third trimester, the structure and composition resembles more a disease-associated dysbiosis as such changes are related to increased risk of metabolic syndrome and weight gain. However, there appeared to be no association with the gut microbiota of the infants. It is speculated that these changes may be beneficial in pregnancy, as they promote energy storage in fat tissue and provide for the growth of the foetus, and potentially other benefits.[125]

A recent exciting development is the role of gut microbiota as an epigenetic regulator. Epigenetics comprise genomic modifications that occur due to environmental factors and do not change the nucleotide sequence. Sequencing of DNA methylomes of pregnant women revealed an association between bacterial predominance and epigenetic profiles.[126] The gut microbiota profiles, with either *Firmicutes* or *Bacteroidetes* as a dominant group, correlated with differential methylation status of gene promoters functionally associated with cardiovascular diseases and also linked to lipid metabolism and obesity, and these findings are consistent with previous studies linking higher levels of *Firmicutes* to obesity. This field is in its infancy but microbiota modification based on diet and prebiotic supplementation before and during pregnancy may offer new directions for improved health in offspring.

Potential of inulin during pregnancy

Although studies for the effect of inulin and oligofructose on the gut microbiome of pregnant women are lacking, it is likely that similar to the general population oligofructose and inulin will elicit a bifidogenic response. This assumption is supported by a study whereby 9 g/d of GOS/lcFOS significantly increased the faecal bifidobacteria level in pregnant women.[127] This study also reported that no changes occurred in a large number of immunological parameters in cord blood in pregnant women when consuming this mixture.

Constipation is common in pregnant women, with reported prevalence of 9-39%, and can develop or increase in severity during pregnancy.[128] The proportion of women requiring treatment with laxatives is much lower.[129] Thus, the classical fibre functionality of inulin of improved bowel frequency and softer stool consistency might be useful during pregnancy. Xylo-oligosaccharides have been shown to improve bowel habit in severely constipated pregnant women,[130] but so far there are no studies with inulin. Constipation in pregnancy is probably caused by rising progesterone levels (resulting in diminished smooth muscle contractility and inhibited gastrointestinal transit, are often implicated.[128,130] There is some evidence that low fluid and fibre intake may also be contributing factors.[128] First-line therapies include increased dietary fibre consumption and/or fibre supplementation, which strongly suggest a role for inulin or oligofructose in pregnancy.

In a study of overweight pregnant women, it was shown that folate status was lower, as well as biochemical parameters related to heart health (triglycerides, cholesterol) and iron status compared to women of normal weight; moreover a higher level of faecal bifidobacteria was associated with an improved folate status in the pregnant women.[131] Folate is important to prevent neural tube defects and is recommended for women planning to become pregnant. Bifidogenic prebiotics like inulin may improve folate status in women planning pregnancy. In addition, the potential of inulin-type fructans to improve or reduce serum triacylglycerols might be considered as an adjuvant therapy in obesity-related conditions and potentially in overweight pregnant women.[132] In an epidemiological study, fibre intake was associated with a reduced risk of preeclampsia, and the relation was evident for soluble and insoluble dietary fibre.[133] Gestational diabetes is considered to have become a global epidemic which can cause difficulties for both mother and unborn child with so far limited success in prevention.[134] Gestational diabetes can increase the risk of birth complications such as babies being large for their gestational age, macrosomia, greater neonatal adiposity, and potentially increasing the risk of metabolic disease later in life for the

Table 7. Effect of fructan-based prebiotics during pregnancy on offspring in experimental animals

Reference	Animals, type of fructan, dosage	Observed effects
Rodent trials		
157	Rats, scFOS, 20%	Moderate reduction of BW of offspring, no effect on pregnancy outcome or *in utero* development
158	Rats, OF/lc-inulin (1:1), 21.6% in feed	Changes in satiety hormones and genes for glucose transport and lipid metabolism in offspring
166	Rats, OF, 10% in feed	Lower BW in offspring, development of pro-inflammatory status in pups
163	Rats, OF/lc-inulin (1:1), 21.6% in feed	High prebiotic diet reduces susceptibility to obesity
162	Rats, OF, 10 % in feed	Lower BW, weight gain and length of offspring (but see (Lina, 2014) for remarks)
159	Mice, GOS/lcFOS (9:1), 4% in feed	Higher BW, more muscle mass, longer colon length in offspring
161	Mice, GOS/lcFOS (9:1), 3% in feed	More tolerogenic immune status in pregnant mice and in fetus
160	Mice, GOS/lcFOS (9:1), 4% in feed	Higher expression levels of tolerance immune biomarkers, no effect on allergy biomarkers
165	Mice, GOS/lcFOS/pAOS (9:1:2), 2% in feed	Lower sensitization to allergy in female offspring
Pig trials		
142	scFOS, 10 g/d (4 w during pregnancy and 4 w during lactation)	Maternal prebiotic supplementation modifies intestinal immune functions in piglets (higher production of sIgA in Peyer's patches) associated with increased colostral immunity (higher level of IgA)

BW: body weight; (s)IgA: (secretory)immunoglobulin; pAOS: pectin derived acidic oligosaccharides

offspring.[135] Fructans have recently been given a positive approval by EFSA for lowering glycaemic response or supporting a lower glycaemic index (GI).[136] Data specific for inulin in women with gestational diabetes are not available. A systematic review comprising eight studies suggested that in pregnancy complicated by gestational diabetes, a low-GI diet may reduce the need for insulin without adverse effects on pregnancy outcomes;[137] subsequent studies on women diagnosed with gestational diabetes supported both low GI and conventional high-fibre diets (and higher GI) reduced the numbers requiring insulin with no compromise of obstetric or foetal outcomes.[138,139] The only study with a prebiotic, i.e. with soybean oligosaccharides, showed alleviation of insulin resistance and reduced oxidative stress in this target group.[140]

To conclude, inulin fibre has the potential to improve the health and well-being of the pregnant woman, notably for bowel habit and for supporting a lower glycaemia index diet, nevertheless well conducted interventions with inulin are required to specifically evaluate these benefits.

Prebiotics during lactation

It is unknown whether prebiotics can have an effect on lactation and data are essentially lacking for women. An increase in interleukin-27 (IL-27) in breast milk with consumption of 8 g/d of scFOS together with higher protein concentrations has been observed.[141] It is not known what the physiological consequences are and whether IL-27 survives digestion in the baby's intestinal tract and thus can become active and affect the baby's immune system. In pig model studies is was shown that ingestion of scFOS led to higher levels of IgA and Transforming Growth Factor β1 in colostrum. Even though this effect disappeared 6 days after delivery it suggests the potential to modulate milk quality by prebiotic consumption.[142] Others have shown in a mouse model that maternal consumption of scFOS lowered the severity of skin inflammation of offspring.[143]

Supplementation of kestose (DP3 FOS) to pregnant and lactating mice led to an increase in total IgA levels in the milk and of the anti-*Bacteroides* IgA level which the authors proposed suggests a link between the gut and mammary gland immune system.[144] An effect on the neonatal immune system was not investigated. Maternal gut microbiota and milk composition could modify offspring microbiota and therefore disease susceptibility. Maternal high-protein or high-prebiotic-fibre diets were shown to modify maternal milk composition in terms of oligosaccharide content and modify gut microbiota in rat dams and their offspring.[145] It is noteworthy that a recent study showed that the oligosaccharide composition, notably high concentrations of fucosylated HMOs, in the breast milk, positively influenced survival of uninfected children born to HIV-infected mothers in Lusaka, Zambia.[146]

Potential of fructans in metabolic programming

Over the years, it has become well established that nutrition at the beginning of pregnancy is an important determinant of pregnancy outcome. Imbalances in nutrition during pregnancy, besides consequences for the mother, can have long-term effects on the health of the offspring into adulthood, a phenomenon called 'metabolic foetal programming'.[147,148] Long-term health and disease risks include later diabetes, blood pressure, or obesity, amongst others. For example, significant under-nutrition during pregnancy can program the foetus for increased weight gain as occurred in offspring of women during the Dutch famine of 1944-45.[149] Profound changes in gene expression are amongst the mechanisms implicated in metabolic programming.[150] Significant natural seasonal differences in the diet of rural Gambian women around the time of

conception and early pregnancy influenced numerous relevant plasma biomarkers which predict systemic epigenetic changes at human metastable epialleles.[151] Maternal over-nutrition may also lead to foetal over-nutrition and reduced energy expenditure.[152,153]

Notably gestational diabetes increases the risk of diabetes in subsequent generations, thereby setting up a cycle of "diabetes begetting diabetes".[134] Observational studies suggest that higher dietary fibre diets show beneficial effects for insulin sensitivity. Even low GI maternal (glycaemic index) diets with a high fibre content as an addition to a typical high fat and sucrose Western diet may reduce levels of insulin resistance,[154] and thus supplementation of low GI products to a Western high GI diet may be supportive. However, the fibre level should not be at the expense of other macro- and micro-nutrients which may give detrimental effects on health.

Besides epigenetics, other factors are implicated in the elevated risk for excess body weight in the child. A healthy birth weight mother, vaginal delivery and breast feeding may protect the child from later gaining excess weight, while overweight mother, caesarean delivery, formula, and unhealthy diet may increase risk for overweight in the offspring.[155]

Recent data suggests that the reduced diversity in Western diets, which are lower fibre and higher fat, may result in loss of taxa in the microbiota of the offspring which is not recoverable.[156] It is proposed that microbiota reprogramming via dietary fibres and prebiotics may be required if microbial losses become linked to disease.

Several rodent trials and a pig study (Table 7) have been performed with prebiotics scFOS, oligofructose, inulin, and/or GOS to study offspring development for example body weight and gain, and also immunity and atopy.[142,157-166] The outcomes were variable although often promising, however, the value of these is difficult to assess as exceptionally high levels of fructans/GOS were sometimes used. Thus prebiotics may have positive effects, but good quality data specific for fructans is scarce. Further fundamental studies in understanding the mechanisms of foetal programming are essential to eventually use such knowledge in application to nutrition for expectant mothers and especially for therapeutic diets in overweight or diabetic mothers.

RECENT DEVELOPMENTS AND CONCLUSIONS

Substantial research effort is aimed at improving the composition of infant and follow-on formulae with the purpose to optimize growth and development of infants. Breast feeding remains the gold standard for infants, but in those cases where breast feeding is not possible, formulae with optimal composition to feed the infant are essential. The infant formula, besides providing ingredients for energy and building blocks for growth and development, contains added non-digestible oligosaccharides that mimic the features provided by HMO present in breast milk as much as possible. One strong feature of the prebiotic HMO in the breast milk is the resulting bloom of bifidobacteria in the infant gut. Inulin and certain prebiotics can mimic this bifidogenic effect. More conclusive evidence is required that bifidobacteria have a certain quantifiable impact on infant health. The microbiome revolution stimulated by high throughput sequencing and post-genomic technologies which seeks to identify the role of the human microbiota and specific individual members on health and disease should ultimately unravel this, and thus continued support of this longer term research is essential.

A range of suitable prebiotic ingredients is available for a bifidogenic effect, and to date native inulin (a natural mixture of short and long chains) represents an economic way to supplement infant formula and follow-on formula with prebiotic oligosaccharides to elicit a bifidogenic response and bring the bowel habit closer to that of human milk. Nevertheless, few of the interventions with inulin or oligofructose on infants and young children have specifically addressed bowel habit as the primary parameter. Remarkably, there are also currently no guidelines on fibre in infant formulas nor follow-on formula for children over 2 years of age. Moreover, intestinal habit issues may be a factor contributing to colic in infants whereby prebiotics could play a role and this aspect deserves further attention.

It is evident that the vast majority of the research on infant nutrition has been performed in the Western world. In view of the fact that especially in the South East Asian region and other Asian countries mal- and under-nutrition with its detrimental effects on infants and children has a high prevalence, the role of prebiotics for these conditions should be investigated further. There is a high prevalence of low birth weight (<2.5 kg) and stunting in the South East Asian region due to mineral and vitamin deficiencies, poor diets in general and frequent infections. It is becoming established that the intestinal microbiota is perturbed in under-nourished infants and children in developing countries and that this 'immature' microbiota can be recalcitrant to recovery even with certain nutritionally rich therapeutic foods. It is noteworthy that certain gut microbiota members were associated with improved nutritional status including bifidobacteria and *F. prausnitzii*, amongst others. Prebiotic-only data are hardly available in these regions; yet if proven beneficial might support a healthier microbiota and improve nutritional status via immune or mineral absorption properties. Moreover, while studies show that inulin-type fructans have immunomodulatory effects, quality studies into the structure-function relationship and dose-effects is required especially for infants and children.[74]

There are still other health aspects whereby prebiotics may play a nutritional or therapeutic role in the future. A new field is the impact of the human gut microbiome on brain health which apparently involves numerous mechanisms such as central nervous system inflammation due to poor intestinal permeability, production of neurotoxic metabolites, production of hormones and neurotransmitters identical to the humans, and direct stimulation of neurons, amongst others.[167] Via these varied routes, the gut microbiota is been implicated in a variety of disorders including mood, cognition, autistic spectrum disorders and anxiety-like behaviours.[168,169] So far there are no such data with prebiotics for infants and children, but as they are in a stage of very rapid development, also for brain function, it is an essential aspect that deserves more attention.

Understanding of the concept of foetal metabolic programming is growing whereby maternal nutrition can directly influence the development and long term health of the offspring. Fundamental studies in understanding the mechanisms of foetal programming are essential to eventually use such knowledge in application to nutrition for expectant mothers and especially for therapeutic diets in overweight or diabetic mothers. However, further fundamental research is required to establish whether prebiotic treatment of the mother or during infancy represents a realistic therapeutic strategy for preventing obesity risk or the cycle of diabetes in offspring, as well as to the potential benefits of fructans of modifying milk of lactating mothers in a positive manner. Human interventions and epidemiology studies will be required before there is reliable scientific support for inulin-type fructans. Stimulating this are increasing findings which prompt the potential for gut microbiota-based therapy or prevention, potentially with prebiotics besides other means, to support health or prevent the development of certain diseases from conception to adulthood.

AUTHOR DISCLOSURES

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Review Article

Fructans in the first 1000 days of life and beyond, and for pregnancy

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果聚糖在出生后 1000 天中的作用及其对远期健康和妊娠的影响

菊粉益生元是一类非消化性多糖，能够影响婴幼儿肠道中微生态的构成，尤其是增强双岐杆菌转化产生短链脂肪酸的作用。菊粉是一个通用的术语，包括 β-(2,1)线性果聚糖（主要从菊苣植物根中分离所得）及其衍生品如低聚果糖和长链菊粉，尽管各自具有不同的生理功能。婴儿出生后第一个 1000 天被认为是影响健康的一个关键期，甚至关系到成年期的健康，其中营养是关键因素之一。在此关键期间营养和肠道微生物群组成之间的联系不断增强和发展，从而影响个体的生命健康。本文总结了对健康新生儿和早产婴儿肠道微生态进行研究的结果，以及在发展中国家对营养不良患儿进行观察的最新进展。这些研究内容包括菊粉或其混合物对婴幼儿和较大年龄儿童的肠道功能和免疫力产生的影响。这些研究结果显示，益生元可以支持营养不良患儿肠道中的有益微生物生长从而抑制感染，因此特别有益于发展中国家的儿童和孕妇的健康。本文提出了有关菊粉益生元在肠道微生态代谢编程方面的重要作用，明确了益生元对儿童长期健康的潜在有益作用。上述研究进展为利用益生元对某些疾病进行肠道微生态疗法治疗和预防的可能性提供了证据。

关键词：回顾、菊粉、婴儿、懷孕、肠道微生态