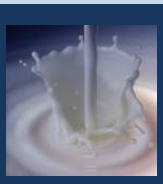
SPLASH! milk science update: May 2012 issue



SPLASH! milk science update
MAY 2012 issue

We are delighted to announce the addition of professional science writer Anna Petherick to our editorial staff. You may already know her through her recently penned review of milk science for Nature.

This month, we bring you a synthesis of recent papers in milk proteomics, a primer on epigenetics in milk production, a review of the ever-expanding superpowers of human milk oligosaccharides, and tantalizing possibilities for mother-to-infant signaling via adiponectin.

Enjoy!

Adiponectin: Mother's fat sends "love letter" to baby via the Milk Express

Venus at a Mirror, by Peter Paul Rubens, 1615

Body fat is not just for buffering us from famine, keeping us warm during winter, and causing our self-recrimination during swimsuit season. Our body fat is also an integral part of our endocrine signaling system. Hormones that are secreted from fat cells- leptin, for example - communicate with the brain triggering hunger (food!) and cravings for calorie rich foods (cheeseburger!). These hormones also contribute to the processes by which we metabolize and assimilate ingested food. The discovery of these hormones occurred relatively recently, and much remains unknown about their function.[1] Among the emerging literature, however, are compelling insights into maternal hormones, their transfer via milk, and their consequences in the developing neonate.

One such molecule that is gaining attention is adiponectin, first described in 2001. This hormone has anti-inflammatory properties and breaks down fatty acids, but adiponectin is particularly important in its role for increasing sensitivity to insulin. Individuals that have healthier metabolisms and lower BMI have higher circulating adiponectin, whereas heavier individuals have lower circulating adiponectin.[2,3] In the mammary gland, adiponectin is secreted directly from the adipose tissue and enters milk. There is only a very low correlation between adiponectin concentrations circulating in blood and the levels found in milk. In humans, about 14% of the variation in milk adiponectin is explained by circulating levels in the blood, suggesting that adiponectin is differentially regulated in the mammary gland than elsewhere in the body. Additionally, humans have adiponectin receptors in their intestinal tract, revealing a pathway by which maternal hormonal signals can influence metabolic pathways in the developing infant.[3] Recently, two studies provided important clues about the milk adiponectin and what it may mean in terms of infant development.

In a large (N=170), well-controlled sample of Canadian mothers, Ley and colleagues[3] showed that in the first week post-partum, adiponectin concentrations in milk are significantly higher than at 3 months post-partum, 50ng/mL vs. 12.3ng/mL. Interestingly, adiponectin concentrations in milk during lactation were not associated with maternal metabolic condition during pregnancy, measured by variables such as body mass index (BMI) and response to oral glucose tolerance test. Other maternal attributes, including socio-economic status, parity, age, ethnicity, physical activity index, and smoking, were covariates in the models. Maternal adiponectin concentrations in serum during pregnancy did predict milk concentrations in the first week post-partum, but only to a minor extent. Similarly, first-time mothers, mothers with a longer gestation, and mothers that had an unscheduled c-section had higher concentrations of adiponectin in milk during the first week post-partum. Why these mothers had higher concentrations of adiponectin was not clear. One explanation is that first-time mothers and women that undergo c-section are more likely to experience delayed onset of copious milk production (aka lactogenesis II or secretory activation). Ley and colleagues did not measure milk volume, but higher adiponectin concentrations may be an artifact if these mothers were producing less milk, and it's likely they were. This may also explain why adiponectin concentrations decrease from the first days of lactation, when many mothers are producing colostrum, to the lower concentrations found in mature milk at 3 months when mothers produce higher volumes of milk.

But what does adiponectin do when ingested by the infant? In an even larger (N=192), similarly well-controlled study, Woo and colleagues[2] investigated monthly adiponectin concentrations in milk across the first 5 months of lactation in a sample of mothers and infants in Mexico City. The first exciting result was that the adiponectin concentrations were substantially higher in the mature milk of the mothers in Mexico City compared to the levels found among mothers in Canada. The sample mean from 1-5 months varied between ~22±5 ng/mL. The Canadian women, on average, were older but of relatively similar parity. Unfortunately, BMI was not reported so could not be compared between the two studies. Inter-population differences could be the result of many factors and warrant further investigation. However, even more fascinating, Woo et al. revealed that infants consuming milk with higher concentrations of milk adiponectin weighed less for their age and length from 1-6 months of age than did infants consuming lower levels of milk adiponectin.[4] But during their second year of life, infants who had consumed higher levels of adiponectin demonstrated accelerated growth; gaining more weight for their age and height than did infants who had consumed low adiponectin milk.

The underlying mechanisms driving the relationship between milk adiponectin and infant growth trajectories are unclear. These data suggest that higher adiponectin in milk slows growth during early development (through reduced appetite?), but then accelerates during the second year of life when they are no longer exclusively breast-feeding and instead consuming primarily solid foods. From an evolutionary perspective, this makes perfect sense. We would predict that milk contains hormonal elements that slow growth or reduce infant appetite in order to reduce the energetic burden on mothers to mobilize body stores for milk synthesis. Every dairy farmer can tell you lactation demands substantial energy expenditure, and the greater the expenditure, the greater the depletion of maternal resources. Depletion delays the ability to conceive subsequently, lengthening inter-birth intervals, and consequently, mothers produce fewer babies in their reproductive career (something that natural selection "frowns upon"). So it could be very adaptive for mother's milk to contain hormones that reduce infant growth when they are reliant on milk but that enable them to catch up when they transition to solid foods and won't be depleting their mother.

However, before I get too carried away getting jazzed about natural selection and adaptations, allow me to exercise some caution. These data are correlational and there may be alternative explanations for the effects described above. For example, adiponectin concentrations in mature milk are negatively correlated with milk glucose[3] (the relationship is weak, but significant), so other milk parameters may be a factor that contributes to infant growth trajectories. Clearly there is much we still need to understand about the relationship among milk constituents and their functions in the developing infant.

** *Neither snow nor rain nor heat nor gloom of night… keeps mammalian moms from nursing their babies. Too bad for reptiles.*

1. Savino F, Liguori SA, Fissore MF, Oggero R. 2009. Breast milk hormones and their protective effect on obesity. Int J Pediatr Endocrinol. Epub 327505. doi: 10.1155/2009/327505

2. Woo JG, Guerrero ML, Guo F, Martin LJ, Davidson BS, Ortega H, Ruiz-Palacios GM, Morrow AL. 2012. Human milk adiponectin affects infant weight trajectory during the second year of life. J Pediatric Gastroenterology Nutr. 54: 532-539

3. Ley SH, Hanley AJ, Sermer M, et al. 2012. Associations of prenatal metabolic abnormalities with insulin and adiponectin concentrations in human milk. Am J Clin Nutr. 95: 867-874.

4. Woo JG, Guerrero ML, Altaye M. et al. 2009. Human milk adiponectin is associated with infant growth in two independent cohorts. Breastfeed Med. 4: 101-109

Contributed by
Prof. Katie Hinde
Human Evolutionary Biology
Harvard University

Do offspring inherit more than genes?

What if the saying "you are what you eat" became "you are what you *and* your parents ate"? The written slate of life's experiences may not be completely wiped clean between generations. How would this knowledge influence our behavior as humans? How would it change livestock production systems?

Emerging research on many fronts is demonstrating that some of the effects of what parents eat and experience in their reproductive lifetimes can be inherited by the next generation. This genetic 'heresy', aptly called epigenetics, or 'above' genetics, potentially has far reaching consequences for understanding disease risk in humans and it may have important implications for the dairy industry.

A recent publication by Singh and colleagues[1] reviews some of the evidence for epigenetic influences in humans, mice, and the dairy cow. Epigenetics is the study of heritable changes in a biological trait caused by molecular alterations occurring at the level of the genome, but not including changes in the underlying DNA sequence.

DNA is the fundamental genetic material of life, which in its entirety is known as the genome of an organism. It contains approximately 25,000 genes in mammals and is unchanged in all cells of an individual. Genes largely code for proteins which are the building blocks and regulators of the biological functions of every cell.

How then does the unchanging DNA sequence present in the zygote, or fertilised egg, generate the huge variety of different adult cell types, such as those in brain, muscle, and mammary cells? The answer to this apparent paradox is epigenetics. This is the process of applying exquisitely detailed programs of molecular marks to the genome which change gene activity during animal development. In this way, each tissue is formed by the activities of different groups of active and silent genes. Importantly, these epigenetic marks are faithfully inherited from one cell division to the next within an animal, yet are usually erased during formation of the gametes, sperm and ova, and during the earliest stages of zygote life. The resetting of the cycle of life is thus complete.

Although animal development is the dominant reason for epigenetic control of gene activity, some epigenetic marks are also modified by environmental influences. These can be acquired early in life and then persist throughout life. In rare instances, these marks are not erased during the formation of new gametes and can be passed onto the next generation. This type of epigenetic mark records life's experiences and can alter gene activity to modify offsprings' responses to changing environmental challenges.

In their recent paper, Singh and colleagues first summarised evidence that epigenetics may play an acute role in regulating the decline in production of milk proteins occurring after cessation of milk removal in dairy cows (mammary involution) and during mastitis. Mastitis induced by different types of bacterial infections caused a shut down of casein protein synthesis in cow mammary tissue and a corresponding change in specific epigenetic marks associated with an important regulator of αS1-casein gene activity. The caseins are the most abundant proteins in milk. The same site in the genome was also similarly epigenetically marked during the early reversible stage of involution when there is pronounced decrease in the activity of this gene. The site then regained its lost epigenetic marks after reinitiation of milking following a period of cessation of milking. Clearly, changes in epigenetic marks in mammary tissue are part of the normal biological adaptations of this tissue to the environmental challenges of mastitis and cessation of milking.

Singh and colleagues next focussed on the evidence for transgenerational epigenetic inheritance of biological traits. Perhaps some of the best documented evidence is derived from epidemiological studies of the Dutch famine of 1944, which is referred to as the Dutch Famine Cohort Study. The study demonstrated that women who were pregnant during this period gave birth to smaller babies who later in life were more susceptible to health problems such as diabetes, obesity, and cardiovascular disease. The children of these children were also smaller, but the effect seems to rapidly diminish in succeeding generations. This study showed there was acquired but waning inheritance of ill health susceptibility traits triggered by an acute environmental challenge, starvation.

This and other information led to the 'fetal origin hypothesis,' or Barker hypothesis (after David Barker who published the theory in 1997), which states that reduced fetal growth is associated with increased risk of a number of diseases later in life. The increased risk results from adaptations by the fetus to the limited supply of nutrients from the mother. It is likely that some of these adaptations involve changes to epigenetic marks at specific sites in the genome, although definitive evidence proving this link is still required.

More compelling is the evidence for transgenerational inheritance in mouse studies. These investigations have, for example, revealed that specific nutritional supplements given to a particular strain of pregnant mice caused changes in the coat colour of adult mice in the next generation. The gene responsible for the coat color change in the offspring was shown to have altered epigenetic marks and altered gene activity, thereby providing a strong link between changes in epigenetic marks and change in coat color.

There is a variety of additional evidence supporting the importance of epigenetics in transgenerational inheritance of biological traits. A mammal contains two copies of each gene, which are resident on paired chromosomes. These paired genes have paternal or maternal origins, but typically their activities are not influenced by the parental origin of the gene. However, for about 100 genes, their activities strictly depend on only a single parent. This pattern of gene activity is called genomic imprinting, and underpinning this effect is a striking program of epigenetic marks in the vicinity of one parental gene but not the other. These genes have strong roles in fetal growth and development and are thought to regulate the delicate balance between the energy demands of the growing fetus and the energy needs of its mother. Parental imprinting of genes can also lead to situations where a single genetic defect can cause two very different diseases in offspring depending on whether the defect is inherited from the mother or father.

Another striking example of these parent-of-origin effects is the cross between a horse and a donkey, which results in either a mule or a hinny, two very different animals, depending on the parental direction of the cross. Moreover, a mutation in the genome of a line of sheep, called callipyge, causes enhanced muscling, leanness, and marked changes in gene activities and epigenetic marks near the site of the mutation. The effects of this mutation only become apparent in offspring when the mutation is inherited from the sire. This is another parent-of-origin imprinting effect. Inappropriate epigenetic marks are also thought to be the cause of the developmental abnormalities appearing in some cloned animals and the condition known as large calf syndrome, which results from the use of assisted reproduction practices. Epigenetic influences are not the biological exception but rather they are at the heart of normal development and animal function.

At present, there is little direct evidence for transgenerational epigenetic inheritance in dairy traits, although there are intriguing hints. Not all of the measured inheritance for dairy traits can be accounted for by simple additive genetic effects. This is not a small deficit as it can account for considerable variability in milk production. Some of this deficit is likely due to epigenetic influences both within and between generations. Further evidence is that a brief period of under-nutrition during gestation can influence subsequent first and second lactations as an adult. This effect is associated with a decrease in use of one particular chromatin mark in late gestation and increased milk protein gene activity during subsequent lactations as an adult. Much more research needs to be undertaken to precisely define the impact of these maternal nutritional influences during gestation on the subsequent milk production of the daughter.

In addition to being the foundation of developmental programs that transform a single fertilised egg into the complexities of an adult animal, epigenetics is also one of the major mechanisms that enable animals to efficiently adapt to changing environmental influences. The latter ability is a huge evolutionary advantage for a species. It may well turn out that the primary inheritance from parents is much more than just their DNA contributions. For the dairy industry, this possibility may provide additional opportunities to enhance milk production through the use of early life nutritional strategies that are designed to impact later in life or even the next generation.

1. Singh et al. Epigenetics: a possible role in acute and transgenerational regulation of dairy cow milk production. (2012) Animal 6, 375-381.

Contributed by
Dr. Ross Tellam
CSIRO Livestock Industries
Queensland, Australia

The Milky500: five hundred worthy proteins

The Indy 500 is perhaps the most famous car race in the United States. Unlike every other sporting competition in the world, the legendary 500 mile car race, held annually in Indianapolis on the last weekend in May, is celebrated with the victor drinking not Champagne, but rather a bottle of fresh milk! Cutting edge research suggests that the term “500” may have more to do with the milk than with the miles.

The quest to completely annotate the milk proteome began nearly a decade ago. The wide dynamic range of milk protein concentrations has so far hindered a comprehensive understanding of its biological role. Impressive analytical advances combined with new separation methods have recently been published in renowned scientific journals and represent a culmination of these efforts, leading to the most comprehensive inventory of milk proteins in history.

It has been known for decades that milk proteins are excellent sources of essential amino-acids, but milk proteins can actually deliver more than just basic nutrition. Many people are aware of bovine and human milk proteins such as immunoglobulins, alpha-lactoalbumin, caseins, and lactoferrin. Recently, increasing numbers of less abundant—but biologically pertinent—proteins are being discovered in other milk “compartments,” within the milk fat globule membrane, for example.

Two new studies published in the last few months deserve special attention considering that when we combined their findings, over 500 proteins were identified in human milk.

First, the study by Gianluca Picariello and colleagues[1] appeared in the "Journal of Chromatography A" in January 2012. This was rapidly followed by the article by Claire Molinari[2] in the "Journal of Proteome Research" in February 2012. Piccariello et al. provided a yet unmatched method to profile low-abundance proteins in human milk. Instead of using the classical electrophoresis-based approach, they first isolated the milk fat globule membrane (MFGM) - a fraction of the milk containing lipids as well as proteins that is typically problematic to study - along with whey protein fractions. Piccariello et al. subsequently employed a shotgun strategy with targeted mass spectrometry. Because of their pre-fractionation strategy, they were able to unambiguously identify 296 proteins, including 165 exclusively present in the MFGM fraction. Because the MFGM proteins are the most genetically conserved milk proteins across species, they are of particular interest from a biological point of view. The identified proteins, which were derived from multiple metabolic pathways, are involved in different physiological functions, such as membrane trafficking, cell signaling, fat metabolism and transport, metabolite delivery, protein synthesis/proteolysis or folding, and immunity-related actions.

The study by Molinari et al.[2] investigated the low-abundant proteins of term and preterm human milk. Similarly to the Piccariello study, they also used various pre-fractionation methods, including a combination of casein depletion and a novel kit designed to enrich low abundance proteins called ProteoMiner, to ensure detection and characterization of the minor protein components. Their use of electrophoretic and separative techniques, combined with advanced analytics (two-dimensional liquid chromatography and MALDI ToF mass spectrometry), led to the identification of 415 unique proteins, 261 of which had not been previously found in human skim milk. The vast majority of proteins identified participate in either immune response or cellular metabolism/growth; some minor components are involved in lipid metabolism and/or nutrient delivery. Among the newly discovered proteins, several growth factors identified may induce nutritional and developmental advantages, but further studies are necessary to assess whether these proteins retain their activity after digestion.

When we superimpose the results from these two studies, we find only 123 proteins in common, bringing a total of 588 different and unique protein entities in human milk.

Recently, D'Alessandro and colleagues[3] provided an updated synopsis of the known bovine milk proteins, merging data from independent studies. After manual removal of redundant duplicates and incomplete proteins, the authors obtained a final list containing 573 non-redundant annotated protein entries, resulting in the broadest bovine milk protein inventory reported to date.

Moving forward, it is noteworthy that all current methods for descriptive analysis of complex proteomes lack protocols that provide a comprehensive snapshot of complex biological systems in a single run. For this reason, specifically designed strategies are required to target common post-translational modifications (e.g. glycosylation) in mammalian milk proteins. Indeed, all studies revealed the protein sequence of numerous previously undiscovered proteins, but to do so, used special enzymes to cleave the carbohydrates (N-glycans) that were coating the proteins. This process, known as N-deglycosylation, is unfortunately accompanied by a significant loss of information. That is, once this method is performed, it is not possible to know neither the extent nor the type of glycosylation. This is a significant drawback because an increasing number of reports demonstrate that protein glycosylation plays an important role in the function of cellular components and processes.

A recent publication by Charles Nwosu and colleagues[4] in the current issue of "Journal of Proteome Research" endorses the fact that it is indeed important to look into the details of glycans attached to the milk proteins. The researchers used nanoflow liquid chromatography coupled with time-of-flight high-resolution mass spectrometry to reveal the first comprehensive N-glycan repertoire of milk. This enables the direct comparison of bovine and human milk glycoproteins as never before. Immunoglobulins, lactoferrin, and casein are all glycosylated proteins, present both in human and bovine milk. They are all thought to have a protective function against pathogenic organisms, therefore playing important roles in preventing gastrointestinal disease. In addition to preventing the proteolytic digestion of proteins in the stomach, it is thought that the glycans participate in additional biological functions.

Nwosu and colleagues were able to determine that human and bovine milk contain over 100 different structures of glycans, corresponding to 38 and 51 unique compositions of isomeric glycan structures, respectively. They observed a pronounced diversity in structures, with only 20 glycan compositions found to be exactly identical in the two milks.
It is important to note that studying glycans is more difficult than studying proteins alone since glycans lack a genetic template, and their compositions are not predictable by bioinformatics methods alone. Studying glycans therefore requires several additional analytical techniques combined with more rigorous analyses.

The combination of all the aforementioned analytical tools for protein and glycan determination may allow for the advancement of our current knowledge of milk bioactives and the characterization of new components, both of which are

vital scientific endeavors for deepening our understanding of the biologically significant components in human and bovine milk. Unbeknownst to them, the Indy500 drivers get a nutritional boost for every mile driven.

1. Picariello, G., et al., Gel-free shotgun proteomic analysis of human milk. Journal of Chromatography A, 2012. 1227: p. 219-233.

2. Molinari, C.E., et al., Proteome mapping of human skim milk proteins in term and preterm milk. J Proteome Res, 2012. 11(3): p. 1696-714.

3. D'Alessandro, A., L. Zolla, and A. Scaloni, The bovine milk proteome: cherishing, nourishing and fostering molecular complexity. An interactomics and functional overview. Mol. BioSyst., 2011. 7(3): p. 579-597.

4. Nwosu, C.C., et al., Comparison of the Human and Bovine Milk N-Glycome via High-Performance Microfluidic Chip Liquid Chromatography and Tandem Mass Spectrometry. J Proteome Res, 2012.

Contributed by
Prof. Daniela Barile & Matthew Lange
Dept. of Food Science and Technology
University of California, Davis

Multi-tasking milk oligosaccharides

Like the moms who produce them, human milk oligosaccharides (HMOs) juggle many different tasks. As we continue to learn about their functions, it seems their to-do list is unending. In a recent review, Jantscher-Krenn and Bode run through the well-established jobs of HMOs and then shift focus onto the ones we're just discovering[1].

HMOs top the list of ingredients that make breast milk distinguishably human. They occur in concentrations of about 15g/L, making them the third most common solid component of breast milk, after lactose and fat. Compared to cows' milk with just 0.05g/L, that is extraordinary. What is also remarkable is the complexity with which HMOs occur. As chains of up to 15 disaccharides hitch to a lactose molecule, there are thousands of theoretically possible structures. And out of those, the enzymes of the mammary gland can generate up to 200-300. Such complexity seems largely explained by the roles of HMOs in clearing pathogens from the gut. A broad variety of structures is needed to combat the great diversity of intestinal germs. It is well known that HMOs act as soluble decoy receptors, adhering directly to 'bad' bacteria and thus smothering their means of grasping the intestinal wall. And, conversely, HMOs attach directly to receptors on gut epithelia, competing with pathogens for a limited number of parking spots.

In their review article, Jantscher-Krenn and Bode focus on several new roles for HMOs.[1] There is growing evidence, the authors assert, that HMOs may influence the development of the intestines, of the immune system, and of the brain. In other words, HMOs are actively and internally--rather than passively and externally--involved in infant biology.
Take, for example, the finding that one sialylated HMO alters the expression of enzymes within CaCo-2 cells in the gut by ultimately modulating the suite of glycans that appear on these cells' surface. Consequently, disease-causing E.coli have a much tougher time of binding to these modified CaCo-2 cells. When tested on different intestinal cell lines in the lab, HMOs changed the growth rates, the differentiation, and the tendency towards apoptosis of these cells.

When it comes to the immune system, some HMOs have been found to induce cytokine production in cord blood mononuclear cells and to tone down the response of allergen-specific T-cells. This suggests some means by which breast milk may lower the odds of an infant developing allergies and autoimmune diseases later in life. In the brain, a greater concentration of sialic acid in gangliosides has been associated with a better ability to learn. Post-mortem examinations reveal less sialic acid in the brain gangliosides of formula-fed infants, who do not regularly imbibe sialylated HMO (a putative source of sialic acid), compared to breast-fed infants.

The important premise to all these reports is that a small percentage of HMO somehow makes the journey from an infants' gut lumen to her urine. That logically implies that these soluble molecules flow freely in the systemic circulation, and can therefore travel all over the body.

This new picture of HMO functionality still contains many holes. Experimenters have looked for HMO in infant blood and somehow come up empty-handed. It isn't clear how HMO might be metabolized or incorporated in the infant brain--nor,

even, the biochemical steps that manufacture them in the mother's breast. Nonetheless, the evidence for an expanded list of the benefits of HMO is impressive. And with that, so is the catalog of potential benefits that formula-fed kids are missing out on. They consume bovine and plant oligosaccharides with very different chemical structures compared to the HMOs that seem to guide various parts of human development. But, if improvements in formula continue as is hoped, that may soon change.

1. Jantscher-Krenn E, Bode L. Human milk oligosaccharides and their potential benefits for the breast-fed neonate. Minerva Pediatr. 2012 Feb;64(1):83-99.

Contributed by
Anna Petherick
Professional science writer and editor
www.annapetherick.com