IMGC VIRTUAL SYMPOSIUM October 13 – 16, 2020

INTERNATIONAL MILK GENOMICS CONSORTIUM

Program Abstracts

Day 1 – Tuesday, October 13, 2020

Microbiome 2.0

Welcome-Knowledge Mining of Lactation as a Therapeutic Strategy

Bruce German, University of California Davis, CA, USA

The 21st Century's challenges to food are clear: make more, make it more nourishing, make it more safe and make it more sustainable. The practical implications of these challenges are daunting: how to feed 10 billion people, how to produce that food sustainably, how to guide 10 billion diets to prevent disease and how to nourish and fuel human performance through a century-long lifespan. Lactation, the genetic secret to the success of mammalia, has emerged from Darwinian selection over hundreds of millions of years for similar challenges. Scientific research and its translation to commercial utility can use the principles learned under this relentless selective pressure of Evolution. The IMGC has been guided by lactation as the Rosetta stone for diet and nourishment. Lactation is not a simple recipe, it is an encoded dictionary and encyclopedia. Both the 'words' and the 'deeds' of nourishment are intrinsic to the subset of mammalian genomes dedicated to lactation. We have made inspiring discoveries of how milk nourishes infants and published literally thousands of peer reviewed publications. Now we must take those lessons to practice. How can our understanding of milk's nourishment of the infant microbiome inspire products and services that guide the adult microbiome? How can the transfer of immunity from mothers to infants serve as a blueprint for protecting adults from infectious pandemic diseases? How can the efficiency of the transfer of maternal essential nutrients ensuring the sustainability of mothers, inform the agricultural systems on sustainability of the environment?

Keynote Speaker: How to Enrich Specific Taxa within the Gut Microbiome? Lessons from Human Milk (0.5 L-CERPs)

David Mills, University of California Davis, CA, USA

Human milk contains numerous components that shape the microbial constituents of the gastrointestinal tract of infants. A prominent feature of milk is an array of oligosaccharides and glycoconjugates that are proposed to enrich a protective microbiota often dominated by bifidobacteria. Different infant-borne bifidobacteria contain specific glycosidases and transport systems required to utilize milk oligosaccharides and glycoconjugates. This suggests a co-evolutionary relationship between mammalian milk glycans, infant-borne bifidobacteria and the infant host enabling a programmed enrichment of a protective bifidobacterial-dominant community. However, two factors cloud this hypothesis. This enrichment could be driven by a "founder effect" given neonates do not have a significantly formed, or resilient, gut microbiome in early life. In addition, milk also contains numerous other effectors—such as lysozyme and lactoferrin among others—that modulate the infant gut microbiota. This makes it difficult to determine

the proportional influence of milk oligosaccharides in the common bifidobacterial enrichment witnessed in humans. We have identified a number of select bifidobacterial strains that grow vigorously on specific milk oligosaccharides. Provision of wild type adult mice with both these strains and cognate milk oligosaccharides resulted in a dramatic, and persistent, enrichment of some strains to a level witnessed in human infants, albeit the effect was strain specific. The synbiotic enrichment also protected mice in a dextran sodium sulfate colitis murine model and resulted in a systemic lowering of inflammation. This work confirms that milk oligosaccharides are a driving factor in bifidobacterial enrichments in neonates and the synergy between oligosaccharides and specific bifidobacterial strains drives lower inflammation and protection. Moreover, this new model provides a path to decipher the specific genetic elements among bifidobacteria that contribute to both enrichment and protection. Further analysis of this naturally evolved system will shed light on effective pre- and probiotic tools that support a protective gut microbiota for at-risk infants and adults alike.

Metagenomic Survey of the Gut Microbiome of Term Infants in the US Reveals Widespread Dysbiosis, Absence of *B. infantis*, and High Abundance of Antibiotic Resistance Genes
(0.5 L-CERPs)
Giorgio Casaburi, Evolve Biosystems Inc., Davis, CA, USA
Infant dysbiosis is associated with the absence of key infant-associated Bifidobacterium (e.g., B. infantis) known to facilitate the utilization of human milk oligosaccharides (HMOs), increased enteric inflammation, increase in potentially pathogenic bacteria and the abundance of antibiotic resistance genes (ARGs) they harbor. Here, we performed a nation-wide metagenomic survey to profile the current status of the US term infant microbiome.Fecal samples from 227 infants (0-6 months of age) collected across five US states were examined using shotgun metagenomics sequencing. Sequences were analyzed to characterize the taxonomic and functional composition following the Human Microbiome Project guidelines. Bifidobacterium are less abundant among term infants than generally assumed (20%). In contrast, potentially pathogenic species were identified to be the highest abundant species overall, including E. coli (12.6%), Klebsiella pneumoniae (7%), Klebsiella oxytoca (2.5%), Enterobacter cloaceae (2.7%), and Streptococcus agalactiae (Group B Strep; 0.01%). These species are associated with increased risk of sepsis, gut inflammation and were widespread distributed amongst US infants, independently of location, age, and diet. Analysis of the ARG load in the infant microbiomes across the US confirmed a high abundance of ARGs in all five states with signatures of unique ARG composition by state. 54% of the ARGs identified were associated with genes conferring multi drug resistance. Furthermore, the gut microbiome of US infants was limited in human milk oligosaccharide utilization capacity. In fact, B. infantis was missing in 97% of the population resulting in the absence of the majority of known HMO genes necessary to fully capture, transport and metabolize HMOs from breast milk.Considering the role of the gut microbiome in early life in shaping the immune system, protecting against pathogen colonization and maximizing nutrition from selective prebiotic fibers (e.g., HMOs), we found that 97% of infants in this survey had microbiomes which failed to provide these services and could be classified as dysbiotic. Future efforts to correct infant gut dysbiosis in early life will need to address current widespread deficiencies to restore full ecosystem services in term infants.

All oral presentations are available as recordings.
https://milkgenomics.org/

2'-fucosyllactose Supplementation Improves Gut Barrier Function and Lipid Metabolism in High-Fat Fed Mice

(0.5 L-CERPs)

Sunhye Lee, Sun Moon University, Asan, Korea

2'-fucosyllactose (2'-FL), the most predominant oligosaccharide found in human milk, acts as a prebiotic with beneficial effects on the host. The aim of this study was to determine the beneficial effect of 2'-FL on intestinal barrier integrity and metabolic functions in low-fat (LF)- and high-fat (HF)-fed mice. Male C57/BL6 mice (n=32, 8/group; 6 weeks old, JAX, CA) were counter-balanced into four weight-matched groups and fed either a low-fat (LF; 10 % kcal fat with 7% kcal sucrose) or HF (45% kcal fat with 17% kcal sucrose) with or without supplementation of 2'-FL in the diet [10% (w/w), 8 weeks; LF/2'-FL or HF/2'-FL; BASF, Germany]. General phenotypes (body weight, energy intake, fat and lean mass), intestinal permeability (ex vivo in Ussing chambers), lipid profiles, and microbial metabolites were assessed. 2'-FL significantly attenuated the HF-induced increase in body fat mass with a trend to decrease body weight gain. 2'-FL significantly decreased intestinal permeability in LF-fed mice with a trend for a decrease in HF-fed mice. This was associated with a significant increase in interleukin-22, a cytokine known to have a protective role in intestinal barrier function. Visceral adipocyte size was significantly decreased by 2'-FL in both LF- and HF-fed mice. 2'-FL suppressed HF-induced upregulation of adipogenic transcription factors peroxisome proliferator-activated receptor gamma and sterol regulatory element binding protein-1c in the liver. Lastly, 2'-FL supplementation led to a significant elevation of lactic acid concentration in the cecum of HF-fed mice, which is known to be a product from beneficial microbes. 2'-FL supplementation improved gut barrier integrity and lipid metabolism in mice with and without the metabolic challenge of HF feeding. These findings support the use of 2'-FL in the control of gut barrier function and metabolic homeostasis under normal and abnormal physiological conditions.

Update from 2019's Most Valuable Presentation- Breast Milk and Gut Microbiome in Term and Preterm Infants

(0.25 L-CERPs)

Christopher Stewart, Newcastle University, Newcastle upon Tyne, United Kingdom

It is an honour to accept the MVP award and I look forward to presenting a summary of my talk from 2019 alongside some new unpublished data. Following birth, the infant gut is rapidly colonized by a range of microbes that play fundamental roles in health and disease. Unlike infants born at term (>37 weeks gestation), extremely preterm infants (<32 weeks gestation) have immature intestinal architecture and an underdeveloped immune system. Because the preterm gut can become leaky, translocation of microbes into the bloodstream and/or intestinal cell death represent major problems in this vulnerable population. However, certain types of bacteria may promote gut and immune maturation. Our group utilises systems biology, combining microbiology, molecular biology, and biochemistry to comprehensively profile clinical samples, including maternal breast milk and infant respiratory and gut samples. The presentation will focus on the development of the gut microbiome in both term and preterm infants, and I will share recent data combining maternal human milk oligosaccharide profiling with infant gut microbiome development.

Building on from these association-based analyses, I will also talk about a state of the art co-culture system being development, allowing human intestinal stem cell derived enteroids ("mini guts") to be tested under physiologically relevant oxygen conditions. Using this ex vivo model will allow for targeted experimentation, exploring how specific microbes and milk components modulate intestinal barrier integrity and function. Understanding how bacteria and gut epithelial cells interact holds exciting possibilities to better predict, diagnose, and treat infants at risk of disease.

Microbiome 2.0 and Immunity/Autoimmunity and Inflammation: the 21st Century's BIG Challenge

Outstanding Mid-Career Investigator Award: Milk, Nose, Gut: Microbiomes in the CHILD Cohort Study (0.5 L-CERPs)

Meghan Azad, University of Manitoba, Winnipeg, Canada

The CHILD Cohort Study (www.childstudy.ca) is following 3500 Canadian families from pregnancy onwards to understand the developmental origins of chronic diseases. We have shown that breastfeeding is associated with reduced risks of childhood asthma and obesity, and these beneficial effects appear to be partly mediated by the infant gut microbiome. Current research in the Azad lab (www.azadlab.ca) is focused on understanding how breastfeeding practices and breast milk components (including bacteria, fungi, oligosaccharides, fatty acids, hormones and cytokines) shape the developing infant nasal and gut microbiomes and contribute to health and disease trajectories.

Early Colonization with Firmicutes is Associated with More Rapid Infant Weight Gain in the STRONG Kids 2 Cohort

(0.5 L-CERPs)

Sharon Donovan, University of Illinois Urbana, Illinois, USA

The gut microbiome and growth trajectories are established in early life and are influenced by mode of nutrition. The aim of this research was to investigate how feeding mode (breastfed [BF], formula-fed [FF], or mixed-fed [MF]) affects growth trajectories and establishment of the microbiome and to explore potential associations between diet, microbiome composition and growth.Heights, weights and stool samples were collected from infants (n=450) enrolled in the Synergistic Theory and Research on Nutrition and Growth (STRONG) kids 2 longitudinal birth cohort at 1 wk, 6 wks, 3 mos, 1 mo after the introduction of solids, and12 mos of age. Wt-for-length Z-scores were calculated and compared to WHO growth standards. Weight trajectories were identified using semiparametric mixture models. Fecal microbial DNA was extracted and microbiome composition was measured by sequencing the V3-V4 region of the 16S rRNA and microbial diversity and taxonomy were established using QIIME2. Multiple regression was used to determine the association between change in WFLZ and diet and microbiome characteristics. Associations between growth trajectories and microbiome was tested using logistic regression.At 1 week, 70% of infant were BF, 21% were MF and 8% were FF. At 12 months, 48% of infants were BF, 30% were FF, 8% were MF and 14% were receiving cow milk. MF infants received between 8% and 95% human milk. Complementary foods were introduced at 5.5 ±1.1 mos. Three growth trajectories were identified and categorized as low-slow (LS), low-high (LH) and midstable (MS). Beta diversity of the fecal microbiota

differed at all time-points and between BF and FF and MF at all time-points; FF and MF differed only at 1 and 6 wks. Alpha diversity was greater in the LH vs. LS and MS at 1 wk, 6 wk and 6 mos. Infants displaying the LH trajectory (e.g. birth weight < 0 Z-score and rapid growth in the first 6 mos) had greater relative abundance of Ruminococcus gnavus and Blautia) at 1 wk and Clostridium inoculum and Lachnoclostridium 6 wks than infants in the LS and MS trajectoriesThese data suggest that early colonization by Firmicutes is a biomarker of rapid postnatal growth in infants. Potential mechanisms include production of short chain fatty acids by cross-feeding with bifidobacteria.

Outstanding Early-Career Investigator Award: *B. infantis* EVC001 Colonization in Breastfed Infants Modulates Cytokine Profile Linked to Autoimmune and Allergic Diseases

(0.5 L-CERPs)

Bethany Henrick, Evolve Biosystems Inc., Davis, CA, USA

The intestinal microbiome plays a critical role in the development of the immune system, including the development of specific CD4+ T cell subtypes, which can have numerous long-term health consequences. Certain proinflammatory cytokines are linked to the development of autoimmune and allergic diseases, whereas IFNβ has been shown to induce immune tolerance. Given the pleiotropic activities of cytokines, we evaluated fecal cytokines from breastfed infants fed B. infantis EVC001 (EVC001) during the first 60 days postnatally. Stool samples collected at Day 6 (baseline) and day 60 of life from exclusively breastfed infants (n=40) randomly selected to receive either 1.8 x 1010 CFU B. infantis EVC001 daily for 21 days starting Day 7 postnatal (EVC001) or breast milk alone (controls) were analyzed using 16S ribosomal RNA, qPCR, metagenomics, and for fecal proinflammatory cytokines using multiplexed immunoassay. Pairwise correlation tests were performed at day 60 of life between the fecal microbial taxonomic composition and specific enteric cytokine concentrations. Clostridiaceae, Enterobacteriaceae and Staphylococceae correlated with increased proinflammatory cytokine concentrations, and negatively with IFNβ levels. Conversely, Bifidobacteriaceae abundance was the only taxa that correlated with increased levels of IFNβ and negatively with proinflammatory cytokines. Comparison between Bifidobacteriaceae abundance and IFNβ show a direct correlation (P = 9.8e-06, rho = 0.65), while Enterobacteriaceae and Clostridiaceae abundance negatively correlated with IFNβ concentrations (P < 0.01r). At a species level, only B. longum abundance at day 21 correlated with IFNβ levels at day 60 postnatal (P = 0.0028, rho = 0.48). Importantly, infants fed B. infantis EVC001 produced significantly increased IFNβ levels compared to controls day 60 postnatal (P = 0.047). These findings suggest a novel immunomodulatory function of B. infantis EVC001 in breastfed infants and suggest this strain of bacteria may induce CD4+ T cell development critically important in the reduction of autoimmune and allergic diseases.

Difference in Levels of SARS-CoV-2 Spike Protein- and Nucleocapsid-Reactive SIgM/IgM, IgG and SIgA/IgA Antibodies in Human Milk

(0.5 L-CERPs)

Veronique Demers Mathieu, Medolac Laboratories/University of Massachusetts Amherst, USA

SARS-CoV-2 is the novel coronavirus 2019 (2019-nCoV) that is responsible for the severe acute respiratory syndrome (SARS) pandemic that began in 2019. This study evaluated the presence and the levels of antibodies reactive to SARS-CoV-2 spike protein and nucleocapsid. The impact of vaccination with influenza and Tdap and the presence of viral respiratory infection in the past year on the antibody levels were also studied.SARS-CoV-2 spike protein- and nucleocapsid-reactive SIgM/IgM, IgG and SIgA/IgA were measured via ELISAs in human milk samples (collected from 02/30/20 to 04/03/20) from 41 women.SARS-CoV-2 spike protein-reactive SIgA/IgA, SIgM/IgM, and IgG were detected in 97.6%, 68.3% and 58.5% in human milk whereas nucleocapsid-reactive antibodies were detected in 56.4%, 87.2% and 46.2%, respectively. The detection of SIgM/IgM reactive to nucleocapsid in human milk was 1.2-fold higher than SIgM/IgM to spike protein whereas IgG reactive to nucleocapsid IgG was comparable with that of IgG reactive to spike protein. The detection of SIgA/IgA reactive to nucleocapsid in human milk was 1.7-fold lower compared with SIgA/IgA reactive to spike protein. The level of SARS-CoV-2 spike protein-reactive SIgA/IgA in human milk was higher than SIgM/IgM and IgG, which were comparable. Spike protein-reactive IgG in milk from vaccinated women was 2.9-fold higher than unvaccinated women and 3.5-fold higher from women that had symptoms of viral respiratory infection during the last year than women without symptom. Binding activity of SARS-CoV-2 spike protein and nucleocapsid-reactive SIgM/IgM, IgG and SIgA/IgA from human milk was completely inactivated after heat-treatment at 100°C for 30 min.The presence of SARS-CoV-2-reactive antibodies in human milk could provide passive immunity to breastfed infants and protect them against COVID-19 diseases. Human milk antibodies with high polyreactive properties against SARS-CoV-2 and other coronaviruses could be useful to prevent future coronavirus pandemics.

Day 2 – Wednesday, October 14, 2020

Immunity/Autoimmunity and Inflammation: the 21st Century's BIG Challenge

Keynote Speaker: Human Milk and Immune Development Early in Life

(0.5 L-CERPs)

Belinda van't Land, Danone Nutricia Research BV, Utrecht, The Netherlands

Postnatally our immune system is being trained to respond to, and modulate its microbiological environment. Together with the development of a stable host-microbe interaction early in life, infant's immune system matures to full strength. Human milk (HM) is unique in its composition as it covers all nutritional and physiological infant requirements during the first months of life. Immunomodulatory components in HM support the infant in this crucial period by, providing nutrients that contain substrates for the microbiome, supporting intestinal barrier function, protecting against pathogenic infections, enhancing immune development and facilitating immune tolerance. Determining the components in human milk that influence infant health outcomes in terms of both short- and long-term sequelae is complicated by a lack of understanding on the interaction of environmental factors that modify HM constituents and thereby influence offspring outcomes. Therefore, studying the biological activity of components derived from human milk is an area of great interest, essential for our understanding of a healthy development of the microbiome, and immune maturation. Recently we have identified specific immune modulating, as well as mucosal barrier supporting properties of human milk oligosaccharides (HMOS). These data

illustrate our current understanding of how isolated human milk oligosaccharides and specific structures can directly interact with the Dendritic cells during maturation. Which is a key starting point in order to educate our immune system early in life and how to deal and process foreign antigens. These novel insights in immune modulatory effects of human milk obtained by in vitro as well as in vivo studies, add to our understanding on how early life nutrition impacts immune development.

Student Award Recipient: Loss of Allergy-Protective Capacity of Raw Cow's Milk After Heat Treatment Coincides with Loss of Immune Active Whey Proteins

Ling Xiong, Wageningen University and Research, Wageningen, The Netherlands

The allergy-protective capacity of raw cow's milk is abolished after heat treatment. The heat-sensitive whey protein fraction of raw milk is therefore often implied to be the source of this allergy-protective effect. This study aimed at investigating the mechanistic relation between heat damage to whey proteins and allergy development.Raw cow's milk was heated for 30 min at 50, 60, 65, 70, 75, or 80 °C and the native whey protein profile of these samples was determined using LC-MS/MS-based proteomics. The allergy-protective effect of differently heated milk samples were tested in a murine OVA-induced food allergy model. Changes in the native protein profile were subsequently related to the capacity of these milk samples to prevent the development of ovalbumin-induced food allergy.The allergy-protective effect of raw cow's milk is lost after heating milk for 30 min at 65 °C or higher. This loss of protection coincided with a reduction in native immunologically active whey proteins. A substantial loss of native whey proteins was observed from 75 °C. However, whey proteins with immune-related functionalities already started to denature from 65 °C, which coincided with the temperature at which a loss of allergy protection was observed in the murine model. Complement component 7, monocyte differentiation antigen CD14, and polymeric immunoglobulin receptor concentrations decreased significantly at this temperature, although several other immunologically active whey proteins also showed a decrease around 65 °C. Heat treatment at 65 °C for 30 min represents low temperature pasteurization, indicating that this treatment may damage the functionality of raw cow's milk.The current study demonstrates that immunologically active whey proteins that denature around 65 °C are important for the allergy-protective capacity of raw cow's milk. The current study provides a better understanding of the mechanistic relation between heat damage to whey proteins and allergy development, which is essential for the development of microbiologically safe alternatives to raw cow's milk that still retain its protective capacity.

The Effect of 50 Years of Breeding on the Ability of Holsteins to Fight Mastitis

John Lippolis, NADC/ARS/USDA, Ames, IA, USA

The objective of this study was to compare a closed herd of Holsteins maintained with genetic traits common in 1964 (unselected) and modern Holstein (contemporary) cows for their ability to respond to an experimental mastitis challenge. Contemporary (n=7) or unselected (n=5) cows were infected with E. coli by intra-mammary infusion in a single quarter. Bacterial counts, somatic cell counts, rectal temperature, and levels of cytokines (IL-1b and IL-6) and BSA found in milk, were used as metrics to determine infection severity. The bacterial counts in the unselected cows were significantly ($P< 0.05$) lower from 0.25 to 3 days post-infection (PI). The peak infection for both groups of cows was 12 hours PI, and the average bacterial counts at that time were 86,247 cfu/mL for the contemporary and 619 cfu/mL for the unselected cows. Contemporary cows also had significantly higher levels of

BSA, IL-1b, and IL-6 in their milk samples 1- and 1.5-days PI (P< 0.05). Both groups of cows suffered a significant loss of milk production at day 1 PI compared to production before infection (P< 0.05). The magnitude of the average day 1 milk losses for the two genetic groups were 43% for the contemporary cows and 13% for the unselected cows. The contemporary cows also had a significant milk loss on day 2 (P< 0.05), the unselected cows did not (17% and 4% respectively). Cows unselected since 1964 had significantly less severe clinical symptoms during an experimental mastitis challenge compared to contemporary dairy cows. Mastitis resistance in cows is known to have a significant genetic component that may be antagonistic with milk production. Determination of the differences between the unselected and modern Holstein and identification of health traits that may have been lost with years of breeding may allow the reintroduction of health traits with the retention of high milk production.

Evidence of a Significant Secretory-IgA-Dominant SARS-CoV-2 Immune Response in Human Milk Following Recovery from COVID-19

(0.5 L-CERPs)

Rebecca Powell, Icahn School of Medicine at Mount Sinai, New York, NY, USA

The nature of the immune response to SARS-CoV-2 in milk is unknown.This response is critical for infants and young children, but perhaps even more significant is the fact that milk antibodies (Abs) could be purified and used as a COVID-19 therapeutic, given they would likely be of the secretory (s) class and highly resistant to proteolytic degradation in the respiratory tissue. In this report, 15 milk samples obtained from donors previously-infected with SARS-CoV-2 as well as 10 negative control samples were tested for reactivity to the viral Spike Receptor Binding Domain (RBD) and the whole Spike protein by ELISAs measuring IgA, IgG, IgM, and secretory Ab.12/15 samples obtained from previously-COVID-19-infected donors exhibited IgA reactivity to RBD. All 12 were also positive for secretory-type Ab reactivity. The IgA and secretory Ab (SC) OD values for undiluted milk were found to be highly correlated ($r=0.81$, $p< 0.0001$ by Spearman correlation test). It was found that all 12 were also positive for Spike binding; as well, 2 samples which were negative for RBD-reactive IgA exhibited reactivity to Spike. Endpoint dilution values were also determined. It was found that 7/15 were at least 5x above the cutoff. Of the 14 milk samples shown to be SARS-CoV-2-reactive, 4 samples also exhibited IgG and IgM reactivity to RBD . An additional 3 samples exhibited positive IgG reactivity but not IgM, and 1 sample also exhibited IgM reactivity but not IgG. Overall, RBD ELISA OD values of undiluted milk obtained from COVID-19-recovered donors and pre-pandemic controls for each assay were grouped and compared, and it was found that the COVID-19-recovered group mean values were significantly greater for IgA ($p< 0.0001$), secretory-type Abs ($p< 0.0001$), and IgG ($p=0.017$).Overall, these data indicate that there is strong sIgA-dominant SARS-CoV-2 immune response in human milk after infection in the majority of individuals, and that a comprehensive study of this response is highly warranted.

SARS-CoV-2, Breastfeeding, and Human Milk

(0.5 L-CERPs)

Michelle McGuire, University of Idaho, Moscow, ID, USA

The COVID-19 pandemic has disrupted all aspects of life, and among issues of great significance is understanding how SARS-CoV-2 is transmitted. Of particular interest is whether SARS-CoV-2 can be transmitted from mother to infant via breastfeeding. Guidance on this was initially based primarily on application of the precautionary principle rather than evidence. Additionally, initial evidence related to the presence/absence of SARS-CoV-2 in milk produced

by infected mothers was riddled with inadequate methods of milk collection and analysis. To date, 40+ studies investigating potential transmission of SARS-CoV-2 during breastfeeding have been published. Emerging data suggest that a small proportion of milk produced by infected women contains evidence of SARS-CoV-2, although its viability has not been demonstrated. Work from our group (which will be presented) suggests that SARS-CoV-2 RNA is not in milk but can be detected in some breast swabs obtained prior to cleaning, which may help explain discrepancy in reported results. Our data also documents that IgA and IgG targeting SARS-CoV-2 and common coronaviruses can be detected in milk within 7 d of maternal diagnosis, and levels of IgA targeting the receptor binding domain is correlated with milk's ability to neutralize the virus. **Galvanizing to Promote Rigor:** Importantly, teams of human milk researchers around the globe have galvanized to not only meet on a regular basis and conduct collaborative science, but also construct and disseminate "best practice" documents outlining milk collection, handling, and analysis methods for both COVID-19 research and that which will be needed for future pandemics. These documents will be presented. **A Call to Action:** We call upon human milk researchers to coordinately demand that public health and funding agencies develop plans to proactively prepare for studying vertical transmission of novel pathogens immediately upon emergence of future outbreaks, epidemics, and pandemics.

Milk Structure-Function: Molecules in the Matrix

Posttranslational Modifications of αS1- and k-Caseins can be Affected by Feed Protein Source and Stage of Lactation

Nina Poulsen, Aarhus University, Aarhus, Denmark

Variations in major milk proteins and their post-translational modifications are largely under genetic influence. Detailed analyses of specific phosphorylation isoforms of both αS1- and αS2-CN have previously revealed that even within the same protein, different regulatory systems may be at play for low and high-phosphorylation forms, respectively (Fang et al., 2016). However, non-genetic factors may also play a role, despite having been less studied. We have examined milk protein composition through a controlled feeding experiment (incomplete balanced Latin square design), addressing the effect of different diets, based on faba beans, rapeseed meal or soybean meal as P and protein sources, under typical Danish management conditions.LC-ESI/MS proteomic analysis was applied for identification and relative quantification of major proteins and their isoforms in milk samples from 24 cows sampled in four periods, where each cow was fed one of the 3 diets in each period. Cows were blocked by lactation stage in early and mid-lactation (23.3 ± 6.7 and 176 ± 15 days in milk, respectively, at the beginning of the experiment). Significant effects of feed protein source were observed on level of αS1-CN 9P, on unglycosylated κ-CN concentrations, as well as for the calculated phosphorylation degree of αS1-CN (PD) and the glycosylation degree of κ-CN (GD). To our knowledge, we are the first to document that feed protein source affects content of αS1-CN phosphorylation isoforms, suggesting an effect on αS1-CN 9P and PD, but not on αS1-CN 8P. Furthermore, although only significant for αS1-CN 8P, we found a lower relative concentration of αS1-CN 8P and higher αS1-CN 9P (and thus higher PD) in cows in mid-lactation compared to cows in early lactation. In depth MS analysis of specific phospho-forms of both αS1-CN and αS2-CN identified additional rare, low abundant isoforms (7P of αS1-CN; 10P, 13P, 14P of αS2-CN) and high heterogeneity of specific κ-CN isoforms (up till 2 phosphorylations and 3 glycosylations). In near future, these components will be related to lactation stage, genotype, and feed protein source.The underlying

mechanisms affecting these forms are again evidently different and our results here suggest that both management and physiological factors can influence PD of the caseins, as exemplified here for αS1-CN.

Student Award Recipient: Milk Peptides in the Intestinal Tract of Breast Milk Fed Infants Have Antimicrobial and Bifidogenic Activity

(0.25 L-CERPs)

Robert Beverly, Oregon State University, Corvallis, OR, USA

Many bioactive milk peptides have been identified with health benefits for the infant. However, for these peptides to be relevant to the infant, they must be released from their parent protein and resist further digestion until they reach their site of activity in the small and large intestine. Little is known about the digestion of milk proteins in the infant intestinal tract. We hypothesized that the natural digestion of milk proteins in the duodenum releases peptides with antimicrobial and bifidogenic activities.Preterm and term infants at Doernbecher Children's Hospital in Portland, OR were fed fortified human milk through a nasogastric tube. Samples were drawn through a nasoduodenal tube in the small intestine as the feed passed through the stomach. Milk peptides were extracted using sterile methods from the intestinal samples. Activity of the bulk peptide extracts was determined by measuring growth of E. coli, S. aureus, and B. infantis after 18 hr incubation with serial dilutions. Milk peptides were identified using mass spectrometry, and the peptide profiles of active and inactive samples were compared to identify candidate bioactive peptides for synthesis. The antimicrobial and bifidogenic activities of synthetic peptides were determined by measuring bacterial growth after incubation with serial dilutions over time.We extracted peptides from 29 intestinal samples collected from 16 infants. The peptides were identified with peptidomics and screened for bioactivity. Five samples had significant antimicrobial activity against S. aureus and six samples had significant bifidogenic activity for B. infantis. From these samples, we narrowed down a list of 6,645 milk peptides to 11 candidate peptides for synthesis from various human casein and whey proteins. These peptides inhibited growth of several pathogenic bacteria and stimulated growth of commensal Bifidobacteria.This study provides vital evidence for the health benefits of bioactive milk peptides in vivo. We have shown that the peptide extracts of human milk-fed infant intestinal fluid can inhibit the growth of pathogenic bacteria and stimulate commensal bacteria. Furthermore, we have identified novel human milk bioactive peptides that are released within the infant gastrointestinal system.

Keynote Speaker: On the Self-Assembly of Milk Lipids During Digestion

(0.5 L-CERPs)

Benjamin Boyd, Monash University, Victoria, Australia

Milk is far from a static material once it is ingested, with digestion of milk triglycerides down to fatty acids and monoglycerides being a key transformation to enable absorption of these fat components and lipid soluble nutrients. From a physical chemistry perspective the formation of these more polar lipids stimulates transformation from disordered triglyceride droplets through a range of highly ordered self-assembled structures. We have developed methodologies to track these transformations directly during lipid digestion using small angle X-ray scattering. The structures that are formed are highly dependent on the source and composition of the milk by virtue of differences in lipid composition, and the phenomena has presented opportunities to determine simplified lipid mixtures that mirror the behaviour of the more complex natural triglyceride mixtures. Enhancing our understanding

of lipid self-assembly in milk and milk-like systems is emerging as an important step towards improved design of nutritional supplements and harnessing key biological interactions with enzymes and potentially the microbiome.

Day 3 – Thursday, October 15, 2020

Milk Structure-Function: Molecules in the Matrix

Outstanding Mid-Career Investigator Award: Milk Fat Globule Size; Mechanism of Regulation and Physiological and Health Implications

Nurit Argov-Argaman, Hebrew University of Jerusalem, Rehovot, Israel

Milk fat globule (MFG) size ranges over three orders of magnitude, from less than 200 nm to over 15 m. The significance of MFG size derives from its tight association between structure and composition which may result in different metabolic response for its digestion.. To explore the physiological and health importance of size, we examined whether MFG size affects skeletal development and metabolism in young rats. Under protein deficient diet, small but not large MFGs improved efficiency of protein utilization and bone architecture. We also examined the effect of MFG size on proliferation, survival and biofilm formation of probiotic bacteria, Bacillus Subtilis and Lactobacillus plantarum. Exposing probiotic bacteria to large MFG induced biofilm formation whereas exposure to small MFG increased survival rates of these bacteria strains. The above mentioned size effect might be attributed to compositional differences between MFG of different sizes. Small MFG have relatively higher content of membrane compared to large globules, and this membrane exerts diverse positive health effects. Studies on the size regulation of MFG are scarce, although two distinct mechanisms were found to regulate size; coregulation of fat content and triglyceride-synthesis capacity of the mammary epithelial cells (MEC), and fusion between intracellular lipid droplets, prior to their secretion from MEC. The latter is partially controlled by the membrane's polar lipid composition and activity and expression of mitochondria enzymes. To conclude, studies on size regulation of MFG suggest that metabolic signals regulating mitochondrial numbers and activity, intracellular lipid droplet assembly and size, and the kinetics of lipid droplet secretion, all play a role in MFG size regulation. The association between size and composition and the effect of structure on MFG digestion, absorption and postprandial metabolism affect probiotics bacteria survival and biofilm formation and the physiology, metabolism and bone health in young animals on deficient diet.

Dose-Dependent Improvement in Glucose Metabolism Mediated by Casein-Sugar Maillard Reaction Products

Robert Ward, Utah State University, Logan, UT, USA

Studies suggest glycated milk proteins may promote metabolic dysregulation. However, in these studies, whole diets are subjected to heat processing, and vitamin degradation or lipid oxidation may contribute to the metabolic stress. Our goal was to provide mice with casein-sugar blends with increasing levels of Maillard Reaction Products (MRPs) and determine the effect on glucose metabolism in diets that were otherwise identical.MRPs were generated via heat processing of casein-sugar blends. MRPs were incorporated into a model rodent Western Diet, and fed to mice for 8 weeks. Oral glucose tolerance was measured at week 6 and insulin sensitivity at week 7. At week 8 mice were sacrificed at cecal contents were collected for short chain fatty acid and microbiome analysis. As

a complementary experiment, whole diet pellets were heated to generate MRPs using protocols from prior rodent experiments, and lipid oxidation was determined.There were no difference in fasting glucose, but the MRP content of the diets was associated with an increase in oral glucose tolerance, and was also associated with increased insulin sensitivity. Systemically, dietary MRPs were associated with lower plasma levels of macrophage inflammatory protein 1 alpha (MIP1a). In fecal samples, MRPs were associated with a dose-dependent decrease in the genus Lactococcus and the genus Akkermansia, but butyrate and propionate levels were increased. Dietary MRPs were associated with elevated hepatic expression of PGC1a, and adipose expression of PPARgamma, which may partially explain the metabolic effects. When diet pellets were baked, lipid oxidation increased substantially.As milk is a food with both proteins and reducing sugars, heat processing can increase the levels of MRPs. Studies with isolated carboxymethyllysine (CML) and baked rodent diets have implicated CML in the promotion of metabolic dysregulation. However, we hypothesize that the results of previous rodent studies were due to lipid oxidation products, and that MRPs, at least in isolated casein, may actually improve metabolism via interaction with the microbiome. Further studies are warranted with other commodity proteins to determine whether this is a specific MRP or casein-derived MRP effect.

Comparative Biology: Mining the Wisdom of Evolution

Outstanding Mid-Career Investigator Award: Co-Organization of Complex Systems: Mother's Milk and BioBehavioral Development in a Non-Human Primate Model

(0.5 L-CERPs)

Katie Hinde, Arizona State University, Tempe, AZ, USA

Variation in maternal behavior has been demonstrated to influence somatic, metabolic, immunologic, neurologic, and behavioral development in offspring. In mammals, virtually all these aspects of offspring development closely depend upon maternal provision of nutrients and bio-actives through milk during infancy. Previous research highlighted that natural variation in milk energy content affects offspring somatic growth and temperament during infancy, but the long-term influences of maternal nutritional effort on offspring phenotype remain underexplored in long-lived socially complex mammals. Here we explored how early life maternal nutrition is associated with brain activity and social behavior in male and female adolescent rhesus macaques. Thirty-two (13 males and 19 females) rhesus macaques were studied during infancy for mother's milk provisioning and for follow-up in adolescence for social behavior and neuroenergetics, specifically the subjects underwent neuroimaging through combined Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) to assess glucose metabolism in areas of the brain that regulate emotional state and behavioral responses. Our results reveal long-term associations between milk received during early development and both social behavior and brain activity in areas known to regulate emotion and behavior. We evaluated glucose uptake in the anterior cingulate cortex, medial prefrontal cortex, amygdala, hippocampus, nucleus accumbens, caudate nucleus, putamen, and hypothalamus. Milk was differentially associated with adolescent neuroenergetics and behavior for males and females. Males were seemingly more sensitive to milk characteristics and a longer window of sensitivity. Additionally, for females, maternally inherited social rank predicted the initiation of social interactions and total activity in the social group, these behavioral outcomes in males were primarily associated with milk received during infancy and neuroenergetics. These new findings suggest early life organization orchestrated by mother's milk may last well after the period of infancy in long-lived social species and extend our understanding of sex-differentiated sensitivities during ontogeny.

All oral presentations are available as recordings.
https://milkgenomics.org/

Prediction and Characterization of Regulatory Long Non-Coding RNAs in Virgin and Lactating Cow Mammary Gland

Yue Xing, Texas A&M University, College Station, TX, USA

Identify and characterize Long-non-coding RNA (lncRNA) regulating cattle lactation. lncRNAs are none-protein-coding functional RNA molecules larger than 200nt that regulate gene expression at transcriptional and post-transcriptional levels.Mammary gland RNA-seq data from Holstein-Frisian animals at peak lactation and virgin were used to predict lncRNAs, using FEELnc. Differential expression analyses were conducted between lactation and virgin. LncRNA overlap with lactation QTL and conservation in other mammals was determined. Weighted gene coepxression network analyses (WGCNA) were conducted to identify correlations between gene modules and 1) lactation or virgin state, and 2) differentially expressed lncRNAs. Furthermore, gene sets containing differentially expressed genes, genes from important WGCNA modules, genes overlapping lactation QTL, and positional partner genes, were used as candidate genes to conduct Regulatory Impact Factor Analysis (RIF) and PCIT analysis. Pathway and GO enrichment analyses were conducted for gene modules, and lncRNA correlation partners from lactation and virgin networks.2210 lncRNA transcripts (1500 loci) were predicted. 88 loci (102 transcripts) were new lncRNA genes. A total of 373 differentially expressed lncRNA genes (636 transcripts) were identified in the two studies. 55 lncRNA and 117 positional partner RNA genes overlapped with lactation QTLs. 39.4% and 53.2% of lncRNA transcripts were conserved in mouse and human respectively. Of these, 45.3%, 53.9% had lncRNA as biotype respectively. 58.9%, and 68.7% of the partner RNA transcripts were conserved in mouse and human respectively, more than 67% of these are protein coding. Based on the RIF-PCIT constructed networks, 69 lncRNA transcripts are key regulators in lactation and 116 in the virgin. Of these, 10 and 16 were transcripts from predicted new lncRNA genes respectively. A proportion of the key regulators' network partners were in the same WGCNA gene module. 100 out of 185 key regulators were conserved in at least one other mammal, several have known mammary gland function. Key regulatory lncRNA regulate genes involved in milk protein and phosphoprotein. We have identified regulatory lncRNA that potentially regulate genes with important functions in lactation and half are conserved in other mammals.

Comparative Biology: Mining the Wisdom of Evolution

Keynote Speaker and Career Award: Exploiting the Evolution of Lactation: Does the Marsupial Provide a Model for Developing Innovative Strategies to Improve Health Outcomes of Preterm Babies?
(0.5 L-CERPs)

Kevin Nicholas, Monash University, Victoria, Australia

Significantly preterm babies in the neonatal intensive care unit (NICU) often fail to thrive and have significant rates of mortality. This is largely due to limited development of functional organs that present demanding challenges for treatment. Examining the evolution of lactation and the role of milk in changing reproductive strategies is providing a better understanding of why mature breast milk together with a nutrition-based fortifier is not optimal to support improved health outcomes for preterm babies. Marsupials such as the tammar wallaby evolved about 150 million years ago. They have a short gestation and give birth to an altricial, ectothermic young with an undeveloped immune system. The newborn lungs are too immature for gaseous exchange and respiration occurs through the skin, CNS development is similar to a 6-week human foetus and the stomach is composed of a simple single layer of columnar epithelial cells. The tammar neonate receives milk exclusively and in contrast to preterm babies

progressively develops into a healthy adult. During the first 100 days after birth the tammar neonate remains in the pouch and attached to the teat. The mother provides milk that slows growth, provides antibacterial molecules to reduce infection and delivers a sophisticated program of changes in milk composition to signal the development and function of primary organs. When the young is more independent the mother changes milk composition to promote significant growth. This is exactly the program required in the NICU for treatment of preterm babies. Significantly, attempts to foster tammar young at an early stage of development with the equivalent of mature milk were unsuccessful. The majority of mammary genes coding for putative signaling molecules in tammar milk during the first 100 days postpartum were also expressed in cells from human colostrum, offering new insights into developing the next generation fortifier that addresses both nutrition and functional development of preterm babies.

Student Award Recipient: Patterns in Milk Oligosaccharides Across Species: Building a Platform to Visualize and Compare Free Oligosaccharide Profiles in the Milk of Humans and Other Mammals

Sierra Durham, University of California Davis, CA, USA

Human milk oligosaccharides (OS) have received considerable attention due to their prebiotic, pathogen decoy and immunomodulatory activities in breastfed infants. Commercially available infant formulas currently contain, at most, a few OS due to the difficulty in synthesizing more structurally complex human milk OS at scale. Other mammalian milks, however, naturally contain a diverse array of OS structures with the potential for commercial isolation and supplementation in infant formula to provide the benefits of human milk OS to infants without access to sufficient breast milk. Our objective was to create a platform to compile milk OS profiles of mammalian species to facilitate their comparison and determine which combination of milks would best mimic the human milk OS profile for potential commercial isolation.A queryable database was constructed to compile the published data on milk OS of 72 mammalian species, and the assembled data was visualized using CmapTools software. Milk OS profiles from 158 studies published between 1970 and 2019 were extracted, parsed into data sets and queried across varied parameters. Particular focus was given to commercially milked species.A total of 485 OS isomers were identified across all profiled species. No two species were found to have identical milk OS profiles. While the milks of some species such as the island flying fox and platypus were identified to have very minimal overlap with human milk OS, others including dromedary camels were found to have milk OS with similar defining structural characteristics as human milk OS.Although there are no individual species with the same pattern and diversity of OS structures as human milk, combining OS isolated from a few commercially milked species could provide a product with considerable homology to human milk OS profiles. Such a product could be used to supplement formula, making it more similar to mother's milk and providing valuable health benefits for the infant. Future versions of this database will include other bioactives, automatic update via natural language processing of articles as they are published, and online queriability and visualization. Such a system will provide end users with guidance for development of precision nutrition food products, as well as novel sources for their biomolecular constituents.

Artificial Intelligence for the Future of Agriculture, Food, and Health

Keynote Speaker: Developing a Virtual Dairy Brain: The Next Big Leap in Dairy Farm Management Using Coordinated Data Ecosystems and Artificial Intelligence

Victor Cabrera, University of Wisconsin Madison, WI, USA

Data pervades the dairy farming industry. However, specific data streams are most often ad-hoc and poorly linked to each other and to decision making processes. It is imperative to develop a system that can collect, integrate, manage, and analyze on- and off-farm data in real-time for practical and relevant actions. Hence, we are developing a real-time, data-integrated, data-driven, continuous decision-making engine: The Dairy Brain by applying Precision Farming, Big Data analytics, and the Internet of Things. This is a trans-disciplinary research and extension project that engages multi-disciplinary scientists, dairy farmers, and industry professionals. We have a four-part strategy: (1) Create a Coordinated Innovation Network (CIN) to shape data service development; (2) Create a prototype Agricultural Data Hub (AgDH) to gather/disseminate multiple data streams relevant to dairy operations; (3) Build the Dairy Brain – a suite of analytical modules that leverages the AgDH to provide insight to the management of dairy operations and serve as an exemplar of an ecosystem of connected services; and (4) Design and execute an innovative Extension program. We will illustrate our Dairy Brain concept with a few practical applications. The vision for the Dairy Brain is a real-time analytical engine capable of performing longitudinal historical analyses and forecasting future from past information in a continuous loop. This is a working task in progress. Here, we prove our concept, with 3 DST as exemplars that demonstrate the feasibility and value added of continuous application of integrated real-time data: 1) Continuous nutritional grouping; 2) Permanent assessment of early risk of clinical mastitis; and 3) Continuous prediction of onset of clinical mastitis. We are successfully implementing a real-time, data-integrated, data-driven, continuous decision-making engine: The Dairy Brain. Our framework includes an AgDH that connects cow, herd, and farm live data streams. We demonstrate our concept with practical dairy farm applications. Tomorrow's dairy industry will be built on the effective capture and integration of more data streams, not fewer. This is a critical moment to develop the structures that can move the industry towards modernized data exchange.

Comparing Machine Learning Algorithms for Prediction of Clinical Mastitis in Early Lactation

Liliana Fadul-Pacheco, University of Wisconsin Madison, WI, USA

The amount of data generated on farms is increasing exponentially with the adoption of new technologies. In addition, techniques as machine learning, are helpful to analyze continuous and large amounts of data to predict outcomes. Therefore, data integration and machine learning algorithms can be used to monitor cows' health. The objective of this study was to compare different machine learning algorithms to daily predict clinical mastitis (CM) in early lactation.Records from 2 different data streams, milking system and management software were integrated in a farm from 2016 to 2018. The analysis was limited to 1 to 150 days after calving and for cows with 2 or 3 daily milkings (n=484,781 records from n=2,563 cows). Lactations were grouped in 1, 2 and 3+. One of the limitations of the data was the low number of CM cases (715) which accounted for only 1% of the records, therefore balancing the data was necessary. With the resulting dataset, three different classification machine learning algorithms were tested: random forest, gradient boosting and support vector machine.Significant variables for the onset of CM were the difference of milk production and milk conductivity between milkings, lactations, days after calving, age at first

calving and previous diseases events (retain placenta, metritis, abortion or ketosis). The random forest showed the best performance overall. Also, best results were achieved using data from the 7 previous milkings before the reported case of CM. For random forest the specificity was 0.88 and sensitivity 0.97, whereas for the gradient boosting and support vector machine results were the opposite, a lower specificity (0.65 and 0.66, respectively) but a higher sensitivity (0.91 and 0.99, respectively). Cows with CM compared to healthy cows had higher absolute mean difference for milk conductivity (0.91 vs. 0.86 mS/cm) and milk production (2.14 vs. 1.95 kg/milking), respectively.Results show that the random forest algorithm outperformed gradient boosting and support vector machine and predicted reasonably well cases of CM 7 milkings before onset. It could be used as a monitoring tool to flag cows that are at risk of CM and follow up them closely in a preventive way. Likewise, the integration of other data streams as genetics, sensors and diet, could help improve the prediction power.

Day 4 – Friday, October 16, 2020

Artificial Intelligence for the Future of Agriculture, Food, and Health

Outstanding Mid-Career Investigator Award: The Future of AI in Nutrition Research

Danielle Lemay, Western Human Nutrition Research Center, USDA, Davis, CA, USA

Software and hardware for artificial intelligence (AI)—machines mimicking human intelligence on defined tasks—have improved dramatically, enabling advances in new areas such as agriculture. We are currently using machine learning algorithms on multiple projects in a USDA nutritional phenotyping study to discover relationships between diet and human health. We use models for both explanatory purposes (e.g., what are the most important dietary predictors of health outcomes?) and predictive purposes (how will a change in diet impact health?). AI is poised to influence two grand challenges in nutrition: to accurately assess what we are eating and to predict the impact of food on our health. Today nutrition researchers use antiquated methods to assess diet, but AI assistance would enable continuous dietary assessment in real-time. In the area of personalized nutrition, machine learning models are being built to predict health outcomes such as glycemic and triglyceride responses to challenge meals. Much more data is needed and many challenges remain, but AI will eventually change what we eat and why.

Artificial Intelligence Reveals Key Biomarkers of Necrotizing Enterocolitis in the Preterm Infant Gut Microbiome

Sufyan Kazi, Evolve Biosystems Inc., Davis, CA, USA

Necrotizing enterocolitis (NEC) is an intestinal disease that affects premature infants, causing an inflammatory process that can lead to intestinal tissue damage and death. NEC is a leading cause of overall infant mortality in the United States, affecting 0.1% of newborns per year in North America while reaching treatment costs of up to $200,000 per patient. Although outcomes related to prematurity have remarkably improved, the mortality rate for NEC has remained constant at up to 50% or more depending on severity. The major limitation in NEC prevention lies in identifying the primary molecular drivers that lead to disease onset and identifying related biomarkers in high-risk premature infants. Recently, gut dysbiosis has emerged as a major contributing factor in the development of NEC, supported by the fact that NEC cannot be reproduced in germ-free animals. Here we present a new, non-invasive approach that combines functional and taxonomical data from infant gut microbiomes coupled with machine

learning techniques to identify biomarkers of NEC.A total of 1,712 raw publicly available shotgun metagenomic datasets were collected, (preterm infants with NEC=253; healthy preterm infants =1,459). Taxonomic and functional analyses were carried out and the dataset was divided based on corrected gestational age (cGA). Several machine learning pipelines were developed to distinguish between NEC and preterm control microbiomes. ML-based ranking algorithms and several statistical methods were implemented to identify core biomarkers of NEC.The 29-32 weeks cGA population reported the highest level of prediction accuracy among models (up to 99.8%). Intersection of multiple models led to the identification of 33 microbial proteins (out of 6.1 million) capable of discriminating NEC from healthy preterm infants with 100% accuracy. The most discriminatory bacterial species were Enterobacter cloacae and Klebsiella spp.This is the first AI model capable of identifying causative NEC biomarkers from microbial signatures with high accuracy and generalizability. Future efforts to minimize the frequency and severity of NEC should focus on reducing exposure to risk factors, including bacterial biomarkers by using safe and stable interventions such as microbial gut modulation.

Innovative Technologies in Milk Science for Human Health

Keynote Speaker: Characterization of Fiber in Human Milk and Weaning Foods

Carlito Lebrilla, University of California, Davis, CA, USA

Human milk contains nondigestible fiber in form of human milk oligosaccharides. As the infant's diet transitions from human milk to weaning foods, the carbohydrates change from human derived to plant derived structures. In this presentation, the development of tools that characterize carbohydrates, in its various form from human milk to weaning food, will be discussed. Recently developed high throughput methods are used to create a map of the food glycome. Monosaccharide compositions provide absolute abundances of different foods. Linkage analysis yields the linkage compositions. Multiple LC-MS methods (3-dimensional LC-MS methods) provide monosaccharide compositions, glycosidic linkages, and polysaccharide compositions of foods. The methods provide unique opportunity to understand the bioactive role of foods by knowing their deep chemical structures.

A One-Year Longitudinal Quantification of Human Milk Oligosaccharides: Insights for Infant Formulas and Milk Banks

Randall Robinson, University of California Davis, CA, USA

Interest in the health benefits of human milk oligosaccharides (HMOs) is leading to the use of synthetic HMOs in infant formulas and the development of human milk banks worldwide. For HMOs to reach their desired efficacy in these applications, the ability to document and mimic the HMO dosage an infant receives from their mother is critical. The objective of this study was to monitor HMO profiles over one year to develop a long-term longitudinal dataset. HMO data was also compared against maternal genotype to deduce relationships with HMO expression. The study used a novel dilute-and-shoot analytical approach to quantify HMOs rapidly and inexpensively.A total of 167 term milk samples were analyzed from 71 mothers from the Cambridge Baby Growth Study, UK. Collection timepoints were at 2 weeks, 6 weeks, 3 months, 6 months, and 12 months postpartum. Milk was diluted, filtered, and analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection. A selection of the most abundant HMOs were quantified, including 2'-fucosyllactose (2'-FL), 3-fucosyllactose (3-FL), lacto-N-fucopentaose I, lacto-N-tetraose (LNT), lacto-N-neotetraose, 3'-sialyllactose (3'-SL), and 6'-sialyllactose.Most HMO concentrations decreased significantly during the first 3 months of lactation, with more gradual changes

following. 3'-SL was among the exceptions to this trend, with concentrations that steadily increased over one year. Maternal genotype significantly influenced 3-FL and LNT concentration and accurately predicted α1-2-linked fucosyloligosaccharide expression, except for two secretor mothers who produced very little 2'-FL. Although HMO extraction steps were minimized, the method provided high recoveries and precision, and the results matched well with an in-house comparison to more common techniques.HMO abundance varied substantially by maternal genotype, with prominent temporal changes occurring during the first 3 months. Other studies, which used more extensive sample clean-up procedures, reported substantially different values for these 7 HMOs. Cross-lab validation studies are needed to reach a consensus on HMO concentrations across lactation and on the analytical methods best suited for HMO analysis and the study of maternal genetics.

Innovative Technologies in Milk Science for Human Health

Keynote Speaker: Omics for Discovering New Value in Milk: New Opportunities and Challenges

Daniela Barile and Gulustan Ozturk, University of California, Davis, CA, USA

Milk has evolved as the ideal food for providing postnatal nutrition for healthy newborn development and, therefore, is a remarkable model to study. Milk bioactive compounds play an important role in modulating gut health and has become an intensive research area. Particular attention is focused on functional compounds including oligosaccharides, glycoproteins, and milk fat globules (MFG). Milk oligosaccharides are highly concentrated in human milk but are much less abundant in other animal milks, and their presence in the dairy marketplace is scarce. With improved production and isolation strategies, these compounds could be recovered from dairy processing streams for use as ingredients in infant formula and adult therapeutics. By using liquid chromatography coupled to mass spectrometry (LC-MS) and other techniques, oligosaccharides can be measured accurately and reproducibly, enabling confident scaling and processing decisions to be made when considering novel ingredient manufacturing. Currently, little is known about variations in oligosaccharides (OS) production among dairy cattle and the factors that impact their abundances, such as breed and diet. Therefore, it is important to develop methods for measuring milk OS abundance in large sample sets by implementing novel techniques for high-throughput profiling. Examining the influence of genetics and diets on bioactive compound formation will provide insights for increasing liquid milk value. Dairy processing streams such as whey protein phospholipid concentrate (WPPC) are currently an under-utilized. A detailed examination revealed that WPPC represents not only a great source of bioactive proteins but also a more functional vehicle for the delivery of small MFG. The combined use of new "*omics*" enables researchers to achieve a molecular-level understanding of functional compounds in milk. It guides the development of engineering methods to capture value from our food side-streams, improving the agricultural enterprise's sustainability. This talk will present some case studies demonstrating how various dairy streams could be valorized, particularly regarding the prebiotic and more functional bioactive compounds content and used to promote health.

Student Award Recipient: Differences in the Self-Assembly of Lipids in Human Colostrum and an Emulsified Colostrum Lipid Mixture During Digestion

(0.25 L-CERPs)

Syaza Binte Abu Bakar, Monash University, Victoria, Australia

Colostrum contains lipids and bioactive proteins that can stimulate the development of organs and prevent

diseases in infants. Surprisingly, there have been no reports of colostrum being assessed in the context of lipid digestion, which is critical in the transport of lipophilic nutrients. Hence, this project aims to understand the differences in the self-assembly of lipids during the digestion of human colostrum and emulsified colostrum-mimicking lipid mixtures and the potential interactions with bioactive proteins.Human colostrum samples were digested under intestinal conditions. The in vitro digestion model was coupled to small-angle X-ray scattering at the Australian Synchrotron, enabling acquisition of phase formation as a function of extent of digestion. Emulsified colostrum-mimicking lipid mixtures were formulated by weighing known amounts of triglycerides and dispersing the lipids with a buffer.Prior to digestion, a lamellar phase was present in human colostrum caused by the formation of calcium soaps due to self-digestion by the breast milk's own bile salt-stimulated lipase (BSSL). In contrast, a lamellar phase was not evident in the emulsified colostrum mixture before the start of digestion due to the absence of BSSL and only grew once digestion was initiated. However, a cubic phase was observed as digestion progressed for the emulsified mimic, but not colostrum. The difference in the phases formed could be related to a greater extent of digestion in the emulsified colostrum mixture leading to the formation of non-lamellar phases.In situ monitoring of the lipid liquid crystalline structures formed during the digestion of emulsified colostrum mixture revealed additional phases formed due to differences in extent of digestion as compared to human colostrum. Further studies to increase extent of digestion and consequent interaction with bioactive proteins will elucidate the role of lipid structuring in the overall function of human breast milk.

Single Cell RNA Sequencing of Human Milk Derived Cells Identifies Heterogeneity Among Mammary Epithelial Cells

Jayne Martin Carli, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Cells present in human milk hold potential for non-invasive investigation into the lactating human mammary gland. It has been reported that milk derived cells comprise luminal and basal mammary epithelial cells (MECs), MEC progenitors, mammary and pluripotent stem cells, as well as immune cells. Single cell RNA sequencing (scRNA-seq) technology applied to the cell populations present in human milk provides a means to understand maternal phenotype during lactation. However, challenges in milk cell collection for these studies exist, including coordinating clinical milk collections with single cell capture and library preparation, and methods to process scRNA-seq data.Here, we explore cryopreservation and cell sorting for live cells as a strategy to increase flexibility in milk cell collection timing and their processing for scRNA-seq application. We comprehensively characterized 4,604 cells from 2-week postpartum milk samples by scRNA-seq.By scRNAseq, we identified primarily LALBA and CSN2 expressing luminal MECs in human milk, which were positive for KRT8 and KRT18. We found very few KRT14+ cells, indicating an absence of basal MECs. Further analysis of luminal MECs identified a subpopulation expressing high levels of FASN as well as milk fat globule docking component transcripts, XDH and BTN1A1, suggesting the existence of professional milk fat globule (MFG) producing MECs. While we demonstrate a small immune cell fraction, we found no expression of transcripts encoding pluripotency markers OCT4, SOX2 or NANOG, suggesting that if pluripotent stem cells exist in milk, they are found in much lower proportions than previously reported. This observation was supported by flow cytometric analyses using cell surface markers EPCAM and CD49f on cells from mature milk samples (2-8 mo postpartum), indicating a negligible EPCAM-/CD49f+ basal population and a small EPCAM+/CD49f+ luminal progenitor population.We report the first milk-derived cells to be analyzed

comprehensively by scRNAseq and we have integrated their transcriptomic profiles with MEC transcriptional signatures derived from MFGs, non-lactating human and lactating mouse mammary glands. Additionally, we address considerations for the use of these cells as liquid mammary biopsies.

Special Feature: Highlights from IMGC's "SPLASH!® milk science update" and Upcoming Hot Topics
Danielle Lemay, Western Human Nutrition Research Center, USDA, Davis, CA, USA
In April 2012, the IMGC began publishing an e-newsletter, "SPLASH!® milk science update," which features four articles on emerging topics in milk science each issue. By the time of the IMGC conference in 2020, we will have published 381 articles! This talk will reveal the editor's choice awards articles published in the previous 12 months. It will also include a behind-the-scenes tour of SPLASH!: who are the current writers and editors, who are our readers and how do they reach our website. The SPLASH! newsletter has helped to grow the IMGC with more than 200,000 annual visits to the website each year. The talk will also cover milk science topics expected to emerge in the coming year and beyond.