Introduction to the Symposium1–3

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On May 16–17th, 2011, the First International Symposium on the Glycobiology of Human Milk Oligosaccharides was held at the Tivoli Hotel in Copenhagen, Denmark. The symposium was supported by Glycom A/S, Kongens Lyngby, Denmark. Human milk oligosaccharides (HMO)7 represent the third most predominant component in human milk but are absent from infant formula (1–3). Thus, oligosaccharide content represents the largest compositional gap between human milk and infant formula. Our understanding of HMO structure and natural variation in human milk has markedly expanded in the past decade, primarily due to advances in analytical methods (4,5). HMO exert a number of important physiological functions, particularly in the early postpartum period, including facilitating the establishment of a healthy microbiota enriched bifidobacteria (6,7), blocking the attachment of pathogens (8), and educating the neonatal immune system (9). Until now, direct study of dietary HMO function has been limited and commercialization has been blocked by lack of available material and high costs. Virtually no HMO were available from extractive sources and only a few synthesized in extremely low quantities and prohibitively high costs. However, due to the enormous biotechnological progress in HMO synthesis, the field is entering a new era focusing on specific biological effects of HMO in animals and humans.

The goal of the symposium was to bring together established and emerging leaders in HMO research to review the current knowledge of the structure, analytical methods, and physiological functions of HMO and to identify future research needs. The symposium was attended by more than 120 participants from 23 countries (Fig. 1). Oral presentations, consisting of 10 plenary lectures (30 min) and 10 talks selected from the abstract submissions (10–20 min) were divided into five themes: Significance of Carbohydrates, HMO Metabolism in Humans, Microbial Colonization and HMO Metabolism by Microbiota, HMO Content and Composition: New Analytical Approaches and Potential of HMOs, and Principal Components in Health and Disease. In addition, seventeen abstracts were presented. The meeting program, including the meeting abstracts is available on the Glycom website: http://www.glycom.com/.

Significance of carbohydrates

In the first session, Clemens Kunz and Hudson Freeze started the meeting with two plenary talks. Dr. Kunz, Institute of Nutritional Sciences, University of Giessen, Germany, kicked off the conference by reviewing the history of HMO research, which was initiated in the late 1800s (10). Next, Dr. Freeze, Sanford Children’s Health Research Center, Sanford-Burnham Medical Research Institute (La Jolla, CA), reviewed the complexity of the implications of congenital disorders of glycosylation for neonatal development through clinical scenarios and examples from animal models. Dr. Pedro Antonio Prieto, Tecnologico de Monterrey, Mexico, and Abbott Nutrition, then described the work conducted in the late 1990s to early 2000s of scientists and engineers employed at Abbott Laboratories to synthesize the HMO
lacto-N-neotetraose (LNnT) and test its biological activities in human clinical trials and animal models (11).

HMO metabolism in humans
In the second session, three plenary lecturers discussed aspects of HMO metabolism. Tadasu Urashima, Obihiro University Agriculture & Veterinary Medicine, Japan, provided evidence that milk oligosaccharides with type 1 structure, containing Gal(\(\beta 1–3\))GlcNAc (LNB), predominate over type 2 structures containing Gal(\(\beta 1–4\))GlcNAc (LacNAc) in human milk (12). The HMO composition appears to be specific to humans, because the milk of other species including apes and monkeys, either contain only type 2 oligosaccharides or the type 2 predominate over the type 1 structures. Next, Clemens Kunz presented results of a study probing HMO metabolism via in vivo labeling of HMO by administration of\(^{13}\)C-galactose to lactating women.\(^{13}\)C-galactose was incorporated into lactose and HMO (13). Milk samples, infant urine, and infant feces were collected. He and his co-workers showed that an in vivo labeling in humans is feasible and should facilitate metabolic studies in infants in the future. In the final talk in that session, Sharon Donovan, University of Illinois, Urbana, reviewed data that she and her collaborators have generated probing host-microbe interactions in the neonatal intestine by integrating infant mucosal gene expression and bacterial metagenomic data to identify important mechanistic pathways affecting intestinal development in the first few months of life (14).

Microbial colonization and HMOs metabolism by microbiota
In the next session, the two plenary speakers summarized relationships between specific HMO and the microbiota. Using sialyl(\(\alpha 2,3\))lactose-deficient mice, Thierry Hennet and colleagues at the University of Zurich showed that milk sialyl (\(\alpha 2,3\))lactose intake during infancy modulated bacterial colonization of the intestine and influenced the susceptibility to dextran sulfate sodium (DSS)-induced colitis in adult mice (15). David Mills, University of California, Davis, provided compelling evidence that \textit{Bifidobacterium longum} ssp. \textit{infantis} is a prototypical bifidobacterial species that can readily utilize HMO as a sole carbon source. This capacity is enabled by encoded glycosidases and transport proteins by which \textit{B. infantis} selectively imports and catabolizes milk oligosaccharides (16). The remaining talks in this session were selected from the submitted abstracts. Motomitsu Kitaoka of the National Food Research Institute, Ibaraki, Japan, presented his discovery of a gene cluster in \textit{Bifidobacterium longum} subsp. \textit{longum} encoding the intracellular pathway specific to the metabolism of galacto-N-biose (Gal\(\beta 1,3\)GlcNAc, GNB) and LNB (Gal\(\beta 1,3\)GlcNAc) (17). Because oligosaccharides containing LNB in their structure (type 1) are predominant in human milk, the possession of extracellular enzymes that liberate LNB from HMO by bifidobacteria explains in part how HMO promote the bifidobacterial growth. David S. Newburg, Boston College, shared results on the in vitro fermentation of HMO showing that co-incubation of human infant feces with HMO resulted in lactic acid production and a reduction in pH of the media. Furthermore, in vitro incubation in the presence of HMO-supplemented media increased Bifidobacteria and Lactobacillus sp. and reduced \textit{Escherichia coli}, \textit{Clostridium perfringens} and \textit{Clostridium difficile} compared to feces incubated with unsupplemented media, suggesting that HMO have prebiotic potential. The final two presentations in this session were given by graduate students. Daniel Garrido, University of California, Davis, described enzymes in \textit{B. infantis} responsible for hydrolyzing HMO. Five genes encode for \(\alpha\)-fucosidases in \textit{B. infantis} (Blon_0248, Blon_0346, Blon_0426, Blon_2335, Blon_2336). Among these, Blon_0248, Blon_0426, and Blon_2336 genes are specific for \(\alpha 1–3\) fucose linkages, whereas Blon_2335 was active on substrates containing \(\alpha 1–2\) fucose. Blon_0346, Blon_2335, and Blon_2336 were induced by growth on...
HMO or lacto-N-tetraose (LNT), the most abundant isomer in HMO. Their results provide a more complete picture of how B. infantis is able to consume HMO and show that the expression of a number of these is genes is responsive to HMO in the environment. Finally, Devon Kavanaugh, Teagasc Food Research Centre, Cork, and the National University of Ireland, Galway, communicated their findings that pre-incubation of B. infantis with 6′sialyllactose (6′SL) enhanced bacterial adhesion to human intestinal cell monolayers. This effect was not seen with 3′sialyllactose (3′SL) and was not associated with a prebiotic activity of 6′SL. Taken together, these presentations provide additional evidence for the unique metabolic and physiologic relationship that has evolved between HMO and bifidobacteria in the infant gut.

The following day, Rudolf Geyer and Carlito Lebrilla delivered plenary talks on new analytical approaches for HMO Content and Composition. Dr. Geyer, University of Giessen, Germany, began by discussing high throughput mass spectroscopic strategies that his laboratory has established for structural characterization of HMO and quantitation of free HMO in milk (18). He described a method used to uncover new HMO that combined fluorescent tagging of glycans with 2-aminoazobenzamide, followed by separation by 2-dimensional HPLC and analysis by different MS techniques. Glycan linkage positions of individual monosaccharides were assigned by digestion with specific exoglycosidases or chemical defucosylation in conjunction with GC/MS linkage analysis. This approach allowed the complete structural characterization of two novel HMO isomers, i.e., a difucosylated octaose and a novel trifucosylated decaose. Next, Dr. Lebrilla, University of California, Davis, reviewed the complexity of the human milk glycome and its analytical challenges (19). He described the various MS approaches used in his lab to define the HMO profiles in the milk of many species, including combining nanoflow liquid chromatography with tandem MS. He also described the annotated library for neutral and sialylated HMO developed by his laboratory. Four oral presentations were selected from the abstract submissions. First, John Klassen, University of Alberta, Canada, discussed the use of direct electrospray ionization mass spectrometry (ESI-MS) assay to quantify the association constants for protein-carbohydrate interactions in vitro. Using an approach known as “catch and release” ESI-MS assay, he screened the interactions between HMO and C. difficile toxins A (TcdA) and B (TcdB). His results revealed that TcdA and TcdB recognize multiple HMOs but binding is uniformly weak (<10^4 mol/L). Similar findings were found for the Shiga toxin B subunit and Cholera toxin B subunit, suggesting that HMO do not inhibit cytotoxic effects of these common pathogens. Next, Dennis Blank, University of Giessen, Germany, described their MS method for rapidly assessing HMO mass profiles and discriminating the Lewis blood group antigens of the mothers using conventional matrix-assisted laser desorption/ionization-Fourier transform ion cyclotron resonance MS (MALDI-TOF-MS) and starting with a 50 μL volume of milk. Shuai Wu, University of California, Davis, shared his work in developing annotated structure libraries for HMO that includes retention times, accurate masses, monosaccharide compositions, and relative abundances for 224 HMO using HPLC-Chip/TOF MS, MALDI FT-ICR and infrared multiphoton dissociation (IRMPD).

In the final presentation of this session, Ute Krengel, University of Oslo, Norway, presented her hypothesis about the molecular origins of blood-group dependence of cholera, based on relevant epidemiological, clinical and molecular data linking these data to oligosaccharide structures in human milk.

The goal of the closing session of the conference was to address the potential of HMO and principal components (e.g., precursors) to impact health and disease. The first two plenary speakers discussed the utilization of sialic acid (SA) and its potential role in neurodevelopment and cognition. Norbert Sprenger, Nestlé Research Center, Lausanne, Switzerland, shared his findings obtained in rodents across the lifespan from infancy to aging (20). In rat milk, >80% of SA is present as 3′SL and 6′SL, suggesting that these HMO could be important dietary sources of SA. In suckling rats, SA was partly catabolized in the gut when SA intake was high, whereas endogenous SA synthesis increased in the gut when SA intake was low. The SA content in the brain of suckling rats seemed unaffected by SA. In aged rats, endogenous SA synthesis capacity increased with age in the large intestine, a major site of SA synthesis, possibly due to low dietary intake because SA synthetic enzymes were normalized by SA feeding. In contrast to suckling rats, SA feeding affected brain ganglioside-bound SA content. Their findings support a functional role for SA that changes over the lifespan. Next, Ping Wang, University of Sydney, Australia, Xiamen University, and Nestlé Research Center, Beijing, P.R. China, reported on her findings that dietary SA supplementation increased the levels of SA in neural tissues, an upregulated expression of two learning related genes, UDP-N-acetylgalcosamine-2-epimerase (Gne) and alpha 2,8 sialyltransferase IV (ST8SiaIV) and enhanced learning and memory in piglets (21). The SA concentration in the frontal cortex of breastfed infants is higher than the levels of formula-fed infants, suggesting that SA synthesis may be a limiting nutrient in the neonatal period, making dietary SA necessary for optimal cognitive development in neonates. In the final plenary lecture, Lars Bode, University of California, San Diego, discussed his findings on the effects of HMO in reducing the incidence of two devastating diseases: amebiasis, caused by the protozoan Entamoeba histolytica and necrotizing enterocolitis (NEC) (22). His lab demonstrated that the HMO, LNT, and the prebiotic galactooligosaccharide (GOS) prevented E. histolytica attachment and cytotoxicity. Using a neonatal rat pup model of NEC, both pooled HMO and a specific isomer of the HMO were identified to be protective. For NEC, GOS was not protective. For both diseases, the mechanism of inhibition was highly structure-specific. The final two talks were short presentations selected from the submitted abstracts. Sørge Kelm, University Bremen, Germany, discussed interactions...
between glycoconjugates and siglecs, which are a family of SA-binding immunoglobulin-like lectins occurring mainly on cells of the immune system. He identified inhibitory bioactive sialooligosaccharides in fractions from bovine milk that bound to Siglec-2 (binds 2,6-linked SA) and Siglec-4 (binds 2,3-linked SA) (23). These findings suggest that bovine milk could be a source of bioactive immune modulators, however, the activity in some these fractions was lost with additional fractionation or heat treatment. The final presentation was by Jasmine Grinyer, Macquarie University, Sydney NSW, Australia. Her presentation focused on the specificity binding of SA-containing glycoproteins in milk and saliva to common oral streptococcal species, S. gordonii and S. mutans. She showed that SA residues are involved in S. gordonii, but not S. mutans, binding to saliva and milk glycoproteins, indicating that these secretions can act as a decoy to protect the oral environment against infection.

In summary, this is an exciting time for HMO researchers as we gain greater insight into the structures of HMO and begin to uncover their varied biological functions. The First International Symposium on the Glycobiology of Human Milk brought together over 100 scientists with expertise ranging from synthetic chemistry to glycobiology to pediatrics. The primary goal of the conference was to review what is known of the structure of HMO, new analytical techniques, HMO physiological functions and potential mechanisms of action. It was also our aim to promote an open exchange of ideas in order to gain different perspectives and to stimulate collaborative research. Currently large quantities of HMO are available for animal and human studies, which has laid the groundwork for new scientific and commercial investigations into HMO.

Acknowledgments

The authors thank Judit Kovacs and Susanne Mau from Glycom AS for their assistance with organizing the symposium. All authors read and approved the manuscript.

References