



### SPLASH! milk science update

August 2014 Issue

This month's issue features articles about the previously unknown diversity of milk sugars, how breastfeeding improves cardiac health in mothers, the search for the dairy gene, and the consequences of a mother's high fat diet on their young.

## Hidden Farmyard Sugars

- **Researchers in Ireland have created libraries of the medium-sized sugar structures present in the milk of common domestic animals.**
- **These sugars are more varied than was previously thought, and are much more acidic than those in human milk.**
- **Among the milks analyzed, pig milk is most similar to human breast milk.**

In recent years, lactation science has paid great attention to the 200-odd medium-sized sugar molecules found in human breast milk. This is because these oligosaccharides (as the sugars are formally termed) have important roles in promoting infant health, and these roles hinge on their structure. It was always understood that the oligosaccharides—oligos for short—of domestic animals are rather dull by comparison. However, a recent survey of the milk of cows, sheep, pigs, horses, goats and dromedary camels has uncovered hitherto unknown diversity. It includes 13 oligos that were thought to be unique to humans (but are not), as well as entirely novel structures.

Simone Albrecht of the National Institute for Bioprocessing, Research and Training in Ireland, and her colleagues, report a suite of generalized distinctions between the oligos in human milk and those in domestic animal milk. For one, the latter contains many more acidic oligos. About 80-90% of the combined pool of medium-sized sugars found in the domestic animal milk are acidic, whereas, in human milk, the majority – around 70% – are neutral. In the domestic animals' milk, one or a small number of molecules, called sialyllactose isomers, are vastly more prevalent than all of the other types of oligos. Another key difference between human and animal milk lies in the phosphorylation of oligo structures. In short, this is rare in domestic animals' milk, but completely absent from human milk.

Why didn't we already know about farmyard milk oligos? To be fair, a good amount has been uncovered about the sugars in cow's milk, but the broader answer to this question is that oligos are much more diluted in the milk of domestic animals than they are in human milk. So the analytical chemistry used to identify them needs to be sensitive to tinier quantities. The advances in this paper are a result of the mixture of techniques that the authors bring together—they use, for example, enzymes from sources as diverse as jack and coffee beans, chicken liver, and calf intestine.

To illustrate the scale of the challenge of identifying these structures in domestic animals' milk, it helps to put some figures on the concentrations involved. A cow that has been lactating for more than a week has a milk oligo concentration of around 0.05mg/l. But a human mother at the same stage of lactation produces milk with oligos present at 12-13mg/l, or at 240-fold higher levels. For this reason, the known variety of oligos in domestic animals' milk is set to rise further in the future, as better techniques are developed to analyze them.



Pigs deserve special mention. Albrecht et al. found that pigs have 40 milk oligos using the techniques that were available to them. This is far below the number that has been found in human milk, and indeed significantly fewer than Albrecht et al. report for camel milk. But pig milk oligos were found to be most similar to human milk oligos, for example in having a higher percentage than the other mammals of neutral, rather than acidic, structures. This makes sense when considering one of oligos' chief roles in infant humans: they aid in the healthy development of the gastrointestinal system. Because both humans and pigs are omnivores (all the other animals in this study are herbivores), with single-chambered stomachs and large intestines stuffed with bacteria that break down food, the developmental guidance required by their gastrointestinal systems is likely to be pretty similar.

However, the similarities only go so far. As with the other creatures discussed here, most pigs' milk oligos contain a kind of chemical bond that classifies them as "type II structures". But the majority of human milk oligos have "type I structures" (a different bond linking the sugars' composite units). Their type I structure is thought to be the reason why certain oligos can be eaten by bifidobacteria, which predominate in a healthy human infant gut. By outcompeting pathogenic bacterial species, bifidobacteria ward off some of the principle killers of human infants, particularly in developing countries.

So when it comes to the usefulness of domestic animals' milk oligos for human purposes, a few reasonable questions arise. It may not make sense to go to the trouble and the expense of extracting oligos from domestic animals' milk in order to make infant formula better at promoting bifidobacteria growth. Yet, on the other hand, some of the oligos in pig's milk might be beneficial to healthy gut development in infant humans. In this way, Albrecht et al's paper has opened the field up to new investigations.

Albrecht S., Lane J. A., Marinõ K., Al Busadah K. A, Carrington S. D., Hickey R. M. & Rudd P. M. (2014) *A comparative study of free oligosaccharides in the milk of domestic animals*. British Journal of Nutrition 111: 1313–1328.

Contributed by  
Anna Petherick  
Professional science writer & editor  
[www.annapetherick.com](http://www.annapetherick.com)

## Maternal High Fat Diet: Consequences for Young

- **Over-nutrition is a form of malnutrition.**
- **Over-nutrition during lactation can dysregulate milk production.**
- **Baby rats nursed by rat mothers eating a high-fat diet show delays in numerous developmental systems.**
- **Maternal diet during lactation affected offspring weight in adulthood.**

Mothers know that they are eating for two during pregnancy and lactation- but more is not always better. In a recent paper, Mendes-da-Silva and colleagues (2014) reported an experiment designed to gain an understanding of how the diet a mother consumed affected offspring development. This research was conducted in the go-to biomedical animal model- the lab rat. Most interestingly, they teased apart the effects of diet during pregnancy from those of diet during lactation by manipulating rat dams either during one status or the other. Feeding the rat mothers a high-fat diet (HFD), particularly during lactation, affected the pups' growth, and physical and neurological development, but not in the way you would think. When more calories were consumed by the mother, pup development was actually delayed.

The negative effects of under-nutrition are well known, but Mendes-da-Silva and colleagues (2014) reveal altered development from dams that are "over-nourished." Specifically, pups nursed by mothers consuming five times the amount of fat in the HFD as in the typical lab rat diet, weighed less than pups nursed by mothers fed a normal diet. Moreover, pups of HFD lactators were shorter in body length for nearly ALL of lactation, managing to catch up with normal pup length the last few days before weaning. In addition, they were delayed in opening their eyes, erupting their teeth and unfolding their ears by 1-2 days. Although a day is nothing to us, and a lifetime for a mayfly, it is an appreciable amount of time for a rat. Indeed, rats are pups for only 21 days before weaning, so multi-day delays are substantial.



The HFD consumed by dams also had consequences for the pups' behavior. Maternal diet during lactation delayed the unconscious reflexes that are critical for responding to environmental stimuli. Pups suckling from HFD mothers were delayed by 1-4 days in their abilities to right themselves "head up" after being placed on a sloping surface, respond to loud noises, twist to land on their feet after being dropped and "vibrissa placing," which is the most boring way ever to say "whisker twitching."

And most problematic, given that globally more people's health is threatened by over-nutrition than under-nutrition, even though these pups were little and delayed while suckling from HFD dams, as adults they were a lot heavier.

How did this happen? Well, unfortunately, Mendes-da-Silva and colleagues (2014) did not directly investigate mammary gland morphology or the specific nutritional or hormonal composition of the milk that might explain the causes of some of these effects. Previous research by others, however, suggests that over-nutrition, like under-nutrition, is a form of malnutrition. But this form of malnutrition is an evolutionary novelty (Hinde and German 2012) that can send physiological systems haywire. Specifically to lactation, over-nutrition can cause metabolic effects that alter mammary gland biology and milk synthesis. Previously reported consequences of over-consuming fat include changes in mammary gland function, milk protein synthesis, and milk volume (reviewed by Mendes-da-Silva et al. 2014). A HFD during lactation increases time spent nursing and fat content in milk from mid to late lactation (Purcell et al. 2011, Rolls et al. 1986).

These findings are largely consistent with those of other studies. A maternal HFD changes the metabolism of young, impairing glucose tolerance and higher circulating leptin- a major regulator of appetite (Sun et al. 2012). Interestingly, unlike the recent Mendes-da-Silva et al. (2014) study in which increased weight of offspring was evident in adulthood, previous research showed that offspring weight gain and tendency toward obesity begins before weaning in both rats and marmoset monkeys (Bayol et al. 2007; Ross et al. 2003).

Mendes-da-Silva and colleagues' paper (2014) is particularly important in several key ways. They demonstrated that maternal diet *during lactation* delays many milestones of rat pup development across multiple developmental domains. Furthermore, their study followed offspring into adulthood, showing that their "catch-up growth" as they approach weaning seemingly set the stage for being overweight in adulthood. Such research should next tackle bioactive molecules in milk that account for these offspring outcomes and better unpack the metabolic pathways involved in both mother and pup.

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Rolls, B. A., Gurr, M. I., Van Duijvenvoorde, P. M., Rolls, B. J., & Rowe, E. A. (1986). Lactation in lean and obese rats: effect of cafeteria feeding and of dietary obesity on milk composition. *Physiology & behavior*, 38(2), 185-190.

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Sun, B., Purcell, R. H., Terrillion, C. E., Yan, J., Moran, T. H., & Tamashiro, K. L. (2012). Maternal high-fat diet during gestation or suckling differentially affects offspring leptin sensitivity and obesity. *Diabetes*, 61(11), 2833-2841.

*Contributed by*

*Prof. Katie Hinde*

*Department of Human Evolutionary Biology*

*Harvard University*

## Breastfeeding Improves Mother's Cardiovascular Health

- **In a prospective trial, women who breastfed for the first five months had lower blood pressure than those who did not.**
- **Mice that do not lactate after giving birth have higher blood pressure than those that do.**
- **Mice with interrupted lactation develop weaker hearts compared with continuous lactators.**

How does breastfeeding alter the odds of developing cardiac diseases later in life? Recently, a small spurt of papers has filled in some important details on this matter. Together, they find that breastfeeding generally promotes a healthy heart. However, if human biology imitates mouse biology, the situation may be more complex for a small minority of women who develop a condition called preeclampsia during pregnancy. For them, it may be that breastfeeding compounds the problem—particularly if they begin and then abruptly stop nursing.



Groer et al. (1) provide strong evidence that breastfeeding broadly promotes cardiac health. Many epidemiological studies on this topic use women with or without heart problems as their starting point, then retrospectively ask about the subject's breastfeeding habits decades earlier. Groer et al. (1), however, used a prospective design. The researchers set about measuring the blood pressure and heart function of women who breastfed continuously for five months, and of women who instead, fed their infants on formula. The resultant pattern was as expected: even when the influence of body mass index was statistically removed- as well as all sorts of other things like income, smoking, and stress levels- women who breastfed had lower blood pressure during the five month period after giving birth.

Poole and colleagues (2), at the University of North Carolina School of Medicine, worked with mice to get a better handle on the known association between breastfeeding and being less likely to develop hypertension down the line. The confounding factors that make this link hard to detect in humans are numerous. Smokers, for example, are both less likely to breastfeed and more likely to experience these exact health problems for other reasons. Obese women are more likely to struggle with high blood pressure and metabolic troubles than women with healthy body mass, and at the same time they also tend to have more difficulties breastfeeding for a sustained period.

To specifically study the effect of terminating breastfeeding early on the odds of these diseases, the team would not have been allowed to work with people. So they randomly assigned female mice to groups with different defined lactation periods. They monitored the animals' body fat, blood pressure, and heart function, showing that interrupting lactation increased the mice's visceral and subcutaneous fat, as well as adversely affected heart function. The hearts of the mice that did not lactate at all seemed to hold up well, but these mice, nonetheless, developed high blood pressure compared with the uninterrupted lactators.

Our understanding of blood pressure's link to breastfeeding also received a recent boost by a team (3) in Japan. This group studied mice that express human versions of a few key proteins, in particular, one called angiotensinogen. This protein is broken down in the liver to form a hormone—angiotensin—that hikes up blood pressure when it is released. The team wanted to understand what is going on inside the bodies of women with preeclampsia, a condition that occurs in about 2–8% of pregnant women. Its symptoms are high blood pressure and proteins appearing in the urine at about the 20th week of pregnancy.

By studying the hearts of mice that lactated and mice that did not (after giving birth), the Japanese team found lactation to play an important role in the onset of problems associated with heart muscle contraction in preeclamptic mice. This was shown using electrocardiograms administered before and during pregnancy, and in the period after the mice had given birth. The molecular underpinnings of these changes are as yet unknown. However, when the answers are revealed, the group's use of human versions of key proteins are more likely to reflect what is happening to the levels of similar molecules in the bodies of pregnant women with preeclampsia.

Do these papers construct a convincing, self-interested argument for choosing to breastfeed, more directly, that is, than merely accommodating the developmental needs of one's infant? Absolutely, for the most part. But they also hint that this is not the case for every woman. It would serve the public interest to understand more about what this means. That would require two of the three findings described above—specifically, those that were performed in mice—to be investigated in humans. Only then should it be considered long-term health advice.

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2. Poole A., Vincent K. L., Olson G. L., Patrikeev I., Saade G. R., Stuebe A. & Bytautiene E. (2014) *Effect of Lactation on Maternal Postpartum Cardiac Function and Adiposity: A Murine Model*. *American Journal of Obstetrics & Gynecology*. Doi: 10.1016/j.ajog.2014.06.004.

3. Murata K., Saito C., Ishida J., Hamada J., Sugiyama F., Yagami K. & Fukamizu A. (2013) *Effect of Lactation on Postpartum Cardiac Function of Pregnancy-Associated Hypertensive Mice*. *Endocrinology* 154(2): 597–602.

*Contributed by*  
*Anna Petherick*  
*Professional science writer & editor*  
[www.annapetherick.com](http://www.annapetherick.com)

## The Search for Dairy Genes

- **Lactation biologists are searching for genes that have a major impact on dairy traits.**
- **Breeds arose as a result of selective breeding that leaves a signature in the genome.**
- **One way to identify signatures is to compare diverse breeds.**
- **Comparing the Korean Hanwoo breed with Holsteins, scientists find highly selected genes.**

The search for gene variants that define a high performing dairy cow continues in earnest with the emergence of tens of thousands of genotypes and an increasing number of genomes sequenced. One of the ways that this can be accomplished is by comparing diverse breeds of cattle. Lee et al. (1) took this approach using the Korean Hanwoo breed, and by comparing this breed with Holsteins, reported findings of highly selected genes.

From the time of domestication, herders and farmers have been practicing selective breeding, eventually giving rise to cattle breeds that today number approximately eight hundred. Breeds that are most numerous include dairy cattle, especially the black and white Holstein-Friesian breed.

The original practice of applying personal preferences for selection evolved into a modern and sophisticated science. The latest genetic tools to apply to this science are “personalized” individual animal genome sequences and SNP genotyping arrays. These same tools are used to evaluate the genetic makeup of existing breeds, with dairy breeds, specifically Holstein-Friesian, as a reference point.



Selective breeding and the formation of breeds produces regions of the genome that have a much higher frequency of characteristic gene variants when compared with ancestors or diverse breeds. They appear within blocks, referred to as haplotypes, within the genome. Along with haplotype blocks, additional SNP variants accumulate over time. So there are haplotype blocks on each of the chromosomes that are characteristic of specific breeds, and within the breeds there are haplotypes that are associated with variation in the phenotype of individuals. However, at some point the most important DNA variants within these blocks- those that have a major impact on a desirable trait- become fixed within the breed or

subpopulation. These genomic features are generically referred to as “selective sweeps,” which together create signatures that can be read from the DNA sequence of each animal. Geneticists working in herd improvement are interested in identifying selective sweeps because they point to the genomic regions within a subpopulation or breed that are most important for production traits. Dairy cattle feature prominently in [these studies](#).

One way to identify selective sweeps is to compare diverse breeds. This was the approach taken by Lee et al. (1) in characterizing selective sweeps in dairy cattle. They contrasted the genomes of Holstein cattle with the Korean Hanwoo breed. Korea is a peninsula, and due to this geography, this breed developed in isolation from ancestral stock over a 4,000-year period. The Hanwoo was originally bred for pulling plows or wagons, but eventually became a domestic beef breed valued for similar meat characteristics to those of Wagyu. Meanwhile, on the other side of the planet, ancestors of the Holstein-Friesian breed developed in Western Europe, initially from ancestral stock brought by migratory tribes around 2,000 years ago. Of course, this breed is now the most productive and widely used dairy breed in the world. So compared with Holstein-Friesian cattle, Hanwoo are about as diverse as any that can be imagined.

There is a series of methods that have been developed for detecting selective sweeps (2-9). Lee et al. (1) initially chose a procedure called the cross-population extended haplotype homozygosity test or XP-EHH. This analysis was able to detect haplotype differences between the two cattle populations by looking for alleles that had become fixed in the respective breeds. Based on these differences, they found over 250 candidate genes that may contribute to the dairy “signature”. When these were compared with candidates from other studies, over 96% were within regions associated with dairy traits. This encouraged the researchers to dig deeper, and what emerged was a consensus of genes from this and previous studies that included casein genes, plus other genes of known function, namely ITGAV, CSRP1, ATP1A2, CASQ1, RAB1A, SULT1E1 and BMP10.

What do we know about these genes? Casein genes have been recognized for many years as markers of selection and dairy cow performance. Most likely this arises from the capacity of dairy cows to produce a high yield of milk protein. The ITGAV gene produces a protein that controls how cells expand and find their place in an organ or tissue. Little is known about the function of the CSRP1 gene product, but it contains domains that are found in other proteins that provide fundamental cellular functions. ATP1A2 is an enzyme that regulates fluid balance by controlling sodium and potassium levels between the interior of a cell and its surrounding fluid. CASQ1 is involved in calcium regulation, most likely through

its capacity for calcium storage. RAB1 is involved in the cellular processes that ensure that proteins are secreted. SULT1E1 is involved in the process of steroid hormone production, and is particularly important for estrogen activity. BMP10 is a member of a family of proteins that have many functions and a complex biology. At this time the precise role of BMP10 is not clear, and we know only of its role in switching between types of muscle protein.

This brief description of functions associated with the genes that were highlighted by Lee et al. (1) provide a good example of the challenges presented by lactation biologists. The proteins produced by nearly all genes function in complex pathways and networks, so individual genes that may have a major effect on the performance of a dairy cow are very rare. It is also much more difficult to find definitive proof of a causal link between a gene variant and a complex trait. This really requires biological experiments that ideally include integrative, functional genomic approaches. Even when these are employed, it is essential to keep an open mind, because sometimes there is not a single gene variant within a haplotype that contributes. A good example of this is the effort to define a role for a single gene in a region on bovine chromosome-6 (10).

Although there is still much work to be done before the mechanisms by which individual gene variants contribute to dairy production traits, what is emerging through convergence of numerous studies based on recent advances in genetics is a common signature that may answer the question, "what makes a dairy cow?"

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Contributed by  
Dr. Peter Williamson  
Associate Professor, Physiology and Genomics  
University of Sydney, Australia

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

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