HMOS AND BRAIN DEVELOPMENT

by Janet Fricker

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Human infants are unique in the animal world in that a large amount of brain development occurs after birth. Head circumference, considered a proxy for brain volume, increases at the phenomenal rate of 1.1 mm/day,[1] growth by far exceeding that of any other organ in the body. Whether underdevelopment of the human brain at birth is due to a narrow birth canal caused by humans being bipedal or limitations of the mother's metabolism is hotly debated;[2] but it is beyond dispute that after birth human babies need optimal nutrition to support brain growth.

"Such rapid brain growth places exceptionally high demands on the supply of nutrients, with failure to meet overall nutrient needs having significant consequences for cognitive development," says Bing Wang, from Charles Sturt University, New South Wales, Australia.

Although babies are born with neurons already formed, synaptic connections between these neurones are largely established after birth.[3] "Nutrients affect multiple brain development processes by regulating neurotransmitter pathways, synaptic transmission, signal-transduction pathways, and synaptic plasticity," explains Wang.

Human breast milk provides the optimal nutrition to support brain development after birth, with many studies demonstrating breast-fed infants show better cognitive performances, memory functions and intelligence quotients (IQ) than formula-fed infants. A meta-analysis of 17 observational studies showed that breast feeding was associated with higher IQ scores by an average of 3.4 points. [4] Intriguingly, a Brazilian birth cohort study found breast feeding had positive associations not only with IQ, but also with adults achieving higher monthly incomes at the age of 30.[5]

Human milk oligosaccharides (HMOs), which after lactose and lipids represent the third most abundant component of human milk, are considered key ingredients for brain development due to the quantity and variety in maternal milk compared to formula.[6] Altogether, more than 150 different HMOs have been identified in human milk,[7 8] ranging from simple structures with three sugar residues to more complex structures comprising 15 monosaccharide units.

HMOs have a core structure consisting of a lactose unit at the reducing end, to which varying combinations of five monosaccharides (glucose, galactose, N-acetylglucosamine, fucose, and sialic acid) are added. HMOs may be classified as neutral or acidic, with addition of fucose (accounting for 50 to 70 of HMOs) providing neutral properties; band addition of sialic acid (accounting for 10 to 30% of HMOs) giving acidic properties. Those containing neither fucose nor sialic acid are neutral (around 10% of HMOs)[9] HMOs may additionally be linear or branched and occur in several isomeric forms.

Such remarkable diversity provides the structural basis for multiple biological functions. "An important point is that HMOs containing the same sugar building blocks but different side chains and branches have completely different properties," explains Lars Bode, from the University of California, San Diego, La Jolla, author of the definitive review of HMOs, 'Human milk oligosaccharides: every baby needs a sugar mama'.[6]

Each mother produces a unique signature of HMOs, [6]with the most extreme variation due to whether women are 'secretors' who produce the gene coding for the enzyme that adds fucose in an alpha-1,2-linkage to the oligosaccharide chain. These mothers consequently have high 2'fucosyllactose (2'FU) content in their milk; while mothers who lack the enzyme (non-secretors) display much lower levels.

HMOs appear to be higher in colostrum (the first milk), and decrease over the course of lactation, with levels ranging from between ~ 21 to 24 g/L in colostrum to ~ 12 to 14 g/L in mature milk.[10] Furthermore, milk of mothers delivering preterm infants has higher HMO concentrations than that found in term milk. [11]"We haven't fully deciphered the HMO biosynthetic pathway, and it's fascinating that there's an entire pathway in the body that has yet to be elucidated," says Bode.

Most HMOs cannot be digested by humans, but instead reach the colon where they act as prebiotics (food for bacteria). The fact only a few Bifidobacterium, Lactobacillus and bacteroides species can digest HMOs, stimulates the development of these bacteria thereby keeping potentially harmful bacteria in check[6]. Accumulating evidence suggests other beneficial effects of HMOs include being soluble 'decoy' receptors preventing pathogens (viral, bacterial and protozoan infections) from attaching to mucosal surfaces, modulators of immune cell response, and lowering the risk of necrotizing enterocolitis (a devastating intestinal illness in premature babies).[6] Now there is a growing body of research exploring the role of HMOs as an essential nutrient for brain development and cognition.

Since sialic acid, a 9-carbon sugar, is an integral structural and functional component of the nervous system, it has long been proposed as the HMO component in breast milk responsible for brain development. Sialic acid is known to be present at high concentrations in the brain as a key component of gangliosides (complex molecules displayed on the surface of neurones).[12] Although humans can synthesize sialic acids endogenously, it is thought the infant liver may not have the full capacity to synthesise sufficient amounts for brain development in early life when demand is highest.[3] Such a view is supported by observations that sialic HMOs are abundant during early milk production but decline over the course of lactation.

Bing Wang, who originally trained as a neonatologist in China, has undertaken much of the research into sialic acid and cognitive development. A clue to the importance of sialic acid in brain development came from her early work showing that human brain cortical tissue has two to four times more sialic acid than that of seven other mammals.[13] Later, the link between breast feeding and sialic brain content was shown in a post mortem study on babies who had died from sudden infant death syndrome. Wang found both ganglioside-bound and protein-bound sialic acid concentrations were 32% and 22% higher respectively in the frontal cortex gray matter of breastfed than formula-fed infants.[14]

Wang believes that pigs represent the ideal translational model for assessing the effect of sialic acid on brain development due to having similar nutritional requirements, comparable gastrointestinal development and similar brain growth trajectories to humans. "Just like humans, new-born piglets are relatively small in relation to their mature size, making them vulnerable to developmental deficits," she explains.

In studies, Wang supplemented three day old piglets with a diet rich in protein bound sialic acid at three different doses, high, medium and low. Results showed those piglets demonstrating the fastest learning on an 8 arm maze using visual clues (associating three dots with milk) had received the higher doses of sialic acid.[15] More recently she demonstrated that beneficial effects of sialic acid supplementation on maze performance could also be achieved with preterm pigs (delivered at 90% gestation).[16]

The mechanism for sialic acid exerting its beneficial brain effects, Wang speculates, is through sialic acid residues on gangliosides interacting with calcium ions, which are important mediators in neuronal responses.[3] Studies have demonstrated that the sialic acid lactoferrin can cross the blood brain barrier via receptor mediated transcytosis.[17] "To my mind there's no question sialic acid crosses the blood brain barrier," says Wang.

Wang is the first to admit that her protein bound source of sialic acid was far from ideal. "The problem is that sialic acid bound to HMOs has not been readily available in sufficient quantities for research," she explains.

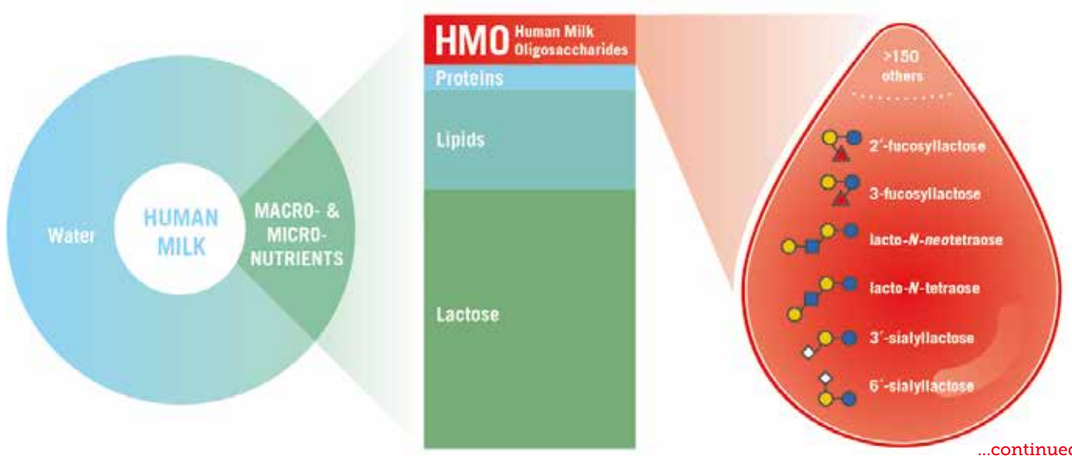
Since 2'FL is the most abundant HMO in the milk of most lactating women (and also one of the smallest comprising just three molecules) it has also been studied in relation to potential brain effects. Much of the work has been undertaken by the R&D department of Abbott Nutrition in Granada, Spain, who in 2015 showed orally administering 2'FL to mice and rats improved long term potentiation and performance in learning behavioural tests. The team additionally showed supplementation increased expression of molecules involved in the storage of newly acquired memories, such as the postsynaptic density protein 95.[18] In a second study, the Abbott team showed rats given oral 2'-FL supplements immediately after birth had better learning and memory (from object recognition at maze tests) at one year than HMO free litter mates,[19] demonstrating that 2'FL cognitive enhancing effects persist into adulthood.

One controversial issue is whether the HMOs themselves (or metabolites from gut bacteria digestion of HMOs) directly influence the brain through the circulation or whether the effect is mediated through the gut brain axis (a bidirectional communication system enabling gut microbes to communicate with the brain). Recent work from the Abbott group strongly suggests that the gut brain axis is the mechanism underlying the effect of dietary 2'FL. In the study, the team showed severing the vagus nerve in rats inhibited the beneficial effects of 2'FL on hippocampal long term potentiation (LTP) and learning.[20] From this finding the investigators concluded that the vagus nerve (the longest nerve in the body that controls the heart, lungs and digestive system) appears to be the main pathway underlying the effects of 2'FL on CNS function.

Gut microbiota appears to play an important role in the axis. A recent study showed that in mice fed radiolabelled 2'FL (13C 2'FL) there was no uptake of 2'FL in the brains of either mice with normal gut microbiota or those with sterile guts (a germ free mouse) demonstrating that dietary 2'FL does not reach the brain.[21] However, dietary 13C enrichment was found in the brains of mice with normal gut microbiota, but not the germ free mice. Such findings suggest that the presence of gut bacteria plays a fundamental role in carbon reaching the brain.

The effect, says Paul Forsythe, a researcher studying the gut brain axis, is likely to be mediated by the enteric nervous system, a mesh-like system of neurons enabling the gastrointestinal tract to operate independently of the brain. "My belief is that bacteria feeding of HMOs make neurotransmitters or other signalling molecules that influence the enteric nervous system, which in turn influences the vagal nerve that directly signals to the brain" says Forsythe, from McMaster University, Canada.

Until recently, HMO availability acted as a bottle neck to performing clinical trials. Extracting HMOs from breast milk was both cumbersome and expensive due to limited access to raw materials. However, innovative technology using bacterial species (like Escherichia coli) as factories to manufacture HMOs has proved the breakthrough opening the way for mass production. The bacteria, which have been genetically engineered to catalyse specific reactions of adding the sugars, allow production of cost effective HMOs in kilogram to ton quantities. Using this fermentation technology Jennewein Biotechnologie GmbH, a German company founded in 2005, can now manufacture a range of HMOs,



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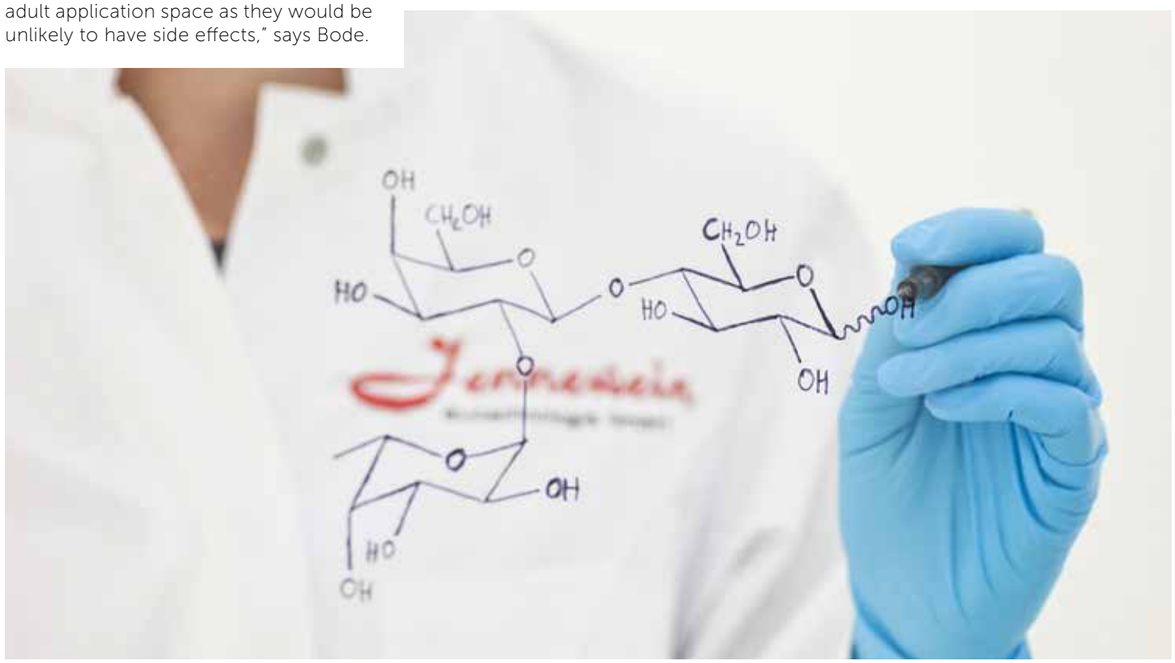
including 2'-Fucosyllactose, 3-Fucosyllactose, 3'-Sialyllactose, 6'-Salyllactose, Lacto-N-tetraose, Lacto-N-triose II, Lacto-N-neotetraose, Lacto-N-fucopentaose I. Perhaps of greatest significance for brain researchers, like Bing Wang, is that Jennewein's portfolio includes sialic acid HMOs (6'sialyllactose, and 3'-siallyllcatose), at last enabling sialic acid products to be used in brain studies resembling those found in human breast milk.

The large scale production process is allowing Jennewein to undertake a clinical trial feeding babies an HMO mix containing the five most common HMOs in breast milk (2'FL, 3-FL, LNT, 3'-SL and 6'SL), selected to allow formula to more closely resemble breast milk. If successful, the HMO mix will join formula products already on the market containing 2'FL that received FDA market approval for use in infant and toddler nutrition in 2015.

Ultimately, industry forecasters believe the HMO market will expand exponentially to include functional foods supplemented with HMOs to combat a range of health conditions including neurodegeneration in the elderly.[22] Evidence for a potential HMO role comes from a study reporting the sialic acid content of the human brain initially increases from infancy to adulthood (achieving maximum levels between 20 and 40 years), but then begins to decrease slowly at >60 years of age, with the most pronounced reduction occurring at > 90 years of age.[23] "It would be really appealing to have compounds from human milk that could be used as a basis for drugs in the adult application space as they would be unlikely to have side effects," says Bode.



References

1. Kristmundsdottir F. Prenatal growth and development. Robertson NRC, ed. Textbook. Neonatol. New York:, 162–9 (1992).
2. Dunsworth H. Metabolic hypothesis for human altriciality. PNAS 109, 15212–16 (2012).
3. Wang B. Molecular Mechanism Underlying Sialic Acid as an Essential Nutrient for Brain Development and Cognition. Adv Nut 3, 465S–472S (2012).
4. Horta B. Breastfeeding and intelligence: A systematic review and meta-analysis. Acta Paediatr Int J Paediatr 104, 14–19 (2015).
5. Victora C. Association between breastfeeding and intelligence, educational attainment, and income at 30 years of age: a prospective birth cohort study from Brazil. Lancet Glob. Heal. 3, e199-205 (2015).
6. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 22, 1147–62 (2012).
7. Wu S. Annotation and structural analysis of sialylated human milk oligosaccharides. J Proteome Res. 10, 856–68 (2011).
8. Wu S. Development of an annotated library of neutral human milk oligosaccharides. J Proteome Res. 9, 4138–51 (2010).
9. Ninonuevo M. A strategy for annotating the human milk glycome. J Agric Food Chem. 54, 7471–80 (2006).
10. Coppa G. Oligosaccharides in human milk during different phases of lactation. Acta Paediatr Suppl. 88, 89–94 (1999).
11. Gabrielli O. Preterm milk oligosaccharides during the first month of lactation. Pediatrics 128, 2011–1206. (2011).
12. Palmano K. The Role of Gangliosides in Neurodevelopment. Nutrients 7, 3891–3913 (2015).
13. Wang B. Sialic acid concentration of brain gangliosides: variation among eight mammalian species. Comp Biochem Physiol A Mol Integr Physiol. 119, 435–9 (1998).
14. Wang B. Brain ganglioside and glycoprotein sialic acid in breastfed compared with formula-fed infants. Am J Clin Nutr 78, 1024–9 (2003).
15. Wang B. Dietary sialic acid supplementation improves learning and memory in piglets. Am J Clin Nut. 85, 561–569 (2007).
16. Obelitz-Ryom K. Bovine Milk Oligosaccharides with Sialyllactose Improves Cognition in Preterm Pigs. Nutrients doi:10.3390/nu11061335 (2019).
17. Kamemori N. Trans-endothelialandtransepithelial transfer of lactoferrin into the brain through BBB and BCSFB in adult rats. J Vet Med Sci 70, 313–15 (2008).
18. Vázquez E. Effects of a human milk oligosaccharide, 2'-fucosyllactose, on hippocampal long-term potentiation and learning capabilities in rodents. J Nutr Biochem. 26, 455–65 (2015).
19. Oliveros E. Oral supplementation of 2'-fucosyllactose during lactation improves memory and learning in rats. J Nutr Biochem. 31, 20–7 (2016).
20. Vázquez E. Dietary 2'-Fucosyllactose Enhances Operant Conditioning and Long-Term Potentiation via Gut-Brain Communication through the Vagus Nerve in Rodents. PLoS One. https://doi.org/10.1371/journal.pone.0166070 (2016).
21. Kuntz S. Metabolic Fate and Distribution of 2´-Fucosyllactose: Direct Influence on Gut Microbial Activity but not on Brain. Mol Nutr Food Res. doi: 10.1002/mnfr.201900035. [(2019).
22. Tay C. HMOs a goldmine of human health applications beyond infant formula: DuPont expert. nutraingredients-asia.com /www.nutraingredients-com/Article/2018/09/12/
23. Svennerholm L. Membrane lipids of adult human brain: lipid composition of frontal and temporal lobe in subjects of age 20 to 100 years. J Neurochem. 63, 1802–11 (1994).