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# Prebiotic carbohydrates in human milk and formulas

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**Abstract**
Human milk oligosaccharides play an important role, as prebiotic soluble fibres, in the postnatal development of the intestnial flora. Infant formulas are virtually free of prebiotic oligosaccharides. As a consequence, formula-fed infants develop an intestinal flora significantly different to the flora of breastfed infants. Due to the complexity of human milk oligosaccharides, it is necessary to use alternative sources of prebiotic ingredients as components of infant formulas. The present review summarizes the data of experimental research and clinical studies with a prebiotic mixture containing 90% short-chain galacto-oligosaccharides and 10% long-chain fructo-oligosacchrides are summarized. The data demonstrate that, with this prebiotic mixture, the growth of bifidobacteria and lactobacilli can be stimulated, the faecal pH can be decreased, and the presence of pathogens can be reduced to levels similar to those of breastfed infants. Thus, prebiotic oligosaccharides such as the studied mixture provide beneficial effects for formula-fed infants.

**Key Words:** *Prebiotics, galacto-oligosaccharides, fructo-oligosaccharides, physiological effects*

## Definition of oligosaccharides

Carbohydrates are usually classified according to their molecular size (degree of polymerization, DP) into monosaccharides and oligosaccharides/polysaccharides; the latter two are made up of monosaccharides by glycosidic linkages. The borderline between oligo- and polysaccharides is not strictly defined. However, the term oligosaccharide is commonly used to refer to defined structures as opposed to a polymer of unspecified length.

Oligosaccharides are major components in human milk and in many natural products such as fruit, vegetables and honey either in a free or combined form (i.e. glycolipids or glycoproteins). Usually, oligosaccharides occur in natural sources as mixtures of compounds with a different degree of polymerization.

## Human milk oligosaccharides

Besides 7% lactose, human milk contains approximately 1% neutral oligosaccharides and about 0.1% acidic oligosaccharides [1]. Therefore, these oligosaccharides make up a large part of human milk composition, similar to the level of proteins. The biological function of these complex oligosaccharides is not yet fully understood. However, there is evidence that human milk oligosaccharides are important for the prebiotic effect (essentially bifidogenic) as well as the anti-infective and allergy-preventive properties of human milk [2,3].

Roughly 130 different neutral and acidic oligosaccharides have been characterized so far. The pattern of human milk oligosaccharides depends largely on the mother's Lewis blood group [4]. Using new analytical techniques, a large variety of different complex oligosaccharides have been detected in human milk. As an example, the new detected masses of the fraction of neutral oligosaccharides are shown in Figure 1.

As different isomeric structures exist for the known masses (see hatched area of Figure 1), the total variety of isomeric structures can be estimated to be higher than 1000. Recently, high-molecular-weight acidic oligosaccharides were also found in human milk by using a combination of liquid chromatography and mass spectrometry. This further confirms the exceptional complexity of human milk oligosaccharides [1].

One characteristic of human milk oligosaccharides is the large amount of galactose. The backbone structure is based on lactose (galactose-glucose) plus a further galactose residue forming the three different galactosyl-lactoses, namely 3′-galactosyl-lactose,

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| Core | 0 Fuc | 1 Fuc | 2 Fuc | 3 Fuc | 4 Fuc | 5 Fuc | 6 Fuc | 7 Fuc | 8 Fuc | 9 Fuc | 10 Fuc | 11 Fuc | 12 Fuc | 13 Fuc | 14 Fuc | 15 Fuc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L | 342 | 488 | 635 | | | | | | | | | | | | | |
| LNT | 708 | 854 | 1000 | 1046 | | | | | | | | | | | | |
| LNH | 1073 | 1219 | 1365 | 1511 | 1658 | 1804 | | | | | | | | | | |
| LNO | 1438 | 1584 | 1731 | 1877 | 2023 | 2169 | 2315 | | | | | | | | | |
| LND | 1804 | 1950 | 2096 | 2242 | 2388 | 2534 | 2680 | 2827 | 2973 | | | | | | | |
| LN12 | 2169 | 2315 | 2461 | 2607 | 2754 | 2900 | 3046 | 3192 | 3338 | 3484 | | | | | | |
| LN14 | 2534 | 2680 | 2827 | 2973 | 3119 | 3265 | 3411 | 3557 | 3703 | 3850 | 3996 | 4142 | | | | |
| LN16 | 2900 | 3046 | 3192 | 3338 | 3484 | 3630 | 3776 | 3923 | 4069 | 4215 | 4361 | 4507 | 4653 | | | |
| LN18 | 3265 | 3411 | 3557 | 3703 | 3850 | 3996 | 4142 | 4288 | 4434 | 4580 | 4726 | 4873 | 5019 | 5165 | 5311 | |
| LN20 | 3630 | 3776 | 3923 | 4069 | 4215 | 4361 | 4507 | 4653 | 4799 | 4946 | 5092 | 5238 | 5384 | 5530 | 5676 | 5822 |
| LN22 | 3996 | 4142 | 4288 | 4434 | 4580 | 4726 | 4873 | 5019 | 5165 | 5311 | 5457 | 5603 | 5749 | 5895 | 6042 | 6188 |
| LN24 | 4361 | 4507 | 4653 | 4799 | 4946 | 5092 | 5238 | 5384 | 5530 | 5676 | 5822 | 5969 | 6115 | | | |
| LN26 | 4726 | 4872 | 5019 | 5165 | 5311 | 5457 | 5603 | 5749 | 5895 | 6042 | 6188 | | | | | |
| LN28 | 5092 | 5238 | 5384 | 5530 | 5676 | 5822 | 5969 | 6115 | | | | | | | | |
| LN30 | 5457 | 5603 | 5749 | 5895 | 6042 | 6188 | | | | | | | | | | |
| LN32 | 5822 | 5969 | 6115 | | | | | | | | | | | | | |

Number of Fucosyl-residues →

= known structures

Figure 1. Masses of neutral human milk oligosaccharides as revealed by MALDI mass spectrometry. Masses of oligosaccharides in the hatched area are equivalent to various isomeric structured identified by other techniques like NMR. Ambiguity of masses due to nearly identical mass increment of 5 fucose- and 2 Gal-GlcNAc residues.

4′-galactosyl-lactose and 6′-galactosyl-lactose. Larger oligosaccharides are formed by repeated units of galactose-N-acetylglucosamine to the core lactose. The backbone is further modified by the specific addition of fucose and sialic acid residues.

## Definition of prebiotics

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that have the potential to improve the host's health. This is in contrast to the concept of probiotics, which are living bacteria in the diet.

Non-digestible oligosaccharides pass the gastrointestinal tract intact and are selectively fermented by the gut flora in the colon. These substances are called prebiotic oligosaccharides. The main types of non-digestible oligosaccharides are: fructo-oligosaccharides (FOS), mainly derived from chicory; $\beta$-galacto-oligosaccharides (GOS), derived from lactose; and $\alpha$-galactosides (raffinose, stachyose), mainly found in legumes.

In general, oligosaccharides can be characterized by MALDI mass spectrometry and HPAE chromatography [1] as shown in Figures 2 and 3.

Since 1980, oligosaccharides have been used to improve the quality of many foods. During the past decade, especially in Japan and Europe, oligosaccharides have attracted interest as food ingredients. Walker and Duffy extensively reviewed the role of prebiotic oligosaccharides such as FOS and GOS as dietary components for bacterial colonization [5].

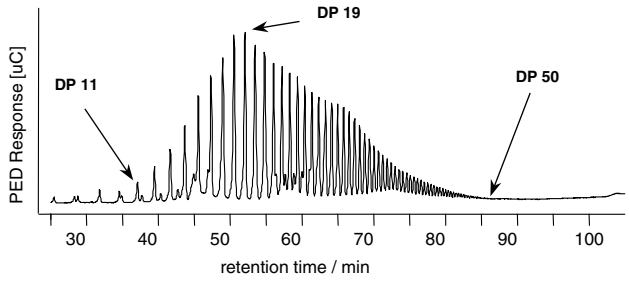


Figure 2. HPAE chromatogram of long-chain fructans. DP is degree of polymerisation, which is equivalent to the number of monosaccharide units of a given molecule.

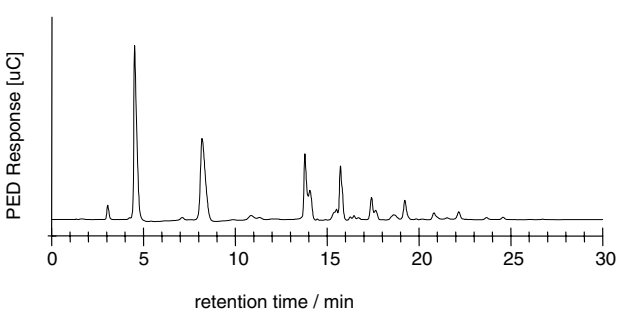


Figure 3. HPAE chromatogramm of galacto-oligosaccharides.

## Concept of prebiotic oligosaccharides in infant milk formulas

Traditionally, in Japan, infant milk formulas are produced containing prebiotic oligosaccharides. Over 90% of Japanese infant formulas have been supplemented with non-digestible oligosaccharides as growth-promoting factors for bifidobacteria for more than a decade.

More recently, based on the investigation of human milk oligosaccharides, a special mixture of available oligosaccharides with proven prebiotic effects for each of the compounds has been developed. This mixture will be used in a concentration resembling the concentration of oligosaccharides in human milk. The size of the molecules and prebiotic effect of the mixture is adapted to that of human milk oligosaccharides [6].

Due to the high amount of galactose in human milk oligosaccharides, we use commercially available galacto-oligosaccharides as one component of the mixture. The second component is fructans with a reduced amount of low-molecular-weight fraction. FOS with a DP greater than 10 show far fewer side effects such as flatulence than small-sized fructans. Galacto-oligosaccharides together with fructans promote growth-beneficial intestinal bacteria in a synergistic way, so that a maximum number of different species, in particular Bifidobacteria and Lactobacilli, can grow.

Galacto-oligosaccharides are side products of lactose hydrolysis by $\beta$-galactosidase (lactase, EC 3.2.1.23). Reports on the investigation of these oligosaccharides date back to the 1950s. The concentration of oligosaccharides in $\beta$-galactosidase-processed milk or milk products can be as high as 25%. In fermented milk or milk products, galacto-oligosaccharides are produced due to the action of $\beta$-galactosidase on lactic acid bacteria. Thus, galacto-oligosaccharides have been used in human nutrition in significant quantities as active components or as side products of processed milk or milk products, and no side effects have been reported. Theoretically, galacto-oligosaccharides may also be produced during normal milk digestion as a result of intestinal $\beta$-galactosidase activity.

FOS (e.g. fructo-oligosaccharides, oligofructose, inulin) are composed of glucose and repetitive fructosyl residues in $\beta$-2-1 linkage or $\beta$-2-6 linkage. FOS are usually extracted from the root of chicory and further enzymatically digested to oligofructose. An alternative is the enzymatic synthesis of oligofructose from sucrose.

## Studies on the prebiotic effects of individual oligosaccharides

FOS as one part of the prebiotic mixture have been studied *in vitro* and *in vivo* (including human studies) most extensively and have been shown to selectively stimulate bifidobacteria with a concomitant inhibition of other species such as bacteroides and clostridia.

As the GOS are the major component of this prebiotic mixture, they will be discussed in more detail below. GOS are one of the most commonly produced prebiotic carbohydrates worldwide. GOS have been studied in term infants. An infant milk formula containing 2% galacto-oligosaccharides was studied and compared to a commercial infant formula and a breastfed group. These trisaccharides were obtained from the treatment of lactose with $\beta$-galactosidase as described above. In these studies, the faecal characteristics of babies fed with the galactosyl-lactose-containing formula were almost similar to those of the breastfed group with regard to stool consistency, sour or sweet-sour odour, colour, and pH. The percentages of bifidobacteria in the faecal flora were 92.7% (breastfed group), 69.3% (galactosyl-lactose group) and 61.1% (commercial formula group) [7]. Further studies have been performed in adults. Administration of 10 g galacto-oligosaccharides in two daily doses for 21 d increased the faecal bifidobacteria and influenced the fermentative activity of colonic flora. Comparable effects on the microflora and their metabolism in healthy adults were obtained with daily doses of 15 g for 6 d. Matsumoto et al. performed breath hydrogen tests on healthy adults who had ingested 30 g of galacto-oligosaccharides, indicating an increase of bacterial metabolism in the gut [7].

Moreover, GOS are non-cariogenic sugars as described in studies including 26 strains of oral Streptococci, six strains of Streptococci, one Lactobacillus and one Actinomyces strain.

The utilization of GOS by intestinal bacteria and different physiological properties of these molecules in a variety of clinical studies with healthy adults have been reviewed extensively several times. A significant increase of bifidobacteria and a decrease of bacteroides were observed by feeding GOS. In a separate study, a significant correlation between the amount of administered GOS and the number of bifidobacteria was found. Also, the number of lactobacilli increased by feeding GOS. In a further study, an increase of bifidobacteria and a decrease of bacteroides and Enterobacteriaceae were observed by feeding a synbiotic product containing galacto-oligosaccharides and Bifidobacterium breve.

Other physiological effects such as regular defecation habits were established for elderly patients with constipation after ingestion of galacto-oligosaccharides. Water content in the faeces was significantly increased when galacto-oligosaccharides were administered to healthy adults.

Burvall et al. [8] studied the *in vitro* digestibility of GOS by human intestinal enzymes. The activity of

small intestine $\beta$-galactosidase on galacto-oligosaccharides was less than 10% of the activity on lactose. Therefore, GOS will pass mostly undigested through the small intestine into the large bowel where bacterial fermentation of the oligosaccharides takes place. Due to their positive effects on the intestinal bacterial flora, GOS are an interesting prebiotic component of infant milk formulas.

## Studies on a new prebiotic oligosaccharide mixture for infant formulas

Based on the experience with GOS and FOS as prebiotic ingredients, a mixture of 90% short-chain GOS and 10% long-chain FOS has been developed. The mixture was designed to mimic the molecular size distribution found in the neutral fraction of human milk oligosaccharides [6].

The first clinical trial in preterm infants demonstrated that this mixture of GOS and FOS stimulates the growth of bifidobacteria [9] and reduces the presence of pathogens in the faecal flora [10]. A study on term infants showed that the effect is dose dependent [11]. Stool pH in a group of term infants fed the GOS/FOS supplemented formula decreased during a 4-wk feeding period similar to that of a breastfed group, whereas in a control group fed a formula supplemented with maltodextrin as placebo the pH increased [11]. More recently, it has also been demonstrated that a formula supplemented with GOS/FOS results in a short-chain fatty acid pattern comparable to that in breastfed infants [12]. The increase in bifidobacteria and lactobaccili, the reduction of clinically relevant pathogens, the metabolic activity of the intestinal flora as described by the short-chain fatty acid profile and pH clearly indicate that the GOS/FOS mixture stimulates the entire flora in the same way as breast milk.

It is widely accepted that the intestinal flora plays an important role in the postnatal development of the immune system. Thus, it can be speculated that a balanced stimulation of the intestinal flora by prebiotics, aiming to resemble the flora found in breastfed infants, will also help to modulate the postnatal development of the immune system. However, this attractive hypothesis still needs to be proved by further investigation.

## References

[1] Boehm G, Stahl B. Oligosaccharides. In: Mattila-Sandholm T, editor. Functional dairy products. Cambridge: Woodhead Publ.; 2003. p 203–43.

[2] Newburg DS, Neubauer SH. Carbohydrates in milks: analysis, quantities, and significance. In: Jensen RG, editor. Handbook of milk composition. San Diego: Academic Press; 1995. p 273–349.

[3] Rudloff S, Stefan C, Pohlentz G, Cunz K. Detection of ligands for selectins in the oligosaccharide fraction of human milk. Eur J Nutr 2002;41:85–92.

[4] Thurl S, Henker J, Siegel M, Tovar K, Sawatzki G. Detection of four human milk groups with respect to Lewis blood group dependent oligosaccharides. Glycoconj J 1997; 14:795–9.

[5] Walker WA, Duffy LC. Diet and bacterial colonization: role of probiotics and prebiotics. J Nutr Biochem 1998;9:668–75.

[6] Boehm G, Fanaro S, Jelinek J, Stahl B, Marini A. Prebiotic concept for infant nutrition. Acta Paediatr 2003;Suppl 441; 64–7.

[7] Matsumoto K, Kobayashi Y, Ueyama S, Watanabe T, Tanaka R, Kan T, et al. Galactooligosaccharides. In: Nakakuki T, editor. Oligosaccharides, production, properties and applications. Japanese Technology Reviews. Geneva: Gordon & Breach Science; 1994.

[8] Burvall AN, Asp NG, Dahlquist A. Oligosaccharide formation during hydrolysis of lactose with Saccharomyces lactis lactase (Maxilact®) Part 3: Digestability by human intestinal enzymes in vitro. Food Chem 1980;4:243–50.

[9] Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, Stahl B, et al. Supplementation of an oligosaccharide mixture to a bovine milk formula increases counts of faecal bifidobacteria in preterm infants. Arch Dis Childh 2002;86:F178–81.

[10] Knol J, Boehm G, Lidestri M, Negretti F, Jelinek J, Agosti M, et al. Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed preterm infants. Acta Paediatr 2005;94(Suppl 449):31–3.

[11] Moro G, Minoli I, Mosca F, Jelinek J, Stahl B, Boehm G. Dosage related bifidogenic effects of galacto- and fructo-oligosaccharides in formula fed term infants. J Pediatr Gastroenterol Nutr 2002;34:291–5.

[12] Knol J, van der Linde E, Wells J, Boeckler H. An infant formula containing prebiotics changes the intestinal microflora of term infants. J Pediatr Gastroenterol Nutr 2003; 436:566.