



Program & Abstracts

Day 1: Tuesday, June 15, 2021

Opening Remarks: Milk, a Unique Source of Nutritional, Sustainable, and Functional Proteins

Kasper Hettinga

Associate Professor, Wageningen University & Research

In recent years, dietary advice has pivoted from a single focus on nutritional recommendations to address sustainability of food production and thereby its impact on the planet. In response to embrace sustainability, we are seeing a flurry of recommendations to replace animal protein with plant proteins. Although such a simple message may seem to make sense, it disregards several unique features of milk proteins and in particular their benefit to human health. To address this ongoing debate, the IMGC VIRTUAL Symposium 2021 will focus on "Future Perspectives on Milk Bioactives and Proteins". Milk proteins serve as the single most important protein source in early human life, being ideally derived from human milk but alternatively from animal milk. Milk proteins not only deliver nutrition by being rich in essential amino acids, but also provide immune protection and positively influence the development (e.g. intestinal tract and brain) of the infant. By studying human milk, we learn more about these functional benefits of its proteins. By simultaneously studying animal milks, from platypus to cow, we can then compare the presence and characteristics of milk proteins among other species, allowing us to gain deeper insight in their unique functions. Part of this understanding of functionality is dependent on studying the digestion of milk proteins, and the resulting peptides and their functions on human health. Where some milk proteins, like caseins, are broken into a wide range of functional peptides, others, like immune-active proteins, are hardly broken down during digestion and impart immune protection, especially in infants. When dealing with the sustainability of milk proteins, we should thus not only focus on outputs such as the carbon footprint per kg of milk protein, but also consider all of the nutritional and unique functional benefits of milk proteins across all stages of life.

Keynote Speaker: Human Milk Bioactives: Nutrition and More

(0.5 L-CERPs)

Bernd Stahl

Director, R&D of Human Milk Research & Analytical Science, Danone Nutricia Research BV

The first 1000 days after conception are a period of rapid growth and development with specific nutritional requirements. The nutritional influence begins parenterally in utero and is affected by the nutritional status and environment of the mother. After birth, human milk (HM) is the preferred nutrition. The composition of HM is uniquely tailored to an infant's specific demands and is affected by diet, lifestyle, genes and health of mother and child. The WHO recommends exclusive breastfeeding (BF) for the first six months, and continued BF with adequate complementary foods for up to two years and beyond. The development of the gastro-intestinal tract and its microbial ecosystem is largely influenced by HM and impacts the child's immune responsiveness and metabolism supporting healthy growth. HM offers nutrients and other bioactive factors which positively affect the physiology and ultimately the health of an infant. More than thousand different HM proteins with hundreds of glycoproteins reflecting variable functional and nutritional needs of an infant. The lipid fraction of HM is composed of variable (long-chain polyunsaturated) fatty acids as part of triglycerides and polar lipids. Those lipids are organized within a complex milk fat architecture driving regulation of energy homeostasis and cognitive development and immunity. Human milk oligosaccharides (HMOS) are

quantitatively the third largest fraction of HM, with more than 200 identified complex molecular structures affecting the microbiota and the developing immune system. By applying a complex mixture of specific short and long-chain prebiotics, the main prebiotic effect of HMOS can be translated into a scientific proven nutritional concept for the first 1000 days. With this approach, a beneficial microbiota, a reduction of pathogens, an improvement of stool characteristics, as well as a reduction in the incidences of infections and allergic symptoms can be achieved. More recently, it was discovered that HM contains low levels of beneficial bacteria and their metabolites (postbiotics). The relevance of these HM biotics (HMOS, pre-, pro-, syn- and postbiotics) and their physiological effects on mother and child are currently still being explored. Future studies on the causes and consequences of these variations in HM nutrients and bioactives will deepen our understanding of the nutritional needs of mothers and their young children during the first 1000 days.

Antigen Shedding in Human Milk: a Key for Immune System Education?

(0.5 L-CERPs)

Valerie Verhasselt

Professor, University of Western Australia

In addition of being a source of nutrients for the developing newborn, human milk contains thousands of bioactive compounds, which influence infant health in the short-term as exemplified by its major benefits on infectious disease prevention. Many of the human milk compounds also have the required characteristics to instruct immune development and guide long-term health. Prebiotics, probiotics, varied antimicrobial molecules, all have the potential to shape the composition and function of the establishing gut microbiota, which is known to be a major determinant of proper immune function. Another and less explored way human milk can instruct long-term immunity, is through antigen shedding. Here, we will review the evidence that antigens from maternal environment and more specifically from allergen sources and microbes, are found in human milk. We will gather the data that highlight the mechanisms underlying the unique ability of breast milk to elicit immune response in offspring and educate the infant immunity towards tolerance or defense as needed. We propose this understanding is fundamental to guide maternal interventions leading to child-tailored vaccination, harmonious microbiota commensalism and lifelong allergen tolerance.

Breastfeeding Promotes Early Neonatal Regulatory T Cell Expansion and Immune Tolerance of Non-Inherited Maternal Antigens

(0.5 L-CERPs)

Gergely Toldi

Neonatologist, University of Birmingham

Breastfeeding is associated with long-term health benefits, such as a lower incidence of childhood infections, asthma, obesity and autoimmune disorders. However, little is known regarding how the maternal and neonatal immune systems interact after parturition when the neonate receives nutrition from maternal breastmilk. We aimed to analyze how immune phenotype and function evolve between birth and 3 weeks of age in breastfed versus formula fed neonates born by caesarean section. We investigated 38 healthy mother and baby pairs in this study. We used flow cytometry to describe the detailed immune phenotype in neonatal and maternal blood samples and mixed lymphocyte reactions to establish the proliferation response of neonatal versus maternal lymphocytes and vice versa. We determined cytokine production by T lymphocytes in the supernatants using a bead-based sandwich immunoassay. We also investigated the microbiome of neonatal stool samples by 16S rRNA sequencing. We show that the proportion of regulatory T cells (Tregs) increases in this period and is nearly two-fold higher in exclusively breastfed neonates compared to those who received formula milk only. Moreover, breastfed neonates show a specific and Treg-dependent reduction in proliferative

T cell responses to non-inherited maternal antigens (NIMA), associated with a reduction in inflammatory cytokine production. We also observed the enrichment of short chain fatty acid producing taxa (*Veillonella* and *Gemella*) in stool samples of exclusively breastfed neonates. Short chain fatty acids play an important role in Treg development and function. These data indicate that exposure of the neonate to maternal cells through breastfeeding acts to drive the maturation of Tregs and 'tolerizes' the neonate towards NIMA. Our results provide an insight into the cellular mechanism of immunological advantages of breastfeeding.

Extracellular Vesicles in Human Milk can Modulate Innate and Adaptive Immune Responses and Epithelial Barrier Function

(0.5 L-CERPs)

Marca Wauben

Professor, Utrecht University

Human milk is nature's first functional food and contains different components playing a role in the development of the infant's gastrointestinal tract and immune system. One of these components are extracellular vesicles (EVs), i.e. cell-derived vesicles used for cell-cell communication. The mode of action of human milk EVs on developmental processes has been poorly studied. In our study we explored the molecular mechanism of milk EV-induced modulation of different cell types present in the oral mucosa. Human milk EVs were purified by differential centrifugation, density gradient floatation and size exclusion chromatography. Effects of milk EVs on epithelial barrier function, Toll-like receptor triggering and T cell activation was studied using different (reporter) cell lines and primary T cells. Functional integrative proteomic analysis was performed based on our previously identified human milk EV proteome. We found that milk EVs promote migration of oral epithelial cells resulting in enhanced re-epithelialization in a gap closure assay. Functional integrative proteomic analysis now unveiled hotspots of regulation in the p38 MAPK pathway involved in migration targeted by milk EV proteins. Besides the influence on epithelial barrier function, milk EVs inhibited agonist-induced endosomal Toll-like receptor 3 (TLR3) triggering of these oral cavity epithelial cells, which coincided with reduced full length and cleaved cellular TLR3 levels and inhibition of TLR3 mRNA. Furthermore, milk EVs inhibited α CD3/ α CD28-induced CD4⁺ T cell activation which could be linked to the presence of EV proteins targeting hotspots of regulation downstream CD28, resulting in cell-cycle inhibition and mTOR stimulation. EVs are bioactive colloid structures of human milk that can modulate canonical signal transduction pathways involved in key processes in the development of the epithelial barrier and the innate and adaptive immunity of the infant.

Student Award Presentation: CD4⁺ T Cell Modulatory Properties and miRNA Cargo of Human Milk-Derived Extracellular Vesicles are Influenced by Allergic Sensitization of the Mother

(0.25 L-CERPs)

Alberta Giovanazzi

PhD student, Utrecht University

Human milk supports post-natal immune development of the infant by providing maternal components, including extracellular vesicles (EVs). EVs are cell-derived vesicles used as cell-to-cell vehicles of biological molecules. It has been demonstrated that milk-EVs contain immune-modulatory miRNAs with potential roles in the development of new-born's immunity. Furthermore, we showed milk-EVs have CD4⁺T-cell modulatory properties. Interestingly, allergic diseases can alter the physiological milk composition, however it is still unknown whether EV function and cargo are affected. In this study we investigated whether miRNA cargo and CD4⁺T-cell modulatory capacity of milk-EVs were different between allergic and non-allergic-lactating women. Human milk (4-13 weeks post-partum) was donated by non-allergic and allergic mothers (serum IgE \geq 50kU/ml and/or positive Phadiatop specific IgE). First, cells and creamy layer were depleted

from milk by centrifugation. Next, EVs were purified by differential centrifugation, density gradient floatation and size exclusion chromatography. EV functionality was assessed by in vitro co-culture with α CD3/ α CD28-stimulated human PBMC-derived CD4+T-cells. Quantitative differences in milk-EV miRNAs were identified by small RNA-seq. CD4+T-cell signaling model based on miRNA-target interactions was built to predict hotspots of milk-EV regulation. Milk-EVs from allergic mothers were less capable of inhibiting CD4+T-cell activation compared to milk-EVs from non-allergic mothers, as indicated by less strong inhibition of activation-associated genes such as CD25, IL6 and JUN. Milk EV-miRNA analysis revealed 30 miRNAs differentially expressed between non-allergic and allergic samples. Our prediction model shows that miRNAs over-represented in non-allergic samples favor the attenuation of T-cell activation-downstream processes such as cell cycle progression, STAT3/IL6 pathway and glucose metabolism, while miRNAs over-represented in allergic samples target pro-apoptotic pathway and negative regulators of cyclins. Milk-EVs from allergic mothers are less potent inhibitors of CD4+T-cell activation compared to milk-EVs from non-allergic mothers and this might be linked to quantitative differences in the miRNA cargo between these EVs.

Keynote Speaker: The Emergent Immunological Functions of Bovine Milk and its Effects on Human Health Early
Joost van Neerven

Professor, Endowed Chair, Wageningen University & Research and FrieslandCampina

Breast milk is the first nutrition that mammals provide to their offspring. Milk contains nutrients for growth and development, but is also an important source of components that support immune function. This is of critical importance for protection of newborns, because they do not yet have a fully developed immune system. Even though there are compositional differences between human milk and bovine milk, many components are present in both. Milk components can support development of the immune system, and can affect immune function directly or indirectly via effects on the gastrointestinal microbiota. Immunologically relevant components include lactoferrin, immunoglobulins, anti-inflammatory cytokines, antimicrobial peptides, and oligosaccharides. The relevance and emerging properties of these components, and the epidemiological findings on the effects of consumption of unprocessed cow's milk will be discussed.

Bovine Lactoferrin Stimulates Neonatal Gastrointestinal and Immune Development in Piglets

Sharon Donovan

Professor and Melissa M. Noel Endowed Chair, University of Illinois, Urbana-Champaign

Lactoferrin (LF) is major iron-binding protein in human milk that exerts a wide-range of biological activities within the infant. The objective of this research was to investigate potential mechanisms whereby orally administered bovine lactoferrin (bLF) modulates gastrointestinal and immune development in the preclinical piglet model. Newborn piglets were fed formula containing 0 (CON), 1 (LF1) or 3.6 (LF3) g/L bLF for 14 days. Fecal bLF and IgA and serum IgG were measured by ELISA. Intestinal morphology, enzyme activity, cellular proliferation, and gene expression were assessed. Immune cells isolated from serum, spleen and mesenteric lymph nodes were phenotyped by flow cytometry. Immune cells were stimulated ex vivo with lipopolysaccharide (LPS) and cytokine secretion measured by ELISA. Dietary bLF did not affect overall piglet growth or intestinal enzyme activity, DNA concentration, and villus length. However, LF3 piglets had 20% greater crypt depth and area, 60% greater cell proliferation and 3-fold higher B-catenin mRNA expression than CON ($p < 0.05$). Intact bLF was present in feces, suggesting that some bLF could interact directly with intestinal epithelial cells. LF3 had 2-fold higher serum IgG than CON. Unstimulated MLN cells from LF1 and LF3 secreted more ($p < 0.05$) IL-6, IL-10 and IFN- γ and unstimulated spleen cells secreted more ($p < 0.05$) IL-10, IFN- γ and TNF- α than cells from CON. LPS further increased all cytokines, with no interaction between dietary treatment and stimulant. Dietary bLF at concentrations in human milk stimulated intestinal cell proliferation, potentially through the Wnt signaling pathway.

Increased cytokine production by mucosal and systemic immune cells isolated from piglets fed dietary bLF indicates potential a priming of the immune system in vivo.

Towards a More Complete Milk Glycome: Advances in Ion Chromatography-Mass Spectrometry (IC-MS) for Improved Separation and Analysis of Milk Glycans

Tian Tian

Staff Scientist, Thermo Fisher Scientific

Glycans form a major component of human milk, occurring both in free form as lactose and oligosaccharides (HMO), and as conjugated to glycoproteins primarily through N- and/or O-linked glycosylation. Glycosylation to milk protein is known to be important for myriad biological processes, such as enabling resistance to proteolytic digestion thereby facilitating the release of encrypted bioactive peptides and promote the growth of gut probiotics. Few tools are currently available to analyze the glycome without derivatization. The objective of this study is to develop a workflow featuring straightforward and IC-friendly sample preparation, and enhanced separation and characterization of milk-derived free oligosaccharides, N-linked and O-linked glycans. The milk sample was diluted with water and centrifuge to skim. Milk protein was precipitated with cold ethanol at -30°C overnight. N- or O- linked glycans were released from collected protein enzymatically or by beta elimination. After deglycosylation, glycans were purified with porous graphitized carbon resin. Subsequently, target analytes were separated using a novel anion exchange stationary phase in a Dionex™ ICS-6000 HPIC™ system fitted with both pulsed amperometric detector and Q Exactive™ HF-X MS. Data were processed with Xcalibur™ software. Annotation of the spectrum was achieved de novo using available structures in UniCarb-DB. This work demonstrated the characterization of the heterogeneous glycans in milk. Overall, near 100 structures were identified, including more than seventy HMO molecules, twenty N-linked glycans, and six O-linked glycans. The novel CarboPac™ PA300-4µm column capitalized the power of HPAE to resolve and detect linkage and positional isomers without the need for derivatization. The highly informative MS spectra with ESI in negative mode facilitate both the sequence and linkage characterization of glycans. Diagnostic fragments from tandem MS enable the assignment of glycan epitopes. To our knowledge, this was the first time that the IC-MS platform was successfully applied to the structural characterization of milk glycans.

The Vaccine-Elicited Immunoglobulin Profile in Milk after COVID-19 mRNA-Based Vaccination is IgG Dominant and Lacks Secretory Antibodies

(0.5 L-CERPs)

Rebecca Powell

Assistant Professor, Icahn School of Medicine at Mount Sinai

No COVID-19 vaccines are yet under investigation for use in infants or young children. As such, the passive immunity of the antibodies (Abs) provided through milk from a vaccinated person may be one of the only ways to protect this population until pediatric COVID-19 vaccines are licensed. Our work examining the milk Ab response after SARS-CoV-2 infection demonstrated that Spike-specific IgA in milk after infection is dominant and highly correlated with a secretory Ab response. Determining if secretory Abs are elicited in milk is critical, as this Ab class is highly stable and resistant to enzymatic degradation in all mucosae. Presently, we describe our analysis of the milk Ab response 14 days after completion of an mRNA-based COVID-19 vaccine regimen among 10 individuals. Levels of SARS-CoV-2 Abs in human milk were measured by ELISA in separate assays measuring IgA, IgG, and secretory-type Ab reactivity. It was evident that IgG dominates after COVID-19 vaccination. One hundred percent of post-vaccine milk contained significant levels of Spike-specific IgG, with 8/10 samples exhibiting high IgG endpoint titers. Conversely, 6/10 (60%) of post-vaccine samples were



positive for Spike specific IgA, with only 1 (10%) exhibiting high IgA endpoint titer. Furthermore, 5/10 (50%) post-vaccine milk samples contained Spike-specific secretory Ab, none of which were found to be high-titer. As our analyses of the immune response in milk to COVID-19 vaccination continues, it will provide a critical opportunity to address huge knowledge gaps, inform the field as to which COVID-19 vaccine, if any, is likely to provide the best milk Ab response, and highlight the need to design improved vaccines with protection of the breastfeeding infant in mind.

Student Award Presentation: Breastmilk; a Source of SARS-CoV-3 Specific sIgA Antibodies, Highly Stable after Pasteurization

(0.25 L-CERPs)

Eva Kontopodi

PhD student, Wageningen University & Research

Since the outbreak of COVID-19, many put their hopes in the rapid availability of effective immunizations. Breast milk containing antibodies against SARS-CoV-2 may serve as protection through passive immunization. We aimed to determine the presence and neutralization capacity of SARS-CoV-2 antibodies in breast milk of mothers who recovered from COVID-19. This prospective case control study included lactating mothers, recovered from (suspected) COVID-19 and healthy controls. We collected serum and breast milk. To assess the presence of SARS-CoV-2 antibodies we used multiple complementary assays, namely ELISA with the SARS-CoV-2 spike protein, receptor binding domain (RBD) and nucleocapsid (N) protein for IgG and bridging ELISA with the SARS-CoV-2 RBD and N protein for total Ig. To assess the effect of pasteurization breastmilk was exposed to Holder and High Pressure Pasteurization. Breast milk contained abundant SARS-CoV-2 antibodies in 83% of the proven cases, in 67% of the suspected cases and in none of the controls. These antibodies were found capable of neutralizing a clinical isolate of SARS-CoV-2 and a pseudovirus. Although after pasteurization of the milk SARS-CoV-2 antibodies were detected with both methods of pasteurization, virus neutralizing capacity of those antibodies was only retained with the HPP approach. High pressure pasteurized breast milk of COVID-19 recovered mothers may represent a safe and effective immunization strategy.

Industry Flash Talk: Composing a Molecular Symphony: BIOMILQ's Cell-Cultured Human Milk Hits All the Right Notes

Leila Strickland

Co-Founder & CSO, BIOMILQ

Since the outbreak of COVID-19, many put their hopes in the rapid availability of effective immunizations. Breast milk containing antibodies against SARS-CoV-2 may serve as protection through passive immunization. We aimed to determine the presence and neutralization capacity of SARS-CoV-2 antibodies in breast milk of mothers who recovered from COVID-19. This prospective case control study included lactating mothers, recovered from (suspected) COVID-19 and healthy controls. We collected serum and breast milk. To assess the presence of SARS-CoV-2 antibodies we used multiple complementary assays, namely ELISA with the SARS-CoV-2 spike protein, receptor binding domain (RBD) and nucleocapsid (N) protein for IgG and bridging ELISA with the SARS-CoV-2 RBD and N protein for total Ig. To assess the effect of pasteurization breastmilk was exposed to Holder and High Pressure Pasteurization. Breast milk contained abundant SARS-CoV-2 antibodies in 83% of the proven cases, in 67% of the suspected cases and in none of the controls. These antibodies were found capable of neutralizing a clinical isolate of SARS-CoV-2 and a pseudovirus. Although after pasteurization of the milk SARS-CoV-2 antibodies were detected with both methods of pasteurization, virus neutralizing capacity of those antibodies was only retained with the HPP approach. High pressure pasteurized breast milk of COVID-19 recovered mothers may represent a safe and effective immunization strategy.



Keynote Speaker: Effect of Thermal and Non-Thermal Processing on the Functionality of Bioactive Milk Proteins

Kasper Hettinga

Associate Professor, Wageningen University & Research

Dairy products receive a lot of attention in relation to their health effects in both science and society. The relation between milk components and human health is, however, very complex. Milk contains numerous components that can have a wide range of physiological effects. The challenge is to understand which milk components are responsible for these physiological effects. As heating of milk changes the functionality of milk, such as its antibacterial capacity, the immune response to milk, heat-sensitive components in milk are underlying several of these beneficial properties of milk. It is therefore of interest to explore the effect of industrial processes on milk protein properties in relation to milk protein functionality. Using state-of-the-art proteomics approaches combined with *in-vitro* functionality assays, the relation between specific milk proteins and the beneficial functions they may have can be better explored. Additionally, the role of milk processing on the functionality of its proteins may be modulated by protein digestion, as heating impacts the gastric digestion of milk proteins and may thereby lead to different digestion products entering the intestine. In this lecture, I will discuss the known effects of thermal processing on milk proteins from both an analytical and functional perspective. To reduce a potential loss of milk protein functionality, either improved thermal processing (applying a lower heat load) or non-thermal technologies (e.g. UV-C, high pressure processing) may be implemented. The possible advantages of such approaches will be discussed in the lecture as well.

Student Award Recipient: Effect of Heating on Milk Protein Digestion and Clot Formation during in vitro Infant Gastric Digestion

Julie Miltenburg

PhD Student, Wageningen University & Research

Heating of milk proteins can influence their gastric digestion, both through differences in whey protein hydrolysis and changes in gastric clot formation. When investigating gastric protein hydrolysis, usually only the soluble part is analyzed, whereas information on the clot is not taken into account, leading to an incomplete picture of protein digestion. Therefore, we aimed to study the effect of heating of milk proteins on in vitro infant gastric digestion by analyzing both the soluble and insoluble part. Raw and heated skim milk (80°C, 30 min) was digested using in vitro infant gastric digestion. After digestion, samples were centrifuged to separate the clot from the supernatant. The nitrogen content in the supernatant was measured to calculate the nitrogen transfer from the clot. To determine which proteins were hydrolyzed, the protein compositions of both the supernatant and clot were analyzed. The nitrogen content in the supernatant from heated milk was lower than from raw milk during the whole gastric digestion, indicating that the clot from heated milk contained more protein. The nitrogen transfer from the clot during gastric digestion was similar for both milks, meaning that proteins dissociated at the same rate, independent of heat treatment. In the supernatants from raw milk, the majority of the whey proteins were present till the end of gastric digestion, while no whey proteins were observed already after 5 min in heated milk. Intact caseins were not present in the supernatants during digestion, but were observed in the clots of both milks along with intact whey proteins and peptides. This shows that caseins and heated whey proteins remained partly intact throughout infant gastric digestion, but were only present in the clot. Analyzing both the supernatant and clot is important to get a complete picture of gastric milk protein digestion. Caseins in raw and heated milk and whey proteins in heated milk were present in the clot and not completely hydrolyzed during infant gastric digestion.

Dose-Dependent Molecular Transcriptome Analysis of Milk Lactoferrin Intervention on Neurodevelopment and Cognitive Function of Neonatal Piglets

Bing Wang

Professor of Physiology and Nutrition, Charles Sturt University

Lactoferrin (Lf), a sialic acid (Sia)-rich milk glycoprotein, can promote early neurodevelopment and cognition in neonatal piglets. The dose-dependency of Lf intervention, however, remains unknown. The objective of this study was to determine the dose-dependency of Lf on genes associated with neurodevelopment and cognition in neonatal piglets provided a pig milk replacer supplemented with Lf at 155 mg/kg/day (low dose) or 285 mg/kg/day (high dose) from postnatal day 3 to 38. Gene expression profiles associated with neurodevelopment, cognition, and their cognate proteins were quantitatively determined. The following are new findings: (1) The rate of piglet learning and long-term memory was higher with the low dose of Lf, as assessed in an 8-arm radial maze; (2) Global gene transcription profiling showed the low dose of Lf up-regulated genes and functions correlated with neurodevelopment and cognition, while the high dose showed a greater correlation with cellular processes modulating neuroprotection; (3) Expression level of genes and proteins associated with BDNF were higher in both Lf groups. In contrast, the low dose of Lf activated genes associated with BDNF signaling, including SLC6A3, IGF-1, and RACN1; (4) Genes differentially expressed in the five highest networks showed the low dose of Lf was more strongly associated with cellular and neurocognitive development, while the high dose correlated with prevention of neurodevelopmental disorders and neurological-associated diseases. Collectively, these unexpected findings show the low dose of Lf intervention enhances neurodevelopmental and cognitive processes, while the high dose shows greater neuroprotection, findings of potential clinical relevance that have not been previously reported.

Student Award Presentation: Effect of Different K-Casein Isoforms on the in vitro Digestibility and Peptidomics of Released Peptides

Bulei Sheng

PhD Student, Aarhus University

Among bovine caseins, only κ -casein (κ -CN) can be glycosylated, which has different genetic variants resulting in amino acid substitutions and varying degree of glycosylation (AA, 46.9%; BB, 50.9%; AB, 50.0%). Besides, the attached glycans represent five different types, of which three include charged sialic acids. Both glycosylation degree and glycan type could influence the digestibility of both whole milk and κ -CN itself, as glycosylation can hinder enzymatic cleavage. The objectives of this study were to investigate the impact of bovine κ -CN genetic variant, glycosylation degree and its sialylation on the in vitro digestibility and peptide release. The in vitro digestibility investigated by INFOGEST 2.0 protocol and analyzed by SDS-PAGE, degree of hydrolysis and peptidomics by LC-ESI/Q-ToF MS/MS. Substrates were 1) κ -CN skim milk representing AA, BB or AB phenotypes, 2) model studies using isolated κ -CN A, B or E genetic variant and inherent isoforms, before and after desialylation using microbial sialidase. The effect of release of bioactive peptides relative to isoform and sialylation will be assessed by in silico analysis using the Milk Bioactive Peptide Database. Skimmed milk pools representing κ -CN phenotype AA had significantly faster in vitro gastric digestion rate compared with BB and AB milk. Model studies using isolated κ -CN variants A, B and E showed that desialylation accelerated the in vitro gastric digestion rate of κ -CN B, complying with the highest glycosylation degree for this variant. In the intestinal phase, desialylation increased the degree of hydrolysis for all three isolated genetic variants A, B and E. The higher glycosylation degree of κ -CN variant B seemed to hinder in vitro digestibility, probably by steric hindrance, both in milk with BB phenotype and for isolated κ -CN B variant. Desialylation on the other hand promoted digestibility. Therefore, both low glycosylation degree and its desialylation can promote digestibility of κ -CN.



Student Award Presentation: Dietary Cross-Species Communication: Context-Dependent Role of Bovine Milk-Derived Extracellular Vesicles in Cancer Progression

Rahul Sanwani

PhD Student, La Trobe University

The idea of cross-kingdom, species and inter-individual transfer of bioactive compounds via extracellular vesicles (EVs) is a recent avenue. However, the bioactivity and bioavailability of these dietary compounds upon consumption is highly debated. It has been proposed that EVs from diet can be absorbed by consuming organisms, be bioavailable in various organs and exert phenotypic changes. Milk is the most vastly consumed beverage and is an abundant source of EVs that may act as signalosomes. Whether these milk-derived EVs can serve as cross-species messengers and have a biological effect on host organism has been poorly understood. Bovine milk-derived EVs were isolated by ultracentrifugation and OptiPrep density gradient centrifugation. The EVs were characterised by TEM, NTA, quantitative proteomics and RNA-Seq. EVs were orally administered to mice models of colorectal, breast and pancreatic cancer. Primary tumor burden was monitored, and the rate of metastases was measured by imaging and qPCR. Immune cells were analysed by FACS. Mechanistic insights were obtained using quantitative proteomics, confocal microscopy and biochemical experiments. We demonstrated that upon oral administration, bovine milk-derived EVs were able to survive the harsh degrading gut conditions and be bioavailable in peripheral tissues. Interestingly, oral administration of milk-derived EVs reduced the primary tumor burden in various cancer models and attenuated cancer cachexia. Intriguingly, despite the reduction in primary tumor growth, milk-derived EVs accelerated metastasis in breast and pancreatic cancer mice models. Timing of EV administration was critical as oral administration after resection of the primary tumor reversed the pro-metastatic effects of milk-derived EVs in breast cancer. Biochemical and quantitative proteomics analysis highlighted the induction of epithelial-to-mesenchymal transition and senescence upon treatment with milk-derived EVs. Taken together, we were able to demonstrate the capacity of bovine milk-derived EVs in mediating cross-species communication and regulating cancer progression in a context-dependent manner.

Keynote Speaker: Decreasing the Environmental Footprint of our Diet – a Modeling Approach Using Optimeal®

Stephan Peters

Nutrition Science Manager, Dutch Dairy Association (NZO)

Replacing animal-based foods with plant-based foods does not necessarily lower the diet's carbon footprint. Sometimes replacing certain foods leads to counter-intuitive results, because a switch of a few food items can affect nutritional value and the carbon footprint significantly. In addition, a healthy and sustainable diet should also be cultural and economic acceptable for consumers. Altogether, this makes composing a sustainable diet a delicate balance between these factors. Modeling with the quadratic programming tool, developed in the Netherlands, Optimeal® helps to understand the impact of changing food choices on health, ecological impact and food prizes. The results show that the general paradigm 'eat a less animal and more plant-based diet' is not a guarantee to reduce the environmental footprint. Nevertheless, reducing the carbon footprint is feasible by complying to ten eating rules based on these calculations.



IMGVC VIRTUAL 20 SYMPOSIUM 21

The Two Protein Transitions: Plant Proteins Versus Dairy

Adam Drewnowski

Professor, University of Washington

The term nutrition transition refers to incomes-driven shift from traditional diets of starchy staples to diets with more animal foods, added sugar, and fat. The term “protein transition” refers to the associated dietary shifts between plant and animal proteins. Two opposing protein transitions are currently taking place. Poor countries are turning away from plants to more animal products including dairy, eggs and meat in order to improve the quality of their diets. Rich countries propose to replace dairy, eggs and meat with plant-based foods, also in order to improve the quality of their diets. The plant-based EAT-Lancet diet was supposed to serve as the global benchmark for healthy dietary patterns, largely plant based, that served both human health and the environment. However, the concept of sustainability goes well beyond environmental impact and includes four key domains: nutrition, economics, society and the environment. First, the Lancet diet was not affordable by the global poor. Second, greenhouse gas emissions are typically calculated per kilogram of food, any food. Kilogram is not a measure of energy or nutrient density; the carbon cost of different foods ought to be calculated per 2000 kcal/day or better, per 100 g of high quality protein. Recalculating standard data on greenhouse gas emissions in this manner, and taking protein quality into account, places milk and dairy products alongside some plant based staples.

Bifidobacteria-Mediated Immune System Imprinting in Early Infancy

Bethany Henrick

Director of Immunology & Diagnostics, Evolve BioSystems Inc.

Immune-microbe interactions early in life influence the risk of allergies, asthma and other inflammatory diseases. Breastfeeding guides healthier immune-microbe relationships by providing nutrients to specialized microbes that in turn benefit the host’s immune system. Such specialized bacteria have co-evolved with humans and human milk components but are now increasingly rare in modern societies. Here we show that a lack of bifidobacteria, and in particular depletion of genes required for human milk oligosaccharide (HMO) utilization from the metagenome, is associated with systemic inflammation and immune dysregulation early in life. In breastfed infants given *Bifidobacterium infantis* EVC001, which expresses all HMO utilization genes, intestinal Th2 and Th17 cytokines were silenced and IFN γ was induced. Fecal water from EVC001 supplemented infants contains abundant indolelactate. *B.infantis*-derived indole-3-lactic acid (ILA) upregulated immunoregulatory Galectin-1 in Th2 and Th17 cells during polarization. This molecular mechanism provides a functional link between beneficial microbes and immunoregulation during the first months of life.

Development of a Bio-Guided Process to Isolate Antimicrobial Peptides from Dairy Streams

Bruna Paviani

Researcher, University of California Davis

Natural bioactive compounds like milk-derived antimicrobial peptides with multifunctional properties are emerging as promising alternatives to conventional antibiotics. Our group previously demonstrated that whey permeate, obtained after protein isolation, is a source of these valuable peptides - despite having been managed as a waste thus far. This work aimed to develop a pilot-scale process to isolate naturally occurring antimicrobial peptides from dairy streams (using whey permeate sourced from colostrum as a model system) and to perform an initial evaluation of these peptides’ antimicrobial properties. Naturally occurring peptides from colostrum whey permeate were isolated through the optimization of an integrated process that favors maximum lactose hydrolysis, removal of whey proteins, and subsequent fermentation of simple sugars before the final concentration of the peptide pool by nanofiltration. The

fermented and hydrolyzed ultrafiltration whey permeate was concentrated by a 700-800 Da nanofiltration membrane until reaching a 9 concentration factor. Peptide sequences and relative abundances of the peptide-rich pool produced by nanofiltration were determined by liquid chromatography coupled with mass spectrometry (Orbitrap Q Exactive Plus mass spectrometer). A specialized milk bioactive peptide database was used to identify bioactive sequences among the peptides in the pool. Absolute peptide quantification was performed using ion-exchange chromatography. Evaluation of antimicrobial potential was assessed using a model of intestinal Caco-2 cells and an enterohemorrhagic E. Coli (EHEC) challenge. The peptide-rich pool produced at pilot-scale resulted in a peptide concentration 3.7 g/L and nearly 3000 peptide sequences were identified. Among the peptides identified, 36 were found to possess multiple functional activities and in particular, 7 bioactive sequences were predicted to have antimicrobial activity. Importantly, the functional study revealed over 50% reduction in the uptake of the pathogen EHEC by epithelial cells pre-incubated with the peptide pool, compared with no treatment, suggesting that the peptide pool successfully attenuated infection. This integrated bio-guided process can be applied to conventional cheese-whey permeate and other dairy streams for isolation of bioactive peptides providing new options for the dairy industry. The isolated peptides can be incorporated into novel products as key ingredients for health-promoting foods.

Outstanding Early Career Investigator Award: Digestive Survival of Human and Bovine Milk Proteins and Release of Antimicrobial and Immunomodulatory Milk Peptides

(0.50 L-CERPs)

David Dallas

Assistant Professor, Oregon State University

Milk proteins have evolved to benefit the suckling neonate. The extent to which most of these proteins survive within the infant (for human milk) and adult (for bovine milk) remains mostly unknown, and thus their bioactive potential is unclear. For many milk proteins, partial digestion releases fragments—peptides—with known antimicrobial, prebiotic, immune-modulating, calcium-delivery, antihypertensive, and pain-modulating activities. The extent to which these peptides survive within the digestive tract need to be further examined to determine their biological relevance. Our objective is to determine the survival of milk proteins and release of bioactive peptides in the intestine of human infants (human milk) and adults (bovine milk). Milk and infant/adult digestive samples were analyzed using mass spectrometry-based peptidomics, protease assays, ELISA and bioactive peptide database searching to determine how proteins are degraded within the digestive system and which potential bioactive peptides are released. Peptides were tested for function with antimicrobial and macrophage assays. In the stomach and intestine of human infants, both infant-produced and milk-derived proteases actively degrade milk proteins, releasing thousands of new peptides while allowing the survival of some milk proteins. Fractions of intestinal peptides had antimicrobial and immunomodulatory actions. In adults, we demonstrated that a key bioactive ingredient from bovine milk, kappa-casein glycomacropeptide does not survive to the intestine. Peptidomic, proteomic and enzyme analyses enable precise characterization of protein digestion and bioactive peptide release in infants (human milk) and adults (bovine milk). We have identified peptides with antimicrobial and immunomodulatory actions.

Most Valuable Presentation 2020: Breastfeeding Support as a Human Universal

(0.25 L-CERPs)

Katie Hinde

Associate Professor, Arizona State University

As 21st Century scientists decode the exceptional properties of human milk for human babies, sustained messaging to "normalize breastfeeding" aims to remedy the declines in breastfeeding in the 20th Century. Re-evaluating our



understanding of human breastfeeding reveals that the difficulties experienced by women in industrialized settings, such as difficulty with latch, pain, and perceived milk insufficiency are experienced by women in traditional settings and are addressed by culturally embedded and sustained lactation support during the perinatal and post-natal period. The breastfeeding support roles were, and in some cultures remain, most typically filled by female relatives, but are increasingly filled by clinicians. Our findings demonstrate that just as it is important to "normalize breastfeeding," essentially we must normalize breastfeeding support as a key aspect of the human adaptive complex. Patient-centered perinatal and post-natal care that incorporates breastfeeding guidance, especially involving the extended family and friend network, are crucial contributions to improving maternal and child health.

Closing Remarks: From Discovery to Practice

Bruce German

Distinguished Professor, University of California Davis

Jennifer Smilowitz

Director of Scientific and Strategic Development, IMGC

Get together with Dr. German and Dr. Smilowitz as they summarize the highlights from symposium's sessions and discuss upcoming events hosted by the IMGC on lactation and milk science.