INTERNATIONAL™
MILK GENOMICS
CONSORTIUM

15th International Symposium on

Milk Genomics and Human Health

November 13–15, 2018

Kimpton Sawyer Hotel
Sacramento, CA

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Dear Participant,

On behalf of the organizing committee, we welcome you to Sacramento for the 15th meeting of the International Conference on Milk Genomics and Human Health. Please take this opportunity to network with your colleagues from around the world and across the scientific spectrum. This meeting is intended to be fully interactive, to build on existing collaborations, and to start new ones.

As always we are going to do our utmost to encourage scientific collaboration using every possible means. We will have various social events as part of the symposium. On Tuesday, we will have a Poster Reception in the Magnolia Foyer sponsored by National Dairy Council. On Wednesday evening, we will have a cocktail hour, a tour of the arena, and a reception-style group dinner at the Golden 1 Center.

The organizers are also delighted to welcome you to the city of Sacramento and to the entire region of Agriculture in Northern California. Sacramento is the state capitol with many opportunities to see California in 'urban' action. You are also encouraged to also visit the University of California-Davis, less than a half hour from the conference, to identify new opportunities.

Welcome to IMGC 2018. This is our 15th anniversary meeting and we are very excited to host this International conference to discuss the exciting discoveries of science related to milk and lactation and to plan for their applications and opportunities for renewed research. Milk and Lactation have proven to be an inspiring and unifying research focus, bringing scientists together from across the physical, chemical, biological and computational sciences. We encourage you to renew acquaintances with colleagues, make new friendships and take a bold leap into the diverse field of milk science.

J. Bruce German & Danielle Lemay
On behalf of the local organizing committee

Tuesday, November 13, 2018

8:00 am **Registration**

9:00 am **Welcome**
Bruce German, University of California-Davis, Davis, CA, USA

Hot Topics

9:20 am **Highlights from IMGC's "SPLASH!® milk science update" and Upcoming Hot Topics**
Danielle Lemay, Western Human Nutrition Research Center, USDA, Davis, CA, USA

9:45 am **KEYNOTE: Maternal Nicotinamide Riboside Enhances Juvenile Development and Adult Neurogenesis**
Charles Brenner, University of Iowa, Iowa City, IA, USA

10:30 am **Coffee Break**

11:00 am **Invited Speaker: The Impact of Dairy Management and Animal Genomics on Product Quality and Environmental Impact: The Need for an Integrated and Interdisciplinary Approach**
John Finley, USDA, Beltsville, MD, USA

11:30 am **What's Normal? Genome-Wide Association Analysis of Human Milk Produced by Healthy Women Across Different Geographic Locations**
Janet Williams, University of Idaho, Moscow, ID, USA

12:00 pm **Lunch**

Genetics and Genomics

1:00 pm **KEYNOTE: Gene Editing and the Future of Bovine Genetics**
Alison L. Van Eenennaam, University of California-Davis, Davis, CA, USA

1:45 pm **GWAS and Expression of Candidate Genes for Oligosaccharide Synthesis in Danish Dairy Breeds**
Nina Aagaard Poulsen, Aarhus University, Tjele, Denmark

2:15 pm **The Genetics of Phosphorylation of Caseins in Bovine Milk**
Marlene Visker, Wageningen University, Wageningen, Netherlands

2:45 pm **Coffee Break**

3:15 pm **Milk Genomics: Variation in Danish Dairy Milk and Its Possible Exploitation in the Dairy Chain**
Lotte Bach Larsen, Aarhus University, Tjele, Denmark

3:45 pm **Beta-Casein Derived Beta-Casomorphin Peptides in Human Milk for Infant Nutrition**
Ashwantha Kumar Enjapoori, Deakin University, Geelong, Victoria, Australia

4:15 pm **Update from 2017's MVP, Benoit Lamarche, Laval University**
Facilitated by *Bruce German, University of California-Davis, Davis, CA, USA*

4:30 pm **Quantitative Proof of Why It's Good to Be Here**
Danielle Lemay, Western Human Nutrition Research Center, USDA, Davis, CA, USA

Evening Sessions

5:30-6:30 pm **Joint Scientific Advisory Council/Steering Committee Meeting (Closed Session)**

6:00-8:00 pm **Poster Reception- Sponsored by National Dairy Council (NDC)**

Wednesday, November 14, 2018

Health Impact of the Gut Microbiota

9:00 am	**Keynote Speaker: A Systems Biology Approach to Characterizing the Gut Microbiome of Breastfed Infants Colonized by B. infantis EVC001** *Steve Frese, Evolve Biosystems, Davis, CA, USA*
9:45 am	**Population Duration of Breastfeeding and Prevalence of Bifidobacterium** *Diana H. Taft, University of California-Davis, Davis, CA, USA*
10:15 am	**Coffee Break**
10:45 am	**Bifidobacterium Longum Subsp. Infantis Stably Restores the Infant Gut Microbiome Over the First Year of Life in Breastfed Infants** *Jennifer Smilowitz, University of California-Davis, Davis, CA, USA*
11:15 am	**Student Travel Award Recipient: Structural and Functional Insights into EchAMP, a Unique Monotreme Antimicrobial Protein Expressed During Lactation** *Alok Kumar, CSIR-Centre For Cellular and Molecular Biology, Habsiguda, Hyderabad, India*
11:35 am	**Moderated Discussion of Morning Session**
12:00 pm	**Lunch**

Milk Microbiomes: Do they matter?

1:00 pm	**The Human Milk Microbiome** *Michelle K. McGuire, University of Idaho, Moscow, ID, USA*
1:30 pm	**Milk Microbiomes in Large-Scale Dairy Processing** *Mary Kable, University of California-Davis, Davis, CA, USA*
2:00 pm	**Panel Discussion- Milk as a Source of Microorganisms: Practical Implications** **Moderator:** *Danielle Lemay, Western Human Nutrition Research Center, USDA* **Panelists:** *Maria Marco (University of California, Davis), Steve Frese (Evolve Biosystem, Davis) and Michele Jay-Russell (Western Center for Food Safety, University of California, Davis)*
2:30 pm	**Coffee Break**

Milk and Dairy Products Impact on Health - Glycans

3:00 pm	**Keynote Speaker: Utilization of Oligosaccharides and Bioactive Carbohydrates from Milk** *Carlito Lebrilla, University of California-Davis, Davis, CA, USA*
3:45 pm	**Human Milk Oligosaccharides Directly Enhance Gut Barrier Integrity to Alter Infection** *Ishita Shah, University of California-Davis, Davis, CA, USA*
4:15 pm	**Student Travel Award Recipient: Evaluate the Effect of Industrial Thermal Treatments on the Enzymatic Release of N-Glycans from Milk Glycoprotein** *Apichaya Bunyatratchata, University of California-Davis, Davis, CA, USA*

Evening Event

5:30-9:30 pm	**Group Dinner (meet in the lobby at 5:20pm to walk to Golden 1 Center)**

Thursday, November 15, 2018

The Lipids of Milk: Globules to Vesicles

9:00 am **Keynote: The Milkfat Globule an Ever Evolving Story**
Sophie Gallier, Fonterra Co-operative Group, Palmerston North, New Zealand

9:40 am **Student Travel Award Recipient: Commercial Bovine Milk Fat Globule Membrane Fractions-Variations Among Sources**
Lauren Brink, University of California-Davis, Davis, CA, USA

10:00 am **Student Travel Award Recipient: The Therapeutic Potential of Bovine Milk-Derived Extracellular Vesicles for Treatment of Osteoarthrits Patients**
Bartijn Pieters, Radboud University Medical Center, Nijmegen, The Netherlands

10:20 am **Coffee Break**

The Digestion of Milk

10:50 am **Milk Protein Digestion and Bioactive Peptide Release in Term and Preterm Infants**
Dave Dallas, Oregon State University, Corvallis, OR, USA

11:20 am **Comparison of Human Milk Immunoglobulin Survival during Gastric Digestion between Preterm and Term Infants**
Veronique Demers-Mathieu, Oregon State University, Corvallis, OR, USA

11:40 am **Differences in Composition and Structure Between Human Milk and Infant Formula: Do They Affect Their Digestion?**
Didier Dupont, INRA-Agrocampus Ouest, Rennes, France

12:00 pm **Closing Remarks**

12:30 pm **Lunch**

Meet the Speakers

Charles M. Brenner, Ph.D.
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A 1993 Ph.D. from Stanford University, Charles Brenner is the discoverer of nicotinamide riboside (NR) as a vitamin and among the world's leading experts on NAD metabolism. In addition to discovering the NR kinase pathway and characterizing unanticipated steps in synthesis and regulation of NAD, Dr. Brenner developed quantitative targeted NAD metabolomics, which has allowed his group to identify diseases and conditions of metabolic stress including obesity, neuropathy, heart failure and central brain injury in which NAD metabolites are under attack. Dr. Brenner has used animal models to show that sequelae of these conditions can be prevented with NR and has worked with ChromaDex, which developed his technologies, to provide the human clinical data that led to FDA notifications for NR as a GMP-produced and commercially available new dietary ingredient that is generally regarded as safe. His current research interests include fatty liver disease, heart failure, neuroprotection, and the maternal and neonatal benefits of NR.

Lauren Brink
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Lauren is a doctoral candidate in the lab of Bo Lönnerdal, Graduate Group of Nutritional Biology at the University of California, Davis. Her dissertation research has primarily focused on the effects of Milk Fat Globule Membrane (MFGM), a component of human milk, on neurodevelopment within a rat pup model.

Meet the Speakers

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Apichaya Bunyatratchata is a Ph.D. candidate in Food Science at UC Davis. After graduating from the University of Massachusetts, Amherst with a B.S. in Food Science and a B.A. in Chemistry, Apichaya joined the Barile Lab at UCD. Her research focuses on understanding the effect of bioactive milk compounds, including oligosaccharides and glycoproteins, on health. She uses advanced mass spectrometry as a tool to reveal glycans' structures and their interaction with the gut microbiota.

David Dallas, Ph.D.
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Dr. David 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.

Veronique Demers-Mathieu, Ph.D.
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Dr. Veronique Demers-Mathieu is completing her postdoctoral training in Nutritional Immunology this year under Dr. David Dallas at Oregon State University. Her research focuses on the remaining adhesion activity of human milk antenatal vaccine-specific (anti-influenza A virus and anti-bordetella pertussis) during the preterm and term infant digestion. Her long-term research goal is to identify the role of immune components (T cells, B cells, macrophages, plasma cells and antibodies) from human milk in the infant gut to improve their immunity and prevent infection.

Meet the Speakers

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Dr. Didier Dupont leads a research group at INRA on the digestion of dairy products and its consequences on human health. He has developed a wide variety of in vitro, in vivo and in silico digestion models. He's the scientific coordinator of INFOGEST, an international network on food digestion of 400 experts from 41 countries. He has written more than 100 peer-reviewed articles, 15 book chapters and given 58 international conferences (40 invited).

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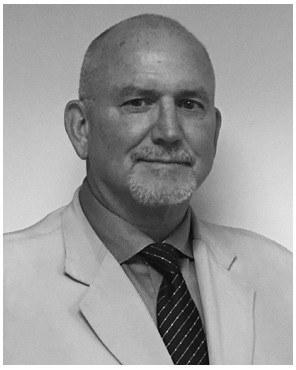
Dr. Ashwantha Kumar Enjapoori is a Research Fellow at Metabolic Research Unit, School of Medicine, Deakin University, Australia. In 2014, Ashwantha received his Ph.D. from the Deakin University, investigated the unique platypus and echidna milk composition. He continued postdoctoral work at Deakin University, identifying the naturally occurring bioactive peptides in milks of various mammalian species with main focus of beta-casein derived beta-casomorphin peptides using mass spectrometry based techniques. His current research is to investigate the individual beta-casomorphin peptides release across human lactation and understanding their biological functions in infants.



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Dr. John Finley serves as National Program Leader for Human Nutrition at the USDA-Agriculture Research Service. In this capacity he is especially interested in developing integrated research and data approaches to solve important nutritional/human health problems as related to the food supply and agricultural production. Dr. Finley has an academic, scientific and professional background in agriculture, food production and human nutrition. His

Meet the Speakers

undergraduate, M.S. and Ph.D. degrees were in Animal Science/Animal Nutrition, and he worked as a research chemist for eighteen years at the USDA-ARS Human Nutrition Research Center in Grand Forks, North Dakota. His research there focused on nutritionally important components of plant and animal foods, how agricultural practices may affect those components and their relation to chronic disease risk. He has also done details with the FDA and served as Corporate Director of Regulatory Affairs for a flavor and extract business. Dr. Finley has contributed over 100 peer-reviewed articles and book chapters to the scientific literature.

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Dr. Steven 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.

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Dr. Sophie Gallier is a Senior Research Scientist in maternal and paediatric nutrition at Fonterra Research and Development Centre in New Zealand. She manages preclinical and clinical research on the role of the milk fat globule membrane during pregnancy and early life, in particular on brain and cognitive development, and the impact of dairy ingredients on growth and development in infancy and on the gut-brain axis across all life stages.

Meet the Speakers

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Professor in Food Science and Technology, Director, Foods for Health Institute, University of California Davis (http://ffhi.ucdavis.edu/), cofounder Evolve Biosystems.

Dr. Bruce German received his Ph.D. from Cornell University, joined the faculty at the UC Davis in 1988 and is currently Director of the Foods for Health Institute and professor, at University of California, 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

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Dr. Michele Jay-Russell is a Research Microbiologist and Manager of the Western Center for Food Safety (WCFS), an FDA Center of Excellence at the University of California, Davis. Prior to joining the university, she worked as an epidemiologist for over 15 years in state and local public health including in the role of California State Public Health Veterinarian, and member of the California Food Emergency Response Team (CalFERT). Her current research activities support the overall mission of WCFS to identify real-world solutions to food safety challenges and support timely communication of new knowledge through outreach and training to the farming community and other stakeholders. Her research explores the interface between production agriculture, wildlife, livestock and the environment. She combines epidemiological and field experiments with molecular techniques to test hypotheses related to the prevalence, survival and growth of foodborne pathogens in the agricultural landscape. Data from her collaborative research program is being used to inform industry guidance documents and training materials, especially related to the FDA's Food Safety Modernization Act, Produce Safety Rule in the areas of domesticated animals and wildlife, biological soil amendments/raw manure, small and medium scale farms, and emerging industries such as aquaponics. She received her D.V.M. and M.PV.M degrees in 1992, and her Ph.D. (Microbiology) in 2011, from UC Davis. She is specialty board certified by the American College of Veterinary Preventive Medicine.

Meet the Speakers

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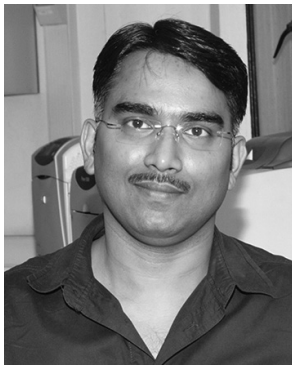
Dr. Mary Kable is a scientist with the USDA-ARS, Western Human Nutrition Research Center where she examines functional relationships between diet, gut microbial community composition and immune health. She received her Ph.D. in Biochemistry and Molecular Biology at the University of California, Davis in 2013 and subsequently completed her postdoctoral work in the Department of Food Science and Technology.



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Mr. Alok Kumar is currently engaged as a graduate student at CSIR-CCMB, Hyderabad, India in the lab of Dr. Satish Kumar. His Ph.D. research is about the evolution of lactation in mammals during the transition from oviparity to viviparity. Using mice as a model and transgenic and gene knockout techniques he aims at understanding how this shift in the temporal pattern of maternal investment into reproduction affected the physiology of lactation and its biological role/significance in mammals.

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Dr. Lotte Bach Larsen is a professor at Department of Food Science, University of Aarhus, Denmark. She leads an active science team "Food Chemistry and Technology", and has published over 120 international, peer-reviewed articles, in addition to book chapters. Dr. Bach Larsen was project leader of the Danish Milk Genomics Initiative. She is using proteomic and peptidomic methods for analyses and profiling of food-based proteins and peptides, protein modifications, natural and process induced. She was a member of Scientific Advisory Board and speaker at the

"11th International Symposium on Milk Genomics and Human Health" in Aarhus (2014), and a recipient, together with the Danish team behind the Milk Genomics Initiative in Denmark, of the Milk Prize awarded by the Danish Dairy Board in 2017 in acknowledge of its contribution to the basic understanding of the relations between cow genes and milk fingerprint and its resulting properties.

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Dr. Carlito B. Lebrilla is a Distinguished Professor at the University of California, Davis in the Department of Chemistry and Biochemistry and Molecular Medicine in the School of Medicine. He received his B.S. degree from the University of California, Irvine and Ph.D. from the University of California, Berkeley. He was an Alexander von Humboldt Fellow and a NSF-NATO Fellow at the Technical University in Berlin. He returned to UC Irvine as a President's Fellow and has been at UC Davis since 1989. He has served as Chair of the Chemistry Department. His research is in Analytical Chemistry focused on mass spectrometry with applications to clinical glycomics and biofunctional food. He has over 360 peer-reviewed publications and was awarded the UC Davis Distinguished Researcher Award in 2018. He is also co-editor of Mass Spectrometry Reviews and has been on the editorial board of Molecular and Cellular Proteomics, Mass Spectrometry Reviews, Journal of American Society for Mass Spectrometry, European Mass Spectrometry, and International Journal of Mass Spectrometry.

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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 and an Associate Adjunct Professor with the Dept. of Nutrition at University of California, Davis. She is the Founder and Executive Editor of "*SPLASH!*® milk science update", the official e-newsletter of the International Milk Genomics Consortium for which she has commissioned and reviewed over 300 lay articles on milk science. 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 gut 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 & Computer Science from MIT.

Meet the Speakers

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Dr. Maria Marco is a professor in the Department of Food Science and Technology at the University of California, Davis. Professor Marco received her B.S. degree in microbiology from the Pennsylvania State University, Ph.D. from the University of California, Berkeley, and worked as a lead scientist at NIZO food research, The Netherlands. She has an internationally recognized research program on lactic acid bacteria and the ecology of food and gut microbiota.

Michelle (Shelley) McGuire, Ph.D.
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Dr. Michelle (Shelley) McGuire, Director of the Margaret Ritchie School of Family and Consumer Sciences and professor of nutrition at the University of Idaho, is fascinated by human milk, which she likes to point out is the only food designed by mother nature to feed humans. Since being introduced to the field of human milk and lactation in 1986 by the late Dr. Mary Frances Picciano and immediately falling in love with everything about it, Shelley has studied a variety of milk components including macronutrients, micronutrients, hormones, immune factors, and most recently bacteria. She is particularly interested in how maternal diet influences milk composition and the health of the maternal/infant dyad and has a long-standing interest in also understanding factors (including breastfeeding and energy balance) related to duration of postpartum anovulation in women. Shelley's recent research and interdisciplinary collaborations have introduced her to the challenges and supreme importance of conducting high-quality nutrition research in non-US populations. Also a seasoned science communicator, Dr. McGuire is coauthor (with Dr. Kathy Beerman) of two college-level, introductory nutrition textbooks (Nutritional Sciences: From Fundamentals to Foods and NUTR, both published by Cengage). In 2018, Shelley received the Excellence in Nutrition Education Award from the American Society of Nutrition. Dr. McGuire lives in Moscow, Idaho with her husband and research partner, Dr. Mark McGuire (University of Idaho). When she isn't engaged in teaching, research, and service, Shelley enjoys spending time with her family and friends, cooking, running, yoga, gardening, playing her flute, caring for her family's 2 Nova Scotia Duck Tolling Retrievers, and traveling (and eating) with her family.

Meet the Speakers

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Bartijn Pieters is a second year Ph.D. candidate at the department of Experiment Rheumatology at Radboudumc in Nijmegen, Netherlands. He performed his undergraduate at the Radboud University, studying medical biology with internships in the field of extracellular vesicles and innate immunity. Bartijn's research interests focus on characterizing and studying the therapeutic efficacy of bovine milk-derived extracellular vesicles in rheumatic diseases. He is a junior founding-member of the recently established Netherlands Society for Extracellular Vesicles (NL-SEV).

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Dr. 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 has been studied as a part of the Danish-Swedish Milk Genomics Initiative.

Ishita M. Shah, Ph.D.
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Dr. Ishita Shah received her Ph.D. at The University of Maryland, Baltimore County where she discovered a new mechanism of transcription activation in bacteria. Her postdoctoral work at Columbia University led to the identification of bacterial cell wall fragments causing an exit from dormancy. At Genentech, she identified and validated targets in pathogenic bacteria in a drug discovery program. At the Foods for Health Institute, UC Davis, Dr. Shah works towards the identification of specific milk bioactives that alter pathogenesis.

Meet the Speakers

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Dr. Jennifer Smilowitz is the Associate Director of the Human Studies Research Program for the Foods for Health Institute at UC Davis. She holds a doctoral degree in Nutritional Biology with an emphasis in Endocrinology from UC Davis. Dr. Smilowitz's education, training and the research program she has built over the past decade have led her to the conclusion that the first 1000 days—from pregnancy through a child's 2nd birthday—is a critical period in life when nutrition largely influences long-lasting health. Dr. Smilowitz's current research program is widely translational and she is currently conducting several ongoing intervention trials involving dietary interventions that support the growth of beneficial gut microbes in infants, children and adults.

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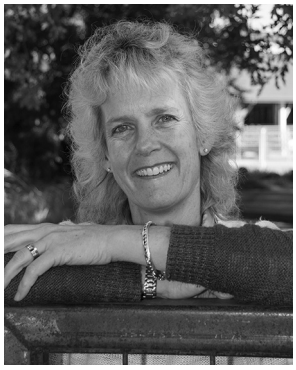
Dr. Diana Taft obtained her Ph.D. in 2014 from the University of Cincinnati where her research focused on the preterm infant gut microbiome and risk of late-onset sepsis. She is currently a post-doctoral researcher at the University of California, Davis in the Mills laboratory where her current research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

Meet the Speakers



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Dr. Alison Van Eenennaam is a Cooperative Extension Specialist in the field of Animal Genomics and Biotechnology in the Department of Animal Science at University of California, Davis. She received a Bachelor of Agricultural Science from the University of Melbourne in Australia, and both an M.S. in Animal Science, and a Ph.D. in Genetics from UC Davis. Her publicly-funded research and outreach program focuses on the use of animal genomics and biotechnology in livestock production systems.

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Dr. Marleen Visker is researcher at Animal Breeding and Genomics of Wageningen University & Research in the Netherlands. She obtained her Ph.D. in plant sciences and performed postdoctoral research at the division of Human Nutrition, both in Wageningen. She works on identification of genes contributing to natural variation in bovine milk composition, and on exploring genetic variation in methane emission by dairy cows. She also works on characterizing genetic variation for natural antibodies in poultry.

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Dr. Janet Williams has a Ph.D. in bioinformatics and computational biology and is currently a senior research scientist in the University of Idaho's Animal and Veterinary Science Department. She has been engaged in human and bovine milk research for over 20 yrs. Janet's goal is to utilize her rich background in lactation physiology with her knowledge of computational methods to understand how genetics, environment, diet, and microbes influence milk composition and maternal and newborn health.

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Presentation Abstracts

Presentation Abstracts

Tuesday November 13, 201

9:20 am **Highlights from IMGC's "SPLASH!® milk science update" and Upcoming Hot Topics**

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

In April 2012, the IMGC began publishing an e-newsletter, "*SPLASH!*® milk science update," which features four articles on emerging topics in milk science each month—that's 48 new articles on milk science each year. By the time of the IMGC conference in 2018, we will have published over 300 articles! This talk will reveal the most exciting milk science topics of the previous 12 months. It will also include a behind-the-scenes tour of *SPLASH!:* who are the current writers and editors, who are our readers and how do they reach our website. The *SPLASH!* newsletter has helped to grow the IMGC with more than 100,000 annual visits to the website each year. Nearly all traffic to the IMGC website is the result of *SPLASH!* content. The talk will also cover milk science topics expected to emerge in the coming year and beyond.

9:45 am **KEYNOTE: Maternal Nicotinamide Riboside Enhances Juvenile Development and Adult Neurogenesis**

Charles Brenner, University of Iowa, Iowa City, Iowa, USA

Ankita Chadda[1], Po-Hien Ear[1*], Marie Migaud[1,2], Sophia Vogeler[1], Johnny Malicoat[1], Hanna Stevens[3] & Charles Brenner[1]*
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**equal contributions*

Nicotinamide adenine dinucleotide (NAD) and NAD-related co-enzymes are the central regulators of virtually all metabolic processes. Despite powerful molecular and physiological networks to maintain homeostasis of the NAD metabolome, levels of NAD+ and/or NADPH are dysregulated under multiple conditions of metabolic stress including obesity, type 2 diabetes, alcohol intoxication, noise-induced hearing loss, sun exposure, oxidative stress, time zone disruption, heart failure, peripheral neurodegeneration and central brain injury. In each of these conditions, depression of NAD+ and/or NADPH is accompanied by stable and/or increased expression of the nicotinamide riboside (NR) kinase pathway, which allows oral NR to protect against these maladaptive stresses. We used mice and rats to test the hypothesis that postpartum is a type of metabolic stress that might dysregulate the maternal NAD metabolome and that increasing systemic post-partum NAD synthesis with NR might benefit mothers and their offspring. Here we show that lactation has a profound effect on hepatic and mammary NAD metabolism as the liver becomes programmed to distribute NAD precursors to the mammary. With NR supplementation, mammary NAD metabolism becomes supercharged, which leads to an increase in lactation and nursing behavior, and increases post-partum weight loss. Offspring of NR-supplemented mothers have advanced development as measured by growth, resistance to fasting-induced hypoglycemia and neurobehavioral phenotypes with respect to offspring of non-supplemented mothers. Remarkably, though we only fed mothers NR-supplemented chow for 21 post-partum days and all pups were returned to normal chow after this intervention, the adult offspring of NR-supplemented mothers retain substantial advantages in physical performance, neurobehavioral functions including spatial memory and resiliency, and in adult neurogenesis. We will reveal our insights into components of the mammary biosynthetic program that are induced by NR, a bioactive factor increased by NR, and the physical and mental advantages of being raised by NR-supplemented mothers.

11:00 am **Invited Speaker: The Impact of Dairy Management and Animal Genomics on Product Quality and Environmental Impact: The Need for an Integrated and Interdisciplinary Approach**

John Finley, USDA, Beltsville, MD, USA

The Dairy Industry faces multiple pressures with lagging consumption of fluid milk and increasing concerns about deleterious environmental effects. Development of dairy products with superior nutritional qualities but less environmental impact would be of great value. Although dairy products are associated with better diets and health, the functional components of milk are not well characterized; moreover, these components are likely altered by animal management and genetics. The industry must maintain/improve animal performance and develop optimized systems reduce environmental impact; this includes development of pasture/forage /feed systems that maintain/improve soil and water quality. These goals cannot be achieved through traditional reductionist approaches; instead they must be addressed through an integrated dairy systems approach that link soil health and ecology, forage production and management, dairy cow genetics and management, and milk production and quality. Such an approach also requires new methods of integrating and linking diverse data. A current inter-disciplinary project joint between multiple groups in the USDA and multiple universities is addressing these issues in an integrated manner.

11:30 am **What's Normal? Genome-Wide Association Analysis of Human Milk Produced by Healthy Women Across Different Geographic Locations**

Janet Williams, University of Idaho, Moscow, ID, USA

Williams JE,[1] Neibergs HL,[2] Kiser JN,[2] McBride K,[3] Brooker SL,[1] Meehan CM,[4] McGuire MA,[1] McGuire MK,[3,5] Murdoch BM,[1] and the INSPIRE Consortium

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The complexity of human milk composition is influenced by many factors including diet, stage of lactation, environment, and even social networks, but little is known about the extent to which maternal genetics plays a role. Although relationships between genetic variation and milk composition have been studied in cattle, very few genetic associations with components in human milk have been investigated. Profiles of human milk oligosaccharides (HMO) have been shown to be related to geographic region and more specifically, to the functionality of genes such as fucosyltransferase 2 and fucosyltransferase 3 (FUT2 and FUT3). Most recently, human milk fatty acid composition has been linked to genetic variation in members of the fatty acid desaturase gene family. Aside from these milk components, however, little is known about how maternal genetic variation impacts milk composition. Therefore, the objective of this study (the INSPIRE study) was to utilize a genome-wide association approach to test if genetic variants are associated with concentrations of selected human milk components. To do this, we collected milk, saliva, and myriad cultural and social data from 412 lactating women in Ghana, The Gambia, Ethiopia, Kenya, Sweden, Spain, Peru, and the United States. Milk was analyzed for a variety of components, including immune factors, protein, lactose, HMO, and microbial community composition. DNA was extracted from saliva, and maternal genotypes were ascertained using the Illumina Multi-Ethnic Global-8 v1.0 array (MEGA). The MEGA array encompasses > 1.7 million single nucleotide polymorphisms (SNPs) and was designed to leverage the detection of variants found in African, American, East Asian, European, and South Asian populations, as well as other subpopulations. After filtering based on sample and SNP call rate, minor allele frequency, and Hardy-Weinberg equilibrium, approximately 1.12 million SNPs were retained. Genome-wide association tests were conducted using a linear mixed model and significance declared at $P < 1x10^{-8}$. Significant associations were identified with total HMO and genetic

variations in FUT2 on chromosome 19, with several SNPs having P-values ≤ 6.24×10^{-20}. Additional associations were identified with protein concentrations and loci in fucose-1-phosphate guanylyltransferase and TNNI3 interacting kinase (FPGT-TNNI3K) and arginine/serine-rich coiled-coil protein 1 (RSRC1) on chromosomes 1 and 3, respectively; whereas, lactose concentration was associated with SNPs on chromosomes 5 and 9. To our knowledge, this is the first genome-wide association study of human milk composition that spans different regions around the world and encompasses a variety of milk components. This study provides evidence that important nutritional and immunologic components in milk are influenced by maternal genetics. Although these analyses have identified numerous genetic loci related to variation in human milk composition, these findings have just begun to help explain the immense variation in the composition of milk produced by women around the world. Only after we gain a better understanding of the various components of human milk and the factors that control them can we begin to understand how this variation affects growth, development, and health of the recipient infant.

Funding: National Science Foundation and the Washington State University Office of Research nutritional genomics grand challenge initiative

1:00 pm **KEYNOTE: Gene Editing and the Future of Bovine Genetics**

Alison L. Van Eenennaam, University of California, Davis, CA, USA

In 2016, the global cattle population of 1.5 billion head produced 6.5 billion tons of cows' milk, and 66 million tons of beef. In the past century, cattle breeding programs have greatly increased the yield per animal with a resultant decrease in the GHG emissions intensity per unit of milk or beef, but this has not been true in all regions. Gene editing using site-directed nucleases (e.g. CRISPR/Cas9) offers an opportunity to precisely edit or change the genetic code. Gene editing could be integrated into conventional cattle selection programs to introduce useful alleles into elite germplasm without the lengthy process of introgressing those same alleles from distant breeds. To date, gene editing research in cattle has focused on disease resistance, production, elimination of allergens and welfare traits such as introducing the polled (hornless) allele from beef breeds into horned dairy cattle breeds. As with earlier genetic engineering approaches, whether livestock breeders will be able to employ genome editing in cattle genetic improvement programs will very much depend upon global decisions around regulation and governance of genome editing for food animals.

1:45 pm **GWAS and Expression of Candidate Genes for Oligosaccharide Synthesis in Danish Dairy Breeds**

Nina Aagaard Poulsen, Aarhus University, Tjele, Denmark

Nina A. Poulsen[1], Randall C. Robinson[3], Bart Buitenhuis[2], Ulrik Sundekilde[1], Danielle G. Lemay[5,6], Daniela Barile[3,4], Lotte B. Larsen[1], Lars-Erik Holm[2]

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4. Foods for Health Institute, University of California, Davis, California, USA
5. UC Davis Genome Center, University of California, Davis, California, USA
6. USDA ARS Western Human Nutrition Research Center, Davis, California, USA

In an earlier study, a total of 15 bovine milk oligosaccharides (OS) were monitored by an optimized LC-MS/MS method. Heritabilities ranged from 0 to 0.68 in Danish Holstein and from 0 to 0.92 in Danish Jersey. A genome wide association study based on the bovine HDchip identified in total 1770 SNPs (FDR < 0.10) for five different OS in Danish Holstein and 7290 SNPs (FDR < 0.10) for eleven OS in Danish Jersey. Only ten of these significant SNPs were found in both breeds. In Danish Holstein, a major overlapping QTL was identified on BTA1 for Lacto-N-hexaose (LNH) and Lacto-N-tetraose (LNT) explaining 24% of the variation in these OS. The most significant SNPs were associated to B3GNT5, a gene encoding a glycosyltransferase involved in OS synthesis.

Other detected candidate genes of interest identified in Danish Holstein were ALG3, B3GALNT2, GLT6D1 and LOC520336 (a hexosyltransferase). In Danish Jersey, a very strong QTL was detected for the OS with composition 2 Hex 1 HexNAc (isomer 1) on BTA11. The most significant SNP had –log10(P-value) of 52.88, which is a missense mutation in the ABO gene, encoding ABO blood group glycosyltransferases. This SNP explains 56% of the OS variation. Other candidate genes of interest identified for milk OS in Danish Jersey were PIGV, MAN1C1, ST6GALNAC6, GLT6D1, GALNT17, GALNT14, COLGALT2, LFNG and SIGLEC.

In the current study, expression of genes associated with significant SNPs are examined using RNAseq data based on mammary gland epithelial cells from 24 additional samples. Preliminary results confirm that several of the candidate genes are expressed in the mammary epithelial cells (including B3GNT5 and ALG3). Currently, the genes within specific QTL regions are explored in more detail. The 24 cows have been genotyped on the EG Bovine LD SNPchip enabling differential expression analysis based on the most promising candidate gene SNPs. Likewise, additional genomic mining in the regions of interest will be performed in the near future.

2:15 pm **The Genetics of Phosphorylation of Caseins in Bovine Milk**
Marlene Visker, Wageningen University, Wageningen, Netherlands

Marleen Visker[1], Zih-Hua Fang[1,2,], Patrice Martin[2] & Henk Bovenhuis[1]*
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**Present address: Animal Genomics, ETH Zürich, CH-8092 Zürich, Switzerland*

Bovine milk contains 3 to 4% protein. The composition of that protein is important, as it contributes to the nutritional value and technological properties of milk. About 80% of total protein in cow's milk is comprised by the four caseins (CN): αs1-CN, αs2-CN, β-CN and κ-CN. Phosphorylation of these caseins occurs after synthesis of the polypeptide chains in the Golgi apparatus of the mammary epithelial cells. This phosphorylation is an
important posttranslational modification, as it allows caseins to aggregate as micelles. The casein micelles enable transport of calcium and phosphorus to the neonate and are relevant for manufacturing dairy products such as cheese and yoghurt.

In bovine milk, αs1-CN and αs2-CN are more highly phosphorylated than β-CN and κ-CN. In addition, αs1-CN and αs2-CN have multiple phosphoserine clusters, whereas β-CN has only one and κ-CN has none. This suggests that αs1-CN and αs2-CN are more relevant for stabilizing internal micellar structure. Relative concentrations of αs1-CN and αs2-CN phosphorylation isoforms in milk are known to vary considerably between individual cows. The aim of our study was to determine if part of that variation between cows is due to genetic variation and to identify candidate genes for such genetic variation.

Genetic parameters were estimated for relative concentrations of αs1-CN and αs2-CN phosphorylation isoforms in milk of 1857 Holstein Friesian cows. Heritabilities were moderate to high (0.48 – 0.89), suggesting genetic control of the phosphorylation of αs1-CN and αs2-CN. In addition, genetic correlations among and between αs1-CN and αs2-CN phosphorylation isoforms suggest that the phosphorylation processes of αs1-CN and αs2-CN are related.

Regions of the bovine genome associated with relative concentrations of αs1-CN and αs2-CN phosphorylation isoforms were identified using 50K SNP genotypes for the 1857 Holstein Friesian cows. A total of ten QTL regions were identified on the bovine genome. Four of these regions were associated with multiple phosphorylation isoforms and enabled identification of candidate genes. The QTL regions

either affected milk protein synthesis, i.e. casein synthesis, or casein phosphorylation, i.e. the relative abundance of αs1-CN and αs2-CN phosphorylation isoforms.

The results of our study demonstrate the possibility to breed for cows with specific αs1-CN and αs2-CN phosphorylation isoforms in their milk. This could be of interest to the dairy industry, as it has been suggested that lower degrees of phosphorylation of αs1-CN and αs2-CN are beneficial for coagulation of the milk, thus enhance its manufacturing properties.

3:15 pm **Milk Genomics: Variation in Danish Dairy Milk and Its Possible Exploitation in the Dairy Chain**
Lotte Bach Larsen, Aarhus University, Tjele, Denmark

Nina A. Poulsen[1], Bart Buitenhuis[2], Thao. T. Le[1], Vivi R. Gregersen[2], Brian Christensen[2], Esben S. Sørensen[2], Lotte B. Larsen[1]
1. Department of Food Science, Aarhus University, Tjele, Denmark
2. Department of Molecular Biology and Genetics, Aarhus University, Tjele, Denmark

Milk genomics identify variations in the genetic background of the cows of significance for the functionality, health properties and composition of milk and provide new opportunities for selective breeding for specific milk quality traits. The activities within the Milk Genomics area on Danish Dairy breeds have so far resulted in more than 40 papers in international peer-reviewed journals. In these papers, major and minor milk components of importance for the technological properties of milk, as well as its health and nutritional value, have been quantified. This includes a large number of milk samples from Danish Holstein (DH) and Danish Jersey (DJ) cows, and therefore gives a unique insight into the variation in these properties in Danish dairy breeds, and how much of this variation that can be assigned to genetic and environmental factors and thereby the opportunities for improving these traits through selective breeding. This has given us a strong documentation platform for the quality of Danish dairy milk and how this can provide added value to milk

in relation to further exploitation during processing at the dairies as well as new perspectives for improving specific milk components through selective breeding, which can potentially also give added value to the dairy farmer.

The milk samples have been analyzed for overall milk composition, fatty acid composition, minerals and trace elements, metabolites, oligosaccharides, pH, conductivity and detailed protein composition including several glycosylation- and phosphorylation-isoforms identified together with underlying genetic variants. Furthermore, coagulation properties have been examined in all the samples and thus the frequency of non-coagulating milk in Danish Holstein and Danish Jersey. Recently absolute levels of α-lactalbumin (α-LA), β-casein (β-CN), and osteopontin (OPN) have been determined in 663 individual cow's milk samples from Danish Holstein. Absolute quantification of OPN was determined by a specifically developed sandwich ELISA, whereas absolute contents of α-LA and β-CN were determined by a Multiple Reaction Monitoring (MRM) method developed on a Triple Quadrupole LC-ESI-MS/MS instrument. The level of OPN varied from 0.4 to 68, with an average of 23 mg/L and has never before been reported in such a large number of individual cow's milk samples. The level of α-LA determined by MRM varied from 0.4 to 1.9, with an average of 1.1 mg/ml. Levels of β-CN varied from 7.5 to 23.4, with an average of 14.9 mg/ml. Parity significantly affected the content of OPN, but not the other traits. Heritabilities for the traits were in the range of 0.05-0.40.

3:45 pm **Beta-Casein Derived Beta-Casomorphin Peptides in Human Milk for Infant Nutrition**
Ashwantha Kumar Enjapoori, Deakin University, Geelong, Victoria, Australia

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Lactation is a specific mammalian function essential for newborn feeding. Recent human milk peptidomic analysis revealed that milk contains naturally occurring milk-derived bioactive peptides in addition to nutritional compounds prior to infant ingestion. Milk-derived proteases play a prominent role in highly specific proteolysis and release cryptic peptides from caseins and whey proteins. These peptides, when ingested by the neonate are known to possess multiple biological functions in the infant's gastrointestinal tract. A group of peptides called 'beta-casomorphins' (BCMs) are proteolytic peptides derived from β-casein in human milk and exert opioid, immunomodulatory, antioxidative and satiety functions in newborns. Human milk is known to contain naturally occurring milk protein derived peptides, but the detection of BCMs in breast milk are limited. It is also unknown how the BCM family of peptides (such as human BCM-4, -5, -7, -8, -9 and -11) are formed throughout human lactation and how the individual BCMs appear relative to one another.

The present study aimed to identify the naturally occurring BCM peptides from beta-casein in human breast milk using liquid chromatography-tandem mass spectrometry (LC-MS/MS). In-depth peptidomics analysis of breast milk samples revealed the presence of BCMs, including BCM-8, -9, -10, -11, precursors and truncated forms of the original peptide, suggesting milk protease activity in the mammary gland generates biologically relevant BCMs. This is the first report to describe the presence of naturally occurring human BCM-10 and BCM-11 in breast milk. Our study provides evidence of beta-casein derived BCM peptides present in human milk before infant digestion. It is likely that proteases present in milk are specific in their proteolysis of beta-casein. Prediction of protease cleavage sites in BCM and BCM precursor peptides was generated by using known human milk proteases including, cathepsin D, elastase, plasmin, proline endopeptidase and thrombin. The identified BCM-8, -9, -10, -11 and BCM precursor peptides meet the structural requirements to elicit opioid, immunomodulatory, antioxidative and satiety functions in newborns.

4:30 pm **Quantitative Proof of Why It's Good to Be Here**
Danielle Lemay, Western Human Nutrition Research Center, USDA, Davis, CA, USA

Abstract not available at time of printing

Wednesday, November 14, 2018

9:00 am **Keynote Speaker: A Systems Biology Approach to Characterizing the Gut Microbiome of Breastfed Infants Colonized by B. infantis EVC001**
Steve Frese, Evolve Biosystems, Davis, CA, USA

Giorgio Casaburi[1], Rebbeca Duar[1], Ryan D. Mitchell[1], Daniel P. Vance[1], Lindsey N. Contreras[1], Claire A. Shaw[1], Stephanie Chew[1], Bethany M. Henrick[1,2], Steven A. Frese[1,2]*
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Extensive work characterizing the utility of bioactive molecules in human milk has highlighted novel functions for its previously underappreciated biological complexity. Beyond gross nutrition, bioactive peptides, functional lipids, enzymes, small molecules, hormones, stem cells and oligosaccharides in human milk are now appreciated for their role in shaping infant health and development. Key among these innovative discoveries is an appreciation for how these bioactive molecules – especially human milk oligosaccharides – interact with, and control, development of the gut microbiome.

Recent evidence suggests that the progressive and generational loss of a key Bifidobacterium species from the infant gut has resulted in profound alterations to the infant gut microbiome from an historical and evolutionary norm. In a recent clinical trial, we showed that restoration of thisspecies in the infant gut resulted in substantial improvements to infant fecal biochemistry and gut function, which resembled historical norms and strong evidence for host-selection of B. infantis as a gut symbiont. Robust evidence from models for host-microbe coevolutionary relationships (and especially among binary symbioses) demonstrate that aposymbiotic hosts show pronounced deficits in developmental growth of gross anatomical structures, deficits in immune system development, and aberrant metabolism in early life and beyond. Notably, among infants in the developing world where B. infantis is still abundant, the conspicuous absence of this organism is associated with profound growth impairment and inadequate immune system development. Further, the extensive work in characterizing the numerous adaptations of B. infantis to human milk demonstrate broad, conserved, and highly efficient features which enable colonization of infants by B. infantis. Together, these results suggest that B. infantis is part of a highly specialized binary symbiosis with humans.

Thus, from an evolutionary and historical perspective, there is insufficient data to characterize this developmentally normal infant gut microbiome (e.g. one colonized by Bifidobacterium such as B. infantis EVC001) in healthy infants as most studies focus on varying degrees of gut microbiome dysbiosis. To address this, we applied a combination of "omics" technologies (metabolomics and shotgun metagenomics) to characterize a developmentally normal infant gut microbiome among infants uniformly colonized in high levels by B. infantis EVC001. We compared the metabolic and metagenomic profiles of infants also fed human milk, but who lacked colonization by this organism or any related gut symbiont.

Substantial deviation was found among infants whose gut microbiome was 'dysbiotic'. Particularly, we observed high variation among individuals and invasion by potential pathogens, inefficient conversion of indigestible dietary fiber to accessible calories, and a lack of adequate provision of a number of critical gut metabolites. Moreover, we identified the causative agents for many of these deleterious effects through shotgun metagenome sequencing and compared results with the stable, high-functioning gut microbiome found among infants colonized by B. infantis EVC001.

9:40 am **Population Duration of Breastfeeding and Prevalence of Bifidobacterium**

Diana H. Taft, University of California-Davis, Davis, CA, USA

Diana H. Taft[1,2], Steve Ho[1], Daniel J. Tancredi[3], Charles Stephenson[4,5], Katie Hinde[6], Erika von Mutius[7,8], Juha Pekkanen[9], Jean-Charles Dalphin[10], Roger Lauener[11], Josef Riedler[12], Ardythe L. Morrow[13], Zachery T. Lewis[1,2], David A. Mills[1,2,14*]*
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4. Nutrition Department, University of California, Davis, California, USA
5. US Department of Agriculture, Western Human Nutrition Research Center, Davis, California, USA
6. Center for Evolution and Medicine, Arizona State University, Tempe, Arizona, USA
7. Dr. von Hauner Children's Hospital, Ludwig Maximilian University, Munich, Germany
8. Institute for Asthma and Allergy Prevention, Helmholtz Centre, Munich, Germany
9. Department of Environment Health, National Institute for Health and Welfare, Kuopio, Finland

10. University of Besançon, Department of Respiratory Disease, UMR/CNRS 6249 Chrono-environment, University Hospital Besançon, France
11. Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland
12. Children's Hospital, Schwarzach, Austria
13. Division of Biostatistics and Epidemiology, Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio, USA
14. Department of Viticulture and Enology, University of California, Davis, California, USA

Introduction: Lack of microbiota-accessible carbohydrates can drive gut commensal extinctions. Formula lacks the microbiota- accessible carbohydrates found in breastmilk, the human milk oligosaccharides (HMOs). As a result, a switch from breastmilk feeding to formula feeding may drive extinctions of infant gut commensals in the population.

Methods: The prevalence of Bifidobacterium longum subspecies infantis was compared in cohorts from Austria, Bangladesh, Finland, Gambia, Germany, Switzerland, and the United States. Deterministic epidemiological models of B. longum ssp infantis prevalence were created for each country to provide insight into the potential effect of changing duration of breastfeeding on the prevalence of this important commensal.

Results: The highest prevalence of infant colonization with B. longum ssp infantis was observed in the countries without a history of disrupted breastfeeding, Bangladesh and Gambia (80% and 91% colonization prevalence, respectively). The remaining countries have a prevalence of infant colonization with B. longum ssp infantis ranging from 0.7% to 14%. The majority of infants in Austria, Finland, Germany, and Switzerland had gut communities frequently dominated by Bifidobacterium (>50% relative abundance Bifidobacterium) despite the low frequency of colonization with B.longum ssp infantis. The R0 of B. longum ssp infantis transmission also varied between countries.

Conclusions: The prevalence of infant colonization with B. longum ssp infantis may be sensitive to breast-feeding duration within populations. Our data supports the concept that lack of microbiota-accessible carbohydrate can drive commensal extinctions, and suggests that deliberate intervention may be needed to restore B. longum ssp infants, a bacteria that evolved to consume HMOs and benefit the infant. More studies of this question are needed, particularly to determine whether species of Bifidobacterium other than B. longum ssp infantis are also sensitive to breastfeeding duration, and to understand the impact of different species and subspecies of Bifidobacterium on infant health.

10:40 am **Bifidobacterium longum subsp. infantis Stably Restores the Infant Gut Microbiome Over the First Year of Life in Breastfed Infants**

Jennifer Smilowitz, University of California-Davis, Davis, CA, USA

Jennifer T. Smilowitz[1,2], Mark A. Underwood[2,3], Steven A. Frese[4], Andra A. Hutton[4], Lindsey N. Contreras[4], Claire A. Shaw[4], Bethany Henrick[4], Carlito B. Lebrilla[2,5], Samara L. Freeman[4], Daniela Barile[1,2], J. Bruce German[1,2], David A. Mills[1,2]
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4. Evolve Biosystems, Inc., Davis, California, USA
5. Department of Chemistry, University of California, Davis, California, USA

Objectives: Breast milk is known to contain oligosaccharides that promote the growth of Bifidobacterium in the infant gut. However, Bifidobacterium species associated with positive health benefits have been found to be substantially lower in breastfed infants from developed compared with developing countries. The objective of this study was to monitor the effects of supplementing activated Bifidobacterium longum subsp.

infantis (B. infantis) on gut microbial composition in infants throughout 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.

Methods: Mothers and their infants were partially randomized to receive lactation support and an activated preparation of activated B. infantis EVC001 or lactation support alone (n = 34 and 32 per group, respectively). Exclusively breastfed infants consumed the preparation for 21 consecutive days starting on Day 7 post-natal supplied in individual daily dose sachets, and mixed with expressed breast milk. Bacterial DNA was extracted from collected infant stool samples, and analyzed by quantitative PCR and 16S rRNA marker gene sequencing. Fecal endotoxin, short chain fatty acids, and pH were also measured.

Results: Infants receiving B. infantis EVC001 were rapidly colonized with B. infantis (>1010 CFU/g feces) and maintained colonization through the first year of age, so long as the diet was primarily breast milk and not infant formula. Colonization by EVC001 was also associated with decreased relative abundances of Enterobacteriaceae and Clostridiaceae, which correlated and significant decreases in fecal endotoxin. Infants supplemented with activated B. infantis had significantly more fecal lactate, acetate, total short chain fatty acids and lower fecal pH.

Conclusion: Infants were rapidly and stably colonized by EVC001 immediately after the start of supplementation. This colonization was stable throughout the first year of age, so long as breastfeeding continued. This colonization had profound beneficial effects on the infant fecal biochemistry and gut microbiome. Infant formula, rather than complementary foods, was associated with gut dysbiosis in supplemented infants and in agreement with previous studies.

11:10 am **Student Travel Award Recipient: Structural and Functional Insights into EchAMP, a Unique Monotreme Antimicrobial Protein Expressed During Lactation**

Alok Kumar, CSIR-Centre For Cellular and Molecular Biology, Habsiguda, Hyderabad, India

Alok Kumar[1], Sadiya Parveen[1,2] and Satish Kumar[1]
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2. Tuberculosis Research Center, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Monotremes, the egg-laying mammals with the ability to lactate are the only living representatives of ancient oviparous mammals with the primitive form of lactation. During evolution, they split from the therian lineage (present days placental mammals) about 166-210 mya and so displays a unique combination of reptilian and mammalian reproductive features. They lay parchment-shelled eggs which upon hatching give birth to altricial young ones developmentally equivalent to a 40-day old human embryo[1]. The mother's milk secreted from the nipple-less mammary patches is the only source of nourishment and protection for these altricial youngs against pathogens present in their environment. Monotreme milk thus is rich in bioactives and can be a novel source of antimicrobial molecules. Echidna AntiMicrobial Protein (EchAMP) is one such protein expressed in the echidna milk[2]. It is the tenth most abundant transcript with conserved secretory signal sequence and multiple putative sites for post-translational modifications[2].

The detailed functional characterization of this protein was hindered because of the exclusivity of echidna as a geographically confined species and poorer protein yields with several eukaryotic expression systems. However, in the present study, we successfully purified the EchAMP protein in optimum quantities using a bacterial expression system. Unlike its eukaryotic counterpart, the recombinant protein from bacteria lacks post-translational modification but shows activity in our antimicrobial assays. Structurally the protein is intrinsically disordered as suggested by tryptophan fluorescence, circular dichroism, and NMR spectroscopy and like most of the IDPs upon thermal melting exhibit propensity to form transient a-helices. The in vitro

antimicrobial assays with the purified EchAMP protein showed activity against both the Gram-positive (Bacillus subtilis, Staphylococcus aureus) and the Gram-negative micro-organisms (Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Enterococcus faecalis). This broad-spectrum antimicrobial activity is species specifically dose-dependent and mostly bacteriolytic as confirmed by live-dead staining and scanning electron microscopy.

Moreover, the observed bacteriolytic activity was relatively higher for bacillus compared to other species used in the assay. Echidna is a terrestrial animal that lives in burrows and so essentially needs protection against spore-forming soil microbes often known to be associated with infections in the lactating glands. Our studies using tryptophan fluorescence and circular-dichroism spectroscopy with amphipathic molecules and bacterial membrane mimics such as SDS, lipopolysaccharide (LPS) and liposomes (SUVs) respectively suggested that like other intrinsically disordered AMPs (Cecropin A and Magainin 2)[3] EchAMP too exhibits a propensity to fold into helices upon interaction with the bacterial envelope. Thereby gaining functionally active conformation and killing cells by compromising their membrane integrity and causing cytoplasm leaking.

To summarize, EchAMP in light of its evolutionary conserved association only with monotremes and its broad-spectrum antimicrobial activity can be a promising candidate of choice against multiple infections associated with the lactating mammary gland in humans and livestock.

References:
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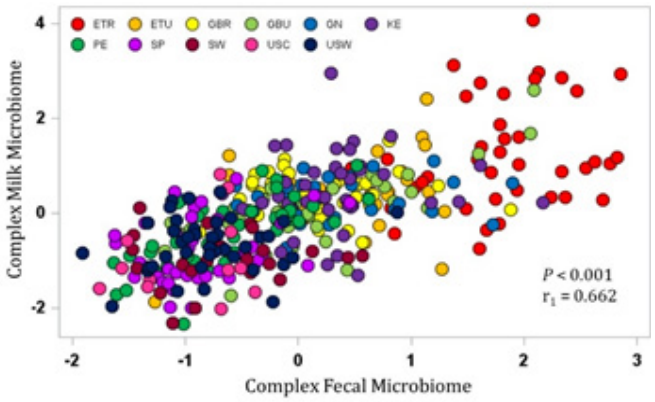
2) Bisana S et.al., Identification and Functional Characterization of a Novel Monotreme-Specific Antibacterial Protein Expressed during Lactation, PloS ONE, January 2013, Volume 8, Issue 1
3) Avitabile C et.al., Circular Dichroism studies on the interactions of antimicrobial Peptides with bacterial cells, Scientific Reports, 4: 4293 | DOI: 10.1038/srep04293

1:00 pm **The Human Milk Microbiome**

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

Once thought sterile unless contaminated or produced by an infected gland, human milk is now known to support a unique and diverse bacterial community. The first report of the human milk microbiome using high-throughput, molecular sequencing suggested that a "core" of 9 genera was present in all milk (Hunt et al., 2011), bacterial community structure of milk was personalized within a woman, and substantial inter-woman variation was evident. Identifying factors driving this variability is now a main focus of many researchers around the world.

For example, to understand "what's normal" in terms of the human milk microbiome, the INSPIRE study was conducted in 11 global locations. Results suggest that all 396 milk samples collected contained Staphylococcus and Streptococcus, but more extensive sets of core bacteria were present in some cohorts (Lackey et al., unpublished). Substantial intra- and inter-cohort variability existed in bacterial community membership within and among cohorts; a finding that supports that of Kumar et al. (2016). For instance, Rhizobium was relatively more abundant in milk from



women in rural Ethiopia than all other cohorts. Milk from the rural Ethiopian cohort was more diverse than milk from Ghana, Peru, Spain, Sweden, and California. Importantly, ecological analyses suggest that variation in the complex microbial community structure of milk is tightly associated with that of the recipient infants' feces (see figure). Variation in milk microbiome is also associated with those of milk-borne oligosaccharides, immune factors, and even the infant's social network (unpublished). This potentially important relationship between social networks and milk microbiome supports previous findings from the Central African Republic (Meehan et al., 2018). Collectively, the INSPIRE study suggests that what's normal in terms of milk microbiome (and other milk components) in one location and culture might not be optimal in another and begs the question as to what genetic, biological, environmental, and behavioral factors drive this variation.

To date, a small but growing literature has examined a handful of such factors. For instance, some studies suggest that both the composition and diversity of the milk microbiome might be influenced by time postpartum (Cabrera-Rubio et al., 2012; Gomez-Gallego et al., 2016; Khodayar-Pardo et al., 2014); although others (Williams et al., 2017) have found only minor changes during the first week postpartum. Delivery mode (Cabrera-Rubio et al., 2012&2016; Hoashi et al., 2016; Khodayar-Pardo et al., 2014; Soto et al., 2014), maternal health (Cabrera-Rubio et al., 2012; Collado et al., 2012; González et al., 2013), and antibiotic use (Soto et al., 2014) also might be important. In addition, maternal diet might affect the HMM. For instance, our data suggest that milk produced by women consuming the highest amounts of carbohydrates (particularly soluble fiber and lactose) produce milk with the highest relative abundance of Firmicutes (Williams et al., 2017). Consumption of a diet high in the essential amino acids seems to be associated with milk having the highest relative abundance of Proteobacteria, and intake of several micronutrients (e.g., vitamins D and E, biotin, iodine, iron, and molybdenum) is also related to variation in some taxa. We posit that these relationships are either due to an alteration of maternal gastrointestinal microbiota (which are thought to be transferred to the mammary gland via an entero-mammary pathway) or modulation of milk nutrient concentrations, which might have local effects on the growth of bacteria in the mammary gland. The former hypothesis is supported by additional data from our group showing multiple associations between a mother's diet and her fecal microbiome (Carrothers et al., 2015), as well as maternal diet and the recipient infant's fecal microbiome (unpublished).

1:30 pm **Milk Microbiomes in Large-Scale Dairy Processing**

Mary Kable, University of California-Davis, Davis, CA, USA

Mary E. Kable[1,2], Yanin Srisengfa[2], Zhengyao Xue[2], Laurynne C. Coates[2] and Maria L. Marco[2]
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2. Department of Food Science and Technology, University of California, Davis, California, USA

Bacteria have a significant influence on dairy quality and safety. Therefore, we set out to characterize the bacterial communities in milk at a large-scale cheese manufacturing plant. In order to determine the bacterial community upon arrival at dairy processing facilities, raw bovine milk from 899 tanker trucks delivering to two dairy processors in the San Joaquin Valley, California were analyzed by community 16S rRNA marker gene high-throughput DNA sequencing. This analysis revealed highly diverse bacterial populations which exhibited seasonal (spring, summer, and fall) differences. Raw milk collected in the spring contained the most diverse bacterial communities, highest proportions of Actinobacteria, and highest total bacterial cell numbers. Even with this complexity, a core microbiota was present in all seasons, consisting of 29 taxonomic groups and high proportions of Streptococcus and Staphylococcus and unidentified members of Clostridiales. In order to determine the effect of onsite storage, milk was sampled from five large-volume silos and from 13 to 25 tankers whose contents were unloaded into the silos on two separate dates. Transfer of the milk to the silos resulted in two community types. One group of silos contained a high proportion of Streptococcus spp. and bacterial populations similar to the tankers that filled them. The community found in the other group of silos

was distinct and dominated by Acinetobacter. Lastly, to determine the impacts of pasteurization, concentration, separation and subsequent storage on the bacterial contents of milk, a total of 142 milk samples were collected from up to 10 pieces of equipment within a single facility for a period spanning 21 h on two collection dates in the spring and late summer of 2014. Milk samples from the late summer were paired such that half were treated with propidium monoazide (PMA) to enrich for living cells prior to DNA sequence analysis. Streptococcus was the most abundant organism in the milk on both sampling dates, irrespective of processing step. Other bacteria including Thermus and Anoxybacillus bloomed during certain processing steps. PMA treatment showed that Turicibacter was proportionally enriched by High Temperature Short Time (HTST) pasteurization, whereas Staphylococcus was significantly reduced by that process. Moreover, we found evidence that the microbiota changed over time after the equipment was last cleaned. Acinetobacter and /or Lactococcus were significantly enriched in silos that were sampled more than 19 h after the last cleaning. Recently cleaned silos and silos with a low bacterial load were more diverse and contained a greater proportion of Bacillus, Turicibacter, Staphylococcus and Streptococcus relative to silos with a high bacterial load. Overall, this study showed that milk contains highly complex and dynamic bacterial communities that fluctuate throughout the course of single days and within individual pieces of equipment. Time of cleaning and low total bacterial cell numbers were not directly correlated with the decreased proportion of bacterial genera that are typically associated with spoilage and therefore are not likely to be good predictors of product spoilage. These findings can be used by dairy processors to inform monitoring programs aimed at maintaining and improving product quality.

2:00 pm **Panel Discussion- Milk as a Source of Microorganisms: Practical Implications**
Moderator: Danielle Lemay, Western Human Nutrition Research Center, USDA
Panelists: Maria Marco (University of California, Davis), Steve Frese (Evolve Biosystem, Davis) and Michele Jay-Russell (Western Center for Food Safety, University of California, Davis)

Milk has microbes. Does it matter? What does this mean for infant formula manufacturers or for donor milk products or for breastfeeding moms? How might microbes in cow's milk affect the dairy industry? What innovations are possible? Come listen to this panel to find out.

3:00 pm **Keynote Speaker: Utilization of Oligosaccharides and Bioactive Carbohydrates from Milk**
Carlito Lebrilla, University of California, Davis, CA, USA

Human and bovine milk contain bioactive carbohydrates that perform a variety of functions, chief among them is the enrichment of specific microbiota in the gut. In this research, we determine how oligosaccharides that are consumed by the host are found in tissues including blood, urine and feces. We show that with recently developed analytical methods, we can determine the oligosaccharides in feces that are the result of degradation through gut microbial enzymes. Monosaccharides that are produced are incorporated into cell lines and tissues. These methods and experiments will provide the fate of carbohydrates in diet.

3:45 pm **Human Milk Oligosaccharides Directly Enhance Gut Barrier Integrity to Alter Infection**
Ishita Shah, University of California, Davis, CA, USA

Ishita M. Shah[1,2], Hai Lu[3], Xi Chen[3] and David A. Mills[1,2]
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2. Department of Food Science and Technology, University of California, Davis, USA
3. Department of Chemistry, University of California, Davis, USA

Complex oligosaccharides present in human milk (HMOs), are the result of millions of years of mammalian evolution to protect the infant from severe infections. While HMOs have many functions, two main roles are

as prebiotics for specific commensal bacteria and reduction in infection through serving as receptor-decoys for pathogens thus blocking their binding to the intestine and reducing rates of infection. However, we have made a recent discovery that pre-incubation of specific HMOs like lacto-N-fucopentaose I (LNFP I) and 3' sialyllactose (3' SL) with intestinal cells directly enhances intestinal barrier integrity. We hypothesize that the well documented HMO-driven reduction in pathogen infection is modulated via two mechanistic routes, one via a direct interaction between HMO and intestinal cells thereby improving barrier function and the second through direct blocking of pathogen binding to the intestine. Pre-incubation of LNFP I and 3' SL with intestinal cells directly increases cell-cell adhesion, structural integrity and dramatically reduces internalization of pathogenic bacteria. Moreover, these HMOs directly increase transepithelial electrical resistance indicating improved tight-junction dynamics and a barrier-strengthening effect prior to pathogen encounter. Direct incubation with these HMOs also results in an alteration in the infection-dependent signaling cascade, and a dramatic reduction in pathogen-mediated chemokine expression. This new evidence suggests a previously unknown protective role for HMOs directly linked to intestinal health—a finding that will enhance understanding of gut health and maturation during breastfeeding.

4:15 pm **Student Travel Award Recipient: Evaluate the Effect of Industrial Thermal Treatments on the Enzymatic Release of N-Glycans from Milk Glycoprotein**

Apichaya Bunyatratchata, University of California-Davis, Davis, CA, USA

Apichaya Bunyatratchata[1], Yu-Ping Huang[1], Gulustan Ozturk[1], Juliana Maria Leite Nobrega De Moura Bell[1], Daniela Barile[1,2]
1. Department of Food Science and Technology, University of California, Davis, USA
2. Foods for Health Institute, University of California, Davis USA

Milk oligosaccharides are indigestible carbohydrates proven to positively influence the shaping of the gut microbiota. Because N-glycans are structurally similar to milk oligosaccharides, they may potentially promote the growth of beneficial strains of bifidobacteria. Preliminary results show a strain-specific prebiotic selectivity of N-glycans after release from bovine whey proteins using a recently discovered Endo-β-N-acetylglucosaminidase (EndoBI-1) enzyme. Hence, there is strong potential for the recovery of N-glycans after dairy processin for subsequent use as a selective prebiotic ingredient in formulations together with bovine milk oligosaccharides. Industrial heat treatments conventionally used on bovine milk (pasteurization and sterilization) may affect whey glycoproteins by unfolding the globular structures differently, potentially modifying the degree of enzymatic release of N-glycans. In this study, we investigated the effects of High Temperature Short Time (HTST: 72°C for 15 sec) and Ultra High Temperature (UHT: 135°C for 3 sec) on the enzymatic release of N-glycans from bovine colostrum glycoproteins. Samples were heat treated using a continuous Microthermics pilot-scale HTST/UHT pasteurizer. Nano-Liquid Chromatography-Chip-Quadrupole-Time-of-Flight Mass spectrometry (Nano-LC-Chip-Q-TOF MS) was used to profile and annotate N-glycans. This work is the first to evaluate the effects of heat treatments on N-glycans release and to demonstrate that HTST represents the ideal combination of time and temperature to maximize the release of all N-glycans classes (neutral fucosylated, neutral non-fucosylated, and sialylated N-glycans) using EndoBI-1. In contrast, the UHT treatment did not favor the enzymatic release of N-glycans. This result might due to unfavorable enzyme accessibility or possible damage to glycoproteins structure due to increased denaturation. Based on these results, we propose that HTST might induce protein unfolding in ways that favor enzymatic accessibility to the N-glycosylation sites and achieve a higher abundance of N-glycans released compared with UHT.

Thursday November 15, 2018

9:00 am **Keynote: The Milkfat Globule an Ever Evolving Story**

Sophie Gallier, Fonterra Co-operative Group, Palmerston North, New Zealand

Van der Zeijden, M.[1,2], MacGibbon, A.[1], Fong, B.[1] and Gallier, S.[1]
1. Fonterra Co-operative Group, Palmerston North, New Zealand
2. Wageningen University and Research, Wageningen, the Netherlands

In bovine and human milk, lipids are present as milk fat globules stabilized by a layer, unique in its composition and structure, called the milk fat globule membrane. The MFGM contains various membrane-specific proteins and polar complex lipids, including phospholipids and gangliosides which have been shown to vary across
lactation. The MFGM structure is a trilayer of phospholipids with the MFGM proteins being asymmetrically distributed, sphingomyelin and cholesterol being complexed as lipid rafts, and glycosylated proteins and lipids forming the glycocalyx. This unique and complex structure potentially plays key roles in the digestion of milk fat.

Milk fat has historically been replaced by vegetable oils to manufacture infant formulas with a fatty acid profile similar to that of human milk fat. Infant formulas commonly contain only residual MFGM components that happen to come from the milk protein ingredients (i.e. skim milk and whey protein powders). However, there is an increasing understanding of the role of the MFGM for infant growth and development, including cognition, gut maturation and immunity. Therefore, MFGM-rich ingredients are a way to provide MFGM at the same level as found in human milk while still matching the fatty acid profile of human milk. There is increasing human clinical evidence that the addition of MFGM-rich ingredients to infant formula enhances cognitive development and reduces the risk of infections in early life. In our recent clinical study in China, we have shown that infants fed an MFGM-supplemented formula from birth to 12 months of age had higher scores of cognitive development than infants fed a non-supplemented formula.

In addition, a proportion of human milk fat globules present interfacial cytoplasmic crescents, filled with a range of vesicles and "sandwiched" between the inner monolayer and outer bilayer of the MFGM. These vesicles most likely play a key role in the development of the immune system of the newborn. We have also found these in bovine MFGM.

This presentation will cover an overview of our understanding of the MFGM composition, structure and role in infant nutrition, including results from our studies, and the potential future development to manufacture infant formula to match more closely the nutritional benefits of human milk using examples from our research on milk and ingredient structure.

9:40 am **Student Travel Award Recipient: Commercial Bovine Milk Fat Globule Membrane Fractions- Variations Among Sources**

Lauren Brink, University of California-Davis, Davis, CA, USA

Lauren R Brink[1], Shasta McMillen[1], Anthony W Herren[2], Karl Frasier[3,4], Heike Schwendel[3], Nicole Roy[4], Bo Lönnerdal[1]
1. Department of Nutrition, University of California, Davis, USA
2. Genome Center, University of California, Davis, USA
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4. Riddet Institute, Massey University, Palmerston North, New Zealand

Milk Fat Globule Membrane (MFGM) is a glycosylated, protein embedded, phospholipid fraction which delivers triglycerides in milk. Commercial bovine sources have recently come to the market as a novel food ingredient. Considering that MFGM is a heterogenous mixture of fat, protein and carbohydrate it can be expected that variations among MFGM products exist. For this reason, our aim was to describe the composition of commercial MFGM samples through proteomic, western blotting and lipidomic analysis. Six bovine milk fractions represented as MFGM fractions or Phospholipid fractions were obtained from various commercial sources. For proteomic analysis, LC-MS/MS was performed in technical replicates on a Thermo Q-Exactive Plus mass spectrometer and protein identifications and intensities extracted with MaxQuant against the Uniprot Bos taurus reference proteome. Relative protein composition within samples as well as between samples was investigated. Various proteins that have been previously described as classical MFGM components were also quantified by western blot. Lipidomic analysis was performed with UPLC-high resolution mass spectrometry followed by peak extraction with the non-targeted peak detection tool XCMS and identification of lipids via LipidSearch. Across the 6 MFGM fractions tested, nearly a thousand proteins were identified with 364 of these having significantly different protein levels. One hundred and thirteen proteins were different by a fold change (fc) of 10 or greater, 14 by an fc of 50 and 2 by a fc of 100. Interestingly, one of the latter two proteins, Mucin 1, is considered a 'classical' MFGM protein. Alpha-lactalbumin, a dominant protein in bovine milk fractions, was not significantly different among the groups and this was confirmed by immunoblotting. A total of 393 lipid species were annotated across positive and negative ionization modes with the major classes detected being triglycerides, sphingomyelins and several phospholipids. Across all samples, triglycerides comprised at least 50% of total lipids, phosphatidylcholine and sphingomyelin were the second and third most abundant lipid class, respectively. This work demonstrates the heterogenous nature of various bovine milk fractions. This variation must be considered when describing potential functional benefits of these products.

10:00 am **Student Travel Award Recipient: The Therapeutic Potential of Bovine Milk-Derived Extracellular Vesicles for Treatment of Osteoarthrits Patients**

Bartijn Pieters, Radboud University Medical Center, Nijmegen, The Netherlands

Bartijn Pieters[1], Onno Arntz[1], Danny Kartoidjojo[1], Anouk Feitsma[2], Joost van Neerven[2], Peter van der Kraan[1], Fons van de Loo[1]
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2. FrieslandCampina, Amersfoort, The Netherlands

Introduction: Bovine milk is a rich source of extracellular vesicles, which are small phospholipid bilayer bound structures that facilitate intercellular communication. It has been shown that these vesicles are able to survive the harsh conditions of the intestinal track and are believed to be taken up into the bloodstream by consumers. We, and others, have highlighted the anti-inflammatory potential of EVs isolated from bovine milk in animal models of experimental arthritis [1,2]. However, little is known how this translates to the human situation. In this study, we investigate the effects of bovine milk-derived EVs (MEVs) on cells of the cartilage (articular chondrocytes) and from the joint capsule (synovial fibroblasts) derived-from osteoarthritis (OA) patients. OA has long been considered a disease of the cartilage due to mechanical stress evoked either by trauma or overloading of the joint especially in combination with loss of chondrocyte function due to aging. Evidence now emerges that in a high percentage of OA patients signs of synovial inflammation can be detected at the early stage of disease. Currently there is no cure and pain killers are the only available drugs for these patients. We investigate whether bovine milk-derived EVs have the potency to reduce joint pathology.

Methods: MEVs were isolated from commercial skimmed cow milk using a standard differential ultracentrifugation protocol. Particle concentration, size and floating density were assessed by NTA analysis and sucrose density gradient, respectively. Articular chondrocytes and primary fibroblast-like synoviocytes (FLS) from OA patients were stimulated for 24hrs and 48hrs with MEVs and gene expression profiles were studied by RT-qPCR. Additionally, short stimulations (2hrs) were performed, in the presence of an anti-TGFβ1,2,3 antibody, to study direct TGFβ-receptor activation.

Results: Stimulation of articular chondrocytes with 10-100µg/ml MEVs was able to effectively reduce expression of cartilage destructive enzymes (ADAMTS5, MMP1, MMP3) and inflammatory mediators (IL6, IL8, TNFα) that play key roles in the progression of OA. Additionally, we observed a significant increase in expression of TIMP3, a potent inhibitor of above mentioned cartilage destructive enzymes. Stimulation of primary FLS showed similar results, with marked reduction of catabolic enzymes (ADAMTS5, MMP1) and also increased in TIMP3 levels. The reduction in inflammatory mediators was however not found, and in contrast IL6 was significantly increased in FLS after exposure to MEVs. Short exposure of chondrocytes to MEVs led to induction of early TGFβ response genes (JUNB, SMAD7, PAI), which was completely blocked using an anti-TGFβ1,2,3 antibody.

Conclusion: Human articular chondrocytes and synovial fibroblasts exposed to MEVs show reduced destructive and inflammatory potential. The induction of early TGFβ response genes after short incubations confirms the presence of active TGFβ, which could explain, in part, the anti-inflammatory and reduced catabolic profiles found. These findings highlight the therapeutic potential of MEVs in osteoarthritis, where inflammatory and catabolic mediators are responsible for joint pathology and subsequent loss of mobility. However, more in vitro work is required to compare different milk sources (e.g. raw milk, colostrum, whey) to find the most potent MEV, and to perform preclinical animal studies before this therapy can be tested in patients.

1. Pieters, BCH et al. "Commercial Cow Milk Contains Physically Stable Extracellular Vesicles Expressing Immunoregulatory TGF-B." PLoS ONE 10.3 (2015).
2. Arntz, OJ, et al. "Oral administration of bovine milk derived extracellular vesicles attenuates arthritis in two mouse models." Mol. Nutr. Food Res., 59 (2015).

10:50 am **Milk Protein Digestion and Bioactive Peptide Release in Term and Preterm Infants**
Dave Dallas, Oregon State University, Corvallis, OR, USA

Background: Preterm infants may not be able to digest breast milk proteins to the same extent as term infants. A lower digestive capacity could be problematic because digestion of milk proteins provides not only nutrition, but also the release of bioactive peptides exhibiting antimicrobial, prebiotic, immunemodulating, calcium-delivery, antihypertensive, and pain-modulating activities. The specific differences in protease activity level and their impact on the infant's ability to break down protein and release bioactive peptides are not known.

Objective/Hypothesis: Determine differences in milk protein degradation and bioactive peptide release in the stomach between term and preterm infants.

Methods: Mother's milk and infant gastric samples were analyzed using mass spectrometry-based peptidomics, protease assays, ELISA and bioactive peptide database searching to determine how proteins are degraded within term and preterm infants and which potential bioactive peptides are released.

Results: Milk protein degradation begins within the mammary gland with milk proteases, releasing thousands of peptides, many of which are bioactive. In the stomach, both gastric pepsin and several milk proteases actively degrade milk proteins, releasing thousands of new peptides. We showed that gastric protein digestion is lower in preterm infants than term infants, releasing different bioactive peptides.

Conclusions: Peptidomic and enzyme analyses enable precise characterization differences in protein digestion and bioactive peptide release between preterm and term infants. Our ongoing research is characterizing which peptides are antimicrobial and immunomodulatory with in vitro assays and preparative liquid chromatography-based peptide fractionation.
Funding source: NIH NICHD K99/R00 (R00HD079561)

11:20 am **Comparison of Human Milk Immunoglobulin Survival during Gastric Digestion between Preterm and Term Infants**

Veronique Demers-Mathieu, Oregon State University, Corvallis, OR, USA

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Immunoglobulins (Igs) are important effectors of the adaptive immune system because of their functions in neutralizing pathogens by binding to their surfaces and in activating T-cells for immune response. During the third trimester, the mother's placenta transports IgG to the fetus via a neonatal Fc receptor and protect the infant for the first 6 months of postnatal age while the infant's own immune system is developing. After birth, human milk provides another form of protection against pathogens for both preterm and term infants, as it contains an array of Igs, including IgA, secretory IgA (SIgA), IgM, secretory IgM (SIgM) and IgG. Due to the early interruption of placenta-fetal IgG transfer caused by preterm birth, preterm infants receive a lower maternal IgG concentration and fewer antibodies against specific pathogens than term infants. The diversity of antibody specificity in preterm infants increases more slowly than in term infants. The lower concentration of maternal antibodies and reduced diversity of antigen-binding sites in preterm infants partially explains their higher risk for bacterial and viral infections compared with term infants.

Milk Igs are known to be important for infant immune development and immunoprotection, yet the extent to which these proteins survive intact within the infant stomach, and the differences in survival between preterm and term infant gastric digestion remain unknown. Therefore, the aim of the present study was to compare human milk total IgA (SIgA/IgA), total secretory component (SC/SIgA/SIgM), total IgM (SIgM/IgM) and IgG between the preterm and term infant stomach as well as their survival across postprandial time in the preterm infant stomach. Human milk, and infant gastric samples at 2 h post-ingestion were collected from 15 preterm (23–32 wk gestational age (GA)) mother-infant pairs and from 8 term (38–40 wk of GA) mother-infant pairs within 7–98 days postnatal age. For preterm groups, gastric samples were also collected 1 and 3 h postprandial. Samples were analyzed via ELISA for Ig concentrations and peptidomic analysis.

Total IgA concentration decreased 60% from human milk to the preterm infant stomach and decreased (48%) but not significantly in the term infant stomach. Release of peptides from IgA in the term infant stomach was higher than in the preterm stomach. Total IgM and IgG concentrations decreased by 33% and 77%, respectively, from human milk to the term infant stomach but were stable in the preterm infant stomach. For the Ig survival across time postprandial in the present stomach, concentrations of total IgA were lower in the gastric contents at 3 h postprandial compared with human milk and gastric contents at 1 and 2 h. Human milk SC/SIgA/SIgM, IgG and total IgM concentrations remained stable in the preterm stomach across postprandial time. Peptide counts from the Ig alpha-chain and the Ig gamma-chain increased in gastric contents from 1 to 2 h postprandial. Peptide counts from the human milk Ig mu-chain, Ig J-chain and SC were stable across

postprandial time. These peptides from Ig-chains were not present in human milk but were released in the stomach due to their partial degradation.

The stability of human milk Igs during gastric digestion differed according to Ig isotype and infant gestational age. As the stomach represents only the beginning of the digestive system, the concentrations of human milk Igs in intestinal samples from preterm and term infants need to be determined to clarify their potential survival during infant digestion. The longer that milk Igs survive through the digestive system, the longer they can act as passive immune system components, which is particularly important in the context of the immune system immaturity of the early postnatal period in preterm and term infants.

11:40 am **Differences in Composition and Structure Between Human Milk and Infant Formula: Do They Affect Their Digestion?**

Didier Dupont, INRA-Agrocampus Ouest, Rennes, France

Didier Dupont[1], Olivia Menard[1], Amelie Deglaire[1]
1. STLO, INRA-Agrocampus Ouest, Rennes, France

Despite all the efforts made by the dairy industry to develop infant formula (IF) mimicking the composition of human milk (HM), some major differences remain between these two foods in terms of composition (nature of proteins and lipids, oligosaccharide, microbiota…) and structure (casein micelle, milk fat globule).
Do these compositional and structural differences affect the behavior of the 2 products when they enter the gastrointestinal tract of the neonate? The answer is yes. For instance, gastric emptying half-time is 54 min for HM and 80 min for IF resulting in major differences in the kinetics of hydrolysis of proteins and lipids.

This presentation will summarize ten years of work conducted at INRA on food digestion leading to the release of more than 40 peer-reviewed articles.

First, gastrointestinal digestion of HM and IF was compared using a dynamic in vitro digestion simulator, the DiDGi®. Caseins were shown to be more extensively broken down in the stomach in IF than HM. In contrast, HM lipids were more degraded in the stomach due to the action of the milk endogenous lipase but were less hydrolyzed in the small intestine. This difference was attributed to the homogenization process applied to IF transforming large milk fat globules into smaller lipid droplets and increasing the specific surface available for digestive lipases.

In a clinical trial performed on pre-term neonates, the effect of pasteurization and homogenization of HM on the kinetics of gastric lipolysis and proteolysis was investigated. Pre-term neonates were fed through a naso-gastric tube that also allowed to aspirate their stomach content at different post-prandial times. Pasteurization was shown to decrease the resistance of lactoferrin that was hydrolyzed faster in the stomach. Homogenization led to an acceleration of lipolysis in the stomach of the pre-term neonates.

In a third project, the effect of the heat-treatment applied to IF manufacture on the casein resistance to gastrointestinal digestion was investigated. Model IF were developed at a pilot plant scale and the effect of 4 heat treatments applied to the protein concentrates (no heating, 80°C/20s, 85°C/3 min, 105°C/60s) on casein digestion was studied. Casein domains carrying post-translational modifications and hydrophobic areas were shown to be the most resistant to the digestive process. The intensity of the heat-treatment was shown to dramatically affect casein resistance to digestion; the more IF were heated, the more resistant the casein were. This resistance was attributed to the thermal aggregation of whey protein that were forming a protective layer around the casein micelle.

Finally, IF were designed at INRA's dairy platform and their in vivo digestion by piglets investigated. IF1 was a control IF with vegetable oil and milk proteins at the interface of the lipid droplet. In IF2, lipid droplets were stabilized by milk phospholipids in order to recreate a structure close to that of the milk fat globule. Finally, IF3 was also stabilized by milk phospholipids and 60% of the vegetable oil was replaced by milk fat. Piglets were fed 28d with one of the three IF. Concentration of caseins and β-lactoglobulin in the jejunum and ileum was higher for IF3. Higher intestinal thickness was also observed with IF3 but was not associated with changes in permeability. IF3 increased IFNγ secretion suggesting an improved intestinal immune system maturation such as observed in sow suckling piglets. Finally, the nature of IF was shown to dramatically affect the intestinal microbiota composition of piglets.

All these data show that IF still exhibit major differences with HM affecting their kinetics of disintegration in the GI tract. Designing a new generation of biomimetic IF is an objective that we are currently trying to reach.

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Poster Presentation Abstracts

Poster Presentation Abstracts

1) **Student Travel Award Recipient: Commercial Bovine Milk Fat Globule Membrane Fractions- Variations Among Sources**

Lauren Brink, University of California-Davis, Davis, CA, USA

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Milk Fat Globule Membrane (MFGM) is a glycosylated, protein embedded, phospholipid fraction which delivers triglycerides in milk. Commercial bovine sources have recently come to the market as a novel food ingredient. Considering that MFGM is a heterogenous mixture of fat, protein and carbohydrate it can be expected that variations among MFGM products exist. For this reason, our aim was to describe the composition of commercial MFGM samples through proteomic, western blotting and lipidomic analysis. Six bovine milk fractions represented as MFGM fractions or Phospholipid fractions were obtained from various commercial sources. For proteomic analysis, LC-MS/MS was performed in technical replicates on a Thermo Q-Exactive Plus mass spectrometer and protein identifications and intensities extracted with MaxQuant against the Uniprot Bos taurus reference proteome. Relative protein composition within samples as well as between samples was investigated. Various proteins that have been previously described as classical MFGM components were also quantified by western blot. Lipidomic analysis was performed with UPLC-high resolution mass spectrometry followed by peak extraction with the non-targeted peak detection tool XCMS and identification of lipids via LipidSearch. Across the 6 MFGM fractions tested, nearly a thousand proteins were identified with 364 of these having significantly different protein levels. One hundred and thirteen proteins were different by a fold change (fc) of 10 or greater, 14 by an fc of 50 and 2 by a fc of 100. Interestingly, one of the latter two proteins, Mucin 1, is considered a 'classical' MFGM protein. Alpha-lactalbumin, a dominant protein in bovine milk fractions, was not significantly different among the groups and this was confirmed by immunoblotting. A total of 393 lipid species were annotated across positive and negative ionization modes with the major classes detected being triglycerides, sphingomyelins and several phospholipids. Across all samples, triglycerides comprised at least 50% of total lipids, phosphatidylcholine and sphingomyelin were the second and third most abundant lipid class, respectively. This work demonstrates the heterogenous nature of various bovine milk fractions. This variation must be considered when describing potential functional benefits of these products.

2) **Student Travel Award Recipient: Evaluate the Effect of Industrial Thermal Treatments on the Enzymatic Release of N-Glycans from Milk Glycoprotein**

Apichaya Bunyatratchata, University of California-Davis, Davis, CA, USA

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Milk oligosaccharides are indigestible carbohydrates proven to positively influence the shaping of the gut microbiota. Because N-glycans are structurally similar to milk oligosaccharides, they may potentially promote the growth of beneficial strains of bifidobacteria. Preliminary results show a strain-specific prebiotic selectivity of N-glycans after release from bovine whey proteins using a recently discovered Endo-β-N-acetyl-glucosaminidase (EndoBI-1) enzyme. Hence, there is strong potential for the recovery of N-glycans after dairy processing for subsequent use as a selective prebiotic ingredient in formulations together with bovine milk oligosaccharides. Industrial heat treatments conventionally used on bovine milk (pasteurization and sterilization) may affect whey glycoproteins by unfolding the globular structures differently, potentially

modifying the degree of enzymatic release of N-glycans. In this study, we investigated the effects of High Temperature Short Time (HTST: 72°C for 15 sec) and Ultra High Temperature (UHT: 135°C for 3 sec) on the enzymatic release of N-glycans from bovine colostrum glycoproteins. Samples were heat treated using a continuous Microthermics pilot-scale HTST/UHT pasteurizer. Nano-Liquid Chromatography-Chip-Quadrupole-Time-of-Flight Mass spectrometry (Nano-LC-Chip-Q-TOF MS) was used to profile and annotate N-glycans. This work is the first to evaluate the effects of heat treatments on N-glycans release and to demonstrate that HTST represents the ideal combination of time and temperature to maximize the release of all N-glycans classes (neutral fucosylated, neutral non-fucosylated, and sialylated N-glycans) using EndoBI-1. In contrast, the UHT treatment did not favor the enzymatic release of N-glycans. This result might due to unfavorable enzyme accessibility or possible damage to glycoproteins structure due to increased denaturation. Based on these results, we propose that HTST might induce protein unfolding in ways that favor enzymatic accessibility to the N-glycosylation sites and achieve a higher abundance of N-glycans released compared with UHT.

3) **Student Travel Award Recipient: Structural and Functional Insights into EchAMP, a Unique Monotreme Antimicrobial Protein Expressed During Lactation**

Alok Kumar, CSIR-Centre For Cellular and Molecular Biology, Habsiguda, Hyderabad, India

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Monotremes, the egg-laying mammals with the ability to lactate are the only living representatives of ancient oviparous mammals with the primitive form of lactation. During evolution, they split from the therian lineage (present days placental mammals) about 166-210 mya and so displays a unique combination of reptilian and mammalian reproductive features. They lay parchment-shelled eggs which upon hatching give birth to altricial young ones developmentally equivalent to a 40-day old human embryo[1]. The mother's milk secreted from the nipple-less mammary patches is the only source of nourishment and protection for these altricial youngs against pathogens present in their environment. Monotreme milk thus is rich in bioactives and can be a novel source of antimicrobial molecules. Echidna AntiMicrobial Protein (EchAMP) is one such protein expressed in the echidna milk[2]. It is the tenth most abundant transcript with conserved secretory signal sequence and multiple putative sites for post-translational modifications[2].

The detailed functional characterization of this protein was hindered because of the exclusivity of echidna as a geographically confined species and poorer protein yields with several eukaryotic expression systems. However, in the present study, we successfully purified the EchAMP protein in optimum quantities using a bacterial expression system. Unlike its eukaryotic counterpart, the recombinant protein from bacteria lacks post-translational modification but shows activity in our antimicrobial assays. Structurally the protein is intrinsically disordered as suggested by tryptophan fluorescence, circular dichroism, and NMR spectroscopy and like most of the IDPs upon thermal melting exhibit propensity to form transient a-helices. The in vitro antimicrobial assays with the purified EchAMP protein showed activity against both the Gram-positive (Bacillussubtilis, Staphylococcus aureus) and the Gram-negative micro-organisms (Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Enterococcus faecalis). This broad-spectrum antimicrobial activity is species specifically dose-dependent and mostly bacteriolytic as confirmed by live-dead staining and scanning electron microscopy.

Moreover, the observed bacteriolytic activity was relatively higher for bacillus compared to other species used in the assay. Echidna is a terrestrial animal that lives in burrows and so essentially needs protection against spore-forming soil microbes often known to be associated with infections in the lactating glands. Our

studies using tryptophan fluorescence and circular-dichroism spectroscopy with amphipathic molecules and bacterial membrane mimics such as SDS, lipopolysaccharide (LPS) and liposomes (SUVs) respectively suggested that like other intrinsically disordered AMPs (Cecropin A and Magainin 2)[3] EchAMP too exhibits a propensity to fold into helices upon interaction with the bacterial envelope. Thereby gaining functionally active conformation and killing cells by compromising their membrane integrity and causing cytoplasm leaking.

To summarize, EchAMP in light of its evolutionary conserved association only with monotremes and its broad-spectrum antimicrobial activity can be a promising candidate of choice against multiple infections associated with the lactating mammary gland in humans and livestock.

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4) **Student Travel Award Recipient: The Therapeutic Potential of Bovine Milk-Derived Extracellular Vesicles for Treatment of Osteoarthrits Patients**

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Introduction: Bovine milk is a rich source of extracellular vesicles, which are small phospholipid bilayer bound structures that facilitate intercellular communication. It has been shown that these vesicles are able to survive the harsh conditions of the intestinal track and are believed to be taken up into the bloodstream by consumers. We, and others, have highlighted the anti-inflammatory potential of EVs isolated from bovine milk in animal models of experimental arthritis [1,2]. However, little is known how this translates to the human situation. In this study, we investigate the effects of bovine milk-derived EVs (MEVs) on cells of the cartilage (articular chondrocytes) and from the joint capsule (synovial fibroblasts) derived-from osteoarthritis (OA) patients. OA has long been considered a disease of the cartilage due to mechanical stress evoked either by trauma or overloading of the joint especially in combination with loss of chondrocyte function due to aging. Evidence now emerges that in a high percentage of OA patients signs of synovial inflammation can be detected at the early stage of disease. Currently there is no cure and pain killers are the only available drugs for these patients. We investigate whether bovine milk-derived EVs have the potency to reduce joint pathology.

Methods: MEVs were isolated from commercial skimmed cow milk using a standard differential ultracentrifugation protocol. Particle concentration, size and floating density were assessed by NTA analysis and sucrose density gradient, respectively. Articular chondrocytes and primary fibroblast-like synoviocytes (FLS) from OA patients were stimulated for 24hrs and 48hrs with MEVs and gene expression profiles were studied by RT-qPCR. Additionally, short stimulations (2hrs) were performed, in the presence of an anti-TGFβ1,2,3 antibody, to study direct TGFβ-receptor activation.

Results: Stimulation of articular chondrocytes with 10-100µg/ml MEVs was able to effectively reduce expression of cartilage destructive enzymes (ADAMTS5, MMP1, MMP3) and inflammatory mediators (IL6, IL8, TNFα) that play key roles in the progression of OA. Additionally, we observed a significant increase in expression of TIMP3, a potent inhibitor of above mentioned cartilage destructive enzymes. Stimulation of primary FLS showed similar results, with marked reduction of catabolic enzymes (ADAMTS5, MMP1) and also increased in TIMP3 levels. The reduction in inflammatory mediators was however not found, and in contrast IL6 was significantly increased in FLS after exposure to MEVs. Short exposure of chondrocytes to MEVs led to induction of early TGFβ response genes (JUNB, SMAD7, PAI), which was completely blocked using an anti-TGFβ1,2,3 antibody.

Conclusion: Human articular chondrocytes and synovial fibroblasts exposed to MEVs show reduced destructive and inflammatory potential. The induction of early TGFβ response genes after short incubations confirms the presence of active TGFβ, which could explain, in part, the anti-inflammatory and reduced catabolic profiles found. These findings highlight the therapeutic potential of MEVs in osteoarthritis, where inflammatory and catabolic mediators are responsible for joint pathology and subsequent loss of mobility. However, more in vitro work is required to compare different milk sources (e.g. raw milk, colostrum, whey) to find the most potent MEV, and to perform preclinical animal studies before this therapy can be tested in patients.

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5) **Release of Milk Peptides within the Infant Stomach and Possible Developmental Relevance**

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Background: Over the course of human milk digestion, native milk proteases and infant digestive proteases fragment intact proteins into shorter peptides. Human milk peptides have previously been identified with bioactivities such as antimicrobial, bifidogenic, immunostimulatory, and anti-inflammatory activity that may be relevant to infant gastrointestinal development. For these peptides to be effective though, they must be released from their parent proteins and survive further proteolysis until they reach their site of action.

Objective/Hypothesis: Measure the release of peptides with and without bioactivity from milk proteins over three hours of gastric digestion in preterm infants.

Methods: Samples of the milk and of the gastric fluid one, two, and three hours post-feeding were collected from 14 preterm infants. The peptide content was extracted and analyzed via Orbitrap tandem mass spectrometry to determine how in vivo peptide release changes over time during gastric digestion and by milk fortification. Bioactivity of the identified peptides was predicted by sequence homology to known bioactive milk peptides.

Results: Thousands of milk peptides and hundreds of potentially bioactive peptides were released over time in the preterm infant stomach, reaching a maximum after three hours. Samples from non-fortified infants contained more unique peptides than from fortified infants, but total peptide content did not differ

between the two. Peptides that survived further gastric digestion after their initial release had structural characteristics that more closely resembled bioactive peptides than non-surviving peptides.

Conclusion: This work is the first to provide a comprehensive profile of the release of milk peptides during gastric digestion over time, which is an essential step in determining which peptides are most likely to be biologically relevant in the infant. Our ongoing research is identifying and characterizing antimicrobial and bifidogenic peptides from in vitro gastric and intestinal human milk digests.

6) **Milk microRNA Composition Depends on Dairy Cow Breed**

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The concept of milk as a healthy food has opened the way of studies on milk components, including macro- and micronutrients, as well as molecules such as microRNAs. The presence of microRNAs in large quantities in milk (including commercial milk) has led the scientific community to focus their attention on their potential role on human health. Some studies suggested that microRNAs present in human milk could affect functions such as immunity, growth, development, cell proliferation, and apoptosis. microRNAs are transmitted from mother to infant and have the potential to act across species and to influence milk's health effects on consumers, as recently documented.

For the first time, Chen et al. (2010) have showed that microRNAs are intrinsic and stable components of milk. Their miRNomes (microRNA composition) have been obtained from several species, such as human and bovine. MicroRNA distribution in milk depends on the stages of lactation.

In that context, we have studied milk microRNA composition variations due to the genetic background. Thus, we have compared milk miRNomes of Hostein and Normande dairy cows. Those two breeds have contrasted lactation performances (milk yield, milk fat and protein yields and contents). We have performed high throughput RNA sequencing on the milk samples from six mid-lactation primiparous cows for each breed. The 40 most abundant microRNAs are the same in the milk of the two breeds. However, our study reveals the presence of 113 microRNAs (known and predicted) in both breeds with a significantly differential level ($p<0.05$). We have also investigated the sequence variations of each microRNAs (isomiRs) and have performed a detailed analysis at the isomiR level, which has never been done before.

microRNAs are differentially distributed in milk compartments such as extracellular vesicles, milk fat, whey and cells (Li et al., 2016; Izumi et al., 2015). Their influence in milk and dairy products must be clarified as their packaging into extracellular vesicles, which provides a protective role for their microRNA cargos. In this study, we have compared the level in extracellular vesicles, milk fat, whey fractions of two microRNA with a significantly differential level in milk of the two breeds.

As we have showed, there is a direct relation between microRNAs expression in the mammary gland and their level in milk (Le Guillou et al., 2012; Laubier et al., 2015). Holstein milk miRNomes obtained here have been compared to Holstein mammary gland miRNomes (Le Guillou et al., 2014). Then, our data have been compared to mammary gland microRNA profiles performed on different cattle breeds (dairy vs beef cattle breeds (Wicik et al., 2015) and Holstein vs Montbéliarde dairy cattle breeds (Billa et al., IMCG 2018, poster)). Interestingly, we have identified few microRNAs common to our comparison and Wicik or Billa studies.

Milk is a source of nutrients for neonate and adults, and its composition influences the health of consumers in both the short and long terms. This composition depends on the genetics, among the variables; microRNAs are a novel class of molecules with broad regulatory properties. Their presence in milk opens a line of investigation to use these as indicators (biomarkers) for downstream consequences of health, physiological or metabolic status, in animals and humans.

7) **Is The Naïve Immune System Affected By Cow's Milk**

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Consuming raw, unprocessed cow's milk in childhood is associated with a decreased incidence of allergy in later life. Cow's milk contains a plethora of immune related bioactive components. Many of these factors are active across species. Being able to supress and enhance immune responses. The abundance and functionality of these components has led to numerous mechanisms being proposed by which raw cow's milk may be protective against allergy. Yet, the potential immunological pathways involved in this protective effect remain to be uncovered.

Work conducted by our group, utilising a mouse model of gastrointestinal allergy, found that a diet containing raw, or pasteurised cow's milk altered the immune response of mice to an unrelated oral antigen (Hodgkinson et. al, Brit J Nutr, 2014). Other groups have demonstrated that raw cow's milk can prevent the development of asthma mouse models (Abbring et.al, Frontiers in Immunology 2017). In this work, the heat-treatment of milk undid these anti-asthma effects. Suggesting that the potential protective factors in raw milk may be heat sensitive. These works demonstrate the abilities of raw and processed milks to effect allergies in mouse models.

To date, no studies have assessed the effects of milk feeding in a naïve non-allergic model. We, thus, hypothesised that the protective effects of raw milk could be evident in naïve animal model; and these effects might be due to raw cow's milk altering the developing immune system. To begin to answer this question we sort to detect the effects of milk feeding, on immune cells within the spleen (splenocytes) of young mice.

Weanling mice were fed either water, raw milk, or processed milk (pasteurised homogenised), for a 7 or 28 day period. After which, augmentations to major immune cell populations, CD4+ T cells, B cells, granulocytes, and monocytes where detected by flow cytometry. Further, CD4+ T cells, being important for the control and orchestration of immune responses, were assessed for their activation status, based on CD44 levels. Additional, affects tested by examining the cytokine milieu of cultured splenocytes. The concentrations of key cytokines involved in CD4+ T cell biology, were analysed by ELISA.

Preliminary findings showed that CD44 high effector/memory T cell numbers were increased in both raw milk and processed milk fed animals at day 28. Augmentations to were found in B cell and granulocyte counts at day 7 and day 28. Antigen presenting granulocytes, based on MHC-II high cells, were increased in mice fed milk compared with those fed water.

Interestingly, splenocyte cultures from raw milk and processed milk fed mice were found to have increased interleukin (IL-)6 concentrations directly ex vivo compared to water-fed controls. Stimulation of splenocyte cultures resulted in a significant increase in IL-17 concentrations in raw milk fed mice.

The significance of these results is that they demonstrate a possible immunomodulatory effect of milk on a young, naïve animal model, at the systemic level. These results show that feeding milk can directly affect the immune cell profile of the spleen. The observed increases in IL-6 and IL-17 concentrations within splenocyte cultures are of particular interest. IL-6 is a pleiotropic cytokine with diverse functions. It's secretion in vivo contributes significantly to the control and prevention of allergic immunity (Mayer et.al., European Journal Immunology, 2014. Hercor et.al., Journal of Leukocyte Biology, 2016). While, IL-17 is a classically pro-inflammatory cytokine produced mainly by activated CD4+ T cells, and has been reported to increase MHC-II expression by granulocytes (Abi Abdallah et.al., International Immunology 2011). Thus, these results suggest that milk, as a whole food, is able to effectuate the immune system, and could have potential effects on allergy mitigation.

8) **Proteomic Analysis and Molecular Characterization of CMP Glycoforms Before and After Desialidation**

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Bovine caseinomacropeptide (CMP) is the third most abundant peptide in whey protein concentrate. It reveals a high degree of heterogeneity as it consists of several glycoforms originating from different genetic variants and is of growing interest due to its reported bioactive and functional properties. CMP has demonstrated beneficial physiological functions including antimicrobial activity. Further, CMP containing glycan moieties (gCMP) has shown positive effects on gut microflora. Functionally, CMP/gCMP in food products may influence microstructure, foaming, gelling and emulsifying properties. Sialic acid containing CMP has additionally been associated with formation of unwanted storage induced aggregates in whey beverages. The objective of the present work was to investigate CMP glycoforms in gCMP isolat (Lacprodan gCMP-20 powder) reconstituted at 7% (w/v), and how these glycoforms were affected by sialidase-induced cleavage of sialic acid residues on gCMP (EC 3.2.1.18, from Clostridium perfringens, Sigma Aldrich). Desialidation treatment of gCMP solutions were confirmed by 2-dimensional gel electrophoresis, where proteins were stained with Coomassie Blue and glycoproteins stained with Periodic acid-Schiff (PAS) confirming the position of gCMP on the gels. A shift in pI was observed after desialidation of gCMP indicating that gCMP with sialic acid in the glycan moieties were located at pI 3 approx. and gCMP without sialic acid were located at pI 3.8. Previous studies have shown that pI decreases 0.07-0.31 for each sialic acid molecule cleaved. The CMP isoforms were on the gels shown as trimers and up to three glycosylations were observed on each CMP. High-pressure liquid chromatography (HPLC) and mass-spectrometry based molecular characterization was used for further analysis of the monosaccharide, disaccharide, trisaccharide and tetrasaccharide chains on CMP. In the present study, all gCMP isoforms detected in the untreated gCMP solutions contained sialic acid. The content of sialic acid containing gCMP relative to the total protein content were 37.3 % in the untreated solutions, whereas the content after sialidase treatment were decreased to 1.5 %. CMP variant A containing NeuAcα(2-3)Galβ(1-3)[NeuAcα(2-6)]GalNAc were identified in three peaks with distinct retention times and CMP variant A containing Galβ(1-3)GalNAc in four peaks on the LC chromatogram, indicating 3-4 different sites of glycosylation and thus different hydrophobicity and retention times.

9) **Accurate Monitoring of Living and Total Bacterial Populations in Milk to Predict Cheese Defects**

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Milk and cheese are microbial ecosystems that can harbor diverse bacterial communities and therefore are generally vulnerable to spoilage and other defects. Cheese defects constitute a considerable burden to the dairy industry, among which slits are one of the most important and common concerns for Cheddar cheese. To advance the goal of producing cheese free of slit defects, we employed high-throughput DNA sequencing targeting bacterial 16S rRNA genes to understand how the bacterial composition of raw and pasteurized milk affects the cheese quality. Included in our DNA sequencing approach is the capacity to distinguish between living and total bacteria by the application of propidium monoazide (PMA) on intact cells. Results from our sampling plan spanning July 2015 to March 2016 showed that the bacterial communities of raw and pasteurized milk varied depending on the collection date and underwent significant alterations at each heating and mixing step during processing, especially among the viable portion of the microbiota. Examination of Cheddar cheese showed a steady decline in alpha diversity of low-abundance bacterial taxa during the 120-day aging period. Proportional changes of these non-starter bacteria were also observed such that Streptococcus and Pseudomonas proportions decreased and the relative abundance of Lactobacillus and Bacillus increased towards the end of aging.

Comparisons between matched milk and cheese samples indicated cheese defects could be associated with the bacterial community composition in pasteurized milk. Cheese that contained slits was made from milk harboring fewer contaminating bacterial cells but a larger number of bacterial species compared to milk that resulted in high-quality cheese. The presence of Bacillus, Brevibacillus, Corynebacterium, Lactobacillus and Turicibacter in pasteurized milk was also correlated with slit development in cheese. Moreover, the abundance of these genera in milk were highly dependent on equipment-cleaning schedules and their proportions were reduced over 3-fold immediately after cleaning. These initial findings have led to new efforts resulting in the selective isolation of thermoduric, putative spoilage bacteria from milk and cheese for validation testing. Overall, our findings show that the slit defects might be due to certain thermoduric bacterial strains or consortia that survive milk pasteurization (HTST) in low abundance but persist and remain metabolically active during cheese fermentation. This work contributes to our understanding of bacterial population dynamics in dairy processing facilities and provides opportunities to modify cleaning and processing protocols to ensure the consistent and reproducible production of high-quality dairy products with optimal sensory and shelf-life characteristics.

10) **B. Infantis Metabolites Protect Human Intestinal Epithelial Cells from Pathogen Induced Inflammation**

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Introduction: It is increasingly clear that breastmilk provides critically important human milk oligosaccharides (HMOs), which are fundamental to the establishment of the infant microbiome. These prebiotics provide a selective carbon source for commensals, particularly Bifidobacterium infantis, to colonize at high abundance in the infant gut and produce greater concentration of acetate and lactate, which alter the gut biochemistry and significantly reduce the burden of potentially pathogenic bacteria. However, little is known about the

anti-inflammatory effects B. infantisderived metabolites have on intestinal epithelial cells and how they might protect and maintain mucosal integrity in the infant gut.

Objective: To investigate whether metabolites from B. infantis grown on HMO and synthetic HMOs can provide protective effects against pathogen-induced inflammation and maintain mucosal integrity compared to other commensal strains that have been previously characterized.

Methods: Bifidobacterium infantis, breve, bifidum, and longum were grown in media containing pooled HMOs, synthetic Lacto-N-neotetraose (LNnT), or fructo-oligosaccaride (FOS) a readily available oligosaccharide commonly used in baby formulas. Four Lactobacillus plantarum strains were also grown in HMO, LNnT, and FOS media. Spent supernatant was collected and filtered after 48 hours of growth when the bacteria reached the stationary phase and evaluated for the remaining oligosaccharide concentration. Human intestinal epithelial cells (IECs; HT-29) were grown to confluency in 96-well plates and exposed to cell medium containing 15% of spent bacterial supernatant for 1 hour at 37°C before media was removed and IECs monolayers were challenged with media containing lipopolysaccharide (LPS) from E. coli O111:B4. After overnight incubation cell supernatant was analyzed by ELISA for reductions in proinflammatory cytokines, including IL-8 and TNF-alpha.

Results: First, growth curves indicated that B.infantis had a selective growth advantage when grown in HMOs compared to other Bifidobacterium and Lactobacillus strains. These data further showed that multiple strains of Bifidobacterium and Lactobacillus grew very well using FOS as a carbon source. Furthermore, HPLC data confirmed that B. infantis growth advantage was due to its ability to utilize HMOs as a carbon source since very low concentrations of pooled or synthetic HMOs could be measured in the spent supernatant. Conversely, high concentrations of pooled and synthetic HMOs remained in the other strains of Bifidobacterium and Lactobacillus strains, which confirms HMOs provide selective growth for B. infantis. All strains tested were able to readily use FOS as a carbon source. Furthermore, IECs exposed to spent supernatant from B. infantis grown on pooled or synthetic HMOs or FOS for 1 hour prior to pathogenic bacterial challenge significantly reduced proinflammatory response compared to medium alone (P=0.015,0.01, and 0.0005, respectively). Moreover, this protective effect was unique to B. infantis compared to other Bifidobacterium and specific Lactobacillus strains used in the study.

Conclusion: We have previously shown that B. infantis supplementation can result in substantial and persistent remodeling of breastfed infants' gut microbiome, which significantly altered gut biochemistry, decreased levels of potentially pathogenic bacteria up to 79%, and provided a 4-fold decrease in fecal endotoxin level. The data provided here confirm and extend our previous findings by providing the first evidence that metabolites produced by B. infantis deliver direct protective effects against pathogen-induced inflammation in the intestinal mucosa that is unique to this strain of bacteria.

11) Improving LC-MS Based B-vitamin Analysis in Human Milk

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Background: Severe matrix effects have been reported when analyzing B-vitamins in human milk using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Lactose, the main milk carbohydrate affects the analysis by rapidly fouling the detector and decreasing method robustness and sensitivity. To-date, none of the LC-MS methods described for human milk B-vitamin analysis have addressed lactose removal to reduce or avoid the impeding lactose effects.

Objective: To examine sample preparation techniques and LC-effluent diversion as methods to remove lactose prior to the detector inlet.

Methods: Solid-phase extraction (SPE; OASIS® HLB) employing a stepwise elution protocol (ammonium formate/acetonitrile (ACN), and acetic acid/ACN); liquid-solid extraction (LSE; acetone, ACN, ethanol, ethyl-acetate, 2-propanol (IPA), methanol (MeOH)) as an additional clean-up step after protein precipitation; and protein precipitation (PPT; acetone, MeOH, IPA) were tested on human milk for their lactose removal capabilities. Also, the possibility of chromatographic separation of lactose from target vitamins to enable in-line/post-column lactose removal was evaluated. Besides human milk, an aqueous B-vitamin standard (100ng/mL) containing thiamin, riboflavin, flavin adenine dinucleotide (FAD), nicotinamide (NAM), and pyridoxal (PL) and a separate aqueous lactose solution (5%) were prepared for preliminary tests.

Results: SPE protocols could not isolate lactose from the B-vitamins. Only 2-propanol removed lactose (>90%) using LSE, but vitamin recoveries in milk under the same conditions were also low (14-64%). The choice of PPT-solvent affected recoveries of FAD, NAM, and PL, but not thiamin and riboflavin, with best results for all vitamins when using MeOH; lactose, however, was unaffected. Chromatographic separation of lactose and the tested B-vitamins was achieved using a 4-minute reversed-phase gradient of aqueous 10mM ammonium formate and ACN on a Waters ACQUITY UPLC HSS T3 column (2.1 x 100mm, 1.8µm). The lactose was efficiently removed by flow diversion using an in-line/post-column two-position switch valve allowing lactose-free detection of the water-soluble vitamins. Matrix effects (ME), process efficiency (PE) and recovery were substantially improved; ME was reduced 6x and PE up to 12x compared to our previous report. Additional B-vitamins (pantothenic acid, biotin and pyridoxine) were quantifiable with recoveries for all B-vitamins between 81.9-118.6%, CV≤11.9%, and without sensitivity impairment or analyte degradation in large (n>120) sample sets.

Conclusion: LC-MS-based analysis has been recognized as the platform of choice in for the simultaneous analysis of water-soluble vitamins in human milk. However, the often superior sensitivity, selectivity, and specificity can be challenged by severe matrix interferences, especially when co-eluting with the target analytes. Routine sample preparation techniques may be unsuitable for efficient removal of matrix components with similar chemical and physical properties, while extensive and time consuming sample preparation may succeed in reducing or eliminating matrix interferences but might also cause analyte degradation. Optimization of the chromatographic conditions coupled with LC-flow diversion allowed simple and efficient removal of harmful lactose and possible other polar matrix interferences from the human milk sample extract while maintaining detector-integrity, allowing for enhanced analysis of human milk B-vitamins.

12) **Reference Values for Human Milk Micronutrient Composition: Gaps and Opportunities**

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The World Health Organization (WHO) recommends human milk as the sole food source for the first six months of life, and it remains an important nutrient source for infants 7-12 months of age. Therefore, its nutritional "quality" is vital for the infant's healthy growth and development, which can be challenging especially in resource-poor settings and were maternal dietary quality is poor.

Despite the importance of human milk, valid data regarding its micronutrient composition is sparse and true reference values are non-existent. Due to the lack of reliable data for the infant's biological response to different levels of nutrient intake, Estimated Average Requirements (EARs) cannot be established. Instead, these recommendations are set as Adequate Intakes (AIs), which are based on reports of mean micronutrient concentrations measured in milk from well-nourished mothers during the first 6 months of lactation and an average milk intake of 0.78L per day. The same measurements are used and extrapolated to set AIs for infants 7-12 months. These data were usually obtained from limited and outdated studies with little to no information regarding the validity of the method applied for analyzing micronutrient content. Additional challenges include inconsistencies in milk collection protocols, stage of lactation, and missing information on the effects of maternal supplementation or environmental factors.

When comparing milk vitamin concentrations to the AIs it appears that human milk in many countries is not adequately supplying the exclusively breastfeeding infant with micronutrients. Even milk obtained from well-nourished mothers in developed countries fails to meet the AI on a regular basis, e.g., vitamin B1 median concentrations in milk from US-American donors only reached 65% of its AI, while vitamin B6 average concentration in milk from Japanese women met only 45% of its AI. Due to the uncertainties of the AI it remains unknown whether or not these milk concentrations are indeed insufficient. Given that very low milk micronutrient concentrations have been shown to cause adverse effects on infant health and development such as growth faltering and micronutrient deficiencies, the importance and need for accurate reference values is undeniable.

While the current AIs are set to constant values, human milk micronutrient concentrations undergo dynamic changes throughout the course of lactation. Thus, nutrients such as trace elements with high concentrations in the first 2 weeks but a rapid decrease thereafter may in fact sufficiently supply the infant needs at each stage of lactation but may be evaluated as inadequate based on the average AI.

These shortcomings of the AI have been recognized by the Institute of Medicine: "the issuance of an AI indicates that more research is needed to determine with some degree of confidence the mean and distribution of requirements for a specific nutrient". The Mothers, Infants, and Lactation Quality (MILQ) Project is a multi-center collaborative study aiming to fill these gaps and to create dynamic reference values for milk nutrient composition from well-nourished, but unsupplemented mothers. Data obtained from milk as well as evaluation of mother and infant status in four countries will enable the creation of reference ranges expressed as percentiles which, will serve as a true benchmark against which status, intervention, and fortification strategies can be evaluated.

13) Lactase Persistence Genetics and Ethnicity Influence Milk Consumption

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Lactase persistence is an autosomal dominant trait in which lactose can be digested through adulthood. Lactase non-persistence can cause lactose intolerance which may influence dairy intake. There are several lactase persistence mutations which occur at varying frequencies in different populations. One of these mutations, rs4988235, is a SNP approximately 14 kb upstream of LCT predominantly found in people of

European descent in which the A allele is associated with lactase persistence and the G allele with non-persistence. To undestand how lactase persistence genetics for rs4988235 influences dairy consumption, we collected food frequency (Block Food Frequency Questionnaire) and genotype data from healthy adults (n=144). Since lactase persistence varies among different populations and diet is related to culture, we also accounted for subjects' ethnic background (Caucasian (n=94), African American (n=10), Hispanic (n=22), and Asian (n=18)). 61% of subjects had lactase persistence genotypes (AA (26%) or AG (35%)). All three genotypes (AA, AG, and GG) were represented in Caucasian and African American subjects. Only the AG and GG genotypes were represented in Hispanic subjects and all Asian subjects were GG. Accordingly, the proportion of subjects with the AA genotype significantly differed between Caucasians and Hispanics (Fisher's Exact Test, Benjamini-Hochberg adjusted (FDR) p = 0.001) and Asians (FDR p = 0.002) as well as the AG genotype between Caucasians and Asians (FDR p = 0.001). No significant differences were observed when comparing to African Americans, although this group had a relatively small number of subjects which may have affected our power. The proportion of subjects with the lactase non-persistence genotype, GG, significantly differed among all ethnic groups. Total estimated dairy intake (cups per day) did not significantly differ among genotypes or ethnicities. While all subjects reported consuming cheese, only 74% of subjects reported consuming cow's milk (i.e. reported intake > 0 cups per day); all of the non-consumers of cow's milk reported drinking an alternative 'milk' instead (soy, almond, or rice). Although the proportion of cow's milk consumers compared to non-consumers did not significantly differ by genotype, the reported servings (cup equivalents) by the GG subjects was significantly lower than that of the AA subjects for those reporting milk intake (Tukey adjusted p = 0.04). Additionally, the reported intake of cheese was significantly higher for African Americans compared to Asians (Tukey adjusted p = 0.031), but there were no further significant differences between other ethnicities. Interestingly, we observed that the mean servings of total dairy and total cheese were lower for the GG subjects compared to the AA or AG subjects although these trends failed to reach statistical significance. Our main goal was to identify how dairy intake relates to genotypes, but since total caloric intake covaries with dairy intake, we also adjusted subjects' dairy intake to total estimated caloric intake (cup equivalents/1000 kcal). Milk intake still significantly differed between the AA and GG subjects but cheese intake no longer differed significantly by ethnicity. We plan to integrate these data with markers of health, such as gut microbial data and metabolites, markers of inflammation, and body composition in future studies to understand how reported dairy intake and genetics relate to health.

14) Infant-Marketed Probiotic Dietary Supplements Differ Substantially from Label Claims

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Background: In the United States probiotics are considered dietary supplements. Thus, probiotic products are not required to undergo FDA approval before marketing and proper quality control requires advanced molecular analyses for proper identification. Many products are branded and sold separately from the manufacturer without proper quality control to ensure label claims are met by ingredient manufacturers. Previous studies (e.g. Lewis et al 2016; Marcobal et al 2008) have shown that the Bifidobacterium strain composition of several commercial probiotic products does not correspond to the product label information. As safety and functionality of probiotics are species, subspecies, and strain-dependent, the objective of this study was to evaluate the label compliance in terms of strain identity and viable cells counts in commercial probiotic products marketed for infants claiming to contain Bifidobacterium.

Methods: A total of 31 probiotic products were evaluated using culture-dependent and molecular approaches. Viable cell counts were determined by quantitative culture on BSIM agar. The identity of 10 randomly selected colonies per product was determined via Sanger sequencing of PCR amplicons of the species-variable internal transcribed spacer region. Using DNA extracted from each product, presence and abundance of five species of Bifidobacterium were determined by quantitative PCR.

Results: Of the products evaluated 11 were capsules, 2 were liquid, 17 were powder, and 1 was an infant formula. Seven products contained a single Bifidobacterium while the majority (25) were multi-strain, with one product only divulging phylogeny to the genus level. Only 22% of the products had the label-claimed bacterial counts and one product failed to list a bacterial count. In 3 of the products tested, only one species was isolated and it was not one named on the product label. Twenty-two products failed to meet live label claims and three with respect specifically to the species of bifidobacteria and in many cases, the specified strains were not detected via culture or PCR.

Conclusions: Our results demonstrate that most infant-marketed probiotic dietary supplements are not accurately represented by label claims and confirm that poor microbiological quality and labeling discrepancies are a common issue in of Bifidobacterium-containing probiotics sold as dietary supplements. Caution should be exercised when selecting probiotic products for clinical applications, or clinical trials using these commercial products, when they are marketed as dietary supplements.

15) **GenCoF: A Graphical User Interface to Rapidly Remove Human Genome Sequence Contamination from Metagenomic Datasets.**

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Improvements in sequencing technologies within the past decade have decreased the cost of shotgun metagenomic sequencing tremendously. Consequently, increased access to such applications has resulted in greater interested across research disciplines to use shotgun metagenomic sequencing data. Although shotgun metagenomic studies are often designed to characterize microbial communities, library preparation methods often fail to eliminate contaminating human DNA, resulting in anywhere from 10 to 50% of total sequencing reads to be human derived. Greater incentives to publish raw sequencing libraries thus results in the publication of unintentionally collected human DNA sequences – whether from subjects in clinical trials without their consent or from technicians during library preparation. Consequently, computational approaches could be employed to identify these individuals or subjects from publicly deposited datasets. Therefore, the first essential step in metagenomics data analysis is the complete removal of host-related sequencing reads prior to analysis or public deposition. In the last few years, different bioinformatics techniques have been applied in metagenomic studies to completely remove human sequences, particularly in clinical trial datasets, and several tools are currently available to perform this task. However, the majority of freely available tools require advanced programming knowledge or present limitations in terms of analysis time and data load due to their server-based nature. In this study, we first compared the most widely cited tools and pipelines for human genomic DNA filtering using both a synthetic and a real metagenomics dataset. Robustness of comparative analysis was assessed with BLASTn due to its superior accuracy of sequence alignment. Finally, we integrated the most efficient pipeline in a graphical user interface made intuitively accessible to the average user, with the intent for broad adoption and implementation of steps to reduce human genomic DNA contamination and reduce inadvertent collection of clinical trial subject information without subject consent.

We present GenCoF, a graphical user interface to rapidly remove human genome contaminants from metagenomic datasets. The application is available as an executable in Unix and Mac OS environments with self-installation without prior knowledge of command line use. In addition to human DNA sequence removal, GenCoF offers the possibility to quality filter sequencing reads and split datasets in smaller sizes to increase manageability of data. Moreover, it allows the interactive modification of any parameter in order to customize the analysis based on user needs. GenCoF is the first non-commercial and freely available tool offering an interactive and easy-to-use interface to filter metagenomics sequencing reads locally for both quality and host associated components. The application is freely available under GPL license at https://github.com/MattCzajkowski/GenCoF.

16) **Sialylated Milk Oligosaccharides Alter Neurotransmitters and Brain Metabolites Optimizing Neurodevelopment in Piglets: An In vivo Magnetic Resonance Spectroscopic (MRS) study**

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Objective: To assess the impact of different sialylated milk oligosaccharides (SMOs) interventions focused on their effect on brain development, brain metabolism and neurotransmitters in piglet.

Methods: Three day-old piglets were randomly allocated to one of three groups and fed either standard sow milk replacer (SMR) alone (n=15), SMR supplemented with sialyllactose (SL), (n=15) or a combination of SL and 6'-sialyllactosamine (SL/SLA, n=16) for 35 days. Brain spectra were acquired in the piglets using a 3T Magnetic Resonance Spectroscopic (MRS) system with a 15-channel human knee radio frequency (RF) coil and a spin echo Point RESolved spectroscopy (PRESS) sequence at short and long echo times (TE = 35 ms, and 270 ms).

Results: SMOs fed piglets were observed to have significantly increased the absolute levels of myo-inositol (Ins) and glutamate+glutamine (Glx). Similar findings were found in the relative amount of these metabolites calculated as ratios to creatine (Cr), choline (Cho) and Nacetylaspartate (NAA) respectively ($P<0.05$). In addition, there were significant positive correlations of frontal lobe Ins, glutathione (Glth), NAA, total NAA, total Cho (TCho), scyllo-Inositol (SI) and total Cr (TCr) with total white matter volume ($P < 0.01$).

Conclusion: In this study, we provide in vivo evidence that milk SMOs can alter many important brain metabolites and neurotransmitters required for optimising neurodevelopment in piglets, an animal model of human infants.

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17) **Dynamic Human Milk Proteolytic System Releases Specific Peptides From α-lactalbumin in Response to pH**

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The use of human milk products is increasing for high-risk infants. Although nutrition of newborn infants has advanced in recent years, meeting the demands of infants born extremely prematurely and those born with very low birth weight is still challenging. New recommendations focus mainly on fortifying human milk with various nutrients; however, the dynamics of human milk and the diversity of gastrointestinal environments have been neglected. Human milk is a dynamic protein-protease system that delivers bioactive peptides to infants. The pH of milk changes from the mother's mammary gland to the infant's digestive tract and varies based on individual conditions. Although the release of human milk peptides has been studied during in vivo or in vitro digestion, these models did not explicitly vary nor observe the effect of pH. The objective of this study was to determine how pH affects the proteolysis of human milk. Fresh human milk samples were incubated at various levels of physiologically relevant pH, and peptides were mapped using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Results showed that the proteolysis of α-lactalbumin in human is pH-dependent. Specifically, α-lactalbumin derived peptides were only found in samples incubated below pH 5. Furthermore, 2-hour postprandial gastric samples from infants were aspirated, and the peptidomic analysis validated the release of peptides from α-lactalbumin in vivo. Identifying how human milk proteins are changed and what peptides are released upon environmental change is important for understanding the complex biological functions of human milk and guiding innovations towards precision design of infant feeding.

18) **The Survival of β-casomorphin-7 Through Gastrointestinal Digestion with Human Enzymes and Porcine Brush Border Membrane**

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The heterogeneity of milk proteins has been connected to both functional properties of milk and effects on human health. The potential nutritional and biological differences between A1 and A2 milk is still much debated. The native sequence of bovine β-casein (β-CN) encrypts the opioid peptide β-casomorphin-7 (BCM7), which is released from both A1 and A2 β-CN during gastrointestinal (GI) digestion. This study aimed to quantify the amount of released BCM7 after ex vivo digestion of A1 and A2 milk and to study the peptide digestion stability to human GI proteases and jejunal brush border membrane (BBM) peptidases.

Bovine milk initially tested homozygous for β-CN variants A1 and A2 was digested according to Minekus et al. (2014) with human GI enzymes. The digests were analysed by LC-ESI-Ion Trap-MS/MS targeting BCM7. Furthermore, a specific multiple reaction monitoring (MRM) method was developed to quantify BCM7. Using this approach, BCM7 was detected and quantified in GI digests from both A1 and A2 milk. However, higher amounts of the peptide were observed after A1 milk digestion. Based on these observations, synthetic BCM7 was subjected to in vitro digestion by GI fluids obtained from human volunteers and downstream degradation with porcine BBM vesicles. BCM7 was sampled at eight time points over 24h after BBM addition. The digests were profiled by either LC-ESI-Q-Orbitrap to monitor BCM7 through digestion or HPLC to quantify unhydrolysed BCM7.

The results showed that small amounts (5%) of BCM7 did survive degradation by BBM up to 24h. The HPLC analysis revealed that 58% of the initial peptide endured 2h BBM digestion, while 21% survived 4h digestion even after supplementation of BBM. LC-ESI-Q-Orbitrap analyses confirmed the presence of intact BCM7 after digestion with human GI enzymes and BBM. To conclude, the opioid peptide BCM7 is released from both A1 and A2 milk and survives GI digestion by human enzymes, substantial amounts of intact BCM7 was also identified after 4h porcine BBM incubation. Further studies should aim to evaluate the ability of BCM7 to translocate across the intestinal epithelium and, eventually, its occurrence in blood following a milk-based meal.

19) Rapid, Low-Cost Identification and Sequencing of Bacterial Genomes Using the MinION Platform

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A decade of DNA sequencing technology advancements has introduced and rapidly improved upon first generation high throughput parallelized genome sequencing technologies (e.g. Roche 454, Solexa). Now, even further advancements in these capacities continue to expand sequence read length, depth of sequencing, and the speed of data acquisition (e.g. Illumina HiSeq, NovaSeq, MiSeq or PacBio). Astonishing decreases to cost of sequencing have also placed these abilities into the hands of a broad, multidisciplinary spectrum of researchers and now allow for rapid and even field-based sequencing approaches.

To evaluate the feasibility of using a novel generation of handheld, long-read DNA sequencing technologies, a workflow for the MinION sequencing platform from Oxford Nanopore Technologies (ONT) was developed for identification and genome sequencing of bacterial isolates. DNA extraction procedures were compared for sequencing, and manufacturer designed protocols were used to test the platform. Library preparation and run times were sufficient to allow same-day genome sequences. Sequencing of a single multiplexed library allowed for sufficient sequencing depth for complete bacterial genomes in a matter of hours in a cost-effective manner. Up to twelve bacterial genomes could be completed (closed) from the sequencing results produced in a single run of a multiplexed library. Cost feasibility was also evaluated.

The ONT software provided with the platform enabled real-time identification of reads from multiplexed sequencing libraries, limited functional prediction, and the application to rapid identification of unknown organisms. The MinION platform (and associated "Pore"-based technologies, e.g. PromethION) may represent a fast, cost-effective approach to rapid screening and identification of bacteria or other microorganisms in a wide variety of dairy, gut, or probiotic applications.

20) Perilipin 2 Dissociates Cytoplasmic Lipid Droplets from The Endoplasmic Reticulum During Milk Fat Secretion

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Perilipin 2 (Plin2, alt. ADRP or ADPH) is highly abundant in the lactating mammary gland, and since it is concentrated on the milk fat globules, it has long been thought to play a role in their secretion. To test its role, we use the following knockout mouse models: (1) a full-body knockout of Plin2 (Plin2null) and (2) a tissue specific knockout that utilizes Cre-recombinase to remove Plin2 specifically in the milk-secreting cells (Plin2 MGKO). Surprisingly, we saw no differences in the growth of a standardized litter of pups nursed on the three dams, and we saw no significant differences between the volume percentage of fat between the three groups: WT (38% +/-5), MGKO (39% +/- 5), Plin2null (33%+/-5). To verify the deletion of Plin2, we immunostained paraffin sections from lactating mammary gland of each line. As expected, we saw no Plin2 reactivity in the Plin2null glands, but increased abundance of perilipin-3 (Plin3), which appeared to be associated with cytoplasmic lipid droplets. We floated and washed milk fat globules and performed proteomic analyses on the protein fraction. We used functional annotation clustering using DAVID 6.8 and String Analysis to show enrichment in two specific categories; The largest node included proteins related to protein folding in the Endoplasmic Reticulum (ER), and the second node was a cluster of ribosomal proteins. Electron microscopy images of lactating mammary epithelial cells from Plin2null glands show an increased association of rough ER with cytoplasmic lipid droplets. Additionally, analysis of the docking complex, Btn:XOR:CideA, showed that docking of the CLD to the apical plasma membrane was partially disrupted by the loss of Plin2. From these data, we conclude that the function of Plin2 in the lactating mammary epithelial cells is to dissociate cytoplasmic lipid droplets from the ER during synthesis and secretion.

21) Protein Digestibility and Peptidomic Profiling of Whey Protein Solutions in Water and Cranberry Juice as Affected by Long Thermal Processing During Simulated Digestion

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The increasing demand for functional foods with high nutritional value has led to the development of beverages containing fruit or vegetable juices and protein. In these beverages, proteins might interact with components of the juice matrix, leading to nutritional and functional product changes. The objective of this study was to understand the possible interactions between proteins, juice polyphenols and their behavior after processing, before and during simulated gastrointestinal digestion.

Whey protein (27 or 54 mg/mL) was dissolved in either cranberry juice or water and used as model systems. Beverages were either non-processed or underwent thermal (Low: 85°C for 1 min, Medium: 99°C for 10 s and Long: 99°C for 5 min) or high pressure processing (600 MPa for 4 min). After processing, beverages underwent oral (30s), gastric (2h) and small intestinal (2h) digestion. During in vitro digestion, protein hydrolysis was monitored by the O-phthalaldehyde (OPA) assay and through SDS-PAGE. Degree of hydrolysis was calculated as the ratio of the amino groups from the OPA assay compared to the total amino groups in the sample. Protein digestibility of □-lactoglobulin was calculated by optical densitometry analysis. Differences between means were analyzed in a three-way ANOVA mixed model with repeated measures. Peptidomic profiling was performed by using Orbitrap mass-spectrometry and ion-exchange chromatography was used to quantify the peptides. Bioactive sequences were identified using a milk bioactive peptide database.
Protein degree of hydrolysis was significantly ($p < 0.0001$) influenced by solvent, protein concentration, digestion time, and the interactions of: solvent* digestion time; protein concentration*digestion time; solvent*protein concentration, solvent*protein concentration*digestion time; protein concentration* processing*digestion time*solvent; protein concentration*processing*digestion time ($p=0.0057$).
β-lactoglobulin digestibility was significantly ($p < 0.05$) influenced by the solvent, processing, digestion time,

and the interactions between: solvent*processing; solvent*digestion time; protein concentration* solvent*digestion time; solvent*processing*digestion time; and solvent*protein concentration* processing*digestion time.

Whey protein dissolved in water had a significantly higher degree of hydrolysis and absolute peptide quantification during intestinal digestion. However, the specific influence of solvent on β-lactoglobulin digestibility depended on the severity of the thermal. Water-protein solutions only had higher protein digestibility during the gastric phase after long thermal processing (99°C for 5 min) treatment. Several bioactive sequences exhibiting antihypertensive, anti-microbial and immunomodulatory effects were identified using a milk bioactive peptide database in the juice samples. A few of the peptide sequences were identified to have multiple bioactive functions. This information can be utilized to optimize the processing and formulation of high-protein juice products to increase both the protein digestibility and bioactive peptide profile after digestion.

22) The Cross-reactivity of Milk Secretory IgA with Bifidobacterium Longum Subsp. Infantis and Escherichia Coli O157:H7 in Colonic Cell Models

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Human milk delivers bioactive compounds to the neonate gut which are involved in a variety of beneficial interactions. Among these compounds is Secretory Immunoglobulin A (SIgA) which has been implicated in many regulatory functions for bacterial colonization and pathogen exclusion to promote gut health while reducing inflammation and disease. This study investigated the cross-reactivity of SIgA with Bifidobacterium longum subsp. infantis (BI) and Enterohemorrhagic Escherichia coli O157:H7 (EHEC) and its ability to alter interactions with colonic epithelial cells. Flow cytometry revealed robust and concentration-dependent binding of SIgA to BI and EHEC independently (up to 81% and 87.5% respectively) and a 3.2-fold increase in aggregation of BI and EHEC when co-incubated with SIgA versus untreated bacteria, confirmed through bright-field microscopy. The introduction of SIgA, BI, and EHEC aggregates to colonocytes altered the expression of inflammatory and tight-junction genes compared to bacteria added alone. These results demonstrate the cross-reactivity of SIgA with EHEC and BI, possibly entrapping both in the mucin layer to prevent EHEC from reaching the epithelium while allowing BI to stay in the mucin, contributing to colonization potential. These data, and the ability of SIgA to mediate gene expression of host colonocytes, suggests a possible method for reducing the pathology of enteric infectious diseases.

23) Genome Sequencing of PL/J, QSi3, and QSi5 Identifies Novel Coding and Truncating Variants for Divergent Litter Rearing Capacity

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An enormous effort has gone into the identification of quantitative trait loci for milk production and composition (QTL) in dairy animals. Prior work by our group has used strains from the mouse diversity panel to both identify phenotypic extremes and to map QLT for lactation-related traits. This work was limited by a lack of genetic data in key strains with high and low litter-rearing capacity. The genomes of females from two low lactation strains, the PL/J, and QSi3, and a high lactation strain, the QSi5, were sequenced to 36-, 43-, and 40-fold coverage, respectively. This data generated a catalog of single nucleotide polymorphisms (SNP) and small insertions and deletions (INDEL) and identify private alleles for each of the strains. There were 33,581, 25,236, and 28,553 private SNP in PL/J QSi3, and QSi5, respectively. Of these, there were 221, 200, and 85 that overlapped with and produced non-synonymous or truncating mutations in the coding regions of 113, 100, and 49 genes, respectively. For these coding region SNPs, there were 9, 7, and 2 that produced radical amino acid changes. Pathway analysis identified enrichment for immune-related pathways in QSi3, Pathway analysis of QSi5 were enriched for transport, lipid metabolism, and Wnt signaling, among others. As a second approach the list of genes affected by all mis-sense mutations present in the low lactation strains but not in QSi5, was intersected with differentially expressed genes identified through a comparison of RNA-Seq data from lactating mammary tissue of PL/J and QSi5. In this subset there were 438 differentially expressed genes. Pathway enrichment analysis of this list identified enrichment for L-cysteine degradation, folate transformations I, Nur 77 signaling in lymphocytes, Th1 pathway, and calcium-induced T lymphocyte apoptosis. Further comparisons will be made by combining the lists of novel alleles in additional high and low lactation strains.

24) **Deep RNAseq reveals miRNome Differences in Mammary Tissue of Holstein and Montbéliarde Lactating Cows**

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Genetic polymorphism is known to influence milk production and composition. However, genomic mechanisms involved in the regulation of milk component synthesis and their secretion are not completely understood. MicroRNAs (miRNAs) are small non-coding RNAs that take part in the regulation of gene expression by base-pairing mRNA that induce their degradation or inhibiting their translation. Recently, 54 differentially expressed miRNAs were detected in mammary tissue from dairy (Holstein-Friesian) compared to beef (Limousin) postpubertal heifers, two breeds with different mammogenic potential. The results of this study suggest that the high developmental potential of dairy cattle MG, leading to high milk productivity, may depend also on a specific miRNA expression pattern (Wicik et al., 2016). The objective of the present study was to identify the genetic influence on mammary gland miRNOme by comparing miRNA profiles of two dairy cow breeds (Holstein and Montbéliarde) with different milk performances by RNAseq analyzes.

Milk production and composition of 19 multiparous mid-lactation (165 ± 21 DIM) cows (9 Holstein and 10 Montbéliarde) were analyzed. Total RNAs were extracted from MG biopsies (n=5 Holstein and n=6 Montbéliarde) with miRVana kit. MiRNAs were sequenced by RNAseq using Illumina HiSeq 4000. The sequence reads were mapped and annotated using miRDeep2 after trimming of adaptor sequences allowing the identification of known and predicted miRNAs. Statistical analyses were performed using DESeq2 package of R. Significance was considered at padj≤0.05 and tendency at 0.05<padj≤0.10. Target genes of

studied miRNA and corresponding putative pathways were investigated using miRWalk and MetacoreTM softwares.

Milk, protein and fat yields were lower in Montbéliarde than in Holstein cows as previously reported (Pomiès et al., 2007). Analyses of miRNA profiles (miRNomes) revealed 623 distinct expressed miRNAs, of which 596 known and 27 predicted miRNAs. Among them, 19 miRNAS were significantly differentially expressed and 15 tended to differ between Holstein and Montbéliarde cow. Among the most abundant 20 miRNA in MG tissue, one miRNA was differentially expressed (miR-186) and one in tended to differ (miR-143) between Holstein and Montbéliarde. Among the 19 differential miRNAs, 7 presented a fold change ≥2. The most abundant among the differentially expressed miRNA was miR-186 known to inhibit cell proliferation (Su et al., 2018) and epithelial-to-mesenchymal transition (Li et al., 2018). Data mining showed that the 19 differentially miRNAs may be involved in 19 KEGG pathways (Padj<0.05) including mTOR signaling pathway and Sphingolipid signaling pathway. Interestingly, cytoskeleton remodeling was the first pathway map identified using MetacoreTM analysis, which could be related to differences in the tissue structure of MG between the two studied breeds which could be related to milk production as shown for dairy versus beef breeds (Wicik et al., 2016).

In conclusion, we showed that MG miRNome differs between Holstein and Montbéliarde cows during established lactation. That opens a way of investigation in genetic regulation of miRNA and their role.
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25) Influence of Caprine alphaS1-casein Genotype on Milk Exosomal miRNAs

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In goats, the gene encoding αs1-casein (CSN1S1) exhibits a large and complex polymorphism including a "naturally occurring KO" with serious consequences to mammary epithelial cell (MEC) and milk composition[1,2]. Extracellular vesicles (EVs) identified in breast milk[3] were later detected in the milk of different species including ruminants[4]. These vesicles contain proteins and mRNA, as well as microRNA (miRNA) known to regulate a large number of biological processes. Evidence of their influence on infant or adult consumers were reported[5,6]. We hypothesized that the absence of CSN1S1 expression may influence milk vesicular cargos, including miRNA. Thus, our objectives were: i) to profile miRNA (miRNome) from caprine milk EVs and ii) to evaluate the impact of the CSN1S1 genotype (AA vs. OO) on this profile as well as potential involvement in the dysfunction of MEC and therefore to speculate on the putative effects of these differences on milk consumers.

Milk exosomes and total RNA were obtained as previously described[7]. RNAseq was performed by Exiqon using an Illumina NextSeq500. Raw data were filtered, mapped on Bos taurus genome (UMD3.1) and

miRBase v20 was used. Differential analyses and multiple testing correction[8] were performed. Bioinformatic analyses were performed using MetacoreTM and Diana MiRPath. In average 7.5 million reads were obtained leading to identify 247 miRNA with ≥ 1 tags per million. A comparison of the most abundant 20 miRNAs between human milk exosomes[9] and the present study revealed 10 miRNA in common.

The comparison of exosomal miRNomes of goat homozygous for strong (A) and null (O) alleles at the CSN1S1 locus, pointed out 15 differentially abundant miRNAs (Padj≤0.05). The integration of potential targeted mRNAs of these 15 miRNAs with the differentially expressed genes at mRNA level (DEG-mRNA) (Bevilacqua et al., in preparation) in the MEC isolated by laser microdissection from the same samples, identified cytoskeleton remodeling as the first pathway map modified. This pathway could reflect the observed differences in +mammary tissue morphology. Another common pathway between mRNA targeted by differentially abundant miRNAs and DEG-mRNA was the apoptosis and endoplasmic reticulum stress response pathway which are linked to the endoplasmic reticulum dilatation observed in MEC of goats of CSN1S1OO genotype. To evaluate a potential effect of the differentially abundant miRNAs between AA and OO genotype, on infant and caprine offspring consuming goat milk, we considered miRNAs exhibiting a fold change>1.5 and with more than 50 counts. Among the most abundant 20 process networks potentially altered, 5 are involved in development. The effects of such networks on infant and caprine offspring consuming goat milk have not been still considered.

In conclusion, differentially abundant miRNAs according to the CSN1S1 genotype in goats were identified in milk exosomes. These differences could be related to the MEC phenotype, already observed[2].

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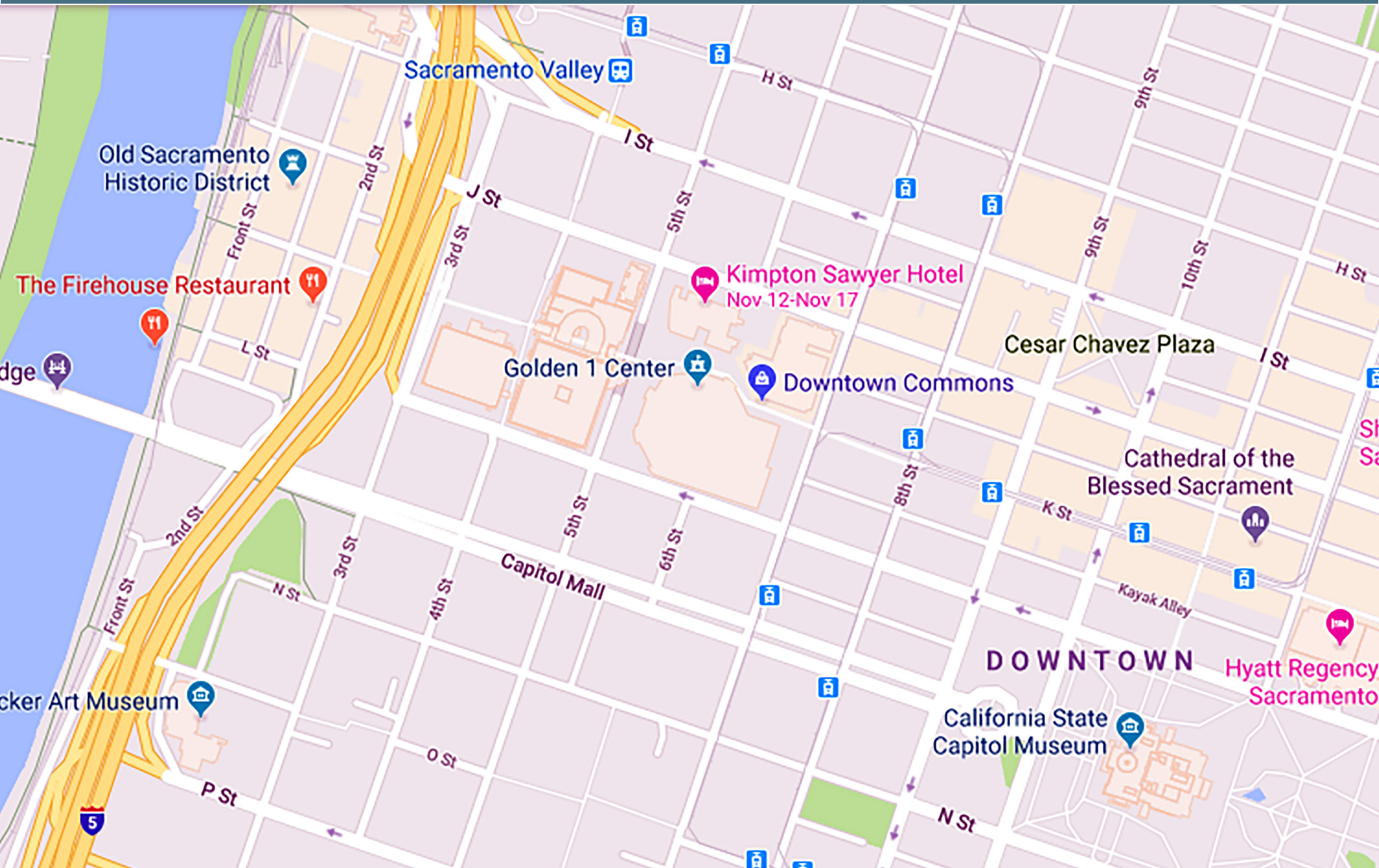
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