Human Milk Oligosaccharide (HMO) Metabolism, Antibiotics, Viruses, Dysbiosis – and Probiotics.

In this analysis, we present some recent interesting papers focussed on the development of a healthy intestinal microbiome, metabolome and virome, as well as a clinical relevance commentary. Links to full text and abstracts are embedded wherever possible.

Background and Transparency: ***Lactobacillus Acidophilus*** **NCFM**, ***Bifidobacterium infantis*** **Bi-26** and ***Bifidobacterium bifidum*** **Bb-06** are the bacterial components of Labinic® Drops, which made by Biofloratech Ltd who commissioned this short report.

Zabel, B., Yde, C.C., Roos, P. et al. Novel Genes and Metabolite Trends in Bifidobacterium longum subsp. infantis Bi-26 Metabolism of Human Milk Oligosaccharide 2'-fucosyllactose. Sci Rep 9, 7983 (2019).

This paper is a detailed analysis of the metabolic effects of *B. infantis* Bi-26 on 2'-FL, which is the most abundant Human Milk Oligosaccharide (HMO) in human milk. Genomic near-identity with *B. infantis* ATCC15697 was noted. *B. infantis* is an early coloniser of the neonatal intestine.

In this study, *B. infantis* Bi-26 produced formate, acetate, 1,2-propanediol and lactate, and cleaved fucose from 2'-FL. These metabolites then "feed" other beneficial commensal organisms, facilitating prolongation of colonisation. *B. infantis* is an efficient metaboliser of HMOs, the non-digestible complex sugars which are abundant in human milk.

The importance of maintaining a favourable, acidic gut environment is noted, and thus the strong associations of antacids such as ranitidine and omeprazole with increased rates of necrotising enterocolitis in preterm infants may be explained by the reduction of gut acidity and change in healthy microbiome organisms. It is known that high levels of probiotic species such as *Bifidobacterium* are important to reduce intestinal dysbiosis.

Thongaram T, Hoeflinger JL, Chow J, Miller MJ. Human milk oligosaccharide consumption by probiotic and human-associated bifidobacteria and lactobacilli.

This paper examined the metabolic effects of a number of different Bifidobacteria and Lactobacillus, including *B. infantis* ATCC 15697 (see homology to *B. infantis* Bi-26 noted above) and also *Lactobacillus acidophilus* NCFM which is a human origin microorganism.

In particular they examined the metabolism of lacto-N-neotetraose (LNnT), which in this study was the only HMO that supported the growth of lactobacilli. This work is interesting because it revealed evidence of synbiotic interactions, where the metabolic output from one probiotic could "feed" another probiotic to facilitate establishment of a metabolome.

Turroni, F., Duranti, S., Milani, C., Lugli, G. A., van Sinderen, D., & Ventura, M. (2019). Bifidobacterium *bifidum*: A Key Member of the Early Human Gut Microbiota. Microorganisms, 7(11), 544.

Duranti, S., Lugli, G. A., Milani, C., James, K., Mancabelli, L., Turroni, F., Ventura, M. (2019). Bifidobacterium *bifidum* and the infant gut microbiota: an intriguing case of microbe-host co-evolution. Environmental Microbiology.

Bifidobacteria are the predominant intestinal bacteria of healthy breast-fed infants. High levels of *B. bifidum* correlate with high overall levels of all *Bifidobacteria*, suggesting a role of *B. bifidum* as a supporter of others in establishing a healthy *Bifidobacteria*l-dominant microbiome in the presence of HMOs.

Evidence for this includes laboratory co-cultivation where other *Bifidobacteria* showed at least two-fold growth in the presence of *B. bifidum* demonstrating the phenomenon of cross-feeding.

This cooperative degradation of HMOs shows, from an evolutionary perspective, the role of *B. bifidum* in the efficient use of resources and energy sources, and its role in developing the sustainability and resilience of the infant intestinal microbiome.

This again supports this concept for cooperative interaction between these particular organisms to co-colonise, cross-feed, and create a persisting microbiome and favourable metabolic environment

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What then are the effects of a healthy bacterial microbiome on the prevailing intestinal virome? This has been gaining deeper understanding of its importance. The interaction between the bacterial population and the viral population could be a key to explaining previously noted associations between breastfeeding and protection against infections such as gastroenteritis.

Liang, G., Zhao, C., Zhang, H. et al. The stepwise assembly of the neonatal virome is modulated by breastfeeding. Nature (2020).

This paper showed that breastfed babies had fewer viruses that infect human cells compared to formula fed babies. Breast milk contains factors such as HMOs, lactoferrin, maternal antibodies and other proteins that inhibit viral colonisation. However, as expected they found that the stool of breastfed babies had higher levels of Bifidobacteria and Lactobacilli.

Phages are viruses that infect bacteria, and there are 2 types. Lytic (virulent) phages infect the bacterial host, injecting their viral genome to produce hundreds of copies, which kills the host bacteria. Lysogenic (or temperate) phages on the other hand integrate into the bacterial chromosome, and as the bacteria replicates so does the phage (which is called a prophage). The bacteria is resistant to further infection from similar viruses.

What is fascinating in this work is that the presence of temperate viruses infecting probiotic organisms was associated with relative inhibition against more pathogenic viruses, perhaps due to the production of inhibiting factors/signals. Viruses infecting human cells were much lower in the stool of babies who were breastfed and had high levels of *Lactobacillus* and *Bifidobacteria*.

This study therefore raises the possibility that virally infected probiotic strains may offer longer term immune protection, and that this protective effect may persist, through bacterial replication, through later life e.g. against viral gastroenteritis.

Azagra-Boronat I, Massot-Cladera M, Knipping K, et al. Strain-Specific Probiotic Properties of Bifidobacteria and Lactobacilli for the Prevention of Diarrhea Caused by Rotavirus in a Preclinical Model. Nutrients. 2020;12(2):498.

This paper investigated the mounting evidence that some *Lactobacillus* and *Bifidobacteria* strains offer protection against rotaviral gastroenteritis. *L. acidophilus* NCFM was the most effective of the 4 microorganisms studied at achieving reduction of rotaviral faecal elimination and reducing duration of diarrhoea.

The three other probiotic bacteria investigated were *Bifidobacterium breve*, *Lactobacillus helveticus* and *Lactobacillus salivarius*. *B. breve* did not achieve reduction in viral shedding. No effect on the humoral immune protective response was seen.

Clinical Relevance

We are interested in intestinal dysbiosis not only in the preterm population (who are frequently exposed to antibiotics) but also in the term and infant population. There is now apparently sufficient evidence of the positive effects of probiotics on reducing necrotising enterocolitis for the many neonatologists and centres that routinely administer probiotics to preterm babies. However, the beneficial impact on other aspects of intestinal and general health are only now starting to be determined.

There is evidence that prenatal and early childhood antibiotic exposure is associated with obesity in later childhood, which includes babies delivered by Caesarean Section (where pre/peri-operative broad spectrum antibiotics are routinely given) and babies who receive antibiotics directly for post-natal sepsis concerns, or indirectly via maternal breastmilk (e.g. Caesarean section, maternal wound infection, mastitis etc). We get anecdotal reports from Paediatricians who follow up these babies that they often appear to have more discomfort, colic, and even green, mucousy stools than babies never exposed to antibiotics. Some of these babies are then (perhaps incorrectly) labelled as having "silent reflux" or suspected cows' milk protein intolerance. They have told us that using Labinic seems to help to improve stool colour and consistency, and reduce abdominal discomfort, as well as avoid the need for medications or dietary restrictions. Perhaps we need an RCT?

Given the current topical viral theme, we have gone back to an older paper from 2009. This was a double blind randomised placebo controlled study entitled "Probiotic effects on cold and influenza-like symptom incidence and duration in children".

Use of a single preparation of *L. acidophilus* NCFM, or combination of *L. acidophilus* NCFM and *Bifidobacteria lactis* in 248 children aged 3-5 years reduced the incidence of fever by 53%-72%, coughing by 41-62%, and rhinorrhoea by 28-59%. In addition, the use of antibiotics was reduced as well. Children were about 30% less likely to miss childcare. Similar studies using *Lactobacillus GG and Lactobacillus reuteri* failed to show respiratory symptom differences, but did show fewer fevers and less antibiotic use. The authors speculated that the mechanism of action might be enhancement of the innate immune system, for example via Toll-like receptor interactions.

It is possible however to speculate that there are more sophisticated interactions at play, including for example boosting the population of lysogenic/temperate phage infected probiotic bacteria at the expense of more pathogenic ones. But why a protective effect in the intestine should offer protection from respiratory viruses is far from clear, unless there is signalling and/or exchange of viral-like particles between the respiratory and intestinal microbiome for example.

Thank you for reading this, we hope you found it interesting. Please feel free to share with healthcare professionals, subject to the transparency and other disclaimers below.