14th International Symposium on

Milk Genomics and Human Health

“Moving Forward with Translational Milk Research
to Advance Health”

September 26–28, 2017

Le Bonne Entente Hotel
Québec City, Canada
Dear Participant,

We are delighted to welcome you to Québec City and to the 14th International Symposium on Milk Genomics and Human Health. Our research community will meet for the first time in Canada and it will be a great opportunity to see each other again, connect and exchange. You will also get to know some of the key researchers and dairy research activities going on in Canada. This symposium is always a unique way to look inclusively at the various research underway in milk science. Concerted interdisciplinary effort is the way to address today’s complex questions, and the International Milk Genomics and Human Health symposium is a key event for cross-fertilizing our specialties. This year’s program showcases young and promising scientists in oral communications and at the poster session.

The local organizing committee, composed of members of the Dairy Science and Technology Research Center (STELA) and the Institute of Nutrition and Functional Foods (INAF), and the Dairy Farmers of Canada, are very happy to welcome you in Québec City, the birthplace of French North America and the only walled city north of Mexico. Québec is an open-air treasure chest. History lovers and nature fans can explore the city and the surroundings and enjoy our region at this colorful season.

Several social events have been planned during the symposium. Everyone is invited to participate in the poster reception at the end of the first day, a unique opportunity to discuss with presenters and speakers. On Wednesday, a group dinner will be held in the heart of Québec City at the Chapel of the French America Museum. On Thursday, for those who want to seize the opportunity to visit the Université Laval campus, we hope that you participate in an afternoon tour of INAF.

On behalf of the local organizing committee, I want to express our pleasure in welcoming you to our wonderful city. We look forward to an intense and fruitful three days of interactions and pleasant encounters, which have made the reputation of this Symposium.

Sylvie Turgeon, on behalf of the organizing committee
<table>
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<td>9:00 am</td>
<td>Welcome</td>
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<tr>
<td>9:15 am</td>
<td>A Brief History of the IMGC</td>
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<td>Gerrit Jan Hiddink, Emeritus Professor, Wageningen University, The Netherlands</td>
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<tr>
<td>9:30 am</td>
<td>The Collaborative Effect of Scientific Meetings: A Study of the International Milk Genomics Consortium</td>
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<td>Danielle Lemay, Western Human Nutrition Research Center, USDA, Davis, California, USA</td>
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<tr>
<td>9:50 am</td>
<td>Invited Speaker: Opportunities to Genetically Improve the Nutritional Value of Milk and Cow Health with Mid-Infrared Spectrometry</td>
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<td>Filippo Miglior, Research and Strategic Development, Canadian Dairy Network, Canada</td>
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<td>10:30 am</td>
<td>Coffee Break</td>
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<td>11:00 am</td>
<td>Annotating the Lactation Genome</td>
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<td>Monique Rijnkels, Texas A&amp;M University, USA</td>
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<td>11:25 am</td>
<td>Genetic Parameter Estimation for Bovine Milk Oligosaccharides in Danish Dairy Breeds</td>
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<td>Nina Aagaard Poulsen, Aarhus University, Denmark</td>
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<td>11:50 am</td>
<td>Dairy Goats: Gene Discovery and Herd Development in the Time of Genome Sequencing</td>
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<td>Klaus Lehnert, University of Auckland, New Zealand</td>
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<td>12:15 pm</td>
<td>Lunch</td>
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<td>1:30 pm</td>
<td>Invited Speaker: Milk Odd- and Branched-Chain Fatty Acids - An Overview of Current Research</td>
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<td>Rachel Gervais, Université Laval, Canada</td>
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<td>2:10 pm</td>
<td>In-Silico Genome-Wide Association Mapping of Loci Associated with Milk Triglyceride, Protein, and Lactose in The Mouse Diversity Panel</td>
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<td>Darryl Hadsell, Baylor College of Medicine, USA</td>
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<td>2:35 pm</td>
<td>Characterization and Valorization of the Genetic Pool of the Canadienne Breed</td>
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<td>Alexandra Carrier, Université Laval, Canada</td>
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<td>3:00 pm</td>
<td>Coffee Break</td>
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<tr>
<td>3:30 pm</td>
<td>Student Travel Award Recipient: Sequencing the Bovine Milk Exosome miRNome – Can the Transcriptome Hint at Efficient Recovery from Mastitis?</td>
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<td>Andrea Zukowski, University of Ottawa, Canada</td>
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<td>3:45 pm</td>
<td>The Human Milk Metabolome - Effect of Gestational and Lactational Age</td>
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<td>Ulrik Kræmer Sundekilde, Aarhus University, Denmark</td>
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<td>4:10 pm</td>
<td>Quantitative Variation in the Proteome of Individual Healthy Cows</td>
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<td>Kasper Hettinga, Wageningen University, The Netherlands</td>
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<td>4:35 pm</td>
<td>Student Travel Award Recipient: Alternative Splicing, a Fortuitous or Genetically Programmed Event to Expand Molecular Diversity of Milk Proteins: Camel CSN1S2, a Relevant Model to Try to Provide some Response Elements</td>
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<td>Ryskaliyeva Alma, INRA, UMR GABI, AgroParistech, Université Paris-Saclay, France</td>
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<tr>
<td>5:30-6:30 pm</td>
<td>Joint Scientific Advisory Council/Steering Committee Meeting (Closed Session)</td>
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<td>6:00-8:00 pm</td>
<td>Poster Reception</td>
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Wednesday September 27, 2017

9:00 am  Keynote Speaker: Impact of Dairy Products on the Gut Microbiota of Animal Models of Cardio-Metabolic Diseases  
André Marette, INAF, Université Laval, Canada

9:40 am  Bifidobacterial Dominance Correlates with Reduced Infant Gut Antibiotic Resistance  
Diana H. Taft, University of California, Davis, USA

10:05 am  Coffee Break

10:25 am  Most Valuable Presentation from the 2016 IMGC Symposium, Davis, California, USA

10:35 am  Rescuing the Infant Gut Microbiome Over the First Year of Life in Breastfed Infants with Bifidobacterium Longum Subsp. Infantis EVC001  
Steve Frese, Evolve Biosystems, Inc., Davis, California, USA

11:00 am  Milk Secretory Immunoglobulin A Protects Lactobacillus Reuteri from Digestion and Aids in Colonization In Vitro  
Vanessa Dunne-Castagna, University of California, Davis, USA

11:25 am  Milk Protein Digestion in Premature Infants and Bioactive Peptide Discovery  
David Dallas, Oregon State University, USA

11:50 am  Milk Bioactive Peptide Database: A Comprehensive Milk Bioactive Peptide Database and Novel Visualization  
Søren D. Nielsen, Aarhus University, Denmark

12:15 pm  Lunch

1:30 pm  Osteopontin and Its Relation to Transcription of the Major Milk Proteins  
Vivi R. Gregersen, Aarhus University, Denmark

1:55 pm  Analysis of Milk Phosphor-Peptides in a Processed Whey Stream  
Peter Williamson, University of Sydney, Australia

2:20 pm  Raw Cow’s Milk Prevents the Development of Airway Inflammation in a Murine House Dust Mite-Induced Asthma Model  
Betty van Esch, Utrecht University, The Netherlands

2:55 pm  Student Travel Award Recipient: Comparative Analysis of Bioactive Oligosaccharide Production in Dairy Cows Using Novel Analytical Techniques  
Randall Robinson, University of California, Davis, USA

3:10 pm  Coffee Break

3:40 pm  Keynote Speaker: How has Saturated Fat Become so Controversial?  
Benoit Lamarche, Université Laval, Canada

4:20 pm  Differential Impact of Cheese Matrix on Postprandial Lipid Response: A Randomized, Crossover, Controlled Trial  
Jean-Philippe Drouin-Chartier, Université Laval, Canada

4:35 pm  Student Travel Award Recipient: Identification of Parameters Involved in Disintegration of Commercial Cheese Matrix and Lipid Digestion by Using an In Vitro Static Digestion Model  
Léa Guinot, Université Laval, Canada

4:50 pm  Milk Fat Globule: New Insights into an Unappreciated Complex Lipid System  
Bruce German, University of California, Davis, USA

6:30-11 pm  Group Dinner
Thursday September 28, 2017

9:00 am  Donor Human Milk for the Preterm and At-Risk Term-Born Infant  
Deborah O’Connor, University of Toronto, Canada

9:40 am  A Role for Human Milk Bioactivity in Improved Health Outcomes for Premature and Low Birth Weight Babies: Lessons from the Evolution of Lactation  
Kevin Nicholas, Monash University, Australia

10:05 am  Maternal Programming of Lung Development During Lactation in the Marsupial Neonate  
Christophe Lefevre, Walter and Eliza Hall Medical Research Institute, Melbourne, Australia

10:30 am  Coffee Break

10:45 am  Lactational Concentration Changes of Individual Human Milk Oligosaccharides in Breast Milk from Chinese and Malaysian Women  
Paul McJarrow, Fonterra Research and Development Centre, Palmerston North, New Zealand

11:10 am  Beyond the Bench: The Future of Human Milk Stem Cells for Preterm Infants  
Carrie-Ellen Briere, University of Massachusetts, Amherst, USA

11:35 am  Student Travel Award Recipient: Milk as a Protein-Protease Delivery System: Collaborative Approaches Inspire Inquiry into the Dynamics and Complexities of Human Milk  
Junai Gan, University of California, Davis, USA

11:50 pm  CLOSING REMARKS

12:00 pm  LUNCH

1:30- 4 pm  Tour of Institute of Nutrition and Functional Foods (INAF) at Université Laval  
If not already registered, please see Laurie Jacobson at the registration table to be added to the list. Bus service will be provided.
Meet the Speakers

Carrie-Ellen Briere, Ph.D.
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Dr. Briere is a new Assistant Professor at the University of Massachusetts, Amherst. She just completed post-doctoral training in a joint appointment with the Connecticut Children’s Medical Center and the University of Connecticut School of Nursing. Her clinical background is as a nurse in a level IV Neonatal Intensive Care Unit. Since learning about stem cells in human milk, Dr. Briere’s research in the last Three years has focused on the bioactive components of milk, specifically stem cells.

Alexandra Carrier
M.Sc. Candidate in Animal Science, B.Sc. Bioinformatics
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Alexandra obtained a bachelor’s degree in Bioinformatics at Université Laval in 2016 and now studies animal sciences as a master’s candidate on Dr. Claude Robert’s team. Her fields of interest are animal welfare, and conservation through the use of genomics and population genetics.

David Dallas, Ph.D.
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Dr. Dallas received his B.A. in Public Health in 2008 from Rice University and his Ph.D. in Nutritional Biology in 2012 from UC Davis. He then completed a post-doctoral fellowship at UC Davis in Food Science. Dr. Dallas is currently an Assistant Professor in Nutrition at Oregon State University where he examines how milk proteins are digested in infants, which bioactive peptides are released, and how limited protein digestion may affect outcomes in premature infants.
Jean-Philippe Drouin-Chartier, R.D
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Jean-Philippe Drouin-Chartier is a Registered Dietitian, member of the Ordre professionnel des diététistes du Québec. He is currently pursuing doctoral studies in experimental medicine at the Institute of Nutrition and Functional Foods with Drs. Patrick Couture and Benoît Lamarche. Jean-Philippe is the recipient of the Frederick Banting and Charles Best Graduate Scholarship from the Canadian Institutes of Health Research. His research is focused on the metabolism of lipoproteins associated with different dyslipidemias, as well as on the impact of consumption of dairy products on cardiovascular health.

Vanessa Dunne Castagna, M.S.
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Vanessa Dunne Castagna is interested in the cross-talk between human nutrition, gut microbiota and immune response. She is pursuing a doctorate in Nutritional Biology under the mentorship of Dr. Bo Lönnerdal and Dr. David Mills (Food Science), investigating milk-derived bioactive molecules and the infant gut microbiota. Her background is in microbiology (B.S., 2001, UC Davis) and molecular biology (M.S., 2004, CSU Sacramento) with extensive laboratory internships in milk microbiology and immunology.

Steven A. Frese, Ph.D.
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Meet the Speakers

Dr. Frese is a microbial ecologist leading research and development at Evolve Biosystems. Evolve has developed the next-generation of live bio-therapeutics based on sound, evidence-based research borne out of nearly 15 years of work by the company’s faculty-founders at UC Davis. In understanding how evolution and ecology shape the mother-infant-microbe relationship, Evolve is bringing to market groundbreaking work in the area of the gut microbiome and infant nutrition with Evivo™, to restore the infant gut microbiome.

Dr. Frese completed postdoctoral training in this field at UC Davis with Dr. David Mills studying this milk-microbe relationship, and earned his Ph.D. with Dr. Jens Walter at the University of Nebraska, where his work showed how different populations of a coevolved gut microbe (Lactobacillus reuteri) have evolved to colonize different host animals, and the molecular mechanisms which have enabled these ecological features.

Junai Gan, Ph.D.(c)
Student Travel Award Recipient
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Junai Gan is a Ph.D. Candidate in Dr. Bruce German’s research group at the University of California, Davis. Her research interest is protein digestion of baby foods. Integrating clinical, analytical and computational resources, she has characterized the proteolysis of human milk under various conditions. She is aiming at a better understanding of protein digestion and a translational strategy for food design to improve infant health.

J. Bruce German, Ph.D.
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Professor in Food Science and Technology, Director, Foods for Health Institute, University of California Davis (http://ffhi.ucdavis.edu/), co-founder Evolve Biosystems.

Bruce German received his Ph.D. from Cornell University, joined the faculty at the University of California, Davis in 1988 and is currently Director of the Foods for Health Institute and professor, at UC Davis. His research interests include the structure and function of dietary lipids, the evolution of lactation and the role of milk components in food and health and the application of metabolic assessment to personalizing diet and health. Bruce and colleagues have published more than 400 papers on milk, lipids and food, metabolism and metabolite measurements and food functions and patented various technologies and applications of bioactive agents. The research articles from the lab are available at: https://scholar.google.ca/citations?hl=en&user=GIAAFkMAAAAJ&view_op=list_works&sortby=pubdate
Rachel Gervais, Ph.D.
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Rachel Gervais obtained her Ph.D. in Animal Science from Université Laval in 2009. Her thesis, under the supervision of Dr. Yvan Chouinard, focused on the Biological effects of conjugated fatty acids and their interest as modulators of milk fat synthesis in dairy cows. Following her Ph.D. Rachel spent a year as a postdoctoral fellow in the lab of Dr. Veerle Fievez in the Department of Animal Production at Ghent University, in Belgium. In 2011, Dr. Gervais returned to Université Laval where she currently is an associate professor of animal nutrition in the Department of Animal Science.

Vivi Gregersen, Ph.D.
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Vivi R. Gregersen is a Postdoc at the Department for Molecular Biology and Genetics at Aarhus University. Her main interests are within Milk Genomics where she focuses on genome-wide association studies and method development for identification of causal mutations affecting milk traits in dairy cattle utilizing NGS sequencing techniques. She has a B.Sc. in Biology, a M.Sc.IT. in Bioinformatics and a Ph.D. in Genetics from Aarhus University.

Léa Guinot
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Léa Guinot is a Ph.D. student member of STELA and INAF, currently working at Université Laval on Professor Sylvie Turgeon’s team. Her thesis is about lipid release and cheese degradation during gastrointestinal in vitro digestion. She obtained a double master’s degree in Food Sciences from both Université Laval and University of Bordeaux.
Meet the Speakers

Darryl Hadsell, Ph.D.
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Dr. Hadsell received his bachelor and master of science degrees from South Dakota State University and Ph.D. from Pennsylvania State University. He then worked as a postdoctoral fellow at the Baylor College of Medicine. Dr. Hadsell joined the division of nutrition in the department of pediatrics at Baylor College of Medicine as an assistant professor in 1995 and is currently an associate professor of pediatrics.

Kasper Hettinga, Ph.D.
Associate Professor
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Kasper Hettinga completed his M.Sc. and Ph.D. in Food Technology at Wageningen University. After his Ph.D., he started research on the protein composition of human and bovine milk. He is currently working as tenured associate professor in the Department of Dairy Science and Technology at Wageningen University. His main research focus is the characterization of the proteome of bovine and human milk, focusing on its role in both calf and infant health.

Gerrit Jan Hiddink, Ph.D.
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Dr. Hiddink received both his M.Sc. in Human Nutrition (1975) and Ph.D. (1996) from Wageningen University. He was an Endowed Professor of Nutrition Communication through Health Professionals at Wageningen University from 2000 until 2017. Dr. Hiddink was the Head of Nutrition Education Dutch Dairy Bureau from 1975-1989 and then the manager of Research Nutrition & Health for the Dutch Dairy Foundation on Nutrition & Health until 2003. He then worked for the Dutch Dairy Association from 2003 until 2016. He was Chairman of the Utrecht Group and has held memberships in the International Milk Genomics Steering Committee, SC Nutrition & Health for IDF and the Program Committee for the IDF World Dairy Congress.
Meet the Speakers

Benoît Lamarche, Ph.D.
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Benoît Lamarche is a professor at the school of nutrition and holds the Université Laval Chair in nutrition. He is also a researcher at the Institute of Nutrition and Functional Foods (INAF) since 1998. Educated in biochemistry, he has published more than 300 articles in specialized publications related to physiology, nutrition, medicine and health and is one of the most cited research scientists in Canada in this field. His recent work is on the Mediterranean diet, dairy products, trans fats and their impact on cardiovascular health, obesity and the metabolic syndrome from a physiological, clinical and epidemiological point of view. Dr. Lamarche has received many awards, including those from the Canadian Nutrition Society in 2011 and the Québec Lipidology, Nutrition and Metabolism Society in 2013. He participated in the Winter Olympic Games as a long-track speed skating athlete in 1984 and 1988. He recently wrote a book with chef Jean Soulard promoting the value of balance and pleasure in nutrition of athletes of all levels.

Christophe Lefèvre, Ph.D.
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Christophe obtained a doctorate degree in Biochemistry from the University of Montpellier in 1984. He went on to conduct post-doctoral research in Southern California (USC, UCSD, UCLA) and Japan (Tokai University). After leading bioinformatics software developments at Gentech in southern France, he joined Organon AkzoNobel, a pharmaceutical company in The Netherlands. In 2002 he moved to Australia, working at the Victorian Bioinformatics Consortium at Monash University and the CRC for innovative dairy products at the University of Melbourne. In 2008 he became Associate Professor of Bioinformatics at Deakin University and in 2015 he joined the Division of Bioinformatics of the Walter and Eliza Hall Institute of Medical Research and the Peter MacCallum Cancer Research Institute.
Meet the Speakers

Klaus Lehnert, Ph.D.
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Professor Lehnert is a functional biologist focusing on the molecular mechanism underlying the expression of genetic variations and mutations into continuous and extreme phenotypes, traits, and disease. Most of his work leverages high-throughput phenotypic screening in combination with in-depth genome analysis, especially genome sequencing. His work applies similar principles to a wide range of interesting biological and medical problems, ranging from Mendelian disease over milk composition and production, to conservation biology.

Danielle G. Lemay, Ph.D.
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Dr. Danielle Lemay is a Research Scientist at the U.S. Department of Agriculture’s Western Human Nutrition Research Center in Davis, California. She is also a faculty member at the Genome Center, the Head of Bioinformatics Initiatives at the Foods for Health Institute, and a Visiting Assistant Professor with the Department of Nutrition at University of California, Davis. Her research program was previously focused on the genetics of milk production, mammary biology, and milk-oriented microbes. In her new lab at the USDA, she is studying the effect of diet on host microbes and gastrointestinal health. She has a Ph.D. and M.S. in Nutritional Biology from UC Davis, and a B.S. in Electrical Engineering and Computer Science from MIT.
Meet the Speakers

André Marette, Ph.D.
Director of the Research Chair on the Pathogenesis of Insulin Resistance and Cardiovascular Diseases; Scientific Director, Institute of Nutrition and Functional Food of Université Laval
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Dr. André Marette graduated from Université Laval in 1990 with a Ph.D. in Physiology and Endocrinology. He is currently full professor in the Department of Medicine, and Scientific Director of the Institute of Nutrition and Functional Foods at Université Laval, Québec, Canada. Dr. Marette is an international expert on the pathogenesis of inflammation, type 2 diabetes and cardiovascular diseases in obesity. His research in the areas of insulin action and insulin resistance, and the mechanisms of inflammation, has advanced the understanding of the cellular/molecular defects leading to diabetes and opened new possibilities for nutritional and pharmacological therapeutic interventions.

He has published more than 190 papers and reviews in high-impact journals and received several national and international research grants. Dr. Marette has received several awards including the Charles Best Lectureship Award of the University of Toronto, in recognition for his outstanding contribution to diabetes research. In addition, he has organized a number of national and international meetings and symposia and has been invited to speak at more than 150 national and international meetings. He is Editor-in-Chief of the American Journal of Physiology: Endocrinology & Metabolism.

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Paul McJarrow has a Ph.D. in Biochemistry from the University of Otago. In 1998 he joined the Fonterra Research and Development Centre with research focused on prebiotics, milk oligosaccharides, sialic acid, and complex milk lipids of the milk fat globule membrane (MFGM). Of particular interest are methods of analysis, mechanistic studies, ingredient development, pre-clinical trials, clinical investigations and commercialization. Paul has co-authored more than 20 papers, one book chapter, and is a co-inventor on three patents.
Meet the Speakers

Filippo Miglior, Ph.D.
Chief, Research & Strategic Development, Canadian Dairy Network
President, Canadian Society of Animal Science
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Dr. Miglior is Chief, Research and Strategic Development at CDN and Adjunct Professor at University of Guelph. He is Senior Editor for Journal of Dairy Science, member of several industry boards, Past President of Canadian Society of Animal Science. He manages all industry priority research projects supported by DairyGen. Dr. Miglior is the recipient of several awards and has authored more than 600 journal/technical articles. Dr. Miglior leads several projects with budgets exceeding $12.6 million overseeing more than 20 graduate students and researchers.

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Kevin Nicholas completed B.Sc. Hons and Ph.D. degrees at the University of Western Australia and subsequently held positions in the USA and Australia in research institutes, industry and universities. His interests focus on the evolution of lactation. He has developed research platforms of comparative genomics and bioinformatics to exploit a range of species to identify signalling molecules in milk that direct the development of gut and lung in the neonate. These studies have potential for improved health outcomes for premature and low birth weight babies.

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Dr. Nielsen’s main research focus is on health-promoting molecules from dairy products. Recently, he has been working on identification of peptides deriving from gastric digestion of milk proteins in term and preterm infants including identification and prediction of bioactive peptides. He also investigated protein changes in lactose-free UHT milk during storage. The proteins were modified by different chemical reactions (e.g. cross-linking) and partially digested by active proteases in the milk.
Meet the Speakers

**Deborah L. O'Connor, Ph.D., R.D.**
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Dr. Deborah O’Connor is a professor in the Department of Nutritional Sciences at the University of Toronto where she holds a Chair in Vitamin Research in Human Milk and Development. She also holds scientific appointments at The Hospital for Sick Children and Mount Sinai Hospital. Her CIHR and NSERC-funded research focuses on maternal and infant nutrition and specific strategies to support provision of mother’s own and donor human milk to vulnerable infants, including the preterm infant.

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Nina Aagaard Poulsen obtained a Ph.D. degree in Conservation Genetics from the Department of Biological Sciences, Aarhus University, Denmark in 2008. She has worked at the Department of Food Science, Aarhus University since 2009. Her research focuses on understanding factors affecting variation in raw milk composition of bovine milk, especially in relation to genetic background, which have been studied as a part of the Danish-Swedish Milk Genomics Initiative.

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Her research interests are milk protein gene regulation, genetic and epigenetic regulation in mammary gland development and disease. She trained at Leiden University, the Netherlands, with Pharming Group NV, and at Baylor College of Medicine. She is currently at the department of Veterinary Integrative Biosciences, College of Veterinary Medicine & Biological Sciences, Texas A&M University. As part of the bovine genome consortium she led the lactation group for the annotation and analysis of the bovine genome.
Meet the Speakers

Randall Robinson

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Randall is a fifth-year Ph.D. student in the department of Food Science & Technology at UC Davis. After earning a B.S. in Chemistry from California State University, Stanislaus, Randall joined the Barile Lab and began characterizing health-promoting food compounds, including peptides and oligosaccharides, using mass spectrometry and novel quantification techniques. Randall hopes that by improving our understanding of milk’s functional properties, we will be able to better utilize those attributes to improve human health.

Alma Ryskaliyeva

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Alma Ryskaliyeva is a third year Ph.D. student at AgroParisTech, University of Paris-Saclay, carrying out a thesis “Exploring the fine composition of Camelus milk from Kazakhstan with emphasis on protective compounds” under a supervision of Dr. Patrice Martin. She is currently working towards this goal at INRA (French National Institute for Agricultural Research), Jouy-en-Josas, France, in "Animal Genetics and Integrative Biology" research unit (GABI UMR), and "Milk Genome Health" (LGS) team.

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Ulrik Kræmer Sundekilde received his B.Sc. and M.Sc. in Biochemistry and Molecular Biology from the University of Southern Denmark, and his Ph.D. in Food Science from Aarhus University. He is currently a Postdoc in the Department of Food Science at Aarhus University. His research interests focus on the development of methods for measuring small molecules in biofluids, examining carbohydrates in complex mixtures using both liquid- and solid-state NMR spectroscopy and food metabolomics.
Meet the Speakers

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Dr. Diana Taft obtained her Ph.D. in 2014 from the University of Cincinnati where her research focused on the preterm infant gut microbiome and risk of late-onset sepsis. She is currently a post-doctoral researcher at the University of California, Davis in the Mills laboratory where her current research is focused on how human milk shapes the healthy, term infant gut microbiome and the implications of milk-microbe interactions in human health.

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Dr. Betty van Esch is a postdoc in immunopharmacology. Her work is aimed at the elucidation of immune modulatory and immune-programming mechanisms by nutrition. Her main focus is (allergen-specific) tolerance induction and nutritional and pharmaceutical intervention studies in allergic diseases using experimental models of food allergy, COPD and allergic asthma.

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Peter has a Ph.D. in Animal Science from the University of Sydney. He was a postdoctoral fellow in immunobiology at the University of Pennsylvania, before establishing a laboratory as a Principal Investigator and a Senior Lecturer at University of Sydney. He moved into dairy genomics as a Principal Research Fellow in Functional Genomics and is currently Associate Professor, Physiology and Genomics. He currently serves on the IMGC Scientific Advisory Committee, and has been chair of the committee on three occasions.
Andrea Zukowski

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Andrea Zukowski is a fourth-year undergraduate student at the University of Ottawa. Under the supervision of Dr. Illimar Altosaar in the Department of Biochemistry, Microbiology, and Immunology, she is currently working on a team of milk experts to devise standardized protocols for milk exosome isolation and to explore the miRNome of both human and bovine milk.
Presentation Abstracts
This presentation will describe in short the foundation of the IMGC in 2004, under the leadership of Dr. Joseph O’Donnell of the California Dairy Research Foundation (CDRF) and its development until now. As one of the founding fathers, I experienced a number of crucial success factors: prerequisite factors and inspirational factors. These include the vision, mission, and structure (consortium management, scientific advisory committee, sponsors) of the IMGC, as well as the actions and services of the IMGC (including the flagship annual International Symposium on Milk Genomics & Human Health).

The scientific curiosity of both the global milk science community (multidisciplinary!) and the dairy sponsors, and their interests in understanding the role of milk in human health, have been unifying factors for the IMGC to create win-win situations. Mutual trust, and the understanding that the scientific members are both the base of IMGC and essential for scientific advancement, have been (and still are), crucial for the success and the sustainability of the IMGC.

The IMGC mission is to: provide a collaborative, interactive and pre-competitive platform for the scientific community and industry; to accelerate the understanding of the biological processes underlying mammalian milk genomics; and to facilitate the transition of that knowledge into usable commercial benefits for the industry. Over the last few years, Dr. Gonca Pasin, the Executive Director of CDRF has given much attention to each aspect of the IMGC mission, and modernized the IMGC in its actions as a platform to communicate cutting-edge milk science into applied industry outcomes. In practice: Short lines from science to application!

Collaboration among scientists has a major influence on scientific progress. Such collaboration often results from scientific meetings, where scientists gather to discuss their research and to meet potential collaborators. Competitive funding, however, imposes a bias that confounds attempts to evaluate the role of scientific inquiry in collaboration decisions and outcomes. To evaluate the effects of scientific meetings on collaboration and progress independent of funding bias, we conducted a study of the annual symposia held by the International Milk Genomics Consortium (IMGC) over a 12-year period. In our study, we conducted permutation testing to analyze the effectiveness of the IMGC in facilitating collaboration and productivity in a community of milk scientists relative to non-attendees. Using the number of co-authorships on published papers as a measure of collaboration, our analysis revealed that scientists who attended the IMGC symposium were associated with more collaboration than were scientists who did not attend. Furthermore, we evaluated the scientific progress of consortium attendees by analyzing publication rate and article impact. We found that IMGC attendees, in addition to being more collaborative, were also more productive and influential than were non-attendees who published in the same field. The results of our study suggest that the IMGC symposium encouraged interactions among disparate scientists and helped increase research productivity, exemplifying the positive effect of scientific meetings on both collaboration and progress.
Invited Speaker: Opportunities to Genetically Improve the Nutritional Value of Milk and Cow Health with Mid-Infrared Spectrometry
Filippo Miglior, Research and Strategic Development, Canadian Dairy Network, Canada

Genetic improvement of fine milk components is of interest to consumers and milk processors, but is limited by the lack of readily attainable phenotypes. Mid-infrared (MIR) spectrometry, a staple in milk recording programs, provides an opportunity to obtain large numbers of phenotypes in a rapid, inexpensive manner. An opportunity exists for the genetic evaluation of novel milk component traits by expanding the use of MIR spectroscopy in milk recording to produce new MIR-predicted traits if calibration equations can be developed.

Mid-infrared spectroscopy can be used to produce large numbers of phenotypes for novel predicted fine milk component traits using the present technology in DHI milk recording. The new phenotypes could permit routine genetic evaluation and selection, as well as future study into explanatory regions of the genome influencing these traits.

Annotating the Lactation Genome
Monique Rijnkels, Texas A&M University, USA

Monique Rijnkels1, Hannah S. Lyman1, Christa Kühn1, Rosemarie Welkard1, Keith Bradnam4, Jessica Elswood1, Adrian Molenaar3, Kuljeet Singh6, Joanne Dobson6, Kim Oden6, Danielle G. Lemay2,7, Ian Korf2, Guosong Wang8, and Clare Gill8
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With the generation of the bovine genome assembly the focus has shifted to identifying the functional elements in the genome, to gain a full understanding of the biology that underlies the connection of phenotype and genotype. An international consortium, Functional Annotation of Animal Genomes (FAANG) consortium, has been organized to coordinate such an effort in several livestock species. Within the FAANG project core assays are being performed to identify the following: Transcribed loci, Chromatin accessibility, Transcription factor binding and Histone modification enrichment.

Our project predates the FAANG effort, but is based on the same premise that an improved understanding and knowledge of gene regulatory networks and functional elements in the mammary gland of cattle would allow for direct genetic selection of traits, particularly those that are expensive to select based on phenotype (e.g. fatty acid composition) or low heritability.

It would also lead to a more complete understanding of mammary gland biology, enabling novel strategies to improve the quality, efficiency, and sustainability of milk production. We set out to identify: 1) Mammary gland functional elements genome-wide, 2) Mammary gland transcriptome, 3) Annotate and characterize mammary gland functional elements by computational analysis, including presence of SNP. To date we have
Presented Abstracts

1:25 am  **Genetic Parameter Estimation for Bovine Milk Oligosaccharides in Danish Dairy Breeds**

**Nina Aagaard Poulsen**, Aarhus University, Denmark

**Nina A. Poulsen**, Bart Buitenhuis, Randall C. Robinson, Daniela Barile, and Lotte B. Larsen

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Bovine milk oligosaccharides were quantified by an optimized LC-MS/MS method. Totally, 16 bovine milk oligosaccharides were characterized in milk samples from 334 Danish Holstein and 300 Jersey cows. Individual milk samples were collected from 40 farms and the selected cows were within their first three parities and in midlactation. The results confirmed previous findings that milk from Danish dairy breeds differs in their oligosaccharide profile. In both breeds, parity significantly affected several oligosaccharides, whereas days in milking was mainly affecting variation in milk oligosaccharides in Danish Holstein. Furthermore, herd or management effects were also found to play a role for specific oligosaccharides.

Apart from milk samples, all Holstein and Jersey cows were genotyped using the bovine high-density single nucleotide polymorphism (SNP) array. Genomic DNA was extracted from ear tissue. The Restricted Maximum Likelihood (REML) approach in DMU was used to perform a genome wide association study and estimate variance components. The SNPs on the bovine HD chip were mapped to the Btau4.0 assembly. In Danish Holstein, heritabilities ranged from 0 for 2 Hex 2 NeuAc and 4 Hex 4 HexNAc 1 Fuc to 0.67 for 3 Hex 2 HexNAc and 0.68 for 4 Hex 1 HexNAc. With an average heritability of 0.36, most bovine oligosaccharides display moderate too high heritabilities, which indicate a very strong genetic influence underlying bovine milk oligosaccharides and a good potential for increasing these through selective breeding.

A genome wide association study identified 2201 significant SNP markers (FDR < 0.10) associated with the bovine milk oligosaccharides in Danish Holstein. The SNP markers were aligned to six bovine milk oligosaccharides. Most significant SNPs were detected for Lacto-N-Hexaose (n = 736). Of these 289 were located on BTA 1, where the most significant SNPs were BOVINEHD0100024179 and BOVINEHD0100024184 with –log10 (P-value) of 20.36 and 20.77, respectively. Very interestingly BOVINEHD0100024179 was also the most significant SNP for lacto-N-tetraose (LNT, –log10(P-value) = 19.77). This quantitative trait locus (QTL) contains the gene B3GNT5 which encodes UDP-GlcNAc:betaGal beta-1,3-N-acetylgalactosaminyltransferase 5, an enzyme involved in glycan synthesis. Likewise, an overlapping SNP between lacto-N-tetraose and Lacto-N-Hexaose was found for ALG3 encoding ALG3 alpha-1,3-mannosyltransferase. This gene is also located on BTA1, and the encoding enzyme is related in N-linked glycosylation. Other candidate genes of interest detected were B3GALNT2 encoding beta-1,3-N-acetylgalactosaminyltransferase 2 on BTA28 (5 Hex 4 HexNAc), GLT6D1 encoding...
glycosyltransferase 6 domain containing 1 on BTA11 (4 Hex 1 HexNAc) and LOC520336 encoding N-acetyl-lactosaminide beta-1.6-N-acetylglucosaminyl-transferase isoform C on BTA23 (lacto_N_Hexaose). The results from Danish Jersey cows are still ongoing, but will be finalized in near future. To our knowledge this is the first study documenting a solid breeding potential for bovine milk oligosaccharides. This can be of high value for the dairy ingredient industry, where isolation of bovine milk oligosaccharides can be valuable for improved infant formulae.

11:50 am  Dairy Goats: Gene Discovery and Herd Development in the Time of Genome Sequencing
Klaus Lehnert, University of Auckland, New Zealand
Ashley Smith¹, Alexandra Ankersmit-Udy¹, Kristen Henty¹, Richard G. Sherloc², Andrew Scott², Mathew D. Littlejohn², Russell G. Snell¹, and Klaus Lehnert¹
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Milks with ‘special’ compositions addressing individual nutritional and health requirements are increasingly attractive to consumers and dairy manufacturers. It is interesting to explore whether milks produced by ruminants other than cows can contribute towards this goal. While goats and sheep have been used over millennia to produce milk for human consumption in Africa and Asia, in most other regions their milks have been predominantly used for cheese manufacture. The recent growth in global dairy consumption and customer demand for a wider array of dairy products has led to a strong growth of sheep and goat dairying.

Supported by centralized breeding and performance recording over many decades, consequent phenotypic, genetic, and more recently, genomic selection, has dramatically improved productivity in the bovine dairy industries. In contrast, comparable systems and technologies are not yet available or not well established in many goat dairy industries. While this suggests that considerable genetic diversity may have survived in these herds, the lack of existing data limits accurate assessment of the risks and opportunities resulting from this history. These industries now face the tremendous challenge of integrating the technological breakthroughs that were introduced step-wise and over long time frames in other animal industries: wide-spread artificial insemination, genetic profiling, individual milk testing and recording, and their combined application in breeding and selection strategies. In some markets, ready access to novel genome editing technologies further complicates a robust assessment of opportunities and risks.

In collaboration with the rapidly developing New Zealand goat dairy industry, we have surveyed the extant goat population to acquire the data required to better inform breeding strategies towards milks supporting dairy products of high nutritional value. We will present the initial insights obtained from genome-wide genetic profiling, milk composition analysis, and genome sequencing of a significant proportion of the extant herd, including its overall genetic structure, genetic variation in milk protein genes, and loci with surprisingly strong effect on overall productivity. In addition, we will report initial findings our use of goats as an ‘experimental system’ for discovery of novel genes important for lipid synthesis.

Following characterization of the genetic basis of overall productivity, it will be possible to integrate genomics-based technologies to develop a robust breeding strategy towards a herd with optimized genetic makeup – improving the nutritional value of its milks, but also maintaining (if not improving) animal welfare, health, and survival. This places these dairy goats at an exciting nexus – likely the first case where genomic technologies can be applied at the onset of centralized breeding and recording in a dairy species.
Presentation Abstracts

1:30 pm Invited Speaker: Milk Odd- And Branched-Chain Fatty Acids - An Overview of Current Research
Rachel Gervais, Université Laval, Canada

Rachel Gervais¹, and P. Yvan Chouinard¹
¹. Department of Animal Science, Université Laval, Canada

Nutritional quality is an important determinant of consumer food choices due to the growing awareness of the association between diet and health. Milk fatty acid (FA) composition is a major component of the nutritive value of dairy products, in addition to being associated with both their physical and organoleptic properties. The FA profile of milk can be significantly altered through feeding of cows, offering the flexibility to respond to consumer demands and public health recommendations. Dairy products are the main source of branched-chain FA (BCFA) in the human diet. These FA are synthesized by ruminal microorganisms and are essential components of their lipid membranes. For humans, health benefits such as prevention of necrotizing enterocolitis, symptom attenuation of some neurological diseases, and anticarcinogenic properties have been associated with these bioactive molecules. A series of experiments carried out in our laboratory provided more insight into the effect of different physiological and nutritional factors on milk BCFA composition in dairy cows. These trials showed that milk fat concentrations of BCFA are affected by lactation stage as well as forage type, level of concentrate, and dietary lipid supplementation. In conclusion, milk fat contributes to human health in many important ways. Nutrition and management of dairy cows offers the possibility to adjust milk FA composition as our knowledge of individual and combined health effects of numerous milk FA develop.

Keywords: branched-chain fatty acids, dairy cow, milk

2:10 pm In-Silico Genome-Wide Association Mapping of Loci Associated with Milk Triglyceride, Protein, and Lactose in The Mouse Diversity Panel
Darryl Hadsell, Baylor College of Medicine, USA

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Although it is largely accepted that milk is one of nature's most perfect foods, there is much that remains to be understood about the nutrients and bio-active factors that are present in this complex emulsion. The regulation of macronutrient concentrations in milk has been an area of commercial interest for many years. In this regard, intensive efforts have been underway to identify genomic factors in dairy animals that regulate the concentrations of nutrients such as fat and protein. However, model systems such as the inbred mouse can also serve as a means to identify genomic factors linked to the regulation of milk composition. Previous work in our laboratory has made use of a collection of inbred mouse strains known as the mouse diversity panel (MDP) to map genomic loci associated with variations in a variety of mammary gland-related traits including those underlying post-natal ductal development, litter-rearing capacity, and milk mineral composition. Analysis of milk samples from second lactation MDP females was conducted for triglyceride, protein, and lactose. For all three traits, strain accounted for a significant (P=2x10^-6, 5x10^-6, and 8x10^-5) proportion of the observed variance. Lactose was found to exhibit the largest amount of inter-strain variation ranging from 22±6 to 50±19 mmole/L. The concentrations of protein and triglyceride ranged from 71±25 to 129±39 mg/ml, and 160±58 to 326±84 mmole/L respectively. In-silico genome-wide association analysis detected 18 loci for lactose.
Characterization and Valorization of the Genetic Pool of the Canadienne Breed

Alexandra Carrier, Université Laval, Canada

Alexandra Carrier¹, Alexandre Bastien¹, Pierre Leclerc¹, and Claude Robert¹

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2. Institute of Nutrition and Functional Food, Université Laval, Canada
3. Department of Animal Science Faculty of Agricultural and Food Sciences, Université Laval, Canada

The Canadienne cattle is the oldest breed of dairy cattle in North America. The harsh environmental conditions of the time gave her a rusticity and longevity that made her an invaluable genetic resource. Today, the Canadienne is heavily threatened because of the crossings that have been made and the population is estimated to be about 1,200 individuals, including less than 300 pureblood animals. Until now, no effort has been made to document the genetic pool of this heritage breed. The hypothesis of this project stipulates that it is still possible to properly manage the current genetic numbers to ensure the durability of the breed. The project aims to describe the genetic pool of the Canadienne cow and to establish a management system for the development of the breed. In order to guide efforts to preserve the Canadienne breed, we propose a distinctive strategy based on genomics. A total of 192 animals belonging to four groups called the “originals”, the “pure-blood”, the “ancients” and the “foreigners” were genotyped (640,000 SNPs per sample) using the Axiom platform (Affymetrix). Data were analyzed using genetic statistics to determine their diversity level.

Results suggest that there is still a possibility for diversifying the breed according to a principal component analysis of the genotypes which identified five genetically distinct subgroups from the population. The work aims to dislodge this heritage breed from folklore and to value it for its genetic characteristics in a healthy and sustainable way, in order to arouse the interest of the breeders and restore confidence in the breed. The data will also support future haplotypes research projects associated with the health and longevity of Canadienne cows.
Presentation Abstracts

3:30 pm  Student Travel Award Recipient: Next-Generation Sequencing of Bovine Milk-Derived Exosomal MicroRNA to Determine Transcriptome Expression for Efficient Recovery from Mastitis Infection
Andrea Zukowski, University of Ottawa, Canada

Andrea Zukowski¹, Jamie Kraft¹, and Illimar Altosaar¹
1. Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Canada

Mastitis, an inflammatory disease of the mammary gland, presents one of the greatest economic challenges to the dairy industry. Milk somatic cell count is the current standard for the detection of bacterial infection, but is not diagnostic, pre-emptive, and is prone to false-positives. Biomarkers, such as microRNA (miRNA), for susceptibility and response to treatment may provide the herdsman with a more significant means of managing mastitis.

Exosomes are extracellular microvesicles (30-150 nm diameter) released by various cell types, including mammary cells. Milk-derived exosomes encapsulate degradation-resistant microRNAs (miRNAs), long non-coding RNA, mRNA, and proteins. These miRNAs are thought to play an important role in intercellular communication, especially during an immune system response.

Prior work suggests that milk exosomal miRNAs differ between healthy cows and those that have been inoculated with pathogenic bacteria. However, post-mastitis bovine milk exosomal miRNA expression has yet to be elucidated. Using next-generation sequencing, within-host miRNA expression can be monitored over the course of treatment for the discovery of novel recovery-mediating miRNAs or time-dependent changes in the levels of these miRNAs. We propose that miRNA expression in cow’s milk may vary between cows that respond quickly to treatment and those that do not. This inter-herd variability can be taken advantage of to treat cows with sub-optimal recovery. The aim of our project is to model the flux of miRNA profiles in milk collected from mastitis-infected Holstein cows at various time-points during and after antibiotic treatment. We will analyze miRNA expression for time-dependent, inter-quarter, and intra-herd differences to identify biomarkers of effective and efficient mastitis recovery.

3:45 pm  The Human Milk Metabolome - Effect of Gestational and Lactational Age
Ulrik Kræmer Sundekilde, Aarhus University, Denmark

Ulrik Sundekilde¹, Eimear Downey¹, James A. O’Mahony¹, Carol Anne O’Shea², Anthony Ryan²,³, and Alan L. Kelly²
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Elucidating the changes in human milk from mothers of different gestational ages and at varying lactation stages is important to understanding the nutritional requirements of neonatal infants. Human milk is known to be the ideal nutritional source for infants delivered at full term, while milk from mothers that deliver prematurely often needs to be fortified to meet the nutritional requirements of pre-term infants. In this study, the metabolite profiles of human milk from the mothers of pre-term infants was compared to that of milk (colostrum and mature) from mothers that delivered at full term. Metabolites present in human milk were analyzed by 1H-NMR spectroscopy and included many oligosaccharides and amino acids of nutritional importance. Principal component analysis (PCA) enabled the discrimination between milks of different gestational ages (pre-term vs. full term) and lactation stages (colostrum vs. mature). Metabolite profiling revealed that levels of valine, leucine, betaine, and creatinine were increased in colostrum from term mothers compared with mature milk, while those of glutamate, caprylate and caprate were increased in mature term...
milk compared with colostrum. Levels of oligosaccharides, citrate, and creatinine were increased in pre-term colostrum, while those of caprylate, caprate, valine, leucine, glutamate, and pantothenate increased with time post-partum. There were differences between pre-term and full-term milk in the levels of carnitine, caprylate, caprate, pantothenate, urea, lactose, oligosaccharides, citrate, phosphocholine, choline and formate. Furthermore, we found that the metabolome of pre-term milk changes within 5-7 weeks post-partum to resemble that of term milk, independent of time of gestation at pre-mature delivery.

Presently, our focus is currently to explore larger cohorts to verify our results in addition to examining longitudinal milk samples from mothers of pre-term infants and term infants to establish how the extent of prematurity and early lactation influences the metabolome, microbiome and lipidome of the milk. Furthermore, the significance of milk metabolites in shaping the milk microbiome and infant gut microbiota are identified.

4:10 pm  

**Quantitative Variation in the Proteome of Individual Healthy Cows**  
*Kasper Hettinga, Wageningen University, The Netherlands*

*Lina Zhang¹, Jeroen Heck², Jacques Vervoort³, and Kasper Hettinga¹,*

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The composition of milk proteins varies between cows. For low-abundant proteins, previous research has mainly focused on variation induced by lactation stage and mastitis. However, variation in the milk serum proteome of healthy cows in mid lactation has not been previously studied. The objective of this study was to look at the differences between individuals on both qualitative and quantitative levels of the bovine milk proteome, taking into account a range of other parameters (parity, feed) that may influence the milk proteome.

Samples from 17 healthy dairy cows in mid-lactation with low somatic cell count (SCC), varying in parity from two to five, and receiving four types of feeding were analyzed by filter aided sample preparation (FASP) and dimethyl labelling followed by LC-MS/MS. Quantitative data analysis was subsequently done using MaxQuant and Perseus. A second dataset was collected of four healthy cows that were sampled five times over a 3-month period in mid lactation and was analyzed with the same methodology.

A total of 183 proteins were identified, of which around two-thirds could be consistently identified in all samples. Less than 10% of the proteins were uniquely identified in a single cow. Parity, type of feeding, and somatic cell count were shown to have almost no effect on the milk serum proteome of healthy cows during mid lactation. However, there was a large variation in the relative standard deviations of the proteins abundance between the individual cows, independent of these parameters. Very little variation was found in the proteins related to nutrient synthesis and transport, indicating the importance of milk in providing nutrients for the neonate. The proteins with the largest variation were those known to be involved in the immune system. The high variation of immune-related proteins may be related to the response of the immune system to changes in the environment and the protection of the mammary gland during stress. The variation may also be partly genetically driven, as it has previously been shown that Holstein cows could be grouped according to either a high or low adaptive and innate immune response, which is similar to what was found in this study. The second independent sample set also showed large variation in immune proteins between individual cows, but small changes over time, indicating a stable pattern of immune proteins in the milk of individual cows.
The milk serum proteome was not significantly influenced by parity and feeding, but differed significantly between individuals, especially in immune-related proteins. This seemed to be a stable cow-specific immune protein fingerprint, indicating that the variation is genetically driven.

**Student Travel Award Recipient: Alternative Splicing, a Fortuitous or Genetically Programmed Event to Expand Molecular Diversity of Milk Proteins: Camel CSN1S2, a Relevant Model to Try to Provide Some Response Elements**

Alma Ryskaliyeva, INRA, UMR GABI, AgroParisTech, Université Paris-Saclay, France

A. Ryskaliyeva¹, G. Miranda¹, C. Henry², B. Faye³, G. Konuspayeva⁴, and P. Martin¹

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3. CIRAD, UMR SELMET, Montpellier, France
4. Department of Biology, Al-Farabi Kazakh State National University, Kazakhstan

Camelids are large even-toed ungulates, strictly herbivorous Mammals belonging to the order Artiodactyla including deer, giraffes, antelopes, sheep, goats and cattle, but differing from ruminants in a number of ways. They have three-chambered stomachs, and uniquely among Mammals, their red blood cells are elliptical. They also have a unique type of antibodies lacking the light chain, besides the normal antibodies found in other Mammals. Their genome is particularly “fragmented” with 74 pairs of chromosomes.

Camel’s milk has proved or supposed therapeutic virtues. It is renowned for its ability to strengthen the immune system, prevent or alleviate autoimmune diseases, including ulcerative colitis and Crohn’s disease. It may also have a prophylactic power on diabetes. In addition, camel milk is extremely rich in vitamin C, and the composition of its protein fraction is intriguing. Camel milk contains large amounts of an antimicrobial protein, the peptidoglycan recognition protein (PGRP), known as an intracellular component of neutrophils, which is present at a very low level in ruminant milks. It contains WAP, like rodents and lagomorphs, whereas Lysozyme C, which is an important component in mare’s milk, is absent.

Up to now the composition of its casein fraction appeared to be relatively well established. However, analyzing milk of camelids originating from Kazakhstan, both in Camelus dromedarius, Camelus bactrianus and their hybrids, with powerful proteomic (LC-MS, LC-MS/MS), genomic (RNA sequencing) and bioinformatic tools, an unexpected complexity was observed, having probable consequences at the technological and nutritional levels. Indeed, a great diversity of molecular species, originating in genetic variants, post-translational modifications but also in the processing of primary transcripts, was highlighted. This situation is particularly conspicuous regarding α-s2 casein for which 3 splicing variants were identified, including exon skipping and cryptic splice site usage, with phosphorylation levels for each of them ranging between 7 and 12 phosphate groups. Such result provides useful novel information for understanding the evolution of casein genes and their expression across Mammals.

With the growing number of genes encoding milk proteins sequenced and displaying complex patterns of splicing, thus increasing the coding capacity of genes, the extreme protein isoform diversity generated from a single gene can no longer be considered as an epiphenomenon. Is it a fortuitous or a scheduled event to expand molecular diversity of milk proteins? Structural diversity and variability in expression level are both responsible for modifications in the organization and, consequently, changes in the physico-chemical properties of the casein micelle. A parsimonious vision of this issue addresses a major question: does this convey any biological significance? Important new insights are expected in this field in the near future.
Bifidobacterial Dominance Correlates with Reduced Infant Gut Antibiotic Resistance

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Human milk plays an important role in shaping the infant microbiome. One mechanism by which breast milk shapes the infant gut microbiota is through human milk oligosaccharides (HMOs); these complex sugars are not digested by the infant but are consumed by Bifidobacterial and Bacteroides spp. Prior to the invention of infant formula and antibiotics, bifidobacteria typically dominated the infant gut community.

This dominance of bifidobacteria may have important implications for a major public health challenge; specifically, the rise of antimicrobial resistance (AMR). As AMR increases, infants are coming to harbor AMR genes. Because AMR is not a common trait in bifidobacteria, the dominance of bifidobacteria in the infant gut community promoted by human milk may reduce the presence of AMR genes in the infant gut community. The objective was to assess if AMR genes are inversely correlated with bifidobacterial dominance in the infant gut.

Stool samples were selected from infants enrolled in a study of infant vaccine efficacy trial in Bangladesh based on 16S rRNA gene sequencing. At most, one sample per infant was included in the present study; 8 samples with low levels of bifidobacteria (<20% relative abundance of genus Bifidobacterium in the 16S results) and 11 samples with high levels of bifidobacteria (>65%). Stool DNA was extracted, fragmented to ~225 bp, and sequenced using Illumina HiSeq 2500 with 150 bp PE reads. Host subtraction was completed using BMTagger. Reads were merged using FLASH then assembled using megaHIT. AMR genes were predicted using ResFinder. Comparisons between the number of unique AMR classes, AMR genes, and total read depth between low and high bifidobacteria samples were made using t-tests. IMG was used to further annotate the assembled sequences, and t-tests were used to compare the number of predicted genes in high and low bifidobacteria samples. BLAST was used on contigs containing AMR genes identified by ResFinder to predict the species of origin for AMR genes.

There was no significant difference in read depth between groups after host subtraction and merging steps (p=0.94, mean read depth high group 18,496,667, mean read depth low group 18,666,616). Samples with high bifidobacteria contained significantly fewer unique AMR genes than samples with low bifidobacteria (p=0.017, mean number genes high group 12.1, mean number genes low group 24.5). The number of unique AMR resistance classes was not significant between samples with high and low bifidobacteria (p=0.073, mean number classes high group 6.2, mean number classes low group 8.0). In addition, the high bifidobacteria samples had significantly fewer predicted genes than the low bifidobacteria samples (p<0.001, mean high 28,722, mean low 71,253). Coliform bacteria were the most common predicted origin of AMR genes. One gene for fosfomycin resistance, fosA, was present in all samples and had a predicted origin of Bifidobacterium longum subsp. infantis.
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Both the total number of predicted genes and the number of AMR genes present in infants with high levels of bifidobacteria were significantly reduced compared to samples with low levels of bifidobacteria. Work is ongoing to identify additional classes of genes that may differ significantly between high and low bifidobacterial samples. This preliminary study suggests that domination of the infant gut microbiota by bifidobacteria during human milk feeding may reduce the level of acquired AMR levels during this critical developmental period.

10:35 am  Rescuing the Infant Gut Microbiome Over the First Year of Life in Breastfed Infants with Bifidobacterium Longum Subsp. Infantis EVC001

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Human milk has evolved to foster not only the growth of the infant, but also to shape the infant gut microbiome via complex human milk oligosaccharides (HMOs). Individual taxa, specifically members of Bifidobacterium, are associated with positive health outcomes in infants, and genetic adaptations to human milk appear to be unique among some of these infant-associated gut microbes. The objective of this study was to longitudinally monitor the effects of supplementation early in life with an activated Bifidobacterium longum subsp. infantis (B. infantis) on gut microbial composition in infants through the first year of life. We hypothesized that colonization by B. infantis would significantly reduce populations of bacteria associated with gut dysbiosis while breastfeeding was maintained.

Women who planned to breastfeed their babies for at least 3 months, and who did not have complications which could preclude breastfeeding, were enrolled along with their infants in the IMPRINT Trial (Smilowitz et al 2017). Subjects were partially randomized to receive lactation support and an activated preparation of B. infantis EVC001 or lactation support alone (n = 34 and 32 per group). Infants consumed the preparation for 21 consecutive days starting on Day 7 post-natal supplied in individual daily dose sachets, and mixed with approximately 5 ml of expressed breast milk. Stool samples were collected from the infants throughout the study, through the first year of life. Bacterial DNA was extracted from stool samples, and analyzed by quantitative PCR and 16S rRNA marker gene sequencing. Metabolic biomarkers were assessed by mass spectrometry.

Infants receiving activated B. infantis EVC001 were rapidly colonized at high numbers by a single strain of the organism (>10^10 CFU/g feces) from the first sample collected after supplementation through the first year of life, so long as the diet was primarily breastmilk. Colonization by EVC001 was also associated with decreased relative abundances of Enterobacteriaceae and Clostridiaceae, with correlated and significant decreases in fecal endotoxin. Infants colonized with B. infantis EVC001 also had a dramatically different fecal metabolome. There was no difference in adverse events between the groups.

Stable colonization by the infant gut symbiont, B. infantis is possible in breast-fed infants whether delivered vaginally or by cesarean section. Infants were rapidly colonized by EVC001 in high numbers. This colonization was stable through the first year of life, so long as breastfeeding continued, and was safe and well tolerated. This colonization had profound effects on the infant fecal biochemistry and gut microbiome with major implications for immune and metabolic development.
11:00 am  **Milk Secretory Immunoglobulin a Protects Lactobacillus Reuteri from Digestion and Aids in Colonization In Vitro**

*Vanessa Dunne-Castagna, University of California, Davis, USA*

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Secretory immunoglobulin A (SIgA) is a bioactive protein derived exclusively from breast milk for the first ten days of life. SIgA serves to both exclude pathogen binding in the intestines through specific F(ab)’ mediated binding and is believed to promote colonization of commensal bacteria through nonspecific N-glycan interactions, though the mechanisms and benefits behind this latter function are poorly understood. The objective of this study was to test the ability of SIgA to maintain viability of the commensal Lactobacillus reuteri during proteolytic digestion, increase its binding capacity to human infant intestinal epithelial crypt cells (HIECs), and confirm the mechanism of commensal SIgA binding.

SIgA was isolated from defatted human milk by affinity chromatography and verified through SDS-PAGE and Western Blot. Flow cytometry showed 27, 48, and 67% SIgA coating on L. reuteri after conjugation with 10, 100 or 1000μg SIgA per 1x10⁸ cfu, respectively. Binding assays were done using SIgA-conjugated L. reuteri added to antibiotic-free Opti-MEM media and then to HIEC cells for 2h. Results demonstrated a 2.5x increase (p<0.005) in the bacterial binding capacity. L. reuteri showed a 15% increase in viability (p< 0.05) when first coated with physiological levels of SIgA prior to in vitro pepsin and pancreatin digestion. To confirm N-glycan mediated binding of SIgA, we digested SIgA with Bifidobacterium longum spp. infantis-derived endo-β-N-acetylglucosaminidase (EndoBI-1) either before or after conjugation with bacteria. Immunofluorescence results showed a marked reduction in fluorescence intensity to near baseline levels, and flow cytometry confirmed an 8% reduction in coating after deglycosylation. Our results demonstrate that milk-derived SIgA can improve the colonization potential for select commensal strains by increasing viability through intestinal transit and potentially by amplifying the binding ability to infant colonic cells through an N-glycan mediated interaction. These results support the possibility of breast milk-derived microbial colonization of the newborn with SIgA protection.

11:25 am  **Milk Protein Digestion in Premature Infants and Bioactive Peptide Discovery**

*David Dallas, Oregon State University, USA*

Though advances in neonatology allow for increased survival of premature infants (<37 weeks gestational age), they remain at high risk for nutrition-associated diseases. Premature infants may not be able to digest breast milk effectively. At birth, the source of infant nutrition shifts from the direct supply of the placenta to, ideally, maternal breast milk that must be digested in the gastrointestinal (GI) system. The GI tract of premature infants must complete fetal development ex utero, and may not be adequately developed to accomplish this transition with the same facility as a term infant. Compared to term infants (>37 weeks in gestational age), preterm infants produce less gastric acid and have lower gastric and intestinal protease activity. This lower digestive capacity is critically important to consider because digestion of milk proteins provides not only nutrition, but also the release of bioactive peptides exhibiting antimicrobial, prebiotic, immune-modulating, calcium-delivery, antihypertensive, and pain-modulating activities. Enzyme activity level and protein digestion likely differ between premature and term infants because of the clear physiological maturation differences; however, the specific differences and their impact on the infant’s ability to acquire nutrition are not known.
Our work uses mass spectrometry-based peptidomics and enzyme analysis of milk, gastric, intestinal and stool samples to determine how proteins are degraded within term and premature infants. We have demonstrated that milk begins to digest itself within the mammary gland with milk proteases, releasing thousands of peptides, many of which are bioactive. We have recently thoroughly characterized the array of milk proteases present. In the stomach, we have demonstrated further digestion via increased peptide abundance and we have determined the relative contributions of breast milk proteases and infant gastric proteases to this digestion. Recently, we have demonstrated via enzyme analysis and peptidomics that protein digestion within the stomach is reduced in preterm compared to term infants. We have constructed a comprehensive database of all literature-identified bioactive milk peptides and use this to examine the in vivo infant digestion peptidomics data.

Our ongoing work examines the peptides and proteases of the infant intestinal tract and the peptides present in infant stool and urine. In addition, we are applying a novel stable isotope approach to examine total protein digestion in these infants. Defining the deficiencies in protein digestion within the premature infant will enable the design of novel strategies for improved nutrition to improve health outcomes.

**11:50 am**  
**Milk Bioactive Peptide Database: A Comprehensive Milk Bioactive Peptide Database and Novel Visualization**  
Søren D. Nielsen, Aarhus University, Denmark

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Milk serves as the primary nutritional source for the mammalian neonate. Beyond basic nutrients, milk has evolved to provide an array of functional compounds to assist the immune system and aid development during the neonatal period. Part of this function derives from the milk proteins, which dissemble into functional peptides during digestion. Besides digestion, functional peptides can be released from dairy proteins by native proteases or during production techniques such as fermentation. These bioactive peptides derived from both casein and whey proteins, have an array of activities including, antimicrobial, angiotensin-converting enzyme (ACE) inhibition, immunomodulatory, opioid agonist and antagonist activities and antioxidative function. Functional peptides may be well suited for applications as food ingredients or supplements and increasingly being investigated for their therapeutic potential, as they are often safer and more selective than small-molecular drugs.

Recent advances in mass spectrometry techniques, including increased sensitivity, spectral acquisition rate and detection range, allow for increasingly comprehensive peptide profiling from dairy and digestive samples. The process of comparing these new identified peptides to the current knowledge on bioactive peptides is an important process to determine possible bioactivities and health benefits. A process currently complicated as this information is scattered across hundreds of scientific journals.

To assist this process, we constructed a comprehensive milk bioactive peptide database by searching scientific citation indexing services, reviews and other databases for bioactive peptides derived from different milk proteins across multiple mammalian species.
More than 700 unique peptide sequence-function combinations were identified in the literature from 10 different species. Most bioactive peptides have been discovered from cow milk proteins (76% of all entries) while most bioactive peptides have been reported from β-casein (36%). ACE-inhibitory peptides have attracted most interest and 327 unique peptide sequences with ACE-inhibitory activity was found and mapped to the parent protein sequence against their activity for novel visualization of the data. Antimicrobial peptides were also widely reported in literature. We identified 177 unique antimicrobial peptides with activity towards 49 different species of bacteria, fungi and parasites, but mostly activity against E. coli or S. aureus was reported. Most antimicrobial peptides derived from lactoferrin and a heatmap across the parent protein sequence was constructed to visualize clustering of antimicrobial peptides. In lactoferrin these peptides clustered around the two sections termed lactoferricin and lactoferrampin which are f(17-41) and f(265-284) of lactoferrin.

The data we collected was also made publicly available as an online database (http://mbpdb.nws.oregon-state.edu/). Our online database offers several benefits to pre-existing databases and are more comprehensive to milk bioactive peptides than other databases. The database can be explored and updated with data from novel research. Our online database tool contains extensive search functions not available to other databases. It can be used to search for single or multiple peptide sequences, protein ID, species, function or any combination of these. Furthermore, the search option for peptide sequence includes three options – searching for bioactive peptides matching a sequence query, searching for a bioactive peptide containing a sequence query or searching for bioactive peptides within a sequence query.

Osteopontin and Its Relation to Transcription of the Major Milk Proteins

Vivi R. Gregersen, Aarhus University, Denmark

Vivi R. Gregersen¹, Esben S. Sørensen¹, Brian Christensen¹, Bart Buitenhuis¹, Nina A. Poulsen¹, Thao T. Le², Lars-Erik Holm¹, Mikka S. Hansen³, Lotte B. Larsen², and Bo Thomsen¹

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As part of an ongoing project aiming at analyzing the breeding potential of specific high value and industrially relevant milk proteins, i.e., α-lactalbumin, β-casein, and osteopontin, we have discovered intriguing results in relation to the osteopontin protein level in bovine milk. To strengthen our analysis to previous studies we included implementation of new methods for absolute quantification of the selected proteins. Osteopontin was determined using sandwich ELISA including a combination of polyclonal and monoclonal antibodies. α-Lactalbumin and β-casein were determined by Multiple Reaction Monitoring (MRM) using Triple Q LC/ESI-MS equipment. The developed MRM quantified the amount of specific peptides (unique parts of the specific protein sequence) from the proteins obtained through reproducible enzymatic cleavage by trypsin. The protein levels (amount per L) in 663 Danish Holsteins were analyzed and absolute quantifications were then used as quantitative traits in order to estimate heritability and perform GWAS. In addition, 24 additional samples with related RNA-seq data obtained from mammary gland epithelial cells were likewise analyzed. As these samples had not been stored at -80° the β-casein measures were not reliable due to coagulation and decay. The two whey proteins were, however, not significantly affected by storage and the measured values was found within the same range as in the 663 samples. The 24 RNA-seq samples were used for differential expression (DE) analyses. In addition, the gene regions of the three proteins were sequenced using the Ion Torrent (Illumina) and TaqMan single SNP genotyping assays were designed for potential functional SNPs.
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The heritabilities were found to be low for β-casein, but moderate for α-lactalbumin and osteopontin. The performed GWAS only identified suggestive QTL regions, which will be further analyzed using a haplotype approach. Moreover, DE analyses were performed on extremes (5 highest vs. 5 lowest) for both α-lactalbumin and osteopontin among the 24 samples with RNA-seq read count data. Prior to this analysis the traits were converted to account for the entire protein amount per milking. Analyzing α-lactalbumin extremes discovered a total of 467 significant DE genes and functional annotation revealed that many of the genes were metal-binding. Moreover, the most significant genes were small non-coding RNAs, which potentially might explain the missing correlation between LALBA mRNA expression and α-lactalbumin protein level due to potential regulatory effects of protein translation. Analyzing osteopontin extremes revealed a total of 616 DE genes including all the major milk genes CSN1S1, CSN1S2, CSN2, CSN3, LALBA and PAEP (FDR p-values < 1.33x10-3; negative log fold change (logFC) values). Previously, a QTL region on chromosome 6 encompassing the SPP1 gene encoding osteopontin was found to affect milk protein percentage and some discussion has been on which gene in the region was causing this effect. Our results point to osteopontin as the candidate gene of this region. The most significant DE gene identified was lactadherin (MFGE8, milk fat globule – EGF factor 8). EGF factor 8 is a bioactive component known to prevent rotavirus infections in babies. In addition, a number of interesting receptors were identified, e.g., PIGR (Polymeric immunoglobulin receptor), PRLR (prolactin receptor), IRS1 (insulin receptor substrate 1), FOLR1 and 3 (folate receptor 1 and 3), GHR (growth hormone receptor), THRβ (thyroid hormone receptor beta) and ESR1 (estrogen receptor 1), all with negative logFC similar to the milk proteins. The analysis so far has shown that most of the highly significant genes are related to mammary gland and lactation.

These preliminary findings lead to two different hypotheses: that osteopontin protein level in milk somehow regulates milk protein synthesis with more osteopontin having a downregulating effect on proteins and receptor activity. Alternatively, osteopontin is regulated by prolactin, insulin and other hormones, but opposite the other milk proteins, indicating a tradeoff between amount of major milk protein and osteopontin level.

1:55 pm  Analysis of Milk Phosphor-Peptides in a Processed Whey Stream
Peter Williamson, University of Sydney, Australia

Dairy milk contains approximately 3-4% protein, mostly as the casein component. However, the restricted protein composition of milk belies the range of bioactivities that exist in the fractions of low abundance proteins, or are embedded within the cryptic component of the major milk protein fractions. Further, the fate of bioactive proteins and peptide fragments during milk processing is not obvious, and in some cases low value streams may contain unexpected biologically valuable components. We have undertaken an analysis of peptides from a whey stream fraction following size-exclusion filtration to identify and evaluate potential bioactive molecules. The crude preparation was analysed for its “natural” peptide component and also enriched for phospho-peptides using ion-exchange chromatography. Peptides were identified using mass spectra according to standard methodology, and by comparison to protein database using MASCOT search tools. When phospho-peptide enrichment was employed, up to 20 prominent protein peptide sources were identified. Identified peptides were derived from major milk proteins (alpha-s1-, beta-casein), milk fat globule (MFG-E8, BTN), and minor milk proteins, including IgG and BLAC. A subset of these peptides was present in the crude fraction without enrichment. Functionally these peptides have been associated with immunomodulatory, tissue repair and angiogenic properties. Further analysis of enriched peptide fractions and potential bioactive properties of this processed fraction will be discussed.
Raw Cow's Milk Prevents the Development of Airway Inflammation in a Murine House Dust Mite-Induced Asthma Model

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Numerous epidemiological studies show an inverse relation between raw cow’s milk consumption and the development of asthma. This protective effect seems to be abolished by milk processing. Evidence for a causal relationship is however still lacking and also direct comparisons between raw and heat treated milk are hardly studied. In the present study we therefore investigated the preventive capacity of raw milk and heated raw milk on the development of house dust mite (HDM)-induced allergic asthma in mice.

Six- to seven-week-old, male BALB/c mice were intranasally (i.n.) sensitized with 1 μg HDM or PBS on day 0, followed by an i.n. challenge with 10 μg HDM or PBS on days 7 to 11. In addition, mice were orally treated with 0.5 mL raw cow’s milk, heated raw cow’s milk (10 minutes, 80°C) or PBS three times a week throughout the study, starting one day before sensitization. At the end of the study (day 14), airway hyperresponsiveness (AHR) in response to increasing doses of methacholine was measured in order to assess lung function and bronchoalveolar lavage fluid (BALF) was examined to study the extent of airway inflammation. T helper (Th) cell subpopulations were quantified in lung cell suspensions using flow cytometry and chemokine and cytokine concentrations were determined in lung homogenates and supernatants of ex vivo HDM re-stimulated lung cells.

Sensitization and challenge with HDM resulted in AHR and pulmonary eosinophilic inflammation. Raw milk prevented both typical features of allergic asthma, whereas heated raw milk did not. Epithelial- and DC-derived mediators, IL-33, CCL20, CCL17 and CCL22, were significantly increased in the lungs of HDM-mice. Both milk types reduced the concentration of CCL17. The percentage of Th2 cells in lung cell suspensions was also significantly reduced by both milk types. Pulmonary concentrations of Th2 cytokines, IL-5 and IL-13 were also increased in HDM-mice, but only raw milk prevented this increase. Upon re-stimulation of lung cells with HDM, both raw and heated raw milk were able to significantly reduce the production of IL-4 and IL-13.

Raw cow’s milk prevents the development of asthma in a murine HDM-induced allergic asthma model. Heat treated raw milk did not show this protective effect. Besides an abundant amount of epidemiological evidence, this study now also suggests a causal relationship between raw cow’s milk consumption and the prevention of allergic asthma.

Student Travel Award Recipient: Comparative Analysis of Bioactive Oligosaccharide Production in Dairy Cows Using Novel Analytical Techniques

Randall Robinson, University of California, Davis, USA

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Milk oligosaccharides (OS) possess a multitude of bioactivities, including the abilities to act as prebiotics and prevent serious diseases in infants. Milk OS are highly concentrated in human milk but are much less abundant in bovine milk, 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. Currently, little is known about variations in OS production among dairy cattle and the factors that impact milk OS abundance. Therefore, this study was developed with the objective of measuring milk OS abundance in a large sampling of dairy cattle by implementing novel techniques for high-throughput milk OS profiling. The OS data has been used for breed-level comparisons between Holstein and Jersey cows to identify differences in OS production. This phenotypic data was then used in a genome-wide association study to identify genes that are potentially responsible for the measured OS variations. Examining the influence of genetics on bioactive compound formation will provide insights into ways that both milk OS abundance and the value of liquid milk production can be increased.

A total of 634 milk samples from mid-lactation Danish Holstein and Jersey cows collected under the Danish-Swedish Milk Genomics Initiative were used for OS profiling and relative quantification. OS were extracted from the milk samples, isobarically labeled, and multiplexed prior to analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to improve instrumental throughput. MS/MS collision energies were optimized to generate reliable reporter ion signals from the isobaric labels that were used as a measurement of relative OS quantities. Relative abundances of individual OS and overall OS profiles were compared between the two breeds.

Analysis of test samples demonstrated that relative OS abundances could be accurately measured in real milk samples with a coefficient of variation below 10% for most OS. The application of isobaric labeling to milk OS analysis, which has been done for the first time in this study, has led to improved signal strength for large fucosylated OS during MS analysis and will allow this class of compounds to be studied in greater detail. Unlike conventional methods that rely on analytical standards and measure relatively few OS, this technique reliably measures abundances for 16 OS in large sample sets without requiring standards. Significant differences in the abundance of 13 OS were identified between the breeds, with most OS having a greater abundance in the Jersey samples. Although Jersey milk had higher average OS abundances, the breed also demonstrated much higher variation between animals in OS production. This variation may represent a potential to harness genetic factors that impact OS abundances. In both breeds, correlations were observed between the abundance of specific OS pairs, which could provide clues into the underlying synthetic pathways.

Once complete, the results of this study will enable improved selection for desirable milk traits by identifying inter- and intrabreed variations in milk OS abundance and correlating these variations with genomic data. This dataset could lead to the development of personalized milk with enhanced bioactive content for specific human health needs.

**Keynote Speaker: How has Saturated Fat Become so Controversial?**

*Benoit Lamarche, Institute of Nutrition and Functional Foods, Université Laval, Canada*

Research over the last decades has provided insightful but sometime discordant information as to the role of dairy foods in health. Because high-fat dairy products contribute significantly to dietary fat and SFA intake, and because SFA are so strongly believed to be involved in the etiology of CVD, many guidelines advocate consumption of low-fat dairy products as opposed to products with higher fat content. Yet, the association between SFA and the risk of CVD remains highly controversial. Several meta-analyses of population studies have in fact failed to find an association between dietary SFA intake and the risk of CVD. Our recent systematic review of evidence from epidemiological studies indicate that intake of total dairy, low-fat dairy, cheese
and specifically may be associated with a lower risk of hypertension. Interpretation of the association between dietary SFA from various dairy foods and health relies on indirect evidence from epidemiological data as well as from a thorough understanding of their impact on many cardiometabolic risk factors, not just LDL-C and blood pressure. In that regard, the focus on SFA as a single nutrient may simply be obsolete. Whole foods in the context of whole diets need to be considered in the diet-heart paradigm.

Differential Impact of Cheese Matrix on Postprandial Lipid Response: A Randomized, Crossover, Controlled Trial

Jean-Philippe Drouin-Chartier, Université Laval, Canada

Jean-Philippe Drouin-Chartier¹, André J Tremblay¹, Julie Maltais-Giguère¹, Amélie Charest¹, Léa Guinot¹², Laurie-Ève Rioux¹², Steve Labrie⁵, Michel Britten¹, Benoît Lamarche¹, Sylvie Turgeon¹², and Patrick Couture¹⁴

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4. Lipid Research Centre, CHU de Québec-Université Laval, Canada

Previous studies from simulated gastrointestinal environments have shown that the cheese matrix resistance to digestive enzymes modulates dairy fat digestion and release of fatty acids. However, the impact of the cheese matrix per se on postprandial lipid response has never been thoroughly evaluated in healthy humans.

The objective of the present study was to compare the impact of dairy fat provided from firm cheese, soft cheese and butter on postprandial triglyceride (TG) response at 4 h (primary outcome) and on the incremental area under the curve (iAUC) of TG and apolipoprotein (apo) B-48 (secondary outcome) in healthy subjects. TG response at 4 h was selected as the primary outcome because of the association between postprandial TG concentrations at 4 h and cardiovascular disease risk.

Forty-three healthy subjects were recruited in this single-blinded, randomized, crossover, controlled trial. In a random order, at intervals of 14 days and after a 12-hour (h) fast, subjects had to ingest 33 g of fat from firm cheese (young cheddar), soft cheese (cream cheese) or butter (control) incorporated in standardized meals matched for macronutrients. Plasma concentrations of TGs and apoB-48 were measured from blood samples collected immediately before the meal and at 2, 4, 6 and 8 h.

The cheddar, the cream cheese, and the butter induced a similar increase in TG concentrations at 4 h (Δ vs baseline: +59% vs +59% vs + 62% respectively, P=0.9 for all). Also, no difference was observed between the 3 meals in TG iAUC (Pmeal=0.9). However, at 2 h, the cream cheese induced a greater increase in TG concentrations than the butter (Δ vs baseline: +44% vs +24%, P=0.002). At 6 h, the response was attenuated with the cream cheese compared with the cheddar (Δ vs baseline: +14% vs +42%, P=0.0004). Although no difference was observed in apoB-48 iAUC between the cheese and the butter (0.08<P<0.7), the cream cheese induced a lower apoB-48 iAUC than the cheddar (Δ vs cheddar: -26%, P=0.01). Differences in apoB-48 response were observed between the cream cheese and the cheddar at 4 h (Δ vs baseline: +42% vs +61%, P=0.02) and 6 h (Δ vs baseline: +14% vs +40%, P=0.0002).

This study demonstrates that cheese matrix modulates the postprandial release of TGs and apolipoprotein B-48 in healthy subjects. However, the cheese matrix does not differentially impact the magnitude of the postprandial peak and response of TGs in comparison with butter.
Student Travel Award Recipient: Identification of Parameters Involved in Disintegration of Commercial Cheese Matrix and Lipid Digestion by Using an In Vitro Static Digestion Model

Léa Guinot, Université Laval, Canada

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Postprandial lipemia is dependent on the bioavailability and bioaccessibility of free fatty acids. The bioaccessibility of fatty acids can be modulated by matrix degradation’s rate which can be impacted by inherent cheese factors as composition or textural properties. The aim of this study is to determine which parameters influence cheese degradation. A static in vitro digestion model has been used on nine commercial cheeses: young and aged cheddar, regular and light cream cheese, parmesan, feta, camembert, mozzarella, and slice processed cheese. At the end of gastric digestion, camembert and mozzarella presented the lowest matrix disintegration whereas aged cheddar, regular and light cream cheeses showed the fastest. All cheeses were disintegrated at the end of duodenal digestion. The free fatty acids release was fast during the first 30 min of duodenal digestion. Correlation between cheese disintegration and its textural and compositional properties was analyzed by partial least square regression and revealed that fracturability was correlated to the rate of cheese disintegration as opposed to springiness, hardness, cohesiveness which were negatively correlated. By modulating cheese texture, it could be possible to influence the matrix disintegration during gastrointestinal digestion which could have an impact on the release of lipids and subsequently on postprandial lipemia.

Milk Fat Globule: New Insights into an Unappreciated Complex Lipid System

Bruce German, University of California, Davis, USA

Nurit Argov-Argaman¹, Ben Boyd⁴, and Bruce German¹,²
1. Department of Food Science and Technology, University of California, Davis, USA
2. Foods for Health Institute, University of California, Davis, USA
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Extensive research on the evolutionary origin of lactation and the composition, structures and functions of milk’s biopolymers illustrates that the Darwinian pressure on lactation selected for biopolymers with considerable complexity and discrete functions within the digestive system (Hinde & German 2012). For example, complex sugar polymers: oligosaccharides possess unique properties in guiding the growth of intestinal bacteria that are not possible by feeding their simple sugars; proteins exhibit enzymatic activities towards other milk components rendering those components both more digestible but also releasing biologically active products. To date however, the most complex structure in mammalian milk, the fat globule has not been effectively examined beyond its simple composition. Our research is revealing that the globules of milk are more dynamic, diverse and functional than previously considered. Milk lipids are a unique biological particle class composed of a triglyceride core bounded by a phospholipid monolayer, assembled in the endoplasmic reticulum, and a complete bilayer structure enrobed around the globule by the mammary epithelial plasma membrane during globule secretion (German 2011; Argov et al., 2008). The size and composition of milk fat globules changes during lactation and as a function of genetics, diet and mammary gland metabolism (Mesilati-Stahy et al., 2011). Fat globules undergo complex disruption during digestion and then these lipids spontaneously self-assemble within the intestine forming unique three dimensional structures. Recent studies on the phase changes during lipolysis have demonstrated that this ensemble of complex lipids forms distinct
cubic structures in real time (Salentinig et al., 2015) whose presence has been linked to successful absorption of a variety of fat soluble nutrients. Thus the lipid globule constitutes the precursors for a complex, higher order, structured delivery system that self assembles within the infant’s intestine facilitating absorption by the infant. This growing body of evidence argues for a broader view of milk composition that includes the complex structures of large biopolymers, their structures as ensembles, their distinct activities within the milk as it is digested and the influence of this structural dimension on the health value of milk within the entire diet.

Arbov N, Lemay D.G. and German JB Milk fat globule structure and function: nanoscience comes to milk production Trends in Food Science & Technology 19, 12, 617-623 (2008)
German JB Dietary lipids from an evolutionary perspective: sources, structures and functions Maternal & child nutrition 7 (52), 2-16

Thursday September 28, 2017
9:00 am Donor Human Milk for the Preterm and At-Risk Term-born Infant
Deborah O’Connor, University of Toronto, Canada

Mother’s milk is the optimal source of nutrition for all infants including very low birth weight (VLBW, <1500 grams) and hospitalized term-born infants. Even in high income countries mother’s milk is associated with a reduction in infant mortality, morbidity (e.g. diarrhea, lower respiratory tract infection), improved neurodevelopment and likely a reduction in overweight and diabetes (Victora C et al Lancet 2016). However, for a variety of reasons including immaturity of the mammary gland, maternal illness and other factors related to preterm birth, up to 70% of infants born VLBW require a supplement to mother’s milk. Until recently this supplement was typically preterm formula. With a growing awareness of the benefits of mother’s milk, use of pasteurized donor human milk as a supplement compared to formula has risen exponentially. At the end of this presentation participants will be able to describe the different sources of donor milk being used in North America. They will be able to describe the milk composition changes that accompany pasteurization (62.5°C for 30 minutes). By the end of this presentation participants will be knowledgeable about the level of evidence in support of supplemental donor milk for VLBW infants—specifically the impact on necrotizing enterocolitis, other serious morbidities, growth and neurodevelopment. Finally, the audience will have a good understanding of some of the nutritional issues that need to be considered in extending the use of pasteurized donor milk as a supplement to term-born infants in and outside the neonatal intensive care unit.

Funded by a Programmatic Grant in Food and Health from the Canadian Institutes of Health Research (CIHR,
Lactation has evolved over the past 200 million years since the appearance of the aplecantal, egg laying monotremes. Subsequently, there has been extensive adaptation to reproduction, particularly in lactational strategies when the Theria split into the Metatheria (Marsupialia) and Eutheria (Placentalia) lineages over 140 Mya. For example, reproduction in an Australian marsupial, the tammar wallaby is characterized by a short gestation (26.5 days), birth of immature young and a long lactation (approximately 300 days) during which the concentration of all the major milk constituents, and many minor milk bioactives progressively change. There is increasing evidence that these changes in milk composition regulate growth of the tammar pouch young, particularly during the first 100 days postpartum when the development of the neonate is similar to that seen in a late stage eutherian foetus. It appears that evolution has not “reinvented the wheel” and therefore the signaling factors from the placenta, amniotic fluid and potentially colostrum involved in the development of the eutherian foetus/neonate are most likely delivered in the milk of marsupials.

Fostering experiments demonstrated that transferring the early phase tammar pouch young to a later phase lactating tammar can accelerate the growth and physical development of pouch young. In addition, continual fostering of a tammar neonate to a tammar at an earlier stage of lactation limited development of the young. More recent studies using in vitro models have shown that milk collected from marsupials during early lactation (day 20-100), but not late lactation (day 100-300) stimulated proliferation and differentiation of cultured whole lung from mouse embryos. Preliminary experiments have also shown that tammar milk can stimulate development of cultured stomach from the mouse foetus. Therefore, the temporal delivery of these bioactives is most likely crucial for the development of the suckled young.

The mammary gland in marsupials is very sophisticated in terms of its capacity for temporal delivery of bioactives for multiple targets. In contrast, the eutherian mammary gland is less sophisticated as many of its previous functions have evolved to be delivered by multiple tissues, particularly a well-developed placenta and the amniotic fluid. Therefore, the composition of milk does not change significantly during lactation apart from the transition from colostrum to mature milk. We have known for some time that both significantly pre-mature and low birth weight babies have acute challenges for survival, largely due to limited development of their lungs and gut, and these babies may show an increased frequency of mature onset disease.

Therefore, many developmental clocks are set in the foetus and any potential disruption to this process may subsequently impact development and the frequency of mature onset disease. It is clear that the marsupial provides a unique opportunity to more easily identify bioactives that potentially play a role in early development of the foetus.
Experiments in our laboratories have focused on RNAseq and microarray analysis of the tammar wallaby mammary gland and human milk cells and tissue to identify the transcriptional profile of genes and miRNA expressed throughout the lactation cycle. Bioinformatic-based comparisons of these databases using the tammar data from early lactation and the human mammary expression profile, and comparisons with publicly available genomic and proteomic databases from human placenta and amniotic fluid have focused on identifying potential protein, peptide and miRNA signaling factors for early development of the human neonate. These studies have been extended to re-evaluate the potential role of human colostrum in this process. It is increasingly evident that the interrogation of the evolutionary history of lactation has great promise for application of comparative approaches for better understanding the role of milk in acute and chronic well-being of the baby. Studies using the tammar wallaby may lead to a new range of human fortifiers that include bioactives with the potential to specifically target the growth and development of tissues in the human neonate to improve outcomes for premature and low birthweight babies.

Maternal Programming of Lung Development During Lactation in the Marsupial Neonate

Christophe Lefèvre, Walter and Eliza Hall Medical Research Institute, Melbourne, Australia

Christophe Lefèvre1,5, Vengamanaidu Modepalli3, Amit Kumar4, Julie A Sharp1, Norman R Saunders4, and Kevin R Nicholas2,3
1. Division of Bioinformatics, Walter and Eliza Hall Medical Research Institute, Melbourne, Australia
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5. Peter MacCallum Cancer Centre, Australia

After a short gestation period, marsupials give birth to immature neonates with lungs that are not fully developed. In contrast to eutherian mammals where lung development occurs predominantly in the embryo, the marsupial neonate partially relies on gas exchanges through the skin at birth and significant lung development occurs during the early lactation period. To explore the mechanisms of postnatal marsupial lung development and to investigate the contribution of milk in its control, we conducted morphological and gene expression analysis of the developing lung in the grey short tail opossum (Monodelphis domestica) and assayed the activity of tammar wallaby (Macropus Eugenii) milk on embryonic mouse lung cultures in vitro and postnatal lung development in vivo by cross-fostering experiments in tammar wallabies.

The transcription program of the developing opossum lung was obtained by RNA-seq at seven time points and the results were analyzed using the genome reference of the opossum and integrated with large public datasets of gene expression microarray of lung development in mice to compare the development programs of a marsupial with a eutherian mammal. Despite the limitations associated with the mapping of genes between species and the challenges of integrating RNA-seq and microarray data, the results show a significant overlap between the overall timing of differential gene expression in both lineages along the morphological phases of lung development and highlight differences due to either 1) the functionalization of the pulmonary vascular system shortly after birth corresponding to different development stages in the two lineages, or 2) the differential phasing and relative amplitude of muscle fiber biogenesis during the perinatal transition from the saccular to the alveolar stages in the eutherian and the maturation stage in the marsupial lung.

The activity of milk from different periods of lactation of another larger marsupial, the tammar wallaby (Macropus Eugenii) was assayed on lung development in vitro using a mixture of mouse lung organ and organoid cultures. The results show a differential effect of milk collected during different lactation period with milk collected from the second half of the early phase 2A (lactation days 60 to 100) having the strongest...
positive effect on: 1) branching morphogenesis and growth in whole lung cultures, 2) organoid formation and, 3) cellular differentiation in epithelial and mesenchymal cell cultures. An apparent inhibitory effect was observed with day 20 milk while day 120 milk had limited effect. This was followed further in vivo by cross-fostering experiments on suckling wallaby neonates. Tammar pouch young at postnatal day 25 were maintained on early lactation day 15 to 25 milk for a total of 20 days by a series of two fostering transfers onto mothers at day 15 lactation for 10 days and compared to a normal neonate at day 45 postnatal. In comparison to controls, fostered pouch young had smaller lungs with reduced airway surface, thicker parenchyma and lower expression of gene markers of lung development. The results therefore support a differential role of the lactation period on development. Interrogation of the differential biogenesis and composition of tammar milk during the course of lactation by mammary gland transcriptomics and milk proteomics experiments identified a number of putative growth factors potentially involved in these bioactivities.

The results therefore suggest the changes in milk composition during lactation in marsupials provide temporal bioactives that can influence diverse aspects of lung development and support further exploitation of the marsupial model toward the identification of maternal milk factors regulating marsupial development and their putative uterine and milk equivalents in eutherians. As the immaturity of the lungs at birth in preterm infants may lead to significant developmental problems and the maturation period of lung development in human extend to the age of 3, the identification of putative milk factors and associated mechanisms influencing lung maturation would be of particular significance for the understanding of the role of milk in the establishment and maintenance of good health.

Lactational Concentration Changes of Individual Human Milk Oligosaccharides in Breast Milk from Chinese and Malaysian Women

Paul McJarrow, Fonterra Research and Development Centre, Palmerston North, New Zealand

P. McJarrow*, X. Liu, JM Hamid Jan, L. Ma, A. Welman, and B. Fong
1. Fonterra Research and Development Centre, Palmerston North, New Zealand
2. Department of Clinical Nutrition, Guangzhou Woman and Children’s Medical Centre, China
3. Nutrition and Dietetics Program, School of Health Sciences, Universiti Sains Malaysia, Malaysia

Human milk oligosaccharides (HMOs) are a significant and diverse component of human milk which are lacking in infant formulas. Research suggests that HMOs can act as anti-infectives, immune system modulators, prebiotics and a source of sialic acid for neural development. Current infant formulations have relied on the use of GOS and FOS to provide the prebiotic function delivered by HMOs. Recent advances in the production of HMOs may enable a limited number of HMOs to be added to infant - and follow-on formulas at human milk concentrations.

Historically, a wide range of extraction and analysis methods have been applied to quantifying HMOs. This assortment of approaches has yielded a diversity of validation statuses with respect to recovery and repeatability of HMOs from human milk. This assortment of approaches has also resulted in a variety of HMO concentrations being reported. In this investigation, we have used a validated extraction and LC-MS analysis approach to investigate the concentrations of HMOs in breast milk from two human cohorts.

The aim of this work was to determine HMO concentration changes in the milk of two human populations over the time-course of lactation. To this end, we have used a HILIC-MRM-MS approach to quantify seven acidic and five neutral major oligosaccharides in the two populations. Additionally, we followed changes in the HMO levels of individual mothers across lactation. The acidic oligosaccharides quantified were 3’ N-acetyl neuraminyl
lactose, 6’ N-acetyl neuraminyl lactose, 6’ N-acetyl neuraminyl N-acetyl-lactosamine, 3’ N-acetyl neuraminyl 3’
fucosyl lactose, disialyl lactose, LS-tetrasaccharide a/b and LS-tetrasaccharide c. The neutral oligosaccharides quantified were Lacto-N-neotetraose, Lacto-N-tetraose, Lacto-N-fucopentaose, 2’ fucosyl lactose and 3’
fucosyl lactose. This presentation summarises our findings and compares the results with historical data.

11:10 am  **Beyond the Bench: The Future of Human Milk Stem Cells for Preterm Infants**

Carrie-Ellen Briere, University of Massachusetts, Amherst, USA

Carrie-Ellen Briere¹,²,³, Todd Jensen⁴, Jacqueline M. McGrath²,³, and Christine Finck³,⁴
1. College of Nursing, University of Massachusetts, USA
2. School of Nursing, University of Connecticut, USA
3. Connecticut Children’s Medical Center, USA
4. University of Connecticut Health Center, USA

Stem cells were first identified in human milk in 2007. Since then there have been discoveries related to their function and potential. Recent research suggests that human milk stem cells may be involved in typical infant health and development. In milk science we understand there are various differences between full-term human milk and preterm human milk and we believe that the stem cell content may differ as well. Our research team is the first known team to enroll mothers of hospitalized preterm infants during the first few weeks of lactation and compare stem cell phenotypes and gene expression to mothers of healthy full-term infants.

Participants were recruited from a Level IV NICU (preterm dyads) and the community (full-term dyads) in the Northeastern USA. Mothers of hospitalized preterm infants (32-34 weeks gestational age at birth – n=7) and mothers of healthy full-term infants (40 weeks gestational age at birth – n=4).

Human milk stem-like cell populations were identified in both preterm and full-term human milk samples. The data suggest variability in the proportion of stem cell phenotypes present, as well as statistically significant differential expression (both over and under-expression) of stem-cell specific genetic markers when comparing mothers’ milk for preterm and full-term births. Specifically, when comparing preterm milk to full-term milk, SOX2 had a fold change (FC) in expression of 8.4 (p<0.0001), Nanog had an FC of 9.2 (p<0.0001), CD90 had an FC of 8.3 (p<0.03), and CD105 had an FC of 1.6 (p=0.001) [indicating these markers were expressed more in preterm samples]. Markers that were under-expressed in the preterm sample included EpCAM with an FC of 0.22 (p<0.0001) and TJP1 with an FC of 0.56 (p=0.007).

Our findings indicate that 1) stem cells are present in preterm human milk; 2) differential expression of stem-cell-specific markers can be detected in preterm and full-term human milk samples; and 3) the percentage of cells expressing the various stem-cell specific markers differ when preterm and full-term human milk samples are compared. This presentation will focus on these findings as well as next steps with our preliminary data in murine research. Additionally, clinical implications for preterm infants will be addressed in relation to this research and the translation of research into practice.
The discoveries that milk contains proteolytic enzymes are compelling evidence that one of milk’s key values has been unappreciated. Milk is not a simple mixture of proteins functioning solely as a source of amino acids. Milk can now be viewed as a protein-protease system delivering specific protein fragments to sites along the gastrointestinal tract. The selectivity, specificity and activity of this system can be studied at the level of complexity necessary to appreciating its diverse values to health, yet deciphering such complex protein-protease delivery system is challenging. Current methods of food protein digestion that examine pure proteins reacted with digestive enzymes and measuring hydrolysis in vitro are incapable of incorporating the complexity of the milk system and the outcomes. Furthermore, exploration of the bioactivity of hundreds of milk protein fragments in infants is daunting. Integrated approaches are needed to investigate the protein-protease system from these various perspectives to capture the value of milk. A broad collaborative effort is the key to reaching this goal. We have found that this breadth of research objectives provides an unusual educational opportunity in team science for young undergraduates. Therefore, we have assembled a team including undergraduate students with various individual perspectives, utilizing analytical, physiological, biochemical and computational tools to study the protein-protease system of human milk. This collaborative research model has been inspiring young students to develop versatile skill sets and to make novel research discoveries about milk and its properties. Using biochemical models, we have studied interactions between milk enzymes and proteins. With LC-MS/MS, we have identified unique human milk peptides released by its ensemble of proteins and proteases that are distinct from those previously studied. With computational tools, we have constructed databases of the naturally occurring peptides annotated for their protein origins and putative enzymes responsible for their cleavages. Using ontology and evolutionary criteria, we have proposed novel bioactivities for specific peptides. These research successes are building a better understanding of milk as a uniquely complex and effective delivery system to change the value proposition of milk for health improvement.
1) **Milk as a Protein-Protease Delivery System: Collaborative Approaches Inspire Inquiry into the Dynamics and Complexities of Human Milk**  
*Junai Gan, University of California, Davis, USA*  
**Student Travel Award Recipient**  

*Junai Gan*, Daniela Barile¹,², Carlito Lebrilla²,³, and Bruce German¹,²  
¹. Department of Food Science and Technology, University of California, Davis, USA  
². Foods for Health Institute, University of California, Davis, USA  
³. Department of Chemistry, University of California, Davis, USA

The discoveries that milk contains proteolytic enzymes are compelling evidence that one of milk’s key values has been unappreciated. Milk is not a simple mixture of proteins functioning solely as a source of amino acids. Milk can now be viewed as a protein-protease system delivering specific protein fragments to sites along the gastrointestinal tract. The selectivity, specificity and activity of this system can be studied at the level of complexity necessary to appreciating its diverse values to health, yet deciphering such complex protein-protease delivery system is challenging. Current methods of food protein digestion that examine pure proteins reacted with digestive enzymes and measuring hydrolysis in vitro are incapable of incorporating the complexity of the milk system and the outcomes. Furthermore, exploration of the bioactivity of hundreds of milk protein fragments in infants is daunting. Integrated approaches are needed to investigate the protein-protease system from these various perspectives to capture the value of milk. A broad collaborative effort is the key to reaching this goal. We have found that this breadth of research objectives provides an unusual educational opportunity in team science for young undergraduates. Therefore, we have assembled a team including undergraduate students with various individual perspectives, utilizing analytical, physiological, biochemical and computational tools to study the protein-protease system of human milk. This collaborative research model has been inspiring young students to develop versatile skill sets and to make novel research discoveries about milk and its properties. Using biochemical models, we have studied interactions between milk enzymes and proteins. With LC-MS/MS, we have identified unique human milk peptides released by its ensemble of proteins and proteases that are distinct from those previously studied. With computational tools, we have constructed databases of the naturally occurring peptides annotated for their protein origins and putative enzymes responsible for their cleavages. Using ontology and evolutionary criteria, we have proposed novel bioactivities for specific peptides. These research successes are building a better understanding of milk as a uniquely complex and effective delivery system to change the value proposition of milk for health improvement.

2) **Identification of Parameters Involved in Disintegration of Commercial Cheese Matrix and Lipid Digestion by Using an In Vitro Static Digestion Model**  
*Léa Guinot, Université Laval, Canada*  
**Student Travel Award Recipient**  

*Léa Guinot*, Laurie-Eve Rioux¹,², Steve Labrie¹,², Michel Britten¹,²,³, and Sylvie Turgeon¹,²  
¹. STELA Dairy Research Centre, Université Laval, Canada  
². Institute of Nutrition and Functional Foods (INAF), Université Laval, Canada  
³. AgriFood Canada, Saint-Hyacinthe, Québec, Canada

Postprandial lipemia is dependent on the bioavailability and bioaccessibility of free fatty acids. The bioaccessibility of fatty acids can be modulated by matrix degradation’s rate which can be impacted by inherent cheese factors as composition or textural properties. The aim of this study is to determine which parameters influence cheese degradation. A static in vitro digestion model has been used on nine commercial cheeses: young and aged cheddar, regular and light cream cheese, parmesan, feta, camembert, mozzarella, and...
slice processed cheese. At the end of gastric digestion, camembert and mozzarella presented the lowest matrix disintegration whereas aged cheddar, regular and light cream cheeses showed the fastest. All cheeses were disintegrated at the end of duodenal digestion. The free fatty acids release was fast during the first 30 min. of duodenal digestion. Correlation between cheese disintegration and its textural and compositional properties was analyzed by partial least square regression and revealed that fracturability was correlated to the rate of cheese disintegration as opposed to springiness, hardness, cohesiveness which were negatively correlated. By modulating cheese texture, it could be possible to influence the matrix disintegration during gastrointestinal digestion which could have an impact on the release of lipids and subsequently on postprandial lipemia.

3) 

**Comparative Analysis of Bioactive Oligosaccharide Production in Dairy Cows Using Novel Analytical Techniques**

*Randall Robinson, University of California, Davis, USA*

*Student Travel Award Recipient*

Randall C. Robinson¹, Nina A. Poulsen², Lotte B. Larsen², and Daniela Barile³

1. Department of Food Science and Technology, University of California, Davis, USA
2. Department of Food Science, Aarhus University, Denmark
3. Foods for Health Institute, University of California, Davis, USA

Milk oligosaccharides (OS) possess a multitude of bioactivities, including the abilities to act as prebiotics and prevent serious diseases in infants. Milk OS are highly concentrated in human milk but are much less abundant in bovine milk, 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. Currently, little is known about variations in OS production among dairy cattle and the factors that impact milk OS abundance. Therefore, this study was developed with the objective of measuring milk OS abundance in a large sampling of dairy cattle by implementing novel techniques for high-throughput milk OS profiling. The OS data has been used for breed-level comparisons between Holstein and Jersey cows to identify differences in OS production. This phenotypic data was then used in a genome-wide association study to identify genes that are potentially responsible for the measured OS variations. Examining the influence of genetics on bioactive compound formation will provide insights into ways that both milk OS abundance and the value of liquid milk production can be increased.

A total of 634 milk samples from mid-lactation Danish Holstein and Jersey cows collected under the Danish-Swedish Milk Genomics Initiative were used for OS profiling and relative quantification. OS were extracted from the milk samples, isobarically labeled, and multiplexed prior to analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to improve instrumental throughput. MS/MS collision energies were optimized to generate reliable reporter ion signals from the isobaric labels that were used as a measurement of relative OS quantities. Relative abundances of individual OS and overall OS profiles were compared between the two breeds.

Analysis of test samples demonstrated that relative OS abundances could be accurately measured in real milk samples with a coefficient of variation below 10% for most OS. The application of isobaric labeling to milk OS analysis, which has been done for the first time in this study, has led to improved signal strength for large fucosylated OS during MS analysis and will allow this class of compounds to be studied in greater detail. Unlike conventional methods that rely on analytical standards and measure relatively few OS, this technique reliably measures abundances for 16 OS in large sample sets without requiring standards. Significant differences in the abundance of 13 OS were identified between the breeds, with most OS having a greater abundance in the Jersey samples. Although Jersey milk had higher average OS abundances, the breed also
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demonstrated much higher variation between animals in OS production. This variation may represent a potential to harness genetic factors that impact OS abundances. In both breeds, correlations were observed between the abundance of specific OS pairs, which could provide clues into the underlying synthetic pathways.

Once complete, the results of this study will enable improved selection for desirable milk traits by identifying inter- and intrabreed variations in milk OS abundance and correlating these variations with genomic data. This dataset could lead to the development of personalized milk with enhanced bioactive content for specific human health needs.

Alternative Splicing, A Fortuitous or Genetically Programmed Event to Expand Molecular Diversity of Milk Proteins: Camel CSN1S2, a Relevant Model to Try to Provide Some Response Elements

Alma Ryskaliyeva, INRA, UMR GABI, AgroParisTech, Université Paris-Saclay, France

Student Travel Award Recipient

A. Ryskaliyeva¹, G. Miranda¹, C. Henry², B. Faye³, G. Konuspayeva⁴, and P. Martin¹
1. INRA, UMR GABI, AgroParisTech, Université Paris-Saclay, France
2. INRA, UMR MICALIS, AgroParisTech, Plateforme d’Analyse Protéomique Paris Sud-Ouest (PAPPSO), Université Paris-Saclay, France
3. CIRAD, UMR SELMET, Montpellier, France
4. Department of Biology, Al-Farabi Kazakh State National University, Kazakhstan

Camelids are large even-toed ungulates, strictly herbivorous Mammals belonging to the order Artiodactyla including deer, giraffes, antelopes, sheep, goats and cattle, but differing from ruminants in a number of ways. They have three-chambered stomachs, and uniquely among Mammals, their red blood cells are elliptical. They also have a unique type of antibodies lacking the light chain, besides the normal antibodies found in other Mammals. Their genome is particularly “fragmented” with 74 pairs of chromosomes.

Camel’s milk has proved or supposed therapeutic virtues. It is renowned for its ability to strengthen the immune system, prevent or alleviate autoimmune diseases, including ulcerative colitis and Crohn’s disease. It may also have a prophylactic power on diabetes. In addition, camel milk is extremely rich in vitamin C, and the composition of its protein fraction is intriguing. Camel milk contains large amounts of an antimicrobial protein, the peptidoglycan recognition protein (PGRP), known as an intracellular component of neutrophils, which is present at a very low level in ruminant milks. It contains WAP, like rodents and lagomorphs, whereas Lysozyme C, which is an important component in mare’s milk, is absent.

Up to now the composition of its casein fraction appeared to be relatively well established. However, analyzing milk of camelids originating from Kazakhstan, both in Camelus dromedarius, Camelus bactrianus and their hybrids, with powerful proteomic (LC-MS, LC-MS/MS), genomic (RNA sequencing) and bioinformatic tools, an unexpected complexity was observed, having probable consequences at the technological and nutritional levels. Indeed, a great diversity of molecular species, originating in genetic variants, post-translational modifications but also in the processing of primary transcripts, was highlighted. This situation is particularly conspicuous regarding α-s2 casein for which 3 splicing variants were identified, including exon skipping and cryptic splice site usage, with phosphorylation levels for each of them ranging between 7 and 12 phosphate groups. Such result provides useful novel information for understanding the evolution of casein genes and their expression across Mammals.

With the growing number of genes encoding milk proteins sequenced and displaying complex patterns of splicing, thus increasing the coding capacity of genes, the extreme protein isoform diversity generated from
a single gene can no longer be considered as an epiphenomenon. Is it a fortuitous or a scheduled event to expand molecular diversity of milk proteins? Structural diversity and variability in expression level are both responsible for modifications in the organization and, consequently, changes in the physico-chemical properties of the casein micelle. A parsimonious vision of this issue addresses a major question: does this convey any biological significance? Important new insights are expected in this field in the near future.

**5) Next-Generation Sequencing of Bovine Milk-Derived Exosomal MicroRNA to Determine Transcriptome Expression for Efficient Recovery from Mastitis Infection**

*Andrea Zukowski, University of Ottawa, Ontario, Canada*

*Student Travel Award Recipient*

Andrea Zukowski¹, Jamie Kraft¹, and Illimar Altosaar¹  
¹ Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Canada

Mastitis, an inflammatory disease of the mammary gland, presents one of the greatest economic challenges to the dairy industry. Milk somatic cell count is the current standard for the detection of bacterial infection, but is not diagnostic, pre-emptive, and is prone to false-positives. Biomarkers, such as microRNA (miRNA), for susceptibility and response to treatment may provide the herdsman with a more significant means of managing mastitis.

Exosomes are extracellular microvesicles (30-150 nm diameter) released by various cell types, including mammary cells. Milk-derived exosomes encapsulate degradation-resistant microRNAs (miRNAs), long non-coding RNA, mRNA, and proteins. These miRNAs are thought to play an important role in intercellular communication, especially during an immune system response.

Prior work suggests that milk exosomal miRNAs differ between healthy cows and those that have been inoculated with pathogenic bacteria. However, post-mastitis bovine milk exosomal miRNA expression has yet to be elucidated. Using next-generation sequencing, within-host miRNA expression can be monitored over the course of treatment for the discovery of novel recovery-mediating miRNAs or time-dependent changes in the levels of these miRNAs. We propose that miRNA expression in cow’s milk may vary between cows that respond quickly to treatment and those that do not. This inter-herd variability can be taken advantage of to treat cows with sub-optimal recovery. The aim of our project is to model the flux of miRNA profiles in milk collected from mastitis-infected Holstein cows at various time-points during and after antibiotic treatment. We will analyze miRNA expression for time-dependent, inter-quarter, and intra-herd differences to identify biomarkers of effective and efficient mastitis recovery.

**6) Influence of Sodium Citrate on DNA Extraction and Bacterial Quantification in Dairy Products**

*Josefa Blaya, Department of Food Science, University of Guelph, Canada*

Josefa Blaya¹, Zoha Barzideh¹, and Gisèle LaPointe¹  
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Culture-independent methods are increasingly being applied in conjunction with culture-dependent methods to study the microbial composition of milk and dairy products. Quantitative PCR (qPCR) has proven to be a reproducible method for quantification of microbial populations on food samples, including milk and cheese. The success of this technique requires the extraction of adequate amounts of DNA free of inhibitors. However, the high concentration of caseins in dairy products may hinder DNA extraction from
bacterial cells. Calcium-chelating agents such as tri-sodium citrate have been in common use to overcome this issue. Nevertheless, the influence of tri-sodium citrate on the suitability of the extracted DNA for PCR amplification of bacteria has not been tested. In this study, qPCR was applied to quantify the populations of lactic acid bacteria (L. lactis subsp. cremoris, L. lactis subsp. lactis and Leuconostoc spp.) in a total of 11 starter culture samples produced in skim milk or whey-based medium. The main goal was to analyze the effect of tri-sodium citrate on the DNA yield and purity obtained, but also on the quantification of bacterial DNA using qPCR. Viable counts were performed as a culture-dependent approach to determine if tri-sodium citrate introduced a bias by inhibiting the growth of bacterial cells from starter cultures and cheese.

Six starter culture samples showed higher DNA yields when sodium citrate was employed. For four of them, low to no DNA was obtained when sodium citrate was not used. Conversely, two samples showed higher DNA yields when sodium citrate was not used, whereas no effect was observed in two other samples. The DNA extraction method employed, UltraClean Microbial DNA Isolation kit (Mo Bio Laboratories Inc.) was consistent in terms of the purity of the DNA extracted.

The levels (in log copy numbers using the 16S rRNA gene by qPCR) of all the quantified bacterial populations were lower in the samples in which DNA yield was affected by sodium citrate. In these cases, sodium citrate may have promoted a premature release of bacterial cells into the suspension. In the remaining samples, tri-sodium citrate affected the quantification of the tested bacteria depending on the microbial composition, which was not correlated with the DNA yield. While the quantification of L. lactis subsp. cremoris was not affected by sodium citrate in any other sample, the levels of L. lactis subsp. lactis were highly affected in two samples and Leuconostoc spp. in one.

Traditional plating of one of the starter culture samples on M17 and Reddy agar media led to 10^8 CFU/mL, while a one-log reduction was observed when tri-sodium citrate was used. A higher reduction was seen when counting Leuconostoc spp. Approximately 10^5 CFU/mL were obtained when sodium citrate was not used, whereas no colonies were obtained when sodium citrate was used. In the case of Cheddar cheese, the effect of sodium citrate was negligible with 18-month-old Cheddar cheese (plated on MRS). However, a one-log reduction was observed when sodium citrate was employed in a younger Cheddar cheese (less than 12 months old). The application of high-throughput sequencing (MiSeq) to both cheeses indicated that whereas lactococci dominated the bacterial population of the younger cheese, the aged Cheddar was mainly composed by lactobacilli. The reduction of the number of isolates on M17 and MRS with vancomycin indicates that SLAB populations are affected by tri-sodium citrate, while lactobacilli may be more resilient.

These results highlight the relevance of high quality DNA for accurate quantification of the microbes in dairy products. This study may serve as a starting point for implementing new measures during DNA extraction to ensure an accurate downstream quantification of the bacteria of interest.

7) Characterization and Valorization of the Genetic Pool of the Canadienne Breed
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The Canadienne cattle is the oldest breed of dairy cattle in North America. The harsh environmental conditions of the time gave her a rusticity and longevity that made her an invaluable genetic resource.
Today, the Canadienne is heavily threatened because of the crossings that have been made and the population is estimated to be about 1,200 individuals, including less than 300 pureblood animals. Until now, no effort has been made to document the genetic pool of this heritage breed. The hypothesis of this project stipulates that it is still possible to properly manage the current genetic numbers to ensure the durability of the breed. The project aims to describe the genetic pool of the Canadienne cow and to establish a management system for the development of the breed. In order to guide efforts to preserve the Canadienne breed, we propose a distinctive strategy based on genomics. A total of 192 animals belonging to four groups called the “originals,” the “pure-blood,” the “ancients,” and the “foreigners” were genotyped (640,000 SNPs per sample) using the Axiom platform (Affymetrix). Data were analyzed using genetic statistics to determine their diversity level.

Results suggest that there is still a possibility for diversifying the breed according to a principal component analysis of the genotypes which identified five genetically distinct subgroups from the population. The work aims to dislodge this heritage breed from folklore and to value it for its genetic characteristics in a healthy and sustainable way, in order to arouse the interest of the breeders and restore confidence in the breed. The data will also support future haplotypes research projects associated with the health and longevity of Canadienne cows.

8) Whey Protein Content and Smoothing Temperature are Tools to Modulate Stirred Yogurt Structure and Rheological Properties
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Yoghurt represents an increasing part of food markets nowadays. Although, a major problem for the dairy industry is to suppress additives in the non-fat product. Indeed, consumers are not always ready to compromise with the loss of texture and increased syneresis that non-fat stirred yoghurt displays. In the present study, milk protein composition and smoothing temperature were investigated as a leverage to control the non-fat yoghurt gel microstructure, textural properties and wheying-off. Reconstituted milk was standardized at 14 % total solid, 0 % fat content, and 3% caseins content. Whey protein content was varied among three different caseins to whey protein ratio 1.5 (Y1.5), 2.8 (Y2.8) and 3.9 (Y3.9). The mixes were then homogenized and heat treated at 95 °C for 5 min. Using a commercial starter, the mixes were fermented (3.5 to 4 h) at 42 °C, until pH 4.6. Then yoghurt gels were pumped through a smoothing pilot system comprising a pre-smoothing conic filter (2 mm screen) followed by a plate heat exchanger set at 15, 20, or 25 °C, and finally a smoothing conical filter (0.5 mm screen). Yoghurt was then packaged in plastic pot and stored at 4°C. Analyses were realized at day 1, 9 and 23 after production. Particle size (yoghurt microgels) was studied using laser light scattering (only at day one), and microscopic image analyses. Syneresis was measured using a classical forced syneresis method, and time domain low frequency nuclear magnetic resonance on protons (1H-LF-NMR). Finally, textural properties were determined by viscosity measurement using a rheometer, and by firmness using a texture analyzer. Protein formulation was the main parameters influencing all the measured properties. Increasing the smoothing temperature increased the gel firmness, and microgel sizes assessed by image analyses, but did not influence the viscosity or microgel sized obtained by laser light scattering. It means that microgel in stirred yoghurt is shaped by whey protein content and the smoothing temperature. However, the structure of microgel due to the smoothing temperature is weak and sensitive to mechanical shears. As expected, the gel evolved with time storage, but the evolution pattern depended on protein
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formulation. Yogurt with the highest whey protein content showed the biggest particles. Moreover, their sizes increased with storage time as opposed to yogurt Y2.8 and Y3.9. Micrographs showed heterogeneous gel density with empty area occupied by serum for Y1.5, while Y2.8 and Y3.9 showed few serum and more disrupted gel embedding microgels. Forced syneresis reduced with whey protein content and time of storage. It was in agreement with 1H-LF-NMR showing less serum mobility with increasing whey protein content and longer storage. However, 1H-LF-NMR also showed higher spontaneous serum separation in Y1.5 and Y3.9 rising over time compared to Y2.8 separated serum which was low and stable. In conclusion, texture and syneresis are controlled by the whey protein standardization and smoothing temperature, the resulting microstructure seems to be a key point. Also, for industry this offers a possibility to reduce the use of additives in non-fat dairy products, by optimizing milk standardization and the smoothing process.

Effect of Milk Protein Preparations on In Vitro Intestinal Wound Healing
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Inflammatory bowel disease (IBD), comprising primarily of ulcerative colitis and Crohn’s disease are pronounced welfare problems. One of the conditions associated with IBD is the occurrence of injuries and wounds at the luminal side of the intestine resulting in a disruption of the protective intestinal epithelial cell layer. Injuries to the epithelial layer can increase the penetration and absorption of factors that triggers an immune response. It is therefore important with rapid resealing of the epithelial surface barrier in the intestine following damage and injuries.

The wound healing mechanism in the intestine, also termed mucosal healing, is a complex process involving different and overlapping stages. At first, epithelial cells migrate from the edge of the wound into the denuded area, a process termed restitution. This process is followed by epithelial cell proliferation to replenish the decreased cell poll in the wounded area. When the wound gap has closed, the new cell layer goes through a final process of maturation and differentiation to reestablish the cell wall barrier. The different stages of wound healing are regulated by a number of different compounds such as growth factors and gut peptides.

Our work comprised analysis of eight commercial milk protein preparations consisting of milk protein hydrolysates or whey protein concentrate. The milk protein preparations were investigated for their potential as functional foods regarding gut integrity and repair using in vitro wound healing assays with rat intestinal epithelial cells of normal origin. The effect of milk protein preparations on cell migration was studied by scratch assay and cell proliferation was determined by a resazurin metabolism assay. The protein and peptide profile of the milk protein preparations was investigated by liquid chromatography-electrospray ionization tandem mass spectrometry ion trap and one-dimensional polyacrylamide gel electrophoresis. We demonstrated that especially two casein-based hydrolysates were very potent stimulators of cell migration, showing a significantly increased migration of up to 50% compared to the control sample in the concentration range 0.01 to 10 mg/mL. One of these casein-based hydrolysates consisted of both protein fragments and peptides ranging between 5 to 16 amino acids in length while the second casein-based hydrolysate consisted mostly of large protein fragments. Both casein-based preparations also stimulated cell proliferation. However, the highest level of proliferation was obtained with a third casein-based hydrolysate, showing nearly 50% increased proliferation compared to the control sample at 1 mg/mL. This casein-based hydrolysate consisted
of small peptides ranging between 5 and 10 amino acids in length. As migration and proliferation are important mechanisms for the integrity of the intestinal epithelial cell layer, both at normal conditions and especially after injury, our results suggest that peptides released from caseins by hydrolysis have beneficial activity on gut wound healing mechanisms.

Metabarcoding of Québec's Terroir Cheeses

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The province of Québec is the major cheese producer in Canada, with 48\% of total cheese production in 2016. The production of speciality cheeses accounts for 30\%, and increases steadily (about 6\% per year). Cheesemaking is known to be a very challenging process, especially regarding the constancy between batches. Depending on both the cheese variety and the plant where they are produced, between 2\% and 20\% of the production can be affected by a microflora disequilibrium, which can lead to undesired development of organoleptic properties in the cheeses. As a consequence, cheesemakers need new tools to control the development of the cheese’s natural microflora. The aim of this study was to develop a metabarcoding approach to determine the composition of the cheese microflora and to better understand the microbiological variations occurring in several terroir cheese varieties. In total, twenty-eight (28) cheeses made from cow milk having or not undergone heat treatment (raw, thermized or pasteurized milk), belonging to different varieties (natural-, smear- or bloomy-rind), and originating from twelve administrative regions of the province of Québec were selected. The first objective of this project was to determine the best molecular targets to assess the microbial diversity of cheese ecosystems through metabarcoding. To do so, four (4) smear-cheeses were selected and the sequencing was performed for comparison on the ITS regions (ITS1 versus ITS2) and the 16S rDNA regions (V3-V4 versus V6-V8) for fungi and bacteria, respectively. The bioinformatics analysis was optimized through QIIME software.

For the bacteria, the V6-V8 region was more effective for characterizing the smear-cheese ecosystems. The metabarcoding results showed that both the starter and the ripening cultures used for smear-cheeses (Lactococcus, Streptococcus, Brevibacterium) were more abundant in the cheese core and on the surface of cheeses in the early days of ripening (day 6). Moreover, the results showed that the bacterial diversity (Inverse Simpson index) and richness (Chao index) were higher on the cheese rind and that they increased along with ripening time. The secondary microflora, mostly found on the rind, included coryneform bacteria (Corynebacterium, Staphylococcus, Arthrobacter/Glutamicibacter, Brachybacterium) and psychrohalophilic bacteria (Halomonas, Pseudoalteromonas, Cobetia, Psychrobacter). In conclusion, the 16S rDNA metabarcoding method is reliable and functional for the characterization of cheese bacterial communities. The analysis for the best ITS target region, the diversity and richness, and the identification and relative abundance of the fungal microflora are still in progress and the metabarcoding method developed will be performed to characterize 24 cheese ecosystems. The characterization of the cheese microflora will allow the identification of microorganisms potentially involved during cheese ripening. It will eventually help Québec cheesemakers to select new ripening cultures and control their inoculation, on the way to produce high quality cheeses constantly.
Breakfast Meal Composition Modulates in vitro Protein Digestion

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Dairy products are known as a very good source of several essential nutrients which are highly bioavailable. Several studies have linked food structure and texture to different kinetics of nutrient delivery. Changes in some nutrients’ release rate such as peptide and amino acids from casein and whey proteins qualified as "low and fast" proteins could induce different physiological effects. However, in a complex meal it is not known if these different behaviors remain. This study aims to understand how the kinetics of proteins bioaccessibility of milk enriched in proteins with different casein to whey protein ratios are influenced by breakfast cereal. Milk enriched in proteins (7.7 %) with different casein: whey protein ratios (80:20, 40:60 and control) were prepared. They were mixed or not with breakfast cereals and an in vitro digestion was performed to assess protein digestion and matrix disintegration. The milk type and the addition of cereals significantly impacted the breakfast meal disintegration. Disintegration of milk with higher amounts of whey proteins was reduced at the end of the oral and gastric step in presence of cereals. The addition of cereals has delayed protein digestion during gastric digestion. Most of the whey proteins were still undigested at the end of the gastric digestion in accordance with previous studies. At the end of the duodenal digestion, each meal (with and without cereals) was completely disintegrated and most of their proteins content were bioaccessible. This study will help understand how a complex meal modifies the release of nutrients unlike the ingredients studied alone. This will improve our knowledge on the impact of the food matrix composition on the kinetics of nutrients release in conditions closer to meal consumption.

Sheep and Cow Milk – Differences in Protein Digestibility and Effect on Intestinal Microbiota in a Rat Model

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Milk compositional variations from different species have recently become more widely known, and it has become apparent that these differences may result in distinct effects on physiological function, and ultimately, on the health of the consumer. Some of these effects are likely related to gut health and comfort. The large intestinal microbial community can be modulated via bacterial metabolism of milk components such as oligosaccharides. Changes in the intestinal microbiota can influence many health outcomes including obesity, inflammation and allergies. Anecdotally, some people that are unable to tolerate cow milk find they are able to better tolerate sheep milk, and vice versa. This may be attributed to differences in sheep and cow milk protein digestibility. However, controlled scientific studies examining these aspects are lacking. To better understand some of the fundamental effects of sheep milk on gastrointestinal health, we conducted two rodent studies to (A) investigate how sheep and cow milk affect the caecal tissue gene expression and microbial community, and (B) examine the apparent ileal amino acid digestibility in rats fed either cow or sheep milk.

For both studies, newly weaned Sprague-Dawley rats were fed a dairy-free rodent chow and provided either raw sheep milk or raw cow milk for 28 days in groups of ten rats per treatment. Caecal tissue and
content samples were collected for microarray gene expression analysis and community profiling by 16S rRNA pyrosequencing for study (A). In the second study (B), the rats were also fed a dairy-free rodent chow and raw sheep or cow milk for 28 days. However, in this study, the rats were housed individually in metabolism cages at days 12-14 and 26-28, at which time they only had access to sheep or cow milk. Amino acid concentrations were measured in the distal ileal content and blood serum at day 28.

There were no significant differences in mean body weight after 28 days of milk feeding in both studies. However, rats fed raw cow milk consumed more solid food than rats fed raw sheep milk but there was no difference in intake volume. This difference is likely to reflect, at least in part, the higher percentage of milk solids present in sheep milk. In the first study (A), microarray analysis of caecal tissue gene expression showed differences in expression of pathways involved in tissue organization and development, with the majority of genes being more highly expressed in the sheep milk-fed rats. Only relatively minor differences were observed in the caecal microbial community, which primarily centred on genera within the Bacteroidetes phylum. In the second study (B), amino acid digestibility in the distal ileum at day 28 was higher in rats fed sheep milk; amino acid intake in sheep milk rats was 1.8 times higher, but the ileal content amino acid concentration was only 1.3 times higher. Differences in amino acid digestibility between the two milks was reflected in serum amino acid concentrations, with significantly higher concentrations of essential amino acids of rats fed sheep milk.

Our results show that sheep and cow milk affect the caecal tissue gene expression and large intestinal microbial community differently. The results also suggest that sheep milk proteins are more readily digested than cow milk proteins in vivo and this difference is reflected in a higher bioavailability of essential amino acids.

13) Natural Solutions to Make Healthy, Safe and Nutritious Dairy Products

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Dairy products with improved taste and texture, but fewer additives and reduced sugar, fat, or overall calorie content are in high demand by modern, health-conscious consumers. As a major player in the global dairy industry, Chr. Hansen A/S is constantly exploring novel and natural ways to improve products to fulfill demanding consumers. This pushes the boundaries of microbial performance and requires the constant development of new dairy cultures with novel properties. As many of the dairy products like yoghurt, cheese, buttermilk and kefir contain living lactic acid bacteria, the development of new improved cultures are today based on natural strategies (i.e. without the use of recombinant DNA technology) such as random mutagenesis, directed evolution and dominant selection.

Here we will demonstrate the use and the progress of natural methods for selection and improvement of dairy bacteria with examples that illustrate all-natural solutions to make attractive dairy products with low residual lactose, low fat, controlled acidity, better texture or improved safety.
14) Study of the Effects of Shear Treatment, Fat Content and Fermentation Time on Rheological Properties of Stirred Yogurts
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Stirred yogurt is the most consumed in Canada. Once the yogurt has been fermented, it is stirred, pumped, cooled and smoothed inducing major gel structural changes. Only limited results are available on the effect of industrial processing on yogurt gel as the process can hardly be simulated at the laboratory scale. The aim of this project was to determine the effect of fermentation time and two cooling processes on the rheological properties of stirred yogurts varying in total solid and fat contents. Yogurt composition was standardized at 16.5% total solids, 4% protein and 0-3.9% fat contents. Three fermentation time were targeted: 3, 4 and 5 hours. A control non-fat yogurt has also been produced at 14% total solid content. Stirring has been performed using a pilot plant system developed in-house allowing yogurt stirring and cooling with either a tubular (low shear treatment) or plate heat exchanger (high shear treatment). Physicochemical analysis (syneresis), texture (firmness) and viscosity were measured 1 or 34 days after storage at 4°C.

Syneresis was lower for yogurts with higher total solid contents. Increasing fat content reduced syneresis and increased firmness and viscosity of yogurts. Shorter fermentation time showed a tendency for lower firmness and viscosity with more syneresis compared with longer fermentation times. Yogurts obtained after tubular cooling were more firm than others cooled by plate heat exchangers but showed more syneresis. There was no effect of cooling process on yogurt viscosity. Firmness and viscosity increased during storage due to structure recovery but there was no change in water retention ability (similar syneresis). The pilot plant system allowed to compare two types of cooling processes using small scale (20L) yogurt production. This information is valuable to identify critical processing steps to optimize yogurt quality.

15) Effect of Fermented Milk from Lactococcus Lactis Subsp. Cremoris Strain JFR1 on Salmonella Invasion of Mucosal HT29-MTX Epithelial Cells
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The process of fermentation contributes to the organoleptic properties, preservation and nutritional benefits of food. Fermented food may interfere with pathogen infections through a variety of mechanisms, including competitive exclusion or improving the intestinal barrier integrity. Salmonella is a major foodborne pathogen causing significant public health concerns with an estimated 93.8 million human cases annually worldwide. In this study, the effect of milk fermented with Lactococcus lactis subsp. cremoris JFR1 on Salmonella invasion of an intestinal epithelial cell line HT29-MTX was investigated. HT29-MTX cells were pretreated for 1 h with Lactococcus lactis subsp. cremoris JFR1 fermented milk before the addition of Salmonella enterica subsp. enterica Typhimurium DT104. Treatment with fermented milk resulted in increased cell membrane integrity shown by monitoring the transepithelial electrical resistance (TEER) of HT29-MTX cells. The TEER values of treated HT29-MTX cells increased during pretreatment and remained constant for the duration of
infection (up to 3 h); illustrating a protective effect. After gentamicin treatment to remove adhered bacterial cells, enumeration revealed a reduction in numbers of > 1 log10 of intracellular Salmonella. However, chemically acidified and coagulated milk (gluconodeltalactone) failed to show the same effect on monolayer integrity and Salmonella invasion. Collectively, these results suggest that milk fermented with Lactococcus lactis subsp. cremoris JFR1 is effective in vitro in the reduction of Salmonella invasion into mucosal HT29-MTX cells. Further research will show how fermented milk could strengthen the epithelial cell tight junctions, with a potential role in maintaining gut barrier integrity.

16) **Regulating the Switch: Cellular Differentiation and Mitochondrial Turnover in the Mammary Gland**

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Characterizing the mechanisms underlying the regulation of cellular differentiation and metabolic crosstalk are critical to understanding the metabolic adaptation to lactation, and functional differentiation of the mammary gland. We have previously shown that bHLH/PAS family member, Singleminded-2s (Sim2s), plays an important role in mammary gland development. Sim2s is temporally regulated, reaching maximal expression in concert with milk protein genes at mid-lactation. Mice over-expressing Sim2s under the mouse mammary tumor virus (MMTV) promoter exhibit precocious alveolar differentiation evidenced by enhanced expression of milk protein genes prior to lactation. Cross-fostered pups nursed by MMTV-Sim2s dams also weigh significantly more by mid-lactation compared to pups nursed by control dams. Transmission electron microscopy of MMTV-Sim2s mammary glands at peak lactation revealed increased accumulation of autophagic structures. Interestingly, reduced mitochondrial content was observed in the mammary glands of these mice as well, suggesting that these structures could be mitophagic. Real-time PCR analysis in HC11 mouse mammary epithelial cells over-expressing Sim2s confirmed upregulation of autophagy genes, including LC3 and Atg7, during lactogenic differentiation. Employing the use of MitoTimer in HC11 cells, increased turnover of mitochondria with the onset of differentiation was visualized and confirmed in real time with over-expression of Sim2s. Together, these data demonstrate that Sim2s expression is associated with enhanced mammary gland differentiation and performance by regulating mitochondrial turnover in response to pup nutritional demand.

17) **Milk Fat Globule Membrane Source Affect Intestinal Microbiota Profile and Short Chain Fatty Acid Production**

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Human and ruminant milk fat globule membranes (MFGMs) contain components such as glycoproteins and long chain fatty acids, some of which are known to reach the infant large intestine. Studies have shown the large intestinal microbiota of rodents fed formula supplemented with bovine MFGM and rodents fed maternal milk have greater similarity than rodents fed un-supplemented formula. Bovine, caprine and ovine MFGMs...
differ in protein and fat composition, and this might affect their digestibility, and consequently their effects on microbial composition and fermentation end-products in the large intestine.

We hypothesized that dietary bovine, caprine and ovine MFGMs differently modulate intestinal microbiota population diversity and fermentation end-products. This study aimed to determine the in vitro effects of undigested and digested bovine, caprine and ovine MFGMs on (1) fermentation by a faecal microbial population sourced from breast-fed infants, (2) fermentation by ileal and caecal microbial populations sourced from piglets, and (3) production of short-chain fatty acids (SCFAs).

Bovine, caprine, and ovine MFGMs and a commercial bovine phospholipid concentrate (PC; Tatura, New Zealand) were predigested using in vitro digestive conditions representative of those found in humans in the first 5 months of life (90 min pepsin, 60 min pancreatin). Digested and undigested MFGMs and PC were then fermented for 6 or 14 h, under anaerobic conditions, using faecal inoculum sourced from 3-5 month old breast-fed infants. Digested MFGMs and bovine PC were also fermented using ileal and caecal microbiota sourced from piglets (ileal inoculum for 2 h, which was then added to caecal inoculum and fermented for 12 h). Changes in the relative proportions of microbial taxa, and production of SCFAs, were assessed at the end of fermentation.

Undigested substrates, infant faecal inoculum: After 6 h fermentation bovine PC increased the relative proportion of Bifidobacterium and decreased the proportion of Alistipes compared to other substrates (P<0.05). Caprine and ovine MFGMs decreased the proportion of Bacteroides compared to bovine MFGM and PC. After 14 h fermentation, these differences in microbial composition were no longer present. Instead, the relative proportion of Alistipes was increased in bovine, caprine and ovine MFGMs compared to bovine PC. The relative proportion of Staphylococcus was also increased in response to caprine MFGM compared to other undigested substrates. Fermentation of caprine MFGM produced more acetic, propionic and isobutyric acids when compared to the other undigested substrates after 6 and 14 h fermentation.

Digested substrates, infant faecal inoculum: After 6 h fermentation, caprine MFGM and PC increased the relative proportion of Bifidobacterium and decreased the proportions of Bacteroides, Lactobacillus, Blautia and Escherichia genera compared to bovine and ovine MFGMs (P<0.05). However, these differences in microbial composition were not present after 14 h fermentation (P>0.05). Fermentation of PC produced less total SCFAs when compared to the other digested substrates after 6 and 14 h. Bovine MFGM produced more acetic and butyric acids after 6 and 14 h fermentation compared to caprine and ovine MFGMs and bovine PC.

Digested substrates, piglet ileal and caecal inoculum: Bovine and caprine MFGMs decreased, and PC increased, the proportion of ileal Escherichia coli compared to the levels found in the initial inoculum. All digested substrates increased the proportion of caecal Bacteroides and decreased the proportions of caecal Clostridium and Coprococcus. No differences in SCFA concentrations were observed after ileal fermentation. Bovine MFGM produced more butyric and acetic acid and ovine MFGM produced more butyric and formic acid after caecal fermentation.

These results demonstrate that origin MFGM affect infant faecal microbiota composition and SCFA production. MFGM from all species, and bovine PC, may also affect the ileal microbiota composition.
Studying the Effect of Bovine Milk Consumption on the Human Gut Microbiota Using a TWINSHIME® (Twin-Simulator of the Human Intestinal Microbial Ecology)

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Bovine milk is an important source of nutrients to the diet, providing high quality proteins and fats, important minerals such as calcium and magnesium, and vitamins such as B12. It also contains lactose and oligosaccharides. Upon gastrointestinal digestion, bioactive peptides from protein digestion, and free fatty acids and monoglycerides from milkfat digestion are produced, which are absorbed by the small intestine. However, recent in vitro results suggested that some of the fat globules, possibly stabilized by protein, remained after intestinal digestion [1, 2] and may possibly enter the large intestine, with or without lactose, where they come into contact with the gut microbiota. The human gut microbiota is a large population of bacteria, composed of 500-1000 species, which reside in the large intestine. It is linked to human health and disease, and is altered by dietary changes. While the consumption of milk is known to provide health benefits, there is limited knowledge on its effect on the gut microbiota. In future studies, the effects of both fat-free and full-fat milk on the gut microbiota will be tested through application of the Twin Simulator of the Human Intestinal Microbial Ecology (TWINSHIME®). The TWINSHIME® is an in vitro system composed of individual bioreactors designed to reproduce the conditions of the entire gastrointestinal tract from the stomach to waste. As a TWINSHIME®, there are two complete systems set up to run in parallel. Each system has individual bioreactors to represent the stomach, and small intestine, and divides the large intestine into the ascending, transverse, and descending regions. The large intestine bioreactors contain cultures of fecal bacteria and have both a luminal and added mucosal phase. In order to utilize the TWINSHIME® for dietary studies, the system must first be evaluated for its ability to produce a stable bacterial community. This is paramount to application of the system because without establishment of a stable community, there is no way to distinguish between the changes occurring due to system variation and the addition of milk components. To produce a stable community, the system was inoculated with fecal homogenate and run for a total of 6 weeks. During this time, samples were harvested every 2-3 days from both the luminal and mucosal phases of each colon reactor. DNA sequencing of the 16S rRNA genes from these samples was performed to determine community composition over time, and SCFA analysis was performed using a GC-MS to determine metabolic production. PCoA analysis based on weighted and unweighted Unifrac distances and SCFA analysis revealed that the gut microbiota in the TWINSHIME® reached stability by day 10 post inoculation, and remained stable until the end of the experiment. Analysis of the community composition for each intestinal region revealed that the populations were divergent from each other, and had developed unique community structures, yet were similar to the fecal sample used for inoculation. The community of the mucosal phase differed from the luminal phase in relative abundance for each intestinal region. In conclusion, the data provides evidence that the TWINSHIME® is able to produce a stable, human gut microbial community. In upcoming studies this system will be used to study the effect of fat-free and full-fat bovine milk on the gut microbiota of the large intestine, in terms of community dynamics, metabolic function, and proteome composition.

Ganglioside and Phospholipid Composition of Human Breast Milk Over Lactation
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Complex lipids, such as gangliosides and phospholipids, play important roles in intra- and inter-cellular signaling, migration, proliferation, neurological development, and inflammatory and immune responses. These complex lipids are also found in various biological fluids, including the milk fat globule membrane of human milk.

Growing evidence shows that complex lipids, such as those from human milk, play an important role in infant development, which has led to studies of human milk composition. Furthermore, it has been suggested that human milk composition may be influenced by diet and population demographics.

The objective of this study was to use a validated liquid chromatography-mass spectrometry (LC-MS) method to determine the ganglioside and phospholipid classes and concentrations in breast milk from a cross section of mothers.

Human breast milk gangliosides consisted of both GM3 and GD3. GD3 was the dominant ganglioside in colostrum and transitional milk, while GM3 was the major ganglioside class in mature human milk. Total ganglioside concentration was highest in colostrum and transition milk before dropping to a lower level during the start of the mature milk period. Over the mature milk period a gradual increase in average total ganglioside (GD3 + GM3) concentration was observed to about 25 mg/L at 6 months.

The human breast milk phospholipids sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) were also measured. Total phospholipid (SM, PC, PE, PI and PS) concentrations were highest in colostrum, before dropping to lower levels at the start of the mature milk period. In the mature milk samples, the average total phospholipid concentration increased gradually over the lactational period to about 220 mg/L.
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