Abstracts

Milk Genomics – Where It All Starts…

Genomics and RuminOmics

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A collaborative, large-scale integrating project named 'RuminOmics' (project no. 289319) was commissioned in 2012 under the EC's Seventh Framework Programme: Food, Agriculture, Fisheries and Biotechnology. Its title is Ruminomics: *Connecting the animal genome, gastrointestinal microbiomes and nutrition to improve digestion efficiency and the environmental impacts of ruminant livestock production*. The RuminOmics project is exploring genomics, metagenomics and metaproteomic technologies to try to understand how the rumen microbial community influences feed efficiency and particularly methane emissions. The large-scale nature of the project means that the sampling phase of the main experiment, involving 1000 dairy cattle, is not yet complete. Smaller demonstrations of host-microbiome involve digesta exchange between reindeer and dairy cows, whereby the ruminal contents of reindeer are removed and replaced by the digesta from cows. The microbial community adapted at different rates, with protozoa maintaining the profile of the donor and bacteria moving towards the profile of the recipient.

Parallel experiments carried out with SRUC in Edinburgh using beef cattle have highlighted the importance of the rumen archaeal community for methane emissions. Both genomic and metagenomic profiles show a link between the abundance of archaeal genes and methane emissions, in contrast to some other published studies. Four pairs of beef cattle were selected on the basis of extreme high and low methane emissions from 72 animals, matched for breed (Aberdeen-Angus or Limousin cross) and diet (high or medium concentrate), for deep-sequencing of their ruminal genomic DNA. High emitters produced 1.88× more methane than low emitters per kg DM intake. 16S rRNA gene abundances indicated that archaea, predominantly *Methanobrevibacter*, were 2.5× more numerous ($P = 0.026$) in high emitters, whereas, among bacteria, Proteobacteria were 4-fold less abundant (2.7 vs. 11.2% of bacteria; $P = 0.002$). The dominant family among Proteobacteria was Succinivibrionaceae. *Megasphaera* were 12-fold less abundant ($P = 0.006$) in high emitters, while *Desulfovibrio* were twice as numerous ($P = 0.001$). KEGG analysis revealed that the abundance of 21 of the most numerous (>0.1%) genes differed significantly ($P < 0.05$) when the pairs of steers were compared. Eight of the nine most significantly ($P \leq 0.026$) differing gene abundances were associated with methane metabolism. Thus, metagenomic analysis of the ruminal microbiota confirms that the abundance of archaea is key in determining methane emissions.

Bovine Genomic Selection Today - Possibilities to Improve Novel Milk Traits through Genomic Selection

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The genetic trend in major dairy cattle breeds have been driven by large progeny testing schemes. Sires of sons were exclusive daughter proven bulls and a very large proportion of sold doses were from this category of bulls. In 2008 54K SNP panels became available for the AI-industry and a large number of daughter proven bulls were genotyped. The blended index, combining pedigree and genomic information, had a considerable higher reliability than the pedigree index and the situation is today very different. 2014 a great majority of the highest ranked bulls are young bulls without progeny proofs. The sires of sons are predominantly young bulls and up to 90% of the sold semen is from this category of bulls.

The breeding goal in all major dairy cattle population is a Total Merit Index. Traits included in the TMI are functional, production and conformation traits and the traits included differ between countries. All traits in the TMI exhibit a genetic variation, are economical important and are recorded on a large number of daughters. Novel milk traits such as fatty acid composition, protein composition and process ability have in general a high genetic variation. But, they are difficult to record in a large scale. However, IR-technique can be an inexpensive solution for receiving indirect measurements for these new traits. Genomics selection tools can be applied to these novel milk traits and depending on the inheritance of the trait either the candidate gene or the whole genome approach can be applied.

Epitope Mapping of αS1-Casein by Microarray Immunoassay Reveals Differences in Ige Binding within Dairy Breeds and Between Water Buffaloes, Sheep, Goats

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The caseins belong to the major allergens in cow milk. Within these proteins, a noticeable genetic variation has been identified in productive and endangered cattle breeds. The genetic variants are characterized by amino acid exchanges or deletions of peptide fragments. Their importance in human nutrition, especially regarding to the allergenic potential, has not yet been adequately exploited. There is an increasing focus on the use of milk proteins from other species to identify an alternative protein sources for patients with cow milk allergy. Nevertheless, due to the high degree of amino acid sequence homology between the proteins from cow and other ruminant species, clinical cross-reactivity exists. This study investigated the influence of the genetic polymorphisms on the IgE binding properties of epitopes from the bovine αs1-casein variants A, B, C, D, E, F, G, H, and I. In addition, differences in IgE binding between epitopes of cow, sheep, goat, water buffalo, and camel were determined. On the basis of the IgE binding epitopes previously identified for αs1-casein of cow and of the corresponding peptides from goat, sheep, water buffalo, and camel, a set of 57 peptides was commercially synthesized and tested by means of microarray immunoassay for IgE binding by using sera from humans with cow milk allergy.

In the 5 αs1-casein variants A, B, C, E, and I, the amino acid substitutions and deletion affected the immunoreactivity of 5 epitopes leading to an abrogation or increase or decrease of IgE binding. Modifications in the immunoreactivity mainly concerned immunodominant epitopes and, in consequence, the allergenicity of the whole proteins is altered.

The majority of sera showed IgE binding to αs1-casein peptides of cow and the homologous counterparts of sheep, goat, as well as water buffalo, whereas peptides of camel were barely recognized. However, in most sera, epitopes from the non-bovine species displayed lower immunoreactivities compared with those from cow. Moreover, IgE antibodies of individual sera reacted only with peptides of sheep or goat or water buffalo, or both, but not with the corresponding peptides of cow, even when the peptides were highly similar.

The results of this study demonstrated that genetic polymorphisms of αs1-casein influence the allergenic potential of IgE binding epitopes. Therefore, genetic variants of milk proteins should be taken into account into the search for a suitable protein source for patients with cow milk allergy. Furthermore, it was confirmed that milk from sheep, goat, and water buffalo harbor an allergenic potential due to cross-reactivity with αs1-casein peptides from cow and, consequently, milk from these species are not an appropriate replacement for cow milk in the nutrition of allergic subjects. On the other hand camel milk might be a promising protein source for patients with cow milk allergy.

Genetic Analysis of Micro- and Macro-elements in Danish Holstein and Danish Jersey Milk

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Minerals in the milk are important to milk technological properties like casein micelle stability, and to the nutritional value of milk. Minerals of milk origin are considered to have a high availability to humans and animals as compared to most other food mineral sources. In this study we try to find the genetic components underlying the mineral content in the milk based on 400 Danish Holstein (DH) and 400 Danish Jersey (DJ) cows in mid-lactation. In total the content of ten different elements (Ca, Cu, Fe, K, Mg, Mn, Na, P, Se, and Zn) were extracted from skimmed milk by acid sonication and identified using inductively coupled plasma mass spectrometry (ICP-MS). The mean (ppm) and standard deviations for the elements in the milk were: Ca (DH:1214±123; DJ: 1465±148), Cu (DH: 0.03±0.01; DJ: 0.05±0.02), Fe (DH: 0.17±0.04; DJ: 0.19±0.05), K (DH: 1470±115; DJ: 1319±105) Mg (DH: 108±11; DJ: 124±13), Mn (DH: 0.02±0.005; DJ: 0.03±0.009), Na (DH: 349±73; DJ:389±101), P (DH: 724±78; DJ:880±93), Se (DH: 0.007±0.002; DJ: 0.01±0.002), Zn (3.4±0.6; DJ: 4.7±0.8). Furthermore, the cows were genotyped using the High Density bovine SNP array. These SNP markers were used to estimate a genomic relationship matrix among the cows which was used in the linear model to estimate the heritability and perform a genome scan. For each breed the heritability was estimated showing that Ca (DH: 0.72; DJ: 0.63), Mg (DH: 0.07; DJ: 0.57), P (DH: 0.46; DJ: 0.29), Se (DH: 0.10; DJ: 0.20) and Zn (DH: 0.49; DJ: 0.57) had medium to high heritabilities. The standard error was between 0.17 - 0.35 for DH and 0.18 – 0.30 for DJ. The elements Cu, Fe, K, Mn and Na had a very small genetic variance resulting in a heritability of 0. A genome wide scan to screen the cattle genome for SNP markers influencing minerals in the milk is in progress. Initial results based on the first 20 cattle chromosomes detected 2 QTL at the chromosome wise level (Bonferroni corrected P<0.05) for Se (BTA8) and Zn (BTA12), and 1 QTL detected at the genome wise level (Bonferroni corrected P<0.05) for Zn (BTA2) in DH. For DJ a chromosome wise significant QTL for Zn was detected on BTA3 and one genome wise significant QTL was detected for P (BTA1). The top SNP markers in the QTL have been assigned to genes based on the physical location of the SNPs on the cattle genome. The top SNP marker on BTA8 for Se in DH was assigned to chromosome 8 open reading frame, human C9orf3 (C8H9orf3). The top SNP marker for QTL on BTA2 for Zn in DH was assigned to PDLIM1 interacting kinase 1 like (PDIK1L) and the top marker for Zn in DJ on BTA3 was assigned to the gene tubulin tyrosine ligase-like family, member 7 (TTLL7). The results show that some of the micro- and macro-elements in milk have a heritable component (Ca, Mg, P, Se and Zn), suggesting that these can be changed through genetic selection, and that it is possible to detect regions on the genome influencing these minerals. This provides opportunities for changing the content of specific micro- and macro-elements in the milk by genetics, which could be relevant in relation to development of natural milk-based products for people with special dietary needs or nutritional status.

Quantification of Individual Fatty Acids in Bovine Milk by Infrared Spectroscopy and Chemometrics: The Cage of Covariance with Total Fat Content

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High-throughput methods to predict detailed milk composition including the fatty acid (**FA**) profile is highly desired as it would open possibilities for e.g. more efficient breeding. Several studies have investigated the potential of predicting the FA profile of milk using Partial Least Squares (**PLS**) regression applied to Fourier transform infrared (**FT-IR**) measurements. In general, these studies found that a large number of FA could be predicted accurately and precise. However, covariations

amongst the individual FA, total fat content and other factors giving rise to distinct absorption patterns in FT-IR (like breed, feeding, etc.) may serve as the enabler of successful, but indirect FT-IR-based predictions of individual FA. These indirect relationships have to our knowledge not been investigated in details before.

Using 890 milk samples (originating from Danish Holstein and Danish Jersey), this study aimed at proposing a method to determine to what extent shared variation among individual FA, total fat content and breed contributed to predictions of individual FA. Moreover, individual FA (C14:0, C16:0 and C18:1 *cis*-9) were spiked to a background of skimmed milk to stress whether signals specific to these individual FA could be obtained from the FT-IR measurements. The present study demonstrates that shared variation among individual FA, total fat content and breed are responsible for successful FT-IR-based predictions of FA in the raw milk samples. This was confirmed in the spiking experiment, which showed that signals specific to individual FA cannot be identified in FT-IR measurements, when several FA are present in the same mixture. Hence, predicted concentrations of individual FA in milk represent expressions of total fat content rather than actual FA concentrations.

Therefore, FA predictions rely on indirect relationships, which are confined in a cage of covariance. While this is not a problem in itself, it may be problematic in terms of prediction accuracy and calibration robustness. To emphasize this point, samples from Jersey were used for calibrating and samples from Holstein were used for validating PLS models to individual FA. The two breeds have different covariance structures between individual FA and total fat content. PLS models calibrated on the Jersey samples should be valid for the Holstein samples, if the PLS models are based on direct FT-IR absorption signals related to the individual FA. The results show that the Holstein (validation) samples exhibit biased and incorrect predictions. Hence, the models are calibrated on indirect relationships in Jersey samples, which are not valid for Holstein samples. This illustrates the problem of models based on indirect relationships; When indirect relationships in the calibration set are not conserved for new samples, incorrect and biased predictions are obtained.

Recommendations on implementing FT-IR based FA models in e.g. milk recording systems must take the universal validity of the indirect relationships into account. Recommendations cannot be done solely on the basis of PLS model performance parameters like R^2 and RMSECV. In contrast to previous studies, we suggest that the indirect FA models may not be useful in milk recording systems nor breeding programs, as the FA models are providing information related to total fat content rather than individual FA. The indirect relationships used to calibrate PLS models may not be conserved for samples of a different nature (different FA profile) and new samples may show incorrect and biased FA predictions.

Protein Quality in a Sustainable context

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Supplying a growing population with sufficient food is one of the world's major challenges. Evaluating the position of dairy in a diet should both take the nutritional and environmental impact in to account. Dairy products are nutrient rich and an important food group for food and nutrient security in the future. Protein is the most important macronutrient with respect to the constraints of limiting land and global warming. Both the quantity and the quality of protein are determinants for resource efficiency. On global basis live stock products provide 35% of the protein in the human diet and due to the high content of indispensable amino acids, animal proteins contribute to diet quality. Increasing the productivity of a dairy cow will improve the feed (energy and protein) efficiency and reduce the connected greenhouse gas emissions. Local conditions are important and in many parts of the world the cow is an efficient converter of human-inedible resources in a nutrient dense food containing essential micronutrients and proteins of the highest quality. The ration of a dairy cow consist for the major part of resources humans cannot or do not consume and ruminants therefor do not directly compete with the human food chain. Although, the overall energy and protein efficiency of a dairy cow may not be higher than 25%, the return on the human-edible part may be more than 400%, as is the case in the Dutch situation. In addition, the cow converts lower-quality proteins, such as grain and soy protein, into proteins of the highest quality. Models that calculate the nutritional and ecological impact of a diet are the preferred approach to conclude on alternative and realistic consumption strategies. These models should incorporate the content of indispensible amino acids as individual nutrients.

In-Silico Genomic Approaches to Understanding Lactation, Mammary Development, and Breast Cancer

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Lactation---related traits are influenced by genetics. From a quantitative standpoint, these traits have been well studied in dairy species, but there has also been work on the genetics of lactation in humans and mice. In addition, there is evidence to support the notion that other mammary gland traits including those describing mammary ductal development as well as risk for breast cancer are also genetically regulated. Previous work in our laboratory using in---silico genome wide association (GWAS) has identified several quantitative trait loci (QTL) that could drive some of the variation in lactation performance that was observed in an inbred mouse mapping panel known as the Mouse Diversity Panel (MDP). Our additional work in the MDP has also identified variations in maternal food intake, body composition, milk macronutrient and mineral composition, and lastly post---pubertal mammary ductal morphogenesis. With regard to milk macronutrient composition we have detected a total of 60 QTL associated with variations in milk lactose, protein, and triglyceride concentrations. For milk mineral composition, we used inductively coupled plasma optical emission spectrometry to measure the concentrations of nine minerals including calcium, copper, iron, potassium, magnesium, sodium, phosphorus, sulfur and zinc. All of the minerals were significantly influenced by genetic background (P=0.03 to P=2x10---16). GWAS of these data detected a total of 20 milk mineral QTL (Mmq) encompassing 15 gene candidates. Among the most significant of the Mmq was an association on MMU3 for milk calcium (P=2x10---9) and magnesium (P=7x10---7). Closer inspection of this region revealed a cluster of three associated SNPs that were located in the first intron of the gene Ppm1l, which encodes for a magnesium---dependent protein phosphatase. These particular SNPs were also very close to, or within, potential binding sites for the chromatin regulator CTCF, suggesting that the expression of this candidate gene could be differentially regulated through the modulation of chromatin structure. With regard to our mammary ductal development work, a second and larger cohort of mice from the MDP was used to document strain---dependent variation in normal post---pubertal mammary ductal development and to relate this to breast cancer in both mouse models and humans. By measuring five quantitative ductal development traits in digital images of mammary whole mounts collected at two different ages, we first demonstrated that the variation in mammary ductal development was higher than previously described on the basis of commonly used breast cancer research strains such as the FVB/NJ and C57BL/6J. We also identified two strains, CZECHII/EiJ and KK/HlJ, which displayed extreme mammary ductal development differences. These differences were highlighted through 3---dimensional imaging. The work also identified correlations between ductal development and mammary tumor latency in a murine breast cancer model based on polyomavirus middle---T. GWAS analysis of this dataset identified 20 mammary development QTL (Mdq) of which five overlapped syntenic intervals found in human breast cancer studies. Within these loci were 43 genes, of which some could be linked to normal mammary gland development as well as breast cancer on the basis of their biology. These studies highlight the opportunities that exist for novel discoveries in understanding lactation, mammary development and cancer, when combining genomic approaches with genetically diverse mice.

Comparative Genomics of Monotremes, Marsupials, and Pinnipeds: Models to Examine the Function of Milk Proteins

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The composition of milk includes all the factors required to provide appropriate nutrition for growth of the neonate. However, it is now clear that milk has many functions and also consists of bioactive molecules that play a central role in regulating developmental processes in the young and providing a protective function for both the suckled young and the mammary gland during milk production. Identifying these bioactives and their physiological function in eutherians can be difficult and requires extensive screening of milk components that may function to meet the demand for foods that improve wellbeing and options for prevention and treatment of disease. In addition, human targets for milk bioactives are focused on specific and changing health needs during the progression from infancy to old age. For example, the need to support growth and development in early life differs from the maintenance of muscle, eyes, and brain function in the elderly. Health issues such as obesity, diabetes, hypertension, and cancer increase with an aging population. Therefore, functional foods that improve both general nutrition and well-being, and also target prevention and treatment of health problems are very attractive. New animal models with unique reproductive strategies are now becoming increasingly relevant to search for these factors.

The lactation cycle is common to all mammals, although monotremes, marsupials, and some pinnipeds have evolved a reproductive strategy that is very different from that of most eutherians. The evolution of lactation has led to new opportunities to better understand the functionality of milk. Monotreme, marsupial and pinniped animal models have an extreme adaptation to lactation and have increased our understanding of the function of milk proteins by revealing mechanisms that are present but often not readily apparent in many eutherian mammals. Eutherians have a long gestation relative to their lactation period, and the composition of the milk doesn't change substantially. This contrasts with reproduction and lactation in the other three classes of mammals, the monotremes (such as platypus and echidna), the marsupials (such as the tammar wallaby) and the pinnipeds (such as the fur seal). These three animal models provide new insights into how the lactation cycle is regulated, and when combined with technology platforms that include genomics, proteomics and bioinformatics can be exploited to identify milk proteins with bioactivity and subsequent application as nutraceuticals and pharmaceuticals.

The Relationship Between Milk Proteins and Extracellular Matrix in Regulating Murine Mammary Gland Involution

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Mammary gland involution is a physiological process associated with rapid apoptosis (programmed cell death) of the lactating mammary epithelial cells followed by remodelling of the mammary gland to a pre-pregnant state. The loss of secretory alveolar epithelium is attributed to a number of factors like mild ischemia as a result of milk engorgement and compression of vasculature, falling levels of prolactin upon cessation of suckling, factors in milk that promote cell death, physical distension of the luminal epithelium and increased activity of the basement membrane degrading enzymes (matrix metalloproteinases). Therefore it is clear that the retention of milk plays an essential role in this process.

Mammary gland involution in the mouse is a 2 step process according to its reversibility. Early involution, occurring within the first 24-48 hours, is reversible and induced by local factors. If weaning is re-initiated during this phase, the process of programmed cell death can be reversed. The second phase, a remodelling programme initiated to return the gland to a pre-pregnant state, is irreversible and requires systemic factors.

Alpha Lactalbumin, a major whey protein secreted by the mammary gland epithelium, may undergo partial unfolding, bind to a fatty acid cofactor like oleic acid and become tumoricidal. HAMLET (Human alpha-lactalbumin made lethal to tumor cells) induces apoptosis in tumor and immature cells, but normal differentiated cells are resistant to its effects. Recent studies (Sharp and Nicholas Laboratory) have shown that a different form of the α-lactalbumin dimer complexed with unsaturated fatty acids has the capacity to program apoptosis in a variety of cells. The objective of this study is to re-evaluate the regulation of mammary gland involution, particularly to study the role of specific milk proteins and the extracellular matrix proteins involved in mechanisms of transition between the reversible and irreversible phases. Analysis of microarray data analysing murine mammary gland during lactation and involution reveals significant changes in the expression of genes coding for secretory proteins that may play a role in phase 2 involution. In order to examine the interplay between the role of milk factors and ECM components on epithelial cell function and fate during involution, an in-vitro model has been established. Mouse mammary epithelial cells have been isolated from day 12 pregnant mice (C57BL/6J) and cultured on extra-cellular matrix extracted from day 15 lactating (day 0 involuting) to day 4 involuting mammary tissue. In contrast to the 3-D acini structures (mammospheres) formed on day 15 lactating matrix, the cells did not polarize and lost the capacity to form these structures when cultured on day 3 and day 4 involuting matrices. When these 3D acini structures were transferred to lactating and early involuting matrices (day 1 and day 2) they depolarized and were programmed into apoptosis. Current studies are examining the relationship of milk proteins, particularly a-lactalbumin and ECM to promote cell death.

Effect of A 48h Food Deprivation on the Lactating Goat Mammary Mirnome Obtained by Deep Sequencing

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Animal nutrition considerably affects milk composition, influencing its nutritional quality for consumers. Milk components synthesis and secretion involve numerous genes some of which are nutri-regulated. Especially, a 48h food deprivation applied to lactating goats modifies the expression of 161 genes, including genes coding for major milk proteins and for lipogenic enzymes related to milk components synthesis and secretion (Ollier et al., 2007). However, the mechanism of their regulation remains unsolved. MicroRNA (miRNA) are small non coding RNA that regulate genes expression at a post-transcriptional level, conferring a crucial role in most of the biological processes. Due to their pleiotropic role, miRNA could provide new possibilities to decipher these regulations. Thus this study aims to identify miRNA which expression is regulated by food deprivation and could control differentially expressed genes (DEG).

Here, we established the exhaustive list of miRNA expressed (the miRNome) of the lactating mammary gland from goat receiving an ad libitum diet and from goat 48h food deprived, using Solexa (Illumina) sequencing. The mammary miRNA were localized and clustered on the caprine chromosomes allowing an enrichment of the annotation of goat genome. The intragenic location of some miRNA genes was showed to be conserved in different species. Then, a differential analysis between the 2 nutritional trials was performed and showed significant changes in the expression of 30 miRNA, 19 miRNA already annotated in others species and 11 putative miRNA. It is the first study reporting miRNomes whose profiles are affected by nutrition in the mammary gland. Links between the nutriregulated miRNA and DEG identified using transcriptomic mentioned above were analyzed, revealing 47 DEG potentially targeted by nutriregulated miRNA.
To decipher the function of some of those nutriregulated miRNA and to determine their targets, their expression will be deregulated in mammary epithelial cells.

Animal Models for Early Infant Nutrition

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Mothers own milk remains the golden standard for feeding newborn infants, but how to formulate the best possible milk diet when mothers milk is not available? This seemingly simple question is still difficult to answer and much research is needed to clarify the role of specific milk components on short and long term infant health at many levels. Due to the difficulties in doing well-controlled studies in infants, studies in appropriate animal models may help. Results from models that are hypersensitive to small modifications in milk composition and its preparation will help to define the most important diet ingredients. We present a series of results from preterm pigs that have proven to be highly gut-sensitive in response to manipulation of diet ingredients (lactose, casein, whey proteins, essential fatty acids, minerals/vitamins, lactoferrin, pre-, pro- and anitibiotics) and diet treatments (e.g. spray-drying, pasteurizations). Intact, raw milk remains a better source of nutrients and gut health than most milk formulas, especially for hyper-sensitive newborns. Lactose, and its interaction with the developing gut microbiota, deserves more attention. Research should continue to better define the nutritive and gut protective factors in natural milk that benefit newborn and growing infants.

TGF-B2 Protects Intestinal Epithelial Cells by Regulation of Proteins Associated with Stress and Endotoxin Responses

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Transforming growth factor (TGF)-β2, an important anti-inflammatory protein in milk, possesses protective effects against intestinal inflammation by limiting the production of inflammatory cytokines. TGF-β2-fortified infant formula has shown potential in reducing the incidence of necrotizing enterocolitis (NEC) and associated inflammation in preterm neonates. However, the molecular mechanisms by which TGF-β2 protects immature intestinal epithelial cells (IECs) remain to be more clearly elucidated before interventions in infants can be considered. Porcine intestinal epithelial cells (IECs) PsIc1 were treated with TGF-β2 (3 ng/mL, close to the concentration in breast milk) alone and in the presence of lipopolysaccharide (LPS). The changes of the cellular proteome were analyzed using two-dimensional gel electrophoresis (2DE) and LC-MS-based proteomics. TGF-β2 alone induced differential expression of 13 proteins, and the majority of the identified proteins were associated with stress response, TGF-β and toll-like receptor 4 (TLR4) signaling (heat shock protein A5 (HSPA5), HSPA8, HSPA9, HSP60, HSP90B1 and RACK1). Furthermore, by LC-MS-based proteomics and Western blot, 20 differentiated proteins were identified following treatment with TGF-β2 in LPS-challenged IECs. Thirteen of these proteins were associated with stress response pathways. Among them, five proteins (GRP58, PDI, cyclophilin A, TIMP3, and PKC-α) were altered by LPS and restored by TGF-β2, while six proteins (HSPA8, HSP90, apoptosis inducing factor, annexin II, calreticulin and tropomyosin α3) were differentiated only by TGF-β2 in LPS-challenged IECs. In addition, the protein-protein interaction network of all identified proteins analyzed by STRING showed all HSPs and stress-associated proteins interacting together in central clusters. As TLR4 signaling, and TLR4 and HSP interaction are indicated to be involved in inflammation and NEC pathogenesis, our data suggest that TGF-β2 of dietary or endogenous origin may modulate the cellular responses against LPS-induced stress and thereby support cellular homeostasis, and the innate immunity in response to bacterial colonization and the first enteral feeds in early life. These mechanisms may play a role in the protection against NEC induced by feeding mother's milk to immune-compromised preterm infants. Further investigation is important to conclude the protective effects of TGF-β2 used as the supplement in preterm infant formula.

Human Milk: Mother Nature's Prototypical Probiotic Food?

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The concept of "probiotic" is generally attributed to Dr. Ilya Mechnikov, who hypothesized that longevity could be enhanced by manipulating gastrointestinal microbes using naturally-fermented foods. In 2001, a report of the Food and Agriculture Organization and World Health Organization proposed a more restrictive definition of *probiotic*: "a live micro-organism which, when administered in adequate amounts, confers a health benefit on the host." As such, answering the fundamental question posed here – "Is human milk a probiotic?" – requires first grappling with the concept and meaning of the term *probiotic*. Nonetheless, one must also be convinced that human milk contains bacteria. Indeed, there are scores of publications providing evidence of a paradigm shift in this regard. Variation in the human milk microbiome may be associated with maternal weight, mode of delivery, lactation state, gestation age, antibiotic use, and maternal health. Milk constituents (e.g., fatty acids and complex carbohydrates) might also be related to abundance of specific bacterial taxa in milk. Whether these bacteria impact infant health is likely, but more studies are needed to test this hypothesis. In summary, a growing literature suggests that human milk, like all other fluids produced by the body, indeed contains viable bacteria. As such, and recognizing the extensive literature relating breastfeeding to optimal infant health, we propose that human milk should be considered a probiotic food.

Determining factors that influence which bacteria are present in milk and if/how they influence the recipient infant's health remain basic science and public health realms in which almost nothing is known. These factors include genetics, environmental microbial exposure, breastfeeding and infant feeding practices, and maternal nutrition (including consumption of fermented foods). An international collaboration of lactation physiologists, milk biochemists, microbiologists, anthropologists, and mathematicians is currently collecting data from mothers and infants around the world to begin to tease out some of these potential associations; this study (and opportunities for collaboration) will be briefly discussed.

NMR Metabolomics of Term and Preterm Milk during the First Month of Lactation

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Human milk is considered the gold standard of infant nutrition, providing both protective and essential nutrients. Many of the large molecules (e.g. proteins) have been characterized in breast milk, but fewer studies have focused on the small molecule metabolites. In order to better understand the composition of low-molecular-weight species in breast milk, NMR metabolomics was used to identify and quantify metabolites at three time points during the first month of lactation. We examined colostrum, transition, and mature breast milk from 15 term mothers and 13 mothers who delivered preterm (gestational age less than 37 weeks). Both milks contain metabolites that significantly change during the course of lactation. Several key metabolites were identified that are significantly different between term and preterm milk at all-time points. These data will help to understand how both term and preterm infants' nutritional needs change during lactation, and may also aid in determining the best fortification strategy for preterm milk.

Comparative Proteomics of Human and Macaque Milks:
Humans Get a Helping Hand during Post-Natal Development

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Student Travel Award Recipient

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Objective: Milk has been well established as the optimal nutrition source for infants, yet there is still much to be understood about its molecular composition in humans and non-human primates. Therefore, our objective was to develop and compare comprehensive milk proteomes for human and rhesus macaques, the most widely used non-human primate in biomedical research. This comparative biology approach highlights differences in early neonatal nutrition between the species.

Methods: Until this point, the high incidence of interfering macronutrients, pervasive post-translational modifications, and abundant casein and whey proteins have been substantial barriers to comprehensive milk proteomics. Existing methods require a large sample volume that has been prohibitive of utilizing milk from non-human primates where sample availability is more limited. Therefore we have developed a small volume proteomics technique to complete the first high throughput molecular comparison of human and rhesus macaque milk proteins.

Whole milk (15ul) is fractionated using gradient SDS-PAGE and gel slicing which effectively removes the interfering macronutrients and isolates high abundance casein and whey proteins into their respective gel fragment. In addition, one of the most predominant post-translational modifications of milk proteins is glycosylation. Therefore, we incubated gel slices with PNGase F to cleave the N-linked glycans exposing more of the peptide chain. In its first utility in milk proteomics, we found it to greatly increase protein detection during tandem mass spectrometry.

Results: Using the aforementioned novel proteomics technique, we identified 1,606 and 518 proteins in human and macaque milk, respectively (~5% protein FDR, < 1% peptide FDR). This is a considerable expansion on the total number of proteins previously identified in human and is the first comprehensive macaque milk proteome. Using a reciprocal best match BLASTp approach and clustering in-paralogs with InParanoid (v 4.1), we identified 396 orthologous protein clusters present in both human and macaque milk. Notably, there were 88 proteins with differential abundance between the two species (p-value < 0.05 after adjusting for multiple hypothesis testing).

Nearly all differentially abundant proteins are higher in human milk relative to macaque. Some of the most differentially abundant proteins include lactoferrin, bile salt activated lipase, polymeric immunoglobulin receptor, alpha-1 antichymotrypsin, 4-trimethylaminobutyraldehyde dehydrogenase and clusterin. Notably, human proteins with 2-fold greater normalized weighted spectra, compared to macaque, are associated with the development of the gastrointestinal tract, the immune system, and the brain.

Conclusions: Overall, we demonstrate the effectiveness of our small volume, high throughput proteomics method to reveal the most comprehensive human milk proteome to date and the first complete macaque milk proteome. The proteins observed are consistent with the perspective of 'secondary altriciality' that has been used to describe the human development stage at birth in which we are adapted to be less developed at birth as a derived feature from our primate relatives. Our human milk proteome analyses suggest that mother's milk nurtures infant maturation through higher quantities of specific proteins associated with development of the gastrointestinal tract, the immune system, and the brain than observed for macaque. These findings imply that infant formula could be improved by supplementation with specific milk proteins.

Milk Genomics from a Technological Perspective

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Within the Danish-Swedish Milk Genomics Initiative one of the important technological properties profiled and studied in detail was the rennet-induced coagulation properties, and how these are influenced by milk composition, genes, storage, calcium addition etc. Milk and tissue samples were obtained from approximately 400 cows of each of Danish Holstein (DH), Danish Jersey (DJ) and Swedish Red (SR) and constituted the basis of overall as well as in-depth profiling of the variances in the composition of Scandinavian dairy milk at the cow level. The analyses included important features of both milk fat, proteins and metabolites, as well as important technological properties, in addition to free oligosaccharides, calcium distribution and vitamins. Furthermore, all cows were genotyped using 777 K Illumina BovineHD BeadChip.

The distinct milk coagulation properties among breeds were related to some extent, but not exclusively, to casein gene polymorphisms. The studies revealed new genetic variants present in Danish dairy milk, including the C variant of α_{S1}-CN, the I variant of β-CN and the E variant of κ-CN. The great variation in coagulation properties among individual cows and breeds were examined more closely using proteomic techniques to identify genetic variants and isoforms in milk proteins, and the importance of post translational modifications (PTMs) for both first and second phase of milk coagulation was shown.

The results showed that the coagulation descriptors rennet coagulation time (RCT) and curd firming rate (CFR) were differentially related to milk compositional parameters, and reacted differently to cold storage of raw milk. In addition, a range of milk metabolites were found to be significantly different between good and non-coagulating milk. Heritability estimation confirmed that milk coagulation is under genetic influence and GWAS identified new genomic regions both in relation to milk coagulation as well as non-coagulation. Besides a major QTL around the casein genes identified in both the SR and DH, additional regions affecting milk coagulation were identified in close proximity to genes influencing glycosylation and phosphorylation, as well as proteolysis. The project has revealed the presence of large biological variation in milk composition and linked this to genetic information, which could potentially be exploited in breeding schemes, either in order to obtain differentiated products, or to achieve an overall improved milk composition in relation to technological properties and exploitation of milk components as ingredients.

Strategies for Improving Milk Coagulation Properties

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Milk coagulation properties are essential for cheese manufacturing. Within the Danish-Swedish Milk Genomics Initiative, rennet-induced coagulation properties in milk from individual cows have been screened in three major Nordic dairy breeds. The screening of coagulation properties showed that there are large individual differences between cows for these traits. Furthermore, milk from Danish Jersey cows had the best coagulation properties, whereas approximately 2 % of the Danish Holstein samples could not coagulate (denoted non-coagulating), and up to 17 % were poorly coagulating. In Sweden, 18 % of the milk samples from Swedish Red cows were found to be non-coagulating, which is a surprisingly high proportion that calls for improvement and elimination.

We found a strong association between milk coagulation properties and known variants at the casein genes on BTA6. In Sweden, common composite genotypes were associated with poor and non-coagulation, and generally Jersey cows had higher frequencies of variants traditionally associated with improved milk coagulation. Our recent research has focused on identifying the underlying causal mutation for rennet-induced milk coagulation and these haplotypes are now being validated in a new sample set.

The results provide good opportunities for improving milk coagulation properties through selective breeding either using a genomic selection approach or gene test strategy. However, this can potentially affect other important milk quality traits and therefore herds producing milk for specific purposes (e.g. cheese herds) might be a more attractive approach than including milk coagulation traits in the national breeding program, which also calls for high throughput phenotyping method using e.g. infrared technologies.

Moreover, current studies focus on milk coagulation properties mainly from midlactating cows using various rheological methods, and insights into the interplay between the cow, its genes and the environment in relation to milk coagulation properties across the lactation period is still scarce.

Milk Phospholipids as Emulsifiers in Infant Formula

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Abstract not available at time of printing

Lipid and Protein Release During *In Vitro* Digestion of Different Cheese Types

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Dairy products are known as a very good source of several essential nutrients and from which they are highly bioavailable. Dairy products represent a wide variety of products ranging from fluid milk, to fermented dairy products and cheeses. Several studies have linked food structure and texture to different kinetics of nutrients delivery. Changes in some nutrients' release rate such as peptide and amino acids from casein and whey proteins qualified as "slow and fast" proteins could induce different physiological effects. However little is known on the contribution of dairy food structure on nutrient release. To better understand the impact of dairy food composition and structure on the digestion process an in vitro model was used to study dairy products (milk, yogurt and cheese).

Results of several studies showing the effects of casein: whey protein ratio of yogurt, calcium content in cheddar cheese, and texture of a variety of cheese on nutrients release from dairy matrices using an *in vitro* digestion model will be presented to highlight the impact of composition and structure. Rate of disintegration, soluble protein, peptide and amino acids and/or lipid releases were determined. An *in vivo* human clinical trial showed that the isocaloric and iso-protein yogurt formulation with increased whey protein content taken as a snack significantly reduced subsequent energy intake compared to its control which could be due to increased satiety. Various commercial cheeses with a wide range of composition and texture were studied. Cheddar-type cheeses were also manufactured from milks standardized with anhydrous milk fat (AMF), olein-AMF (low-melting point) and stearin-AMF (high melting-point). Cheeses were divided and salted with or without $CaCl_2$. Experimental cheeses had a similar structure and composition, differing only in fat type and mineral profile. During *in vitro* digestion, lipolysis was higher for calcium-enriched cheeses due to its lipase-enhancing role. In addition, commercial cheeses showed a modulated protein and lipid release based on their textural properties. These results show that nutrient bioaccessibility can be regulated by calcium present in cheddar cheese and depends on structural attributes. This project highlights the nutritional impact of the dairy matrix and its composition. Further research in this area will lead to a better understanding of the role dairy foods play on the health of individuals.

Obesity and the Metabolic Syndrome

Dairy and weight management: a review of the evidence

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Accumulating evidence from observational studies indicates an inverse association between dairy and body weight and body fat mass. Some randomised controlled trails (RCT) have been reported, and meta-analyses of observational studies support the role of dairy for weight control, particularly during energy restriction. However, though several of the reported RCT's show beneficial effects of dairy for body weight the mechanisms by which dairy influences energy balance are not entirely clear. However, dairy protein and calcium may play certain roles.

In the Diogenes trial, we have shown that a slight increase in total protein (~25 % of energy), a corresponding reduction in total carbohydrate, and a relative increase in low-glycemic index carbohydrates, promotes weight control both in adults and in obese children. Dairy protein has a very high quality in terms of amino acid composition, and its effects on satiety and muscle anabolism are similar or better than those from other sources. Protein has an important role for weight control, and in combination with calcium there is a role for a specific reduction in abdominal obesity and metabolic syndrome. In Diogenes the diet was also found to have a positive effect on blood pressure, blood lipids, and inflammatory markers.

Increased dietary calcium intake has been proposed to affect both sides of the energy equation, i.e. both energy intake and energy utilization, at least in subjects with low habitual intake. It has been shown that increased dairy calcium intake produces a decrease in fat digestibility, presumable due to formation of insoluble calcium-fatty acid soaps and binding of bile acids. Based on a meta-analysis we have estimated that an increase in dairy calcium intake of ~1200 mg/day produces an increased fecal fat excretion of 5.2 g/day. Finally it has been suggested that low dietary calcium intake may affect appetite regulation and lead to an increased food intake, and this effect has recently been substantiated by a meta-analysis.

In conclusion, a high intake dairy is a natural part of a nutrient dense diet that provides benefits for weight control, and the prevention of type 2 diabetes and cardiovascular disease.

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Effect of Milk Protein And Butter with Different Fat Composition on the Observed Metabolites in Human Urine and Blood – Assessed by an Untargeted Metabolomics Approach

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The Metabolic Syndrome (MeS) is a clustering of components that reflect over nutrition, sedentary lifestyles and resultant obesity. MeS includes the clustering of abdominal obesity, insulin resistance, dyslipidemia and elevated blood pressure1. The prevalence of the MeS is globally increasing to epidemic proportions and is associated with an approximate doubling of the cardiovascular disease risk and a 5-fold increased risk for type 2 diabetes.

Observational studies suggest that dairy foods consumption may prevent the development of MeS and its related disorders2; both whey and casein proteins stimulate insulin secretion and have the potential to improve tissue glucose uptake and suppress postprandial blood glucose3. The milk protein effect on postprandial lipaemia and chronic dyslipidaemia is not well defined as studies have produced different findings4.

Although some studies show beneficial effects of milk fat especially rich in short- and medium-chain fatty acids on weight control, glucose and lipid metabolism5, 6, results are still conflicting.

To gain further insight into the nutritional impact of milk protein and milk fat metabolomics is proposed as a pioneer approach. It may help to increase our understanding of the influence of milk components e.g. on MeS by the advent of comprehensive analysis of low-molecular-weight compounds (metabolites) present in complex samples such as human biofluids collected from an intervention study.

Liquid chormoatography- and gas chromatography-quadrupole time of flight mass spectrometry (LC-MS and GC-MS Q-ToF) untargeted metabolomics were applied to develop multivariate models to identify biomarkers related to the intake of milk lipids with high or low content of medium-chain saturated fatty acids (MCFA), and milk proteins (whey vs. caseins) on metabolic profile in 52 subjects with abdominal obesity. Serum (fasted and 240 min after meal) and urine (fasted and 360 min after meal) samples were collected before and after the 12-weeks randomized, controlled, parallel, iso-caloric dietary intervention. Participants were randomized to one of four groups according to the intervention diet; low amount of MCFA (L-MCFA) + Whey, high amount of MCFA (H-MCFA) + Whey, L-MCFA + Casein, H-MCFA + Casein.

Using LC-MS Q-ToF on blood samples yield both post prandial and long term effects on the metabolic profile of blood. A clear separation in the metabolic profile was observed for the group with H-MCFA + Casein compared to the three other groups while no effect on the metabolomics profile in urine was observed. Using GC-MS Q-ToF we have found a separation of all four groups when analysing the long term effect on the metabolic profile in urine, and a separation between the two groups H-MCFA + Casein and H-MCFA + Whey in serum, which could indicate a synergic effect between dairy protein and H-MCFA. By combining LC-MS Q-ToF with GC-MS Q-ToF data thus secured optimal analysis of more metabolites, which now are being identified and related to the intake of dairy products and their effect on obese individuals.

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Milk Protein Fractions and Body Weight Regulation –
Mechanistic Studies Using Metabolomics in a Mouse Model

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Diets rich in proteins are generally considered to have a beneficial effect on adiposity and body weight regulation. However, proteins from different sources may exert different effects. In a number of experiments we have investigated the role of different milk proteins on weight gain and adipose tissue mass using a mouse model and here we present novel insight into the metabolic mechanisms that may explain how milk proteins impact body weight regulation.

Male C57BL/6J mice were fed a high fat diet for 8 weeks with whey, casein or hydrolysed casein, respectively, as sole protein sources. We found that mice fed whey proteins and hydrolysed protein gained significantly less weight and adipose tissue than mice fed intact casein. In order to shed light on the underlying mechanisms, LC-MS- and NMR-based metabolomics were used to map the metabolic phenotypes associated with this difference in adiposity.

The urinary metabolome reflects the whole body metabolism and over time metabolic end-products accumulate in the urine. Therefore, urine is a very useful tool for assigning metabolic alterations after a dietary intervention. Using a combination of NMR and LC-MS analyses, the urinary metabolome revealed altered secretion of intermediates from the citric acid cycle along with metabolites reflecting the amino acid composition of the diets. Intriguingly, these results indicated that whey proteins induce an increased secretion of energy-rich citric acid cycle intermediates and it is possible that this process removes substrates for anabolic processes, such as lipogenesis, which in turn may explain the reduced adipose tissue mass in whey-fed mice. Furthermore, the NMR platform showed that urinary lactate concentrations were

higher for casein-fed mice. This could indicate an increased glycolysis and thus make a contribution to the higher fat deposition in casein-fed mice.

Intake of hydrolysed casein also reduced weight gain as compared to intact casein and the metabolomics platforms pointed at multiple altered pathways that could explain this finding. Comparison of mice fed intact and hydrolysed casein showed that protein hydrolysis resulted in an altered carbohydrate metabolism and an altered metabolism of sulphur-containing amino acids. This can possibly be linked to a shift in the secretion of Phase II metabolites (glucuronic acid and sulphate conjugates) and it will be discussed how this could link intake of hydrolysed casein with decreased liver lipogenesis.

Through a combination of NMR and LC-MS based metabolomics and gene expression data, the present work demonstrates that several metabolic pathways are altered after an intervention with different protein sources; lipid and carbohydrate metabolism are central players, but the citric acid cycle and Phase II metabolism are also likely targets for the weight-regulating effects of milk proteins.

Biological Effects of Milk Constituents

Biological Effects of Milk Peptides/Proteins – State of the Art

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Novel dairy fractions have been isolated and are now commercially available for potential inclusion into infant formula. α-Lactalbumin is a good source of tryptophan, often the first limiting amino acid in milk-based infant formulas. It is digested into smaller peptides with various functions, such as inhibiting growth of pathogens, stimulating growth of beneficial microorganisms, modulating the immune system and acting as enhancers of mineral absorption. Lactoferrin (Lf) provides a multitude of bioactivities including anti-bacterial and antiviral effects, regulation of immune function, stimulation of intestinal proliferation and differentiation, and facilitating iron absorption. Several functions of human Lf are mediated by its binding to intestinal Lf receptors, but recent studies suggest that some forms of bovine Lf may also bind to the Lf receptor and exert bioactivities. Osteopontin (OPN) is a heavily phosphorylated and glycosylated protein found at a high concentration in human milk. Recently, OPN has been purified from bovine milk and bovine OPN has many structural similarities to human OPN. We have conducted a clinical trial on infants fed formula with added bovine OPN showing beneficial effects on inflammatory cytokines and a reduction in fever. Milk fat globule membranes (MFGM) is a dairy fraction that has previously been excluded from infant formulas. We have recently shown that infants fed formula with MFGM had improved cognitive function and less otitis media infection than those fed regular formula. Further, inclusion of MFGM into complementary foods given to Peruvian infants has shown a reduction in the incidence of diarrhea. In conclusion, milk proteins can successfully be incorporated into infant formula and improve health and development.

Osteopontin – A Bioactive Milk Protein with Immunological Properties

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Osteopontin (OPN) is a multifunctional bioactive protein involved in numerous physiological processes including mineral binding, bone remodeling, cellular adhesion and migration and several immune functions. OPN is encoded by a single gene, but due to extensive post-translational modification, such as phosphorylation, glycosylation and proteolytic processing, it exists in many different isoforms. OPN contains more integrin binding motifs used for interaction with cells and it has cytokine-like properties and is a key factor in the initiation of T helper 1 immune responses. OPN is present in most tissues and body fluids, with the highest concentrations being found in milk.

We have characterized the posttranslational modifications of OPN from various tissues and cell types and found that OPN from milk is the most modified form known. Likewise, we have shown that the modification patterns of human and bovine milk OPN are very similar.

We have measured the OPN concentrations in human breast milk, pooled bovine milk, and infant formulas. The OPN concentration in human milk was ~138 mg/L, which corresponds to 2.1% (wt/wt) of the total protein in human breast milk. This is considerably higher than the ~18 mg/L measured in bovine milk, which corresponds to ~0.005% (wt/wt) of total protein in bovine milk and the ~9 mg/L measured in commercial infant formulas. Likewise, we have measured the OPN concentrations in plasma samples from the umbilical cord, 3-mo-old infants, and pregnant and nonpregnant adults. The OPN level in plasma from 3-mo-old infants and umbilical cords was found to be 7-10 times higher than in adults indicating that OPN plays a role in the development of the infant. In cellular studies, we have shown that bovine milk OPN can induce expression of the cytokine IL-12 in human lamina propria mononuclear cells isolated from intestinal biopsies. Moreover, we have shown that OPN binds to monocytes, but not resting T cells, NK cells, or B cells, and mediates chemoattraction of IL-1-activated human monocytes. We have also shown that OPN binds in a specific manner to all known serotypes of the two bacterial species *Streptococcus agalactiae* and *Staphylococcus aureus* and thereby opsonizes these bacteria for phagocytosis by immune cells.

Taken together, the high levels of OPN in human breast milk and infant plasma, the effects on human immune cells and the interaction with bacteria suggest that OPN plays a role in the activation and action of the infant immune response in the gut. Moreover, very recent studies have shown that orally administered bovine milk OPN can suppress tumor growth and prevent alcohol-induced liver injury in mice, further indicating an immunological role for milk OPN.

Consumption of 2-Fucosyllactose by Infant Borne Bifidobacteria

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INTRODUCTION

Human milk and its assembled glycome is known to enrich specific bifidobacterial populations in the infant gastrointestinal tract. Inactive alleles of the fucosyltransferase 2 gene (FUT2; termed "secretor" due to its role in the expression of ABO blood types in secretions) are common in many populations. Some bifidobacteria (common infant gut commensals) are known to be able to consume 2' fucosylated glycans, such as the oligosaccharides found in the breast milk of a secretor (FUT2+) mother. This work aimed to test whether there is a difference in the ability of bifidobacterial isolates from both secretor-fed infants and non-secretor-fed infants to grow on 2'-fucosyllactose (2FL) as sole carbon source. We also compared the representation of bifidobacterial species isolated from feces to that of data obtained using a Bifidobacterial-specific terminal restriction fragment length polymorphism (Bif-TRFLP) method to measure the bifidobacterial species content of fecal samples.

METHODS

We isolated and characterized bifidobacterial species from a cohort of breast-fed infants. 106 infant fecal samples from the UC Davis Lactation study cohort representing four time points after birth (days 7, 21, 71 and 120) were chosen. Diluted feces were plated on bifidobacterial-selective medium and up to 10 colonies from each sample were isolated and passaged twice using streak-plate technique to ensure purity. These isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, MALDI-TOF MS (Biotyper). Bif-TRFLP was also utilized to identify which species were present in the fecal samples, using DNA extracted from feces by the Zymo Fecal Miniprep kit. Ninety-seven unique isolates were tested for growth on 2'-fucosyllactose, including isolates from both secretor- and non-secretor-fed infants. Isolates were cultured in modified MRS media with 3% 2FL, 3% lactose (positive control), and without carbohydrate (negative control). Their growth was monitored by plate reader in anaerobic chamber by measuring optical density at 600 nm.

RESULTS
382 bifidobacterial isolates were obtained from the feces of 38 brest-fed infants, across 78 samples. Isolates were identified by the MALDI Biotyper as *B. longum, B. breve, B. pseudocatenulatum, B. catenulatum, B. gallinarium, B. bifidum, B. dentium,* and *B. angulatum*. *B. breve* isolate relative abundance increased over time in non-secretor-fed infants while *B. longum* group increased in secretor-fed infants. It was discovered that approximately 25% of secretor fed infant isolates and 7% of non-secretor fed infant isolates were able to consume 2FL as sole carbon source. The species of the fecal isolates were overall representative of the Bif-TRFLP data, with *B. breve* proportionally overrepresented and *B. pseudocatenulatum* proportionally underrepresented.

DISCUSSION
Differences in bifidobacterial population were observed in the feces of secretor-fed and non-secretor-fed infants, in particular in the abundance of *B. breve* and *B. longum*. More isolates from secretor-fed infants were able to consume 2'fucosyllactose than isolates from non-secretor-fed infants, reflecting the enrichment of species that can consume the oligosaccharides found in the mother's milk. There were differences in the relative species abundance identified by the Bif-TRFLP and isolation methods, although isolates were not obtained from all samples.

Role of Protease Inhibitors in Breast Milk in Protection against Allergy

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Background Besides general nutritional benefits, breastfeeding is also important for gut maturation and modulation of inflammatory response. Breastfeeding has been linked to a reduction in the prevalence of allergy and asthma. However, studies on this relationship unfortunately vary in outcome. These differences in study outcomes may partly be related to a different breast milk composition of allergic and non-allergic mothers.

Objective The objective of this study was to explore differences in the proteome of breast milk from individual allergic and non-allergic mothers. The differences observed in the breast milk proteome will be used to generate hypotheses on proteins associated with offspring allergy.

Materials & methods Milk samples from 20 mothers, 10 with house dust mite (HDM) allergy and 10 without allergy, were obtained from the PIAMA project, which is a prospective birth cohort study on incidence, risk factors, and prevention of asthma and inhalant allergy. Non-targeted proteomics technology, based on liquid chromatography and mass spectrometry, was used to compare breast milk from allergic and non-allergic mothers.

Results & discussion Twenty-one proteins, out of a total of 351 proteins identified and quantified in breast milk of allergic and non-allergic mothers, differed significantly in concentration. Remarkably, within this group of 21 proteins, protease inhibitors were found to be present in much higher concentrations in breast milk of allergic mothers. This is an unexpected result as the major HDM allergen, Der p 1, is a protease and its proteolytic activity has been linked to the mechanism of the allergic response. Der p 1 is also known to degrade antiprotease-based defence proteins such as mucosal α_1-antitrypsin, which protects the respiratory mucosa against serine proteases. In addition, it was previously shown that serine proteases and its inhibitors are involved in the maintenance of the epithelial barrier of the skin and airways, and that an imbalance of protease-protease inhibitors allows easier penetration of allergens. Because this proteolytic activity is considered relevant to the pathogenesis of asthma and allergy, protease inhibitors have been suggested as potential therapeutics for these diseases. Breast milk of mothers with an allergy may thus naturally provide components to the offspring that protect against the development of allergy.

Conclusions The non-targeted milk proteomics analysis employed has provided new targets for future studies on the role of breast milk composition in the prevention of offspring allergy, especially with regard to protease inhibitors.

Biotransfer of Milk Mirnas to the Baby: A New Functional Component of Milk?

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MicroRNAs (miRNAs) are small (18-22 nt) RNAs that negatively regulate gene expression by the process of RNA interference. They have recently been demonstrated to occur outside of cells, packaged in exosomes, in many biological fluids - richest of all in milk. A large proportion of milk-derived miRNAs are predicted to have targets on immune genes and pathways and miRNAs are known to have a potent biological effect when transferred between immune cells. Could it be that miRNAs are specifically packaged in milk exosomes so that the mother can influence the development of the neonate by biotransfer of this genetic material?

The first critical step in the hypothesis that milk miRNAs undergo active biotransfer from mother to infant is complicated by the fact that the neonate has the genetic capacity to make these miRNAs itself (although they may be locked by developmental programming in the neonate). To investigate this we have used a transgenic mouse expressing an artificial miRNA in the mammary gland that is present as ~1% of the total milk miRNA population. This artificial miRNA is being used as a biomarker in a cross-fostering experiment. We are studying milk miRNA biotransfer in early, mid and late lactation, as well as post-weaning to determine whether miRNAs that undergo biotransfer persist in the serum and tissues after weaning.

This unique mouse model also presents an opportunity to determine the impact of an artificially introduced miRNA knock-in on natural milk miRNA profiles. In our studies we also contrast the miRNA profile of milk and that of the mammary gland to elucidate the mechanism for milk miRNA packaging. This information will be valuable for future targeted introduction or manipulation of milk miRNAs either for biological studies or for enhanced infant formula.
These studies will broaden our understanding of the functional genomics of lactation and the exciting potential of a post-natal genetic impact of the mother on the infant's development.

Early Supplementation of Phospholipids and Gangliosides Affect Brain and Cognitive Development in Neonatal Piglets

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Background: As human breast milk is a rich source of phospholipids and gangliosides and breastfed infants have improved learning compared to formula fed, the importance of dietary phospholipids and gangliosides for brain development is of interest.

Objective: Herein, we sought to determine the effects of phospholipids and gangliosides on brain and cognitive development.

Methods: Male and female piglets from multiple litters were artificially reared and fed formula containing 0% (control), 0.8%, or 2.5% LACPRODAN® (PL-20, Arla Foods Ingredients, DK), a phospholipid/ganglioside supplement, from postnatal day (PD) 2 to PD28. Beginning PD14, performance in a spatial T-maze task was assessed. At PD28, brain magnetic resonance imaging data were acquired and piglets were euthanized to obtain hippocampal tissue for metabolic profiling. *Results:* Diet affected maze performance with piglets receiving 0.8% and 2.5% PL-20 making fewer errors than controls (80% vs 75% correct on average) (P=<0.05) and taking less time to make a choice (3 seconds vs 5 seconds per trial) (P<0.01). Mean brain weight was 5% higher for piglets receiving 0.8% and 2.5% PL-20 (P<0.05) compared to control; and voxel-based morphometry revealed multiple brain areas with greater volumes and more gray and white matter in piglets receiving 0.8% and 2.5% PL-20 compared to controls. Metabolic profiling of hippocampal tissue revealed that multiple phosphatidylcholine related metabolites were altered by diet.

Conclusion: In summary, dietary phospholipids and gangliosides improved spatial learning and affected brain growth and composition.

Presentation of Award for 2013 IMGC Symposium MVP (Most Valuable Presentation)
Emerging Hot Topics in Milk Science: A Behind-The-Scenes Tour of "*SPLASH!* milk science update"

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Each month, the IMGC publishes an e---newsletter, "*SPLASH!* milk science update," which features four articles on emerging topics in milk science—that's 48 new articles on milk science each year. When publication of *SPLASH!* began, it was questionable whether hot topic possibilities would soon be exhausted, but the content continues to be fresh into its third year. Milk delivers. This talk will highlight some of the most exciting milk science topics and reveal the inner workings of the publication: who are the writers and editors, how topics are selected, and the basics of our publication cycle. The *SPLASH!* newsletter has helped to grow the IMGC: IMGC contacts increased 72% in its first year of publication and by an additional 95% in its second year of publication. Nearly all website traffic is the result of *SPLASH!* content. A vision for the future will be presented with suggestions solicited from the audience to further increase the impact of IMGC publications.