



SPLASH! milk science update NOVEMBER 2012 issue

Did you miss the IMGC Symposium in October? Or were you overwhelmed by all of the amazing science presented? This month, we provide a brief summary of the work presented at this year's meeting, along with articles about the health value of the glycolipids found in milk, recent groundbreaking research in stem cell research (hint: if it's in this newsletter, milk is involved!), and the very real possibility of beta-lactoglobulin-free milk. What does all of this mean? Scroll down to find out!

Enjoy!

Gems from the 2012 IMGC Symposium

Prof. Johan van Arendonk and his local organizing team from Wageningen University assembled a record 28 presenters to speak over two and a half days, as well as a poster session and dinner at a castle. At risk of offending all of the speakers, here are years of their work compressed into a couple of minutes of yours:

1) The Dutch, French, Swedes, and Danes continue to relentlessly genotype and phenotype their favorite breeds of dairy animals to discover genetic sources of economically important milk traits (Boichard, Larsen, Jensen, Bouwman, Duchemin, Krag, Visker, Eskildsen, Lu, Sundekilde).

2) Like sign posts on the DNA, epigenetic marks help milk cells remember how much milk protein to produce (Devinoy).

3) The nutritive advantages of milk are so substantial that human mutants who can drink it into adulthood have been spreading offspring like fire in a dry forest (Thomas).

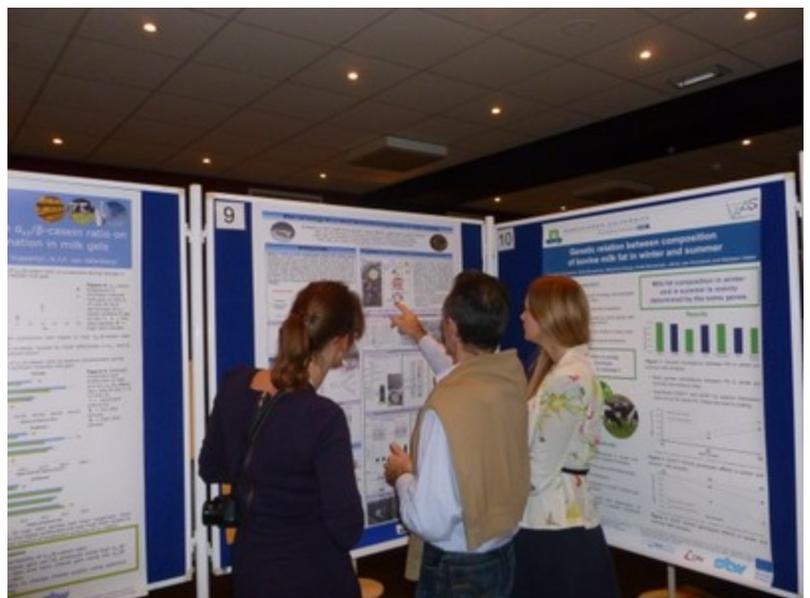
4) The milk of all mammals contains tiny, tiny RNAs (microRNAs); some common, some species-specific (Lefevre).

5) The secret to managing allergies and asthma lies in raw milk, if only there was a way to circumvent those occasional pesky life-threatening infections (von Mutius, German).

6) If you're a woman or a pig, but not a mouse, the mating of estrogen and prolactin produces beautiful mammary glands (Schennink).

7) Fat mice have another reason to spite their thin friends: they have greater difficulty producing milk. Why? Fat mice have increased inflammation and aberrant endocrinology (Hadsell).

8) Whether from the rumen of dairy cows or our own innards, microbes quietly rule the world. We had better learn their language (German, Morgavi, Kleerebezem).



9) How do milk-producing cells move lipid droplets from inside the cell into the milk? Three proteins—one in the cytosol, one in plasma membrane, and one on the outside of the lipid droplet—work together to get the job done (McManaman).

10) Weird Australian animals provide a platform for discovery. How do wallaby mammary glands know whether they're feeding a newborn or a juvenile? Signals from the goo around the milk-secreting cells (Wanyonyi). Echidnas and platypuses (platypi?) produce large amounts of a novel anti-microbial protein in their milk (Bisana).

11) The RNAs of a few milk proteins dominate the total pool like political ads just before an election (Beck, Lemay).

12) Human milk is self-digesting. Imagine you ordered a steak for dinner that cut itself up into tiny bites for you. Milk is that nice (Dallas).

Whew! Got all that? A serious technical review of this year's symposium, as well as an analysis of potential commercial opportunities, will be available to paying sponsors of the IMGC in December. So if you want to know more, pay up.

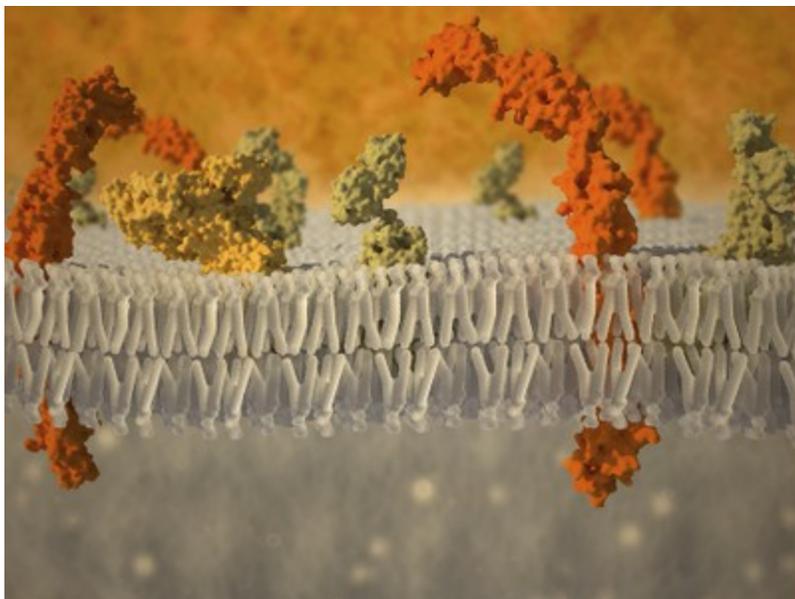
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Buttermilk as a source of protective glycolipids

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- **Healthy human intestines are coated with protective glycolipids.**
- **People with intestinal inflammation/diseases don't have enough of these glycolipids.**
- **Milk is the only known food source for the glycolipids.**
- **Buttermilk made from colostrum could potentially provide a concentrated source.**



Is fat really bad for you? Or could some fat actually be good for your tummy? The common answer might be "That's impossible." But is it? It turns out that one type of milk fat only recently being studied may be especially beneficial for intestinal health. Here is the story.

The human intestinal surface is covered with a layer of complex sugars that act in part as signaling molecules. That is, they transmit information between the cells of multi-cellular organisms. In the sugar layer of the intestines, there are abundant membrane lipids also covered with sugars, and they represent 30-40% of total lipids in the intestinal mucosa. These hybrid molecules that contain both lipids (fat) and glycans (carbohydrates) are called glycolipids. Although originally discovered in the brain, glycolipids are found in virtually every vertebrate tissue, but are especially abundant on the surface of intestinal mucosa.

Recent observations from animal studies show that an inflamed intestinal mucosa has lost many of those glycolipids compared to a healthy intestinal mucosa [1]. Unfortunately, the rapid degradation of glycolipids in the intestine, typical of inflammatory intestinal diseases, is associated with a further increase in pro-inflammatory signaling,

inflammatory markers, and susceptibility to pathogens. Conditions like necrotizing enterocolitis (in infants), inflammatory bowel diseases, and Crohn's disease (in adults) are becoming a new health problem, affecting over 1 million Americans each year. Because neither surgical nor drug interventions cure these diseases, there are increasing demands for new treatments.

One clinical study has already shown the positive role of dietary glycolipids in pre-term infants using glycolipids extracted from porcine brain [2]. However, the estimated average intake of glycolipids by a population not consuming brains is below levels believed to provide therapeutic benefit. Could these precious glycolipids be extracted from brains? Not without exhaustive safety testing that would require decades of investment. A limiting factor in moving this field to the marketplace is the inability to find commercially viable sources of these materials that will allow researchers to move into clinical trials and demonstrate *in vivo* bioactivities.

So, researchers are asking the new question: is it possible to find glycolipids, identical to our protective lipids in the intestinal mucosa, in the food that we eat? Could these dietary glycolipids replace those lost during development inflammatory diseases and re-establish the protective activity? The initial answer is: it appears to be indeed possible.

Glycolipids are also found in milk. The positive effects from nutritional supplementation of glycolipids' in newborns are most likely related to the prebiotic functions of glycolipids, as well as their contribution to an intestinal immune response and service as decoys for toxins [3]. Milk glycolipids have been studied for several years by researchers worldwide; despite their importance to health, isolating them and analyzing their structure is exceptionally challenging. Only recently have we acquired the ability to really understand their structure in detail and to prove that they are identical to the intestinal membrane glycolipids [4]. New results from data collected through advanced mass spectrometry indicate that milk glycolipids have the same structure as those in the human intestinal mucosa, GM3 (NeuAca2-3Gal β 1-4Glc β -Cer) and GD3 (NeuAca2-8NeuAca2-3Gal β 1-4Glc β -Cer), respectively. Therefore, restoring proper glycolipid abundance and function in the intestine through the consumption of milk could resolve inflammation, increase resistance to infection, and improve gut integrity to induce remission of all gut inflammatory diseases.

Will drinking store bought milk provide the desired benefits? The answer to that question is, unfortunately, no. The amount of glycolipids in store-bought milk is too low to elicit bioactive action. However, there is another possibility for recovering and concentrating these molecules from milk.

During industrial milk processing, the fat is disrupted and, as a result, the glycolipids move from the "fat" part of the milk to the "watery" part of the milk. When butter is made, the "watery" part is a byproduct that can then be used to make buttermilk. It is from this buttermilk that glycolipids could be extracted. It turns out that buttermilk can also be produced from colostrum - the early milk - that is naturally richer in glycolipids compared to mature milk. Colostrum contains as much as 63 mg/kg of glycolipids [5]. Therefore, buttermilk made from colostrum could be a rich source of glycolipids.

If buttermilk were to be used in clinical studies to demonstrate enhanced quality of life for people suffering from intestinal diseases, it could become the new star of the dairy industry!

1. Park EJ, Suh M, Ramanujam K, Steiner K, Begg D, Clandinin MT. (2005) Diet-induced changes in membrane gangliosides in rat intestinal mucosa, plasma and brain. *J Pediatr Gastroenterol Nutr.* 40: 487-95.

2. Rueda R, Sabatel JL, Maldonado J, Molina-Font JA, Gil A. (1998) Addition of gangliosides to an adapted milk formula modifies levels of fecal *Escherichia coli* in preterm newborn infants. *J Pediatr.* 133: 90-4.

3. Rueda R. (2007) The role of dietary gangliosides on immunity and the prevention of infection. *Br J Nutr.* 98 Suppl 1: S68-73.

4. Lee H, An HJ, Lerno LA, Jr., German JB, Lebrilla CB. (2011) Rapid profiling of bovine and human milk gangliosides by matrix-assisted laser desorption/ionization fourier transform ion cyclotron resonance mass spectrometry. *Int J Mass Spectrom.* 305: 138-150.

5. Lee H, Barile D, Lebrilla CB, German JB. (2012) High-throughput quantitative analysis of bovine milk gangliosides by ultra-high performance liquid chromatography-tandem mass spectrometry. *American Society of Mass Spectrometry (Vancouver, British Columbia).*

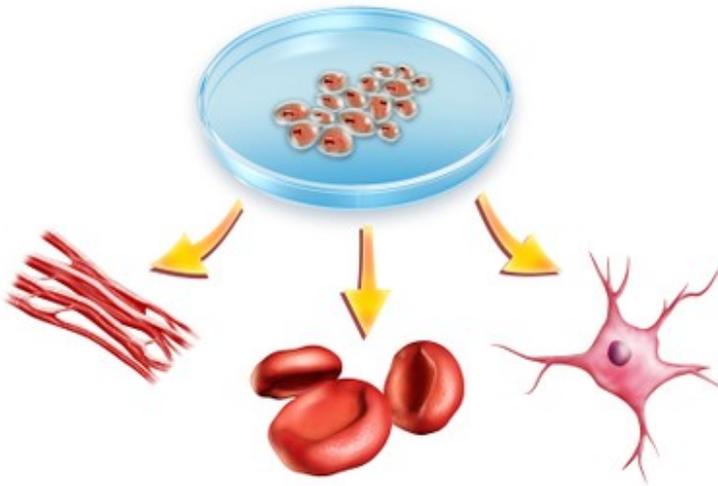
Contributed by

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Human milk contains PLURIPOTENT stem cells

- Stem cells are present in human breast milk.
- These cells can become many different kinds of cells.
- The cells can be non-invasively collected and studied *in vitro*.
- These findings have implications for infant development and regenerative medicine.

Last month during the Bi-Annual Meeting of the International Society for Research in Human Milk and Lactation in gorgeous Trieste, Italy, one could hear a pin drop when Dr. Foteini Hassiotou presented her and colleagues' ground-breaking work on human stem cells in breast milk.



Most of us are familiar with embryonic stem cell research as potentially revolutionary for medical science and human health. This is because during embryonic development, all of our adult tissues derive from three initial germ layers - the endoderm, the mesoderm, and the ectoderm. In this way, embryonic stem cells are pluripotent, which means that the cells have the capability to develop into any of the 200 cell types in our body. However, significant controversy surrounds embryonic stem cell research, constraining research efforts on this topic. Adult stem cells exist, but they are generally more limited in terms of the types of cells they can become- known as multipotent. Although the presence of adult stem cells had been known to occur in mammary tissue, the presence of stem cells in breast milk was established by Cregan and colleagues in 2007. Their initial research suggested that these cells were multipotent and could develop into a limited number

of subsequent cell types. In 2010, the multipotent features of stem cells in breast milk was confirmed by researchers in India (Patki et al., in 2010).

Dr. Hassiotou and colleagues have now established that pluripotent stem cells are active in the lactating breast and can be non-invasively collected from breast milk (2012). Embryonic stem cells have a “core-circuitry of self-renewal” through the transcription of particular genes (OCT4, SOX2, NANOG, SSEA4, & three transcription factors (TFs)) (Hassiotou et al., 2012). hBSC show similar patterns of gene activity that allow for the stem cells to replicate. hBSC were not found in nonactive mammary tissue. Rather, hormonal cues during pregnancy and lactation seemingly activate the stem cells within the mammary gland. Moreover, hBSC are localized within particular areas of the lactating breast. For example, cells expressing TFs were more prevalent in the myoepithelial layer, but much less prevalent in the lumen, ducts, or alveoli. Most excitingly, *in vitro* investigation of hBSC revealed that cells differentiated into cell types of all three germ layers, suggesting pluripotency. For example, hBSC can become neural cells and cells that express insulin, including many others!

In general, discussion of stem cells usually turns to regenerative medicine. Proponents for developing stem cell therapies hypothesize that stem cells could be used to treat patients with spinal injuries, neurodegenerative disorders such as Parkinson's, or Type I diabetics, whose islet cells in the pancreas no longer produce insulin. However, from a developmental biological perspective, I am most intrigued about what these hBSC may do when ingested by the infant. Hassiotou and colleagues suggest that hBSC may behave similarly to immunofactors in the infant, crossing into the infant's bloodstream and playing a role in tissue repair and development.

The discovery of pluripotent stem cells in human milk is a game changer, whether your perspective is regenerative medicine or developmental biology. Research on pluripotent stem cells can now potentially rely on hBSC collected non-invasively, reducing reliance on human embryonic stem cell research. Within the neonate, these stem cells ingested via breast milk may contribute to developmental programming for health and metabolism later in life. We can further hypothesize that stem cells in breast milk may be critically important for tissue development and repair in pre-term and NICU infants. Although there are only a handful of studies on this topic, the implications of this discovery cannot be overstated. I know I am not alone among my colleagues in eagerly anticipating the next discoveries in human breast milk stem cells.

Hassiotou F, Beltran A, Chetwynd E, Stuebe AM, Twigger AJ, Metzger P, Trengove N, Lai CT, Filgueira L, Blancafort P, Hartmann PE. (2012) Breastmilk is a novel source of stem cells with multilineage differentiation potential. *Stem Cells*. 30:2164-74.

Patki S, Kadam S, Chandra V, Bhone R. (2010) Human breast milk is a rich source of multipotent mesenchymal stem cells. *Hum Cell*. 23:35-40.

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Designer cows may help improve human health

- **Around 2-3% of human infants are allergic to β -lactoglobulin (BLG), a protein found in cow's milk.**
- **Using cow microRNAs, scientists in New Zealand have created a method for eliminating BLG from cow's milk.**
- **Feasibility, safety, quality, and health benefits of BLG-free milk need to be addressed.**

Designer jeans are fashioned to suit the individual needs of each human body shape. A good pair of well-cut jeans makes all the difference – they can be tailored to make a person comfortable at an informal BBQ or a theatre premiere. The versatility of the primary design of jeans allows a good fit for one and all, and this is the key to their perpetual success.

Like jeans, the same basic design for milk is used by each mammalian species; milk is just formulated differently to suit the specific needs of the young of each species. The processes of evolution through the action of natural selection have 'designed', or shaped, milk to be exquisitely formulated to meet the unique developmental needs of the newborn of each mammalian species.

The reason for this varied composition of milk is the young of some species, such as the cow, are born in a very advanced developmental state, while for other species, such as man, the young are born in a much less developed state. The different developmental stages of the newborns of these species require different formulations of the basic components of milk to optimise newborn growth and health. Occasionally, the milk from some species contains components not found in the milk of other species.

Allergy to a protein in cow's milk

Cow's milk contains a natural protein, β -lactoglobulin, not present in human milk. The biological role of this protein, apart from making available an additional source of protein to the growing calf, is unclear. About 2-3% of human infants from developed countries are allergic to this cow milk protein. This is not a scientific surprise. The allergy manifests as diarrhoea, vomiting, and to a lesser extent, respiratory issues and skin rashes.

To date, the allergy to β -lactoglobulin in some infants has been minimised by the specialised manufacturing of enzymatically hydrolysed whey proteins, a treatment of milk that converts β -lactoglobulin protein into smaller, less immunogenic fragments. However, this is not a perfect process, and some β -lactoglobulin fragments can still induce allergies in susceptible infants. Removing β -lactoglobulin from cow's milk to make it more like human milk is the obvious alternative.

Cow's milk containing no β -lactoglobulin

New Zealand researchers¹ have now 'designed' a cow that produces little, if any, β -lactoglobulin in its milk. The research was recently published in the prestigious *Proceedings of the National Academy of Science*. The research described in this scientific paper is groundbreaking and opens up the possibility of the production of cow's milk that does not induce an allergic response to β -lactoglobulin in at-risk humans. This new milk potentially fills a niche market designed to satisfy the specific and unique needs of a small section of the population. However, caution and considerable additional research are still required.

There is still a long way to go before this hypoallergenic (low-allergy) milk could be available to the public. First, the milk will need rigorous and long-term testing for safety and quality, initially in animal models and then in humans. Second, the postulated health benefit of this hypoallergenic milk has to be proven in the at-risk human population. Third, suitable herds will need to



be raised and the milk supply line from these cows will likely need separation from mainstream unmodified milk. The welfare of the animals must also be considered. Fourth, there will be a requirement for public acceptance of the technology used to engineer these cows. The latter issue is likely to be a major hurdle, as it involves acceptance of genetic engineering of the cow.

Genetic engineering technology employed to increase agricultural production in animals per se, where the primary benefit goes back to the producer or processor, is unlikely to be accepted by the public in the foreseeable future. However, the use of the technology to produce a proven, safe, and quality food product that, additionally, has strong health benefits to sections of the consumer population, may be accepted and highly successful. After all, diabetics have been using human insulin, produced through genetic engineering of microbes, for a considerable time, and there are many other examples of the use of this technology for human health applications.

A very smart scientific approach

The scientific strategy taken by the New Zealand researchers to remove β -lactoglobulin from cow's milk is very smart. In the future, we are likely to see much more implementation of this general strategy, potentially leading to a wide range of new applications and health products.

The usual scientific strategies for removing a protein are to either eliminate the protein in the processing stage, as already described, or use gene 'knock-out' technology. The latter technology is well-established and routinely used in mice. However, for reasons unclear, gene knock-out technology does not work well in livestock. In an alternative but highly effective, approach, the researchers exploited the recent discovery of microRNA to suppress the production of β -lactoglobulin in cows. This discovery, initially made about 15 years ago, has profoundly increased scientist's understanding of a fundamental regulatory system used by plants, animals, and some viruses to control gene activity.

When a gene is active, it produces, or transcribes, a specific messenger RNA (mRNA) that ultimately encodes the information for making each protein. An mRNA is the carrier of the protein design information encoded within an active gene. A microRNA is a small RNA molecule that targets a specific mRNA, and in doing so, the microRNA blocks the cellular manufacture of the corresponding protein.

The New Zealand researchers first identified ten microRNAs which inhibited the synthesis of cow β -lactoglobulin protein from its mRNA. This research was performed in test tubes using cultured African green monkey skin cells. The best of the ten miRNAs inhibited synthesis of β -lactoglobulin by 98%. Next, the research team used a transgenic mouse engineered to over-produce cow β -lactoglobulin in the mouse mammary gland (mice do not naturally make β -lactoglobulin). By also over-producing a combination of the discovered microRNA with β -lactoglobulin in the mouse mammary gland, the researchers proved that the microRNA could inhibit production of the cow β -lactoglobulin protein in mouse milk by an amazing 95%.

After demonstrating the feasibility of this approach in mice, the researchers undertook a similar experiment in a cow named Daisy. Analysis of the milk from Daisy revealed the complete absence of β -lactoglobulin from her milk. Moreover, Daisy was designed to only produce the microRNA in the mammary gland, and only during lactation, thereby greatly minimising the chances of unintended effects in other tissues, although this expectation has not yet been proven.

A few caveats

The scientists also highlighted a few additional noteworthy features of their research. They showed the removal of β -lactoglobulin from cow's milk corresponded with an approximate three-fold increase in the total casein content of the milk. In particular, there was considerable increase in the κ -casein content (about four-fold), possibly leading to smaller casein micelle size and greater ability to bind calcium. These observations suggest the milk may have retained increased calcium, which is both a strong health benefit to humans and suggests this milk would be very suitable for cheese manufacturing. However, these are only predictions.

The researchers also reported unanticipated features of the technology. Only one cow, Daisy, was born from five pregnancies. The reasons for this are not clear. Daisy was also born without a tail, but this was likely due to the engineering technology itself and not the specific design features that led to the inhibition of β -lactoglobulin protein synthesis, the authors explained. Clearly, considerable future research is still required.

Identifying the biological role of β -lactoglobulin

Scientists can also investigate the biological roles of β -lactoglobulin in cows' milk as they now have suitable animal models and molecular tools. The old concept that the only function of β -lactoglobulin in cow's milk is to supply additional protein to the calf may be simplistic. If this concept is true, it begs the question of why the ancestors of the modern cow

didn't just produce more casein in milk during the evolutionary process. Research showing that β -lactoglobulin binds small molecules in cow's milk, including retinol and vitamin D, suggests there may be a few surprise discoveries in future research in this area. There is also the possibility that cow β -lactoglobulin may have undiscovered positive health benefits for humans.

Milk is uniquely designed for the needs of each mammalian species, and this feature has probably helped underpin the success of mammals as a group. There is much more we can learn about milk by understanding the biological reasons for its design differences between species. Some of this information is likely to lead to new products beneficial to humans.

¹Jabed A, Wagner S, McCracken J, Wells DN, Laible G. (2012) Targeted microRNA expression in dairy cattle directs production of β -lactoglobulin-free, high casein milk. *Proc Natl Acad Sci USA*. **109**:16811-16816.

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