SPLASH! milk science update: January 2014 issue

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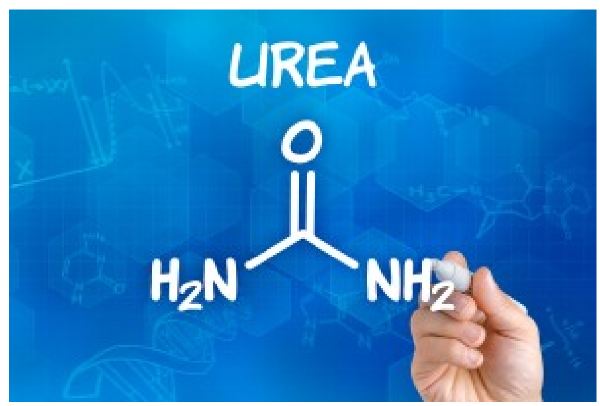
JANUARY 2014 issue

Welcome to SPLASH! 2014! We're jumping off into 2014 with articles about milk metabolites, milk and memory, the healing power of milk, and how rising environmental temperatures affect milk production in cattle!

Milk Makes the Most Out of By-Products

- **Milk contains an assortment of very small molecules called metabolites.**
- **Detailed analysis detected 65 different metabolites in human milk.**
- **Some of these were highly conserved between people.**
- **Interestingly, many of these are normally considered to be waste products.**
- **Milk recycles metabolic by-products to promote health in newborns.**

How do infants get their nutritional and developmental needs without having fully developed intestinal systems? Newborn babies are still developing in many ways, and although maturation of their gut is a high priority, it takes a while for their digestive system to work as it does in an adult. Meanwhile they need to be supplied with nutrients, growth factors, and both the beneficial gut microbes and the ingredients they need to thrive (so that they remain with that person over a lifetime!), all while breastfeeding. To achieve this, breast milk has a complex mixture of components that support all of these requirements. Of these components, we know a lot about the proteins, fats, and carbohydrates, but more recently it is the microcomponents that have come into focus. These are collectively referred to as metabolites, and in a recent issue of the *Journal of Nutrition*, Jennifer Smilowitz and her colleagues from the University of California, Davis, report a detailed analysis of the metabolites in breast milk (1). Surprisingly, when milk samples from nursing mothers were compared, many of the metabolites were found to be present at very similar levels.



Dr. Smilowitz collected breast milk samples from 52 mothers at the same stage of lactation, day 90 postpartum. Along with her colleagues, she then applied nuclear magnetic resonance (NMR) techniques to identify the small molecules present in the milk samples. NMR is the same technology that is used clinically for MRI but applied on a laboratory scale. Using this approach they detected 65 metabolites in breast milk. Many of the metabolites were simple sugars, with lactose present at greater than 40 times the next most abundant metabolite. Some were amino acids, the basic building blocks of proteins, or fatty acids that result from the breakdown of fats. Others were the byproducts of the biochemical processes that generate energy in cells, while the remainder were dominated by members of the vitamin family and DNA derivatives.

The scientists then reasoned that those components most similar between the samples, that is the ones that appear to be conserved, are likely there for a specific purpose. They found that the most highly conserved metabolites are lactose, urea, glutamate, myo-inositol, and creatinine. Lactose is a ready supply of energy for the infant, and it is also known to be responsible for drawing water into milk, thereby helping the milk maintain a consistent composition. They noted an additional degree of conservation in the total level of sugars. However, when the sugars were examined more closely, Smilowitz and colleagues found a distinct division into two groups—those that produce sugars with fucose in a certain orientation and those that do not.

Even more intriguing was the conserved levels of urea in breast milk. Urea is usually considered a nitrogen waste product that is excreted via the kidneys, so why is it conserved at relatively high levels in milk? The authors propose that urea in milk may provide an essential supply of nitrogen for gut microbes. This would promote the growth of those very valuable bacteria that are so critical to a healthy digestive system and important for establishing a healthy immune system in the newborn.

So once again our investment in creating a much deeper understanding of milk has been rewarded. Clearly, breast milk has evolved to be much more than a source of energy and nutrients for the newborn; just as important are the elements it contains for completing physiological development and producing a healthy human.

Smilowitz JT, O'Sullivan A, Barile D, German JB, Lonnerdal B, Slupsky CM. 2013. The human milk metabolome reveals diverse oligosaccharide profiles. *J Nutr* **143**: 1709-1718.

Contributed by
Prof. Peter Williamson
Associate Professor, Physiology and Genomics
Associate Dean of Research
Faculty of Veterinary Research
University of Sydney, Australia

Of Mice and Milk, Mind and Memory

- **Tumor necrosis factor alpha (TNF) in milk affects memory and cognition in mice.**
- **Knockout and cross-foster experiments in mice revealed TNF's role in milk.**
- **Absence of TNF increased the number of cells and their connectivity in a learning area of the brain.**

Model animal systems allow researchers different opportunities for understanding biological phenomena. Depending on the study system, precise pathways from gene to protein to phenotype can be investigated. *C. elegans* and *Drosophila melanogaster* are the heavyweight champions in this domain, but when it comes to lactation, as non-mammals they are of limited use. Enter the lab rat, or mouse in this case. Mice allow researchers to systematically investigate mechanistic pathways through which mother's milk influences offspring neurobiology, immune function, and behavior. And a recent paper in *Nature Neuroscience* by Liu and colleagues (2013) investigated these in concert with the precision and dedication of a neuroscientist Sherlock Holmes.

The target of their research was maternal tumor necrosis factor alpha (TNF), one of the traffic controllers of the immune system. TNF is a proinflammatory cytokine that stimulates the acute phase reactions of the immune response system, regulating the actions of other immune cells. Generally always present at low levels, TNF increases physiologically during immune response. Although primarily associated with the peripheral immune system, TNF is present in the brain and seemingly affects neurons in both positive and negative ways depending on circumstances. Importantly, breast milk contains TNF at low levels, mediating the presence and levels of downstream immunofactors, very small signaling proteins called chemokines.

The researchers created different combinations of knockout (KO) mice to study. Researchers could inactivate the expression of TNF in different tissues by selective deletion of alleles. These KO mice could then be compared with wild-type (WT) mice. There were combinations of heterozygosity (WT(H)) and KO(H)) as well as WT(WT) and KO(KO) homozygotes. Knocking out and reducing TNF level in the mammary gland led to lower levels of immune-modulating chemokines in milk. The pups that ingested lower levels of these chemokines had differential neurodevelopment in their hippocampus. The hippocampus is in the middle of the brain. (Imagine a line through your head from one ear to the other, and a line from the bridge of your nose intersecting that line. That intersection is approximately the location of your hippocampus, in an evolutionary "ancient" part of the brain). Briefly, the hippocampus is crucial for memory, learning, and spatial navigation. The pups of KO moms had more cells in the hippocampus (technically a subpart known as the dorsal dentate gyrus) at 14 days of age. There were no differences once they reached adulthood. In other words, for a small window of time during development, no (or less) TNF and specific chemokines in milk increased the number of cells in the memory center of the brain.

Sounds minor, right? Except that this small change in neurodevelopment had big consequences for memory and behavior. As adults, mice of moms that were KO (or heterozygous) had better spatial memory and spent less time in a freezing behavioral position during a challenge test. And this was specific to the milk they ingested, not their own genotype. They cross-fostered pups among mother types and showed that WT offspring reared by KO moms had the same brain development and behavioral phenotype as KO offspring. Two additional confirmatory manipulations showed that these consequences for offspring were due to the effects of TNF in the mother and its influence chemokines in milk. Blocking TNF production in WT moms with a specific antibody had the same effect as if those moms were genetic KOs. And an artificial cocktail of chemokines mediated by TNF was fed to pups reared by KO moms, recovering normal WT phenotype. These extinguishing, replacing, and cross-fostering manipulations are key components to establishing "lactocrine programming" (Bartol et al., 2008). These gold standard manipulations were suggested and described by Peaker and Neville over twenty years ago (1991), but will be of great value to researchers exploring the pathways by which mother's milk influences infant neurodevelopment and behavior.

Why does the reduction of TNF produce what could be considered a better outcome of improved memory? TNF is sensitive to stress and exercise; it decreases under these energetically adverse conditions. Liu and colleagues hypothesize that TNF may be a reliable source of information about the mother's environment (and therefore the offspring's environment) and lower TNF may reflect more travel to get food, more tangles with other mice to get the food, or a higher density of predators. In such a landscape, spatial memory and less freezing may be very useful traits, useful in the sense that it leads to better survival and reproduction. For mice and other mammals that have very short lifespans, better ability to respond to signals of maternal condition and environment may better prepare them for the world they will experience. This is known as predictive adaptive programming, and the supporting evidence has been equivocal (Wells, 2012). There may be a tradeoff for this better spatial memory though, as the reduction of chemokines ingested through milk may have negative consequences for the offspring's immune system (Liu et al., 2013).

That is the best thing about research–every study gives us more speculations, I mean hypotheses, to study.

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Liu B, Zupan B, Laird E, Klein S, Gleason G, Bozinoski M, Gal Toth J, Toth M. (2014) Maternal hematopoietic TNF, via milk chemokines, programs hippocampal development and memory. *Nat Neuro* **17**:97-105.

Peaker M, & Neville MC. (1991). Hormones in milk: chemical signals to the offspring? *J Endocrinol* **131**:1-3.

Wells JC (2012). A critical appraisal of the predictive adaptive response hypothesis. *Int J Epidemiol* **41**: 229-235.

Contributed by
Prof. Katie Hinde
Department of Human Evolutionary Biology
Harvard University

A Purpose for Spilled Milk

- **Mothers leak milk in the first weeks after giving birth.**
- **For the treatment of diaper rash, mother's milk was as effective as hydrocortisone cream.**
- **Mother's milk was more effective than antibiotics against bacteria that can cause blindness in newborns.**

In the first weeks after giving birth, mothers are a mess of dripping milk. Babies learn how to feed while the mother's mammary glands learn how much milk to produce during what can be a roller coaster of too-much and too-little as the milk supply adjusts. Milk commonly spills on the baby and can even spray the baby's face when he or she unlatches after the flow has started. Does getting milk all over the baby have a purpose? As crazy as that sounds, maybe. Recent studies explored the use of human milk for treatment of diaper dermatitis and eye infections—both potential advantages of spilled milk.

Diaper Rash

Newborn skin is fragile, especially the skin of premature infants. While diapers are a modern invention, the promoters of diaper dermatitis—dampness, friction, fecal enzymes, and some microbes—are ancient enemies. In recent studies, researchers have treated baby's rashes with human milk and compared the treatment against commonly used anti-inflammatory and barrier creams.

In the matchup of milk vs. steroid anti-inflammatory cream (1% hydrocortisone) in otherwise healthy infants, the two were equally effective at resolving diaper rash (Farahani et al., 2013). This is good news given over-exposure to topical steroids can have many harmful side effects, ranging from minor irritation to major hormonal disruptions. Overexposure to milk is not really possible, is it?

In a matchup of milk vs. a barrier cream (40% zinc oxide with cod liver oil formulation) in newborns in the neonatal intensive care unit, the barrier cream won when the dermatitis was moderate-to-severe (Gozen et al., 2013). It seems that milk can only do so much. To limit unnecessary exposure of newborns to chemicals, the authors of the barrier cream study recommend applying human milk as soon as any slight redness of the skin is spotted.

Milk in the Eye

In another study, researchers tested the milk of 23 mothers for its ability to combat pathogens that commonly infect eyes (Baynham et al., 2013). They compared human milk treatment to two other controls—no treatment and a broad-spectrum antibiotic (polymyxin B sulfate/trimethoprim)—on the inhibition of pathogen growth in laboratory plates. Human milk was significantly better than no treatment for three of the nine pathogens while the antibiotic was significantly better than no treatment for all pathogens.

Surprisingly, human milk was better than antibiotics for one particular pathogen: *N. gonorrhoeae*. This pathogen is responsible for the sexually transmitted disease gonorrhea. When an infected mother gives birth, the baby is exposed to *N. gonorrhoeae* in the birth canal and can develop eye infections and subsequent blindness. The ability of milk to combat this pathogen is important, especially because babies whose mothers have inadequate health care are uniquely at risk of untreated gonorrhea and the outcome for the baby can be serious. Furthermore, some strains of *N. gonorrhoeae* are resistant to antibiotics.

The researchers also tested the 23 human milk samples for the presence of various bacteria and found some potentially pathogenic microbes in the milk. The authors suggest that application of human milk to babies' eyes could therefore cause a second infection. However, some of the microbes they implicate as pathogenic are also found in the eyes of uninfected pediatric patients (Weiss et al., 1993) so they may not be particularly pathogenic.

As a practical matter, eye infections should be treated with antibiotics when available. However, in resource-limited settings, human milk is a worthwhile treatment for newborn eyes, particularly given its efficacy for a bug that causes blindness.

Evolutionary Perspective

It's possible that a mother spilling milk on her baby is just another inconvenience of being human. Considering our seemingly useless, but inflammation-prone appendix, and women's unfortunate loss of uterine lining each month, evolution doesn't always yield the smartest design. But given the millennia of evolutionary selection, the products of evolution are always worth a second look.

From an evolutionary perspective, extra milk spilled on a baby may help with protection against pathogens to the extent that it influences survival and reproduction. While a baby is nursing, milk often leaks from the non-nursed breast onto the lower half of the baby, perhaps healing the baby's umbilical cord site or further washing the nether regions. The surprising finding that milk is uniquely honed to combat a pathogen that causes blindness in newborns further suggests that milk spray and leakage is not without purpose. Milk's unique ability to inhibit the growth of this pathogen suggests this particular pathogen has been with humans for a long time and that combating it via milk influenced offspring survival and reproduction. So while spilled milk may be of little consequence in modern nations where antibiotic ointment is immediately applied to every newborn's eyes, at least some of our ancestors depended on it. That's one less reason to cry over spilled milk.

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2. Gozen D, Caglar S, Bayraktar S, Atici F. (2013) Diaper dermatitis care of newborns human breast milk or barrier cream. *J Clin Nurs* [Epub ahead of print] PubMed PMID: 23506257.

3. Baynham JT, Moorman MA, Donnellan C, Cevallos V, Keenan JD. Antibacterial effect of human milk for common causes of paediatric conjunctivitis. (2012) *Br J Ophthalmol* **97**:377-379.

4. Weiss A, Brinser JH, Nazar-Stewart V. (1993) Acute conjunctivitis in childhood. *J Pediatr* **122**:10-14.

Contributed by
Danielle G. Lemay, PhD
Assistant Professional Researcher
Genome Center
University of California, Davis

Farms Feel the Heat

- **Many dairy herds around the world should expect a changing climate in the decades to come.**
- **Often this will mean more days when cows experience heat stress, which reduces milk production.**
- **Better barns and dietary supplements are potential solutions, as is selective breeding, for which some relevant gene variants have already been identified.**

For many regions of the world home to large dairy herds, climate change models predict substantial shifts in the environment. These shifts look likely to harm milk production. The hunt is on, therefore, to define the causal mechanisms by which climatic variations lower milk yields and to figure out ways to keep the cattle of the future comfortable.

The Murray dairy region is a luscious patch of southeast Australia divided among about 1,800 farms. About a fifth of Australia's milk production is pumped from its udders—and there is more pumped per udder than is average for Australia because of Murray's temperate climate, according to Dairy Australia, a government-sponsored dairy organization. In early 2013, Dairy Australia and a group of researchers from CSIRO, the national science agency, published some predictions made by combining climate models and milk production data. They anticipate[1] that in 2015 Murray's cattle will experience 12-15 additional days of moderate to severe heat stress compared to what they typically experienced between the years 1971 and 2000. By 2050, there will be 31-42 additional hot days by the same measure.

Murray is far from the only farming community fearing its milk output will drop. A cursory scan of the literature shows that countries as diverse as Israel, Italy, and Thailand are grappling with how climate change is likely to effect their dairy industries.

By how much are we taking? A really thorough examination of this question can be found in a 2013 study[2] on almost 24,000 cows in Luxembourg, which considered six different indices of heat and humidity stress. By analyzing how milk output correlated with the weather (as recorded by the country's public weather stations) between the years 2000 and 2011, the authors found that a drop-off in daily milk production began when the various thermal indices reached scores of a little over 60. But milk fat decreased at even lower scores, and dropped off more noticeably when thermal indices hit 60.

This is somewhat worrying. A score of 72 (on the most commonly used index), which corresponds to 22˚C (72˚F) and 100% humidity, has become accepted in the academic literature as the point at which thermal stress starts to impact milk production[3]. And that, in turn, suggests that calculations based on those findings might tend to underestimate the problem. The Luxembourg study found that heat-stressed cows produce up to two kilos less milk per day, depending on the degree of heat stress.

Perhaps the most obvious option for places like Murray is to build better barns that can cool their cattle even on the hottest of days. This can be highly effective. In a paper[4] last year, a group at the University of Florida, Gainesville, measured circulating insulin (important in early lactation glucose allocation) in two groups of cows, only one of which had been cooled with sprinklers and fans during the heat-stressed days of their dry period. The dry period is when farm milking is reduced and then stopped in advance of calving. It is also when new udder tissue is formed.

The Gainesville group report that at 42 weeks after giving birth, the cows that were cooled during their dry period produced much more milk (34.0 kg per day) than cows that spent their dry periods in the Florida heat (which produced 27.7 kg per day). The cooler cows also showed reduced circulating insulin 42 weeks after giving birth.

As well as building cooler barns, some researchers propose giving hot cows dietary supplements, such as fatty acids or the essential amino acid, glutamine, which both help reduce the decreased fat yield observed in the milk of heat stressed cows[5]. But a change in genetics—in other words, in breeding strategy—is a longer-term solution. For many decades at least, warm climate breeds like Zebu and Sanga have been crossbred with mega-milk-making Holsteins. These days researchers have the tools to work out why exactly Zebu don't mind the heat, and what it is about the dairy herds in places like Luxembourg causes them to be so sensitive.

Those working in this field have already pointed to a number of important genes for keeping milk production up during heat stress. A study[6] on more than a thousand Chinese Holsteins published in 2011 identified relevant variants in a gene called ATP1B2, which encodes a subunit of a key cellular ion pump. Under heat stress the gene variant denoted by "C2833T" was significantly associated with cows' body temperature, with the potassium ion levels inside their red blood cells, and with milk yield.

A more recent paper[7] in the journal *Gene* looked at the activity of a gene that encodes a heat shock protein, a type of protein that protects cells from heat stress. The particular protein they studied, Hsp70, is especially temperature sensitive and abundant[8]. The researchers found patterns relating how well the 200 cows—all Frieswal cross breeds—handled hot weather to the genetic code in a promoter region (or "on switch") of a heat shock protein gene. One single base deletion from this promoter region, even if it occurred heterozygously (in only one of the two copies of the gene), was linked to significantly lower milk yield in cows chewing cud under the Indian summer sun.

Both studies—and others like them—conclude that the results could be useful in selectively breeding cattle to produce lots of milk in climatic conditions that are currently a struggle for many herds. Much more work is needed in this field. And the dairy industry in regions like Murray should pay close attention.

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

Editorial Staff of "*SPLASH!* milk science update"

Funding provided by California Dairy Research Foundation and the International Milk Genomics Consortium