Human milk oligosaccharides promote a healthy microbiome

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The human body is colonized by trillions of microbes: viruses, bacteria and eukaryotic cells. The composition of the human microbiome is highly variable, differing between ethnic groups and age groups, and undergoing dynamic changes from birth to maturity. The composition of the microbiome is also greatly affected by environmental factors, and in particular the gut microbiome is determined by the diet.

The human microbiome plays a key role in our health. In particular, the intestinal microbiome is recognized as a complex metabolic organ, composed of a large number of microorganisms interacting with each other and with the host. Intestinal bacteria synthesize a huge range of metabolites that can modulate human physiology, e.g. by influencing cell-to-cell communication or the secretion of signaling molecules. Certain gut bacteria can synthesize B vitamins and vitamin K-12, which are essential for the healthy development of newborns (LeBlanc et al., 2015). Other bacterial groups produce conjugated linoleic acids, providing diverse health benefits including the prevention of diabetes and obesity, and the stimulation of the immune system.

Anaerobic intestinal bacteria ferment carbohydrates to produce lactate and short chain fatty acids (SCFAs) such as acetate, propionate and butyrate. SCFAs, particularly butyrate, are needed to maintain the gut epithelium by inducing the proliferation of colonocytes, and to regulate gut barrier integrity thus reducing the risk of inflammation (Morrison and Preston, 2016). SCFAs also regulate many aspects of metabolism, including intestinal and hepatic glucose homeostasis, and lipid metabolism, and even regulation of immune response is dedicated to SCFAs. SCFAs modulate neuronal activity and visceral reflexes directly via receptors expressed on neurons of the peripheral, autonomic and somatic nervous systems. The shortest SCFA lactate (L) is suggested to have direct effect on central appetite via its effect on the hypothalamus (Chambers et al., 2015). SCFAs are therefore involved in the so-called microbiome/gut-brain axis, a bidirectional communication between the microbiome and the host, integrating neuronal pathways and endocrine mechanisms. While the gut bacteria affect the synthesis of cytokines and neurotransmitters such as serotonin, dopamine and acetylcholine, the brain influences the microbiota by causing changes in gut motility or the secretion of signaling molecules into the gut lumen.

Until recently, human fetuses were thought to be sterile. The microbiome was thought to form following the first contact with environmental bacteria during birth. Accordingly, the gut microbiome of neonates following vaginal delivery mostly resembles that of the maternal vaginal and gut microbiome, dominated by the genera Lactobacillus, Bacteroides, Bifidobacterium and Prevotella. In contrast, the gut microbiome of babies delivered by Cesarean section resembles the skin flora and is influenced by the hospital environment. The lower microbial diversity in the microbiome of these babies during the first 90 days of life contributes to a weaker immune system, increasing the risk of allergies, asthma and metabolic disorders (Rutayisire et al., 2016). However, there is now evidence that microbial colonization may begin already during pregnancy via bacterial transfer from the amniotic fluid and placenta to the fetal intestine, although this is controversial (Rautava, 2017). However, this assumption is supported by reports of bacterial life in the amniotic fluid and in the placental tissue. After birth, colonization of the neonate gut is mainly influenced by diet. Human breast milk is not sterile, it contains diverse bacteria, including lactic acid bacteria and Bifidobacterium species, which are ingested by the baby. In healthy, pregnant women, gastrointestinal bacteria are transported by several cell types to the mammary gland (Witkowski-Zimny and Kamińska-El-Hassan, 2017).

In addition to essential nutrients such as lactose and fatty acids, human breast milk is a rich source of bioactive compounds such as immunoglobulins, lactoferrin, lysozymes, cytokines and a large fraction of complex carbohydrates. The latter are known as human milk oligosaccharides (HMOs). They are the third most abundant components of human milk and can reach concentrations of up to 25 g/L just after birth. HMOs contain up to five different types of monosaccharides: D-glucose, D-galactose, L-fucose, N-acetylgalactosamine and N-acetylmuramic acid, the latter also known as sialic acid. About 200 different HMOs are known thus far, containing 3–15 monosaccharide units. The carbohydrate chains may be linear or branched, and are classified as neutral or acidic (the latter contain at least one sialic acid unit). HMOs are prebiotics, i.e. they are not digested by human enzymes so they pass through the gastrointestinal tract intact and arrive unmodified in the colon. However, they do provide nutrition to gut microbes and therefore influence the composition of the microbiome. Directly after birth, the neonatal gut is colonized by facultative anaerobes, such as enterobacteria, streptococci and enterococci. These bacteria consume oxygen and favour the growth of strict anaerobes, such as the genera Bacteroides,
Bifidobacterium and Lactobacillus. In breastfed babies, the gut microbiome is dominated by Bifidobacteria which are regarded as beneficial, whereas formula-fed babies have a much more diverse microbiome. The dominance of Bifidobacterium species in breastfed infants is tightly connected to the abundance of HMOs, which act as carbon source and provide energy for species such as *B. longum*, *B. infantis*, *B. breve* and *B. bifidum*. The different Bifidobacterium species have evolved diverse strategies to metabolize HMOs, e.g. specialized transport systems to import complex sugars across the cell membrane into the cell where the carbohydrate chains are digested by enzymes collectively known as glycosylhydrolases.

Terminal fucosyl acid and galactose residues are removed by fucosidases, sialydases and enzymes collectively known as glycosylhydrolases. Other Bifidobacterium species excrete glycosylhydrolases and digest the HMOs outside the cell. These species then import and metabolize the less-complex, partially or fully digested carbohydrates, including disaccharides and monoosaccharides (Thomson and Garrido, 2016). *B. longum* sp. and *Lactobacillus* species benefit from the digested HMOs, either importing partly-digested molecules and metabolizing them further or utilizing the monoosaccharides liberated by extracellular glycosylhydrolases, a phenomenon known as cross-feeding.

The colonization of the gut by Bifidobacterium species is accelerated by 2′-fucosyllactose, which is the most abundant HMO if the mother is among the 80% of women with the so-called secretor phenotype. This natural genetic variation is characterized by the presence of an active alpha-1,2-fucosyltransferase 2 (FUFT2) enzyme, which creates the chemical linkages necessary for the synthesis of 2′-fucosyllactose and more complex lacto-N-fucopentaose I, missing in milk of non-secretors. The dominance of Bifidobacterium species in the infant microbiome takes longer to establish in babies breastfed by non-secretor mothers, whose milk lacks 2′-fucosyllactose (Lewis et al., 2015).

The ability of bacteria to digest HMOs goes together with their ability to produce SCFAs, reducing the pH in the gut lumen and inhibiting the growth of harmful bacteria such as pathogenic *Escherichia coli*. However, the presence of HMOs can also help to deter pathogens. The first step in an infection often involves the pathogen binding to carbohydrates expressed on the surface of epithelial cells. The bound pathogens can then invade into the cells and proliferate. HMOs structurally resemble these cell-surface glycans and act as decoy receptors to prevent bacterial and viral pathogens binding to the host cells in the gastrointestinal, urogenital and respiratory tracts. This antiadhesion effect has been confirmed in vitro for the diarrhea-causing pathogens *Campylobacter jejuni*, *Vibrio cholerae*, and *Salmonella spp.*, but also for *Pseudomonas aeruginosa*, *Helicobacter pylori*, and *Pathogenic E. coli* strain. Furthermore, some studies suggest that HMOs can also prevent infections with pathogenic yeast, such as *Candida albicans*, and eukaryotic parasites (*T. gondii* and *B. bovis*, 2016). HMOs, and in particular 2′-fucosyllactose, also reduce the risk of infection with *Norovirus* and *Rotavirus*, and inhibit the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Escherichia coli* and *Acinetobacter baumannii*, as well as their ability to form biofilms (Ackerman et al., 2017).

According to the World Health Organization, breastfeeding is the best source of nourishment for infants and young children. However, breastfeeding is not possible for all mothers, which is why infant formula has been developed as an essential substitute. Although formulas have undergone continuous improvement we learn more about nutrition, most are still based on bovine milk. The milk of other mammals does contain oligosaccharides, but these are less complex and diverse than the HMOs in human milk, they are present at lower concentrations, and their composition differs substantially. For example, sialylated oligosaccharides are predominant in bovine milk, whereas fucosylated HMOs are predominant in human milk. Because HMOs are not produced by other mammals and have not been available, infant formulas have been supplemented with other prebiotics including galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS). Recent studies have shown that these substitutes promote the growth of different ranges of microbial species compared to HMOs such as 2′-fucosyllactose. For example, FOS favours the growth of *Lactobacillus acidophilus* whereas 2′-fucosyllactose, as stated above, promotes the dominance of *Bifidobacterium* longum spuus. *Infants* change in bacterial composition in turn modifies the spectrum of SCFAs that are produced by the microbiome, because L. acidophilus mainly produces lactate whereas *Bifidobacterium* species mainly produce acetate (Medina et al., 2017).

Indirectly, the provision of different prebiotics therefore affects the composition of the microbiome, its metabolic capabilities and the impact of these bacterial metabolites on host metabolism, including glucose homeostasis and appetite regulation.

Jennewein Biotechnologie GmbH is the first company to manufacture HMOs on a multi-ton scale. The first approved product (2′-fucosyllactosyl) of the company is produced by metabolically engineered bacteria and is manufactured by fermentation scale. Jennewein 2′-fucosyllactose was granted GRAS status by the FDA in 2015 and has been approved for US markets since 2016. In November 2017, the European Commission similarly decided that Jennewein 2′-fucosyllactose powder and liquid concentrate may be placed on the European Union market as a novel food ingredient. The first HMO to be confirmed as a novel food authorization and thus market approval under EU law. The high-quality product is manufactured under ISO 22000, defining the requirements for food safety management systems, and also conforms to kosher and halal standards.

References:


