Literature/Narrative Review

The role of human milk oligosaccharides, and mode of feeding and delivery in the development of necrotising enterocolitis in the neonatal gut

Kamal El-Badawi
Year 4, Medicine, University of Plymouth
Email: kamal.el-badawi@students.plymouth.ac.uk

Abstract

The aims of this review are to explore the role that human milk oligosaccharides (HMOs), mode of delivery and method of feeding play in the prevalence of necrotising enterocolitis (NEC). Articles from PRIMO and PubMed were reviewed, with inclusion and exclusion criteria added to identify papers specific to HMOs, Bifidobacteria, breast feeding or bottle feeding and the role of HMOs in the development of NEC. The review found that certain HMOs such as disialyllacto-*N*-tetraose (DSLNT) are protective against the development of NEC. HMOs are consumed by bacteria and metabolised via a fermentation pathway; this pathway is commonly used by Bifidobacteria species. Such findings allow for potential prebiotic formulas to be made, which can be used in infants not consuming breast milk. Studies show vaginally birthed neonates have greater proportions of bacteria which are known to inhibit pathogenic bacteria which cause NEC. However, the risk of NEC was only increased in C-sections of multiple pregnancies. Breastfed and bottle-fed neonates have different levels of beneficial bacteria such as Bifidobacteria, which are involved in outcompeting bacteria that can cause NEC, such as *Staphylococcus aureus*. The use of synthetic or natural HMOs, such as DSLNT, in formulas has shown promising results. These results aid in our understanding of the importance of breastfeeding and vaginal birth in preventing NEC development. Future work can be done to aid in prebiotic formulation and increase evidence identifying factors involved in the occurrence of NEC.

Abbreviations

ABC - ATP-binding cassette
ATP - Adenosine triphosphate
DSLNT - Disialyllacto-N-tetraose
F6PPK - Fructose-6-phosphoketolase
HMO - Human milk oligosaccharide
LNFP - Lacto-N-fucopentaose
LNnT - Lacto-N-neotetraose
LNT - Lacto-N-tetraose
NEC - Necrotising enterocolitis
TLR4 - Toll-like receptors

Introduction

Human milk oligosaccharides (HMOs) are complex carbohydrates that play an integral role in the development of the neonatal gut microbiome. HMOs can't be digested by infants yet are abundant in human breast milk.[1] The mode of feeding and delivery impacts the development of necrotising enterocolitis (NEC) in neonates.

HMOs act as immunomodulators, thus preventing disease propagation in the neonatal gut.[2] They also act as prebiotics by providing energy and nutrients necessary for commensal bacteria to proliferate and colonise the gut.[1] Energy is provided through HMO metabolism using the bifid shunt fermentation pathway, allowing

commensals to thrive. Certain HMOs such as disialyllacto-*N*-tetraose (DSLNT) have been shown to be protective against NEC.[3] Breastfed neonates consume these natural HMOs as opposed to synthetic oligosaccharides in formula, consumed by bottle-fed neonates.[4]

NEC is a disease where tissue lining the bowel becomes inflamed and eventually dies, leading to bowel perforation and contents of the bowel entering the abdominal cavity.[5] Bacteria leaking into the abdominal cavity can cause irritation and a local inflammatory response, or a systemic inflammatory response, known as sepsis, due to spread throughout the body via the blood.[6] NEC can be caused by pathogenic bacteria thriving in the gut lining, a lack of beneficial bacteria provided by the breast milk or potentially because commensals do not have enough nutrients to grow.

In comparison to bottle fed neonates, breastfed neonates have lower incidences of NEC.[7] The differences between NEC rates amongst neonates who have been breastfed, compared to those who have been bottle fed has been attributed to the absence of certain bacteria that metabolise HMOs. Further research is needed to explore the use of synthetic HMOs and their potential role in decreasing rates of NEC.

This review aims to explore the role of HMOs in the neonatal gut and their role in the development of NEC. Additionally, the effects of mode of delivery and feeding will be explored.

What are HMOs?

Breast milk is widely considered the best source of food for a new-born baby. It introduces essential nutrients into the body such as proteins, lipids, minerals, and carbohydrates as well as immunoglobins (IgA) and cytokines which are involved in fighting infection. A main component of breast milk is HMOs. These complex carbohydrates are present in breastmilk and act as prebiotics, encouraging the growth of good bacteria in the infant gut such as *Bifidobacterium infantis, Bacteroides fragilis* and *Bacteroides vulgatus*.[8,9] Over 100 different HMOs have been identified.[10] The most common being lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT) and 2'-fucosyllactose (2'FL) with a combined percentage 37.1% of all HMOs.[11]

All HMOs are made up of the same five basic monosaccharides: glucose (Glc), galactose (Gal), *N*-acetylglucosamine, fucose (Fuc) and sialic acid (Sia).[12] The structure of HMOs follows the same template, with there being a lactose group (made from galactose and glucose with a β1-4 bond, written as Galβ1-4Glc) bonded to either lacto-*N*-biose (type I structure) or *N*-acetyllactosamine (type II structure). **Figure 1a** shows the basic structure of HMOs. The addition of lacto-*N*-biose, shown in **Figure 1b**, elongates the molecule and causes the termination of the molecule. However, the addition of *N*-acetyllactosamine allows the addition of other monosaccharides, either fucose (fucosylation) or sialic acid (sialylation) to the molecule of lactose or the elongated lactose molecule (**Figure 1c** and **Figure 1d**, respectively)

Type I HMOs have a lacto-*N*-biose group (disaccharide with a Galβ1-3GlcNAc bond) bonded to lactose with a β1-3 bond, whereas type II HMOs have an N-acetyllactosamine group (disaccharide with Galβ1-4GlcNAc) bonded to lactose with a β1-6 bond.

In human breast milk, the concentration of type I HMOs, including LNT and LNFP I is higher than that of type II HMOs such as LNnT, LNFP III and DSLNT; this potentially sets humans apart from other mammals.[13] With greater knowledge regarding the structure of HMOs, research can be carried out in tailoring interventions for neonates to provide them with a healthy gut microbiome.

NEC

Multiple factors lead to the proliferation of a healthy gut microbiome, including mode of delivery and feeding.

NEC is a common gastrointestinal pathology in premature babies and carries a risk of causing neonatal sepsis if left untreated.[14] The pathophysiology of the disease involves the Toll-Like receptors (TLR4) present on enterocytes in the small and large bowels being activated by gram-negative bacteria (e.g. enterobacteria) which enter the infant gut following birth. The mucosal lining deteriorates, surrounding cells become inflamed and enterocytes undergo apoptosis. This causes a perforation in the bowel lining, allowing bacteria to enter the abdominal cavity and the blood stream, increasing the risk of peritonitis (inflammation of the peritoneum) and sepsis, respectively.[6] In addition, bacterial binding to TLR4 causes a reduced blood flow to enterocytes, leading to ischaemia and eventually necrosis.[5] Through a clear understanding of the pathophysiology of NEC, the role that mode of delivery and feeding have on its occurrence can be better understood. These factors therefore have potential for being utilised in the prevention of NEC.

DSLNT

A study carried out on rats identified HMOs as being protective against NEC, increasing the survival rate of neonatal rats from 73.1% to 95.0% compared to formula fed rats. In particular, an isomer of DSLNT was found to be protective against NEC.[3] However, this study was carried out in rats, therefore it cannot be assumed that the findings are applicable to humans. Another study in human infants found that those who had been breast-fed milk with lower levels of DSLNT developed NEC.[7] This supports the link between the lack of DSLNT and the development of NEC.

HMOs (including DSLNT) are not present in formula feeds given to infants. DSLNT promotes the growth of Bifidobacteria in the infant's gut, one of the most common bacterial species found in the gut.[15] Such bacteria inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus* and *Clostridium perfringens*. Bifidobacteria does this by outcompeting the pathogenic bacteria for nutrients and preventing their adhesion to the epithelial cells lining the gut,[16] thus, ensuring pathogenic bacteria cannot colonise nor thrive in the gut.

Bifidobacteria and the bifid shunt pathway

Exclusive to the genus *Bifidobacterium* is the catabolic fermentation of HMOs by the phosphate phosphoketolase pathway (known as the bifid shunt pathway).[17] The enzyme fructose-6-phosphoketolase (F6PPK) is integral to the functioning of this pathway, and the final products of the process (lactic acid and acetate) are transported through the ABC transporters. The key product of this fermentative pathway is ATP. This pathway theoretically yields 2.5 moles of ATP for every 1 mole of glucose fermented, providing energy needed for bacterial growth and reproduction, eventually leading to bacteria colonising the infant gut.[18,19] 24 different strains of Bifidobacteria were analysed with regard to usage of the bifid shunt pathway, all of which tested positive for the presence of the enzyme F6PPK.[20] Therefore, the bifid shunt pathway may be the main method through which HMOs are metabolised.

Identifying the role of the F6PPK enzyme in the bifid shunt pathway allows prebiotics to be tailored to Bifidobacteria, thus preventing colonisation of pathogenic bacteria, such as *Staphylococcus aureus* which may cause NEC.[21] However, more studies on different Bifidobacteria strains are needed to determine whether the bifid shunt pathway is the most effective and main fermentation pathway involved in HMO metabolism.

A study by Hall *et al* from 1990 provides a different perspective on the argument.[22] The study was one of the earliest on the topic, therefore it gives a view of how the science has progressed over the years. Its conclusion is not consistent with that of updated studies from 2020 like that by Ma *et al*.[23]

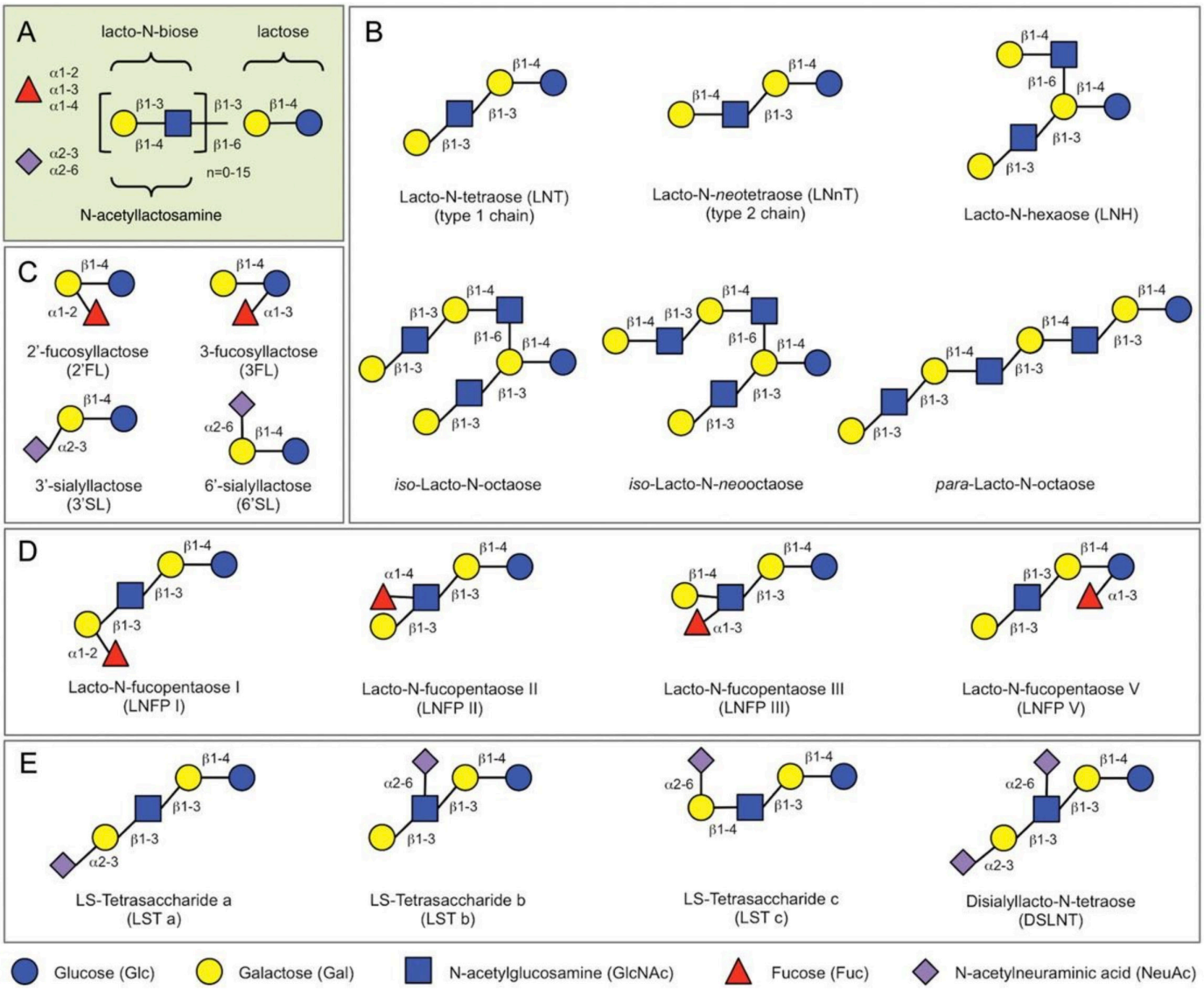

Figure 1. Different HMO structures. (a) Basic structure of an HMO. **(b)** Addition of lacto-*N*-biose to lactose. **(c)** Sialylation and fucosylation of lactose (by sialic acid and fucose, respectively). **(d)** Fucosylation of elongated lactose-forming multiple lacto-*N*-fucopentaose molecules. **(e)** Sialylation of elongated lactose (by N-acetylneuraminic acid, the most common sialic acid).[12] Reprinted from Bode (2012),[12] by permission of Oxford University Press.

Breast fed vs formula fed neonates

The consumption of breast milk or formula in infants has been linked to variation in the rates of NEC.[24] Breast fed infants have a 6- to 10-fold lower risk of developing NEC compared to formula-fed infants which is likely due to the absence of HMOs in formula.[7]

The difference between formula-fed and breastfed neonates commences when the baby takes its first breaths. Aerobic and facultative anaerobic bacteria, such as enterobacteria and streptococci species enter the gut and begin to colonise. These bacteria take up oxygen, allowing the natural proliferation of anaerobic bacteria, such as Bifidobacteria.[25]

Studies have shown Bifidobacteria has a much higher dominance in the guts of breast-fed neonates than in formula-fed neonates.[23] The gut microbiome of 4-week-old breast-fed neonates was found to be comprised of 69.96% *B. longum*, compared to 34.17% in formula fed infants.[16] These results show that bacteria unique to breast milk are introduced to the infant gut by breastfeeding. However, the results may not be representative of the general population, due to the study only being carried out on Korean infants, as there may be potential ethnic variations in *B. longum* populations. An opposing study carried out by Hall *et al* in the United Kingdom showed that there was no significant difference in the presence of Bifidobacteria between bottle-fed and breast-fed infants.[22]

The study by Lee *et al*.[16] using Korean infants had a smaller sample size compared to that used by Hall *et al*,[22] favouring the latter study due to the lower chance of including anomalous data. To combat this issue, the study by Sang *et al* should be repeated using a larger sample size, to accurately compare the two studies.

Many modern infant formulas contain supplementary plant oligosaccharides, which lead to less proliferation in Bifidobacteria colonies, compared to that of their mother's HMOs.[15] New-borns need these bacteria to improve their health, giving them the best opportunity to prevent the growth of bacteria which may cause infections or NEC.

Caesarean section vs vaginal birth

A Caesarean section (C-section) can either be elective or emergency. Elective C-sections are defined as procedures carried out "solely at the wish of the other, without any medical indication".[26] Studies have shown no difference in neonatal outcomes between elective C-section and vaginal deliveries.[27]

Bacteria are present on all parts of the skin and different modes of delivery expose different bacteria to the baby at the start of its life. Most of the differences can be normalised after consuming breast milk. Despite this, there may still be lifelong impacts of mode of delivery on the development of the gut microbiome.

Babies are exposed to different bacteria depending on if they are born via C-section (exposed to bacteria on the skin) or vaginally (exposed to bacteria in the vaginal canal). A study by Hällström *et al* suggested that there was a positive correlation between the mode of delivery and the onset of NEC.[28] With neonates who developed NEC, higher numbers of Enterococcus and *Candida albicans* were detected in stool samples. Compared to those delivered via C-section, vaginally birthed neonates were found to have an increased amount of Lactobacillus, which inhibit the growth of candida, protecting against NEC.[29] Supporting this is a study by Riskin *et al*, in which the risk of NEC was increased with C-sections, but only with multiple pregnancies.[30] However, the study by Riskin and colleagues was carried out using very-preterm (24-31 weeks gestational age) and very-low birth weight babies (<1500g), NEC is more likely in this demographic than in infants born at term or normal birth weight.

Another study, carried out by Son *et al* found no relationship between the mode of delivery and NEC development, contradicting the findings of Riskin *et al*.[31] Son *et al's* study was carried out using a larger population, reducing the likelihood of anomalous results. Considering both studies, there is an apparent increase in the risk of developing NEC for babies born by C-section in multiple pregnancies. However, due to the incongruence of conclusions, more research needs to be carried out to confirm an association between mode of delivery and onset of NEC.

Conclusion

HMOs are components of breast milk that are involved in the growth of beneficial bacteria and prevention of NEC. Studies identifying HMOs, such as DSLNT, as being protective against NEC were carried out on rats, therefore more research is needed into the role of DSLNT in the neonatal human gut microbiome. Plant saccharide supplementation has limited effect on NEC prevention and trying to mimic breast milk may show more potential in preventing NEC.

Regarding the mode of delivery and its effect on the prevalence of NEC, studies showed that babies born by C-section may be more likely to develop NEC. However, the study was carried out exclusively on pre-term infants, therefore, more studies must be carried out on term infants to confirm this.

Additionally, more research is needed to gain conclusive evidence regarding the effects of mode of delivery on rates of NEC before determining the need to reduce elective C-section rates. Once more research has been carried out, measures such as patient education of the risks of elective C-sections could be trialled, potentially reducing the prevalence of the practice and allowing infants to be exposed to vaginal bacteria.

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