

SPLASH! milk science update: April 2012 issue



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Welcome to IMGC's monthly newsletter. We find and translate the best, recent discoveries in milk science and human health.

Save Time, Read *SPLASH!*



Imagine if you spent every minute of every day reading scientific articles that have the keyword "milk" associated with them. Suppose you read one article per hour, 24 hours a day. Even with this impossible regimen, you could not cover even half of the milk-related articles published each year. In 2011, there were over 20,000 journal articles published with the keyword "milk" in the PubMed, CAB Abstracts, Agricola, and FSTA databases.

The purpose of "*SPLASH!*", IMGC's innovative newsletter, is to make it easier for you to stay up-to-date with the scientific literature. Much easier. Our goal is to keep you current on the hottest topics in milk science and human health in less than an hour per month by featuring the most important articles in today's scientific journals and explaining why they're important.

"*SPLASH!*" has an all-star team of contributors including a co-leader of the Bovine Genome Sequencing and Analysis Consortium, an anthropologist/primatologist who directs the Comparative Lactation Laboratory at Harvard University, the leader of the Dutch Milk Genomics Initiative, a food scientist and chemist with a research program focused on bovine milk functional glycomics, and a co-leader of the Bovine Lactation Genome Consortium. You can read more about us at [IMGC Newsletter Editorial Staff](#). Join us, your milk science experts, for captivating insights and reviews of milk research during 2012.

-[Danielle G. Lemay](#), Executive Editor

The Many Faces of Lactoferrin

The Many Faces of Lactoferrin – variation is the name of the game!

Fresh out of the womb, a newborn baby is challenged with armies of disease-causing microbes. How does he survive this onslaught? In some parts of the world, he doesn't. Millions of babies die each year in the first few months of life from common infections.

A recent publication by Barboza and colleagues¹ reinforces the importance of mother's milk as a major factor protecting a newborn from infection. Milk is much more than just a convenient source of high quality nutrition for the newborn. The researchers have now expanded our knowledge about the biological defenses in mother's milk by focusing on the antimicrobial properties of a highly abundant human milk protein, lactoferrin. They characterised the amazing versatility of this protein, both in terms of its structure and antimicrobial activities.

The newborn has an immature immune defence system. Consequently, it strongly depends on maternal factors to help protect it against bacterial and viral infections. This first line of defence against infection must rapidly neutralise threats and discriminate between foreign and self as well as pathogenic (disease-causing) and non-pathogenic bacteria. While milk provides the newborn with the fundamental building blocks for growth and development, including amino acids derived from the digestion of milk proteins, and energy originating from the metabolism of fatty acids, it has a number of other biologically important functional roles, too. Components in milk directly promote maturation of the newborn gut mucosa, the growth of beneficial bacteria that aid digestion, and protection of digestive epithelial cells against attachment and invasion by pathogenic bacteria.

Barboza and colleagues investigated the finer structure and antimicrobial activities of one of the most abundant human milk proteins, lactoferrin. This protein is an iron-binding glycoprotein that is found in the milk of most mammals. A glycoprotein is simply a protein that has sugars, often complex sugars, attached to specific regions of the protein. Researchers have long known that a protein component of lactoferrin, a peptide called lactoferricin, has antimicrobial activity. This peptide binds bacteria and uses the iron bound by lactoferrin to produce toxic peroxides which then destroy the bacteria.

The researchers have now uncovered remarkable and previously unrecognised variation in the antimicrobial activities of lactoferrin that are separate from those of lactoferricin and mediated by an array of complex sugars attached to different regions of the protein. The big surprises from the results of this research are the extent and variability of these complex sugars as well as the range of their antimicrobial activities.

Complex sugars are often attached to proteins secreted from cells. The general view asserts these complex sugars have simple physical roles that help stabilise protein structure and increase protein solubility. However, emerging research indicates that complex sugars provide other services as well. In the case of lactoferrin, it plays key roles in generating a diversity of protective antimicrobial activities.

Barboza and colleagues first identified the spectrum of different types of complex sugars attached to human lactoferrin using a highly sensitive and selective technique called mass spectrometry – a technique that uses a targeted laser beam to energise small molecules which are then accelerated in a strong magnetic field thereby enabling their masses and structures to be determined. The initial revelation Barboza and colleagues made about lactoferrin using mass spectrometry was the considerable diversity of complex sugars attached to lactoferrin.

They then analysed these complex sugars, isolating them from the milk from five mothers over a time period from one day until 10 weeks after birth. The researchers showed the complex sugars attached to lactoferrin were very dynamic both in type and overall quantity during this period, which paralleled the lactational transition from colostrum to milk production in the mothers. There was also considerable variation of the complex sugars between milk samples from individual mothers at the same lactation time.

Another result from Barboza and colleagues pertains to the mode of bacterial infiltration into the body. Some pathogenic bacteria can adhere to digestive epithelial cells before gaining entry into the cell and thereby entry into an individual. Barboza and colleagues showed that the different types of complex sugars attached to lactoferrin help prevent enteropathogenic bacterial attachment and in some cases invasion of colonic epithelial cells. However, the most remarkable discovery they made is that different complex sugars have selective effects on attachment to and invasion by different microbial species. Thus, the complex sugars attached to lactoferrin are exquisitely variable and specific in function, presumably enabling efficient neutralisation of the unpredictable onslaught on a newborn by a wide variety of bacterial challenges.

The new information about lactoferrin is very interesting as it emphasises the advantages of structural variation and provides us a glimpse into a fundamental evolutionary strategy designed to improve the survival chances of a newborn. Apart from demonstrating how little we know about even one of the most studied milk proteins, this research hints at future applications and investigations.

Although most proteins are strongly conserved in mammals, one of the greatest differences between species relates to the types of complex sugars attached to glycoproteins. Thus, lactoferrins from the milk of other species, such as cow, may have unique antimicrobial properties moulded by the specific microbial challenges in the environment of that species. Some of these specific antimicrobial activities could be very useful to humans.

If lactoferrins of other mammals possess different types of antimicrobial activities, it may be possible to isolate these other lactoferrins, such as those from a cow, and use them to specifically boost the antimicrobial properties of human infant formula, or to produce a food product that acts as a prophylactic to prevent specific gastrointestinal diseases prevalent in at-risk individuals, such as travellers. There may be many additional potential uses for lactoferrins from other mammals as well.

The source of maternal variation in the complex sugars is also of great interest. Is it genetically based, or can it be manipulated by diet, perhaps even before lactation and during pregnancy? Other questions raised by these results relate to mechanism. How do these complex sugars exert their antimicrobial activity? Are they just decoys that prevent pathogenic bacteria from binding to their target cells or do they have more direct protective roles? Lactoferrin is clearly a multifaceted protein par excellence. The name represents an array of physical forms that confer a spectrum of antimicrobial activities. Lactoferrin probably has many more functional faces yet to be discovered.

Contributed by
Dr. Ross Tellam
Senior Principal Research Scientist
CSIRO Livestock Industries
Queensland, Australia

The "Ripened Cheese" Treatment for Obesity and Diabetes

Brie cheese lovers everywhere have reason to rejoice. Researchers from the University Catholique de Louvain in Belgium found that eating ripened cheese decreased blood sugar levels and fat tissue in obese/diabetic mice [1]. The study conducted by Cani and colleagues comes at a crucial time for our public health. According to the National Institutes of Health, more than 65 percent of American adults are overweight or obese. Obesity is the second leading cause of preventable death in the US, with nine million children and teens ages 6-19 already overweight. Type 2 diabetes, a condition usually associated with adults, has been described as a new epidemic in the American pediatric population, yet no specific treatments are available for pediatric patients with type 2 diabetes.

Obesity and type 2 diabetes are both associated with insulin resistance. Insulin is a hormone that normally mobilizes glucose, a sugar that is a crucial source of energy for the body, into cells, thereby providing fuel for our cells. In patients suffering from obesity or type 2 diabetes, insulin works less efficiently. This means glucose remains in the bloodstream rather than being transported into cells. The body's fuel is collecting outside of the cells rather than being utilized by them.

Another disease associated with obesity and type 2 diabetes is nonalcoholic fatty liver disease. It affects as many as 30% of adult Americans and occurs in people who consume little or no alcohol. Nonalcoholic fatty liver disease is characterized by a marked accumulation of fat inside liver cells. This fat accumulation causes liver failure, similar to that observed in alcoholic cirrhosis. Unfortunately, there is no cure for fatty liver disease, and treatments are aimed only at reducing underlying risk factors such as obesity, diabetes, and hyperlipidemia, a condition in which a high level of lipids (energy storing molecules) collect in the blood.

For patients suffering from obesity, type 2 diabetes, and nonalcoholic fatty liver disease, cheese may be part of the solution. Although in the past cheese and all dairy products containing milk fat have been incriminated as a source of unhealthy saturated fatty acids, recent studies challenge the notion that unsaturated fatty acids in dairy products are deleterious to one's health. Epidemiological studies show that consumption of milk and other dairy products is actually associated with a reduced incidence of obesity, insulin resistance, and type 2 diabetes [2-4].

Among these studies is one recently published in the *Journal of Agricultural and Food Chemistry* by a Belgian group from the University Catholique de Louvain. The research team, led by Dr. Cani, discovered that feeding obese and type 2 diabetic mice with cheese ripened for over a month significantly improved glucose tolerance, decreased lipid levels in the liver, and also decreased cell damage in the mice. The results of this study lead the researchers to believe the duration of cheese ripening is an important factor in the physiological impact of dairy product ingestion.

During their month long study, Cani and colleagues compared the effects of feeding mice cheese ripened for 35 days, 15 days, or non-ripened control cheese. Only the cheese ripened for 35 days significantly reduced levels of glucose in the blood without changing insulin secretion.

Now Cani and colleagues are working to understand the mechanisms behind their important findings. They speculate the interaction of different microbes in the ripened cheese may contribute to the beneficial effects observed. During the ripening process, interactions between specific bacterial communities lead to the final composition of fermented dairy products. Each microbial population produces metabolites and enzymes that break down cheese proteins into bioactive components. Cani's group and others have demonstrated intestinal bacteria, including those ingested with the ripened cheeses used during the experiment, interact to control lipid levels in fat tissue and the liver.

Scientists in this line of research hope to discover specific mechanisms that will help them decipher the complex interaction between dairy product fermentation and human health. The ultimate goal is to deliver successful therapeutic solutions for those living with type 2 diabetes, obesity, and other diet related conditions. Until then, enjoy your Camembert.

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- 2) Pereira, M. A.; Jacobs, D. R. Jr.; Van, H. L.; Slattery, M. L.; Kartashov, A. I.; Ludwig, D. S. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. *J. Am. Med. Assoc.*(16), 2081
- 3) Choi, H. K.; Willett, W. C.; Stampfer, M. J.; Rimm, E.; Hu, F. B. Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study. *Arch. Intern. Med.* 2005 165(9), 997-1003.
- 4) Fumeron, F.; Lamri, A.; Abi, K. C.; Jaziri, R.; Porchay-Balderelli, I.; Lantieri, O.; Vol, S.; Balkau, B.; Marre, M. Dairy consumption and the incidence of hyperglycemia and the metabolic syndrome: Results from a french prospective study, Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care.* 2011 34 (4), 813-817.

Contributed by

Prof. Daniela Barile
Assistant Professor
Dept. of Foods Science & Technology
University of California, Davis

Building Baby's Brain: Milk does the Heavy Lifting

Milk makes a baby grow, including the baby's brain. DHA, for example, is an omega-3 fatty acid that is a critical structural component of the brain. DHA, and other LCPUFA (long-chain poly unsaturated fatty acids), naturally occur in mother's milk and are ingested by the infant during critical periods of neurodevelopment. More fatty acids are thought to enhance neurodevelopment which in turn produces better cognitive function. A number of studies supported these predictions and influenced companies to start adding fatty acids to infant formulas starting about a decade ago. But there is now a growing body of evidence that milk contains numerous bioactive micro-constituents beyond LCPUFA that are critical for the development of the infant's brain.

For example, in their recent paper, Serpero and colleagues (2012) outlined the functional properties of "brain" proteins found in human breast milk- specifically Activin A and S100B. Activin A is important for brain cell growth and development. Additionally, Activin A shields brain cells from damage. S100B is part of the calcium-binding protein family and is also a brain specific protein that regulates communication between cells, as well as cellular energy metabolism and growth. In addition to characterizing the functions of Activin A and S100B, Serpero and colleagues address two important issues when thinking about milk and milk science applications; developmental priorities of the neonate and enhancing milk formula.

Developmental Priorities of the Neonate

In each species, unique properties of milk are specific to the developmental needs of the infant of that species. For example, compared to other mammals our size, humans have a brain that is seven times larger. Most of our brain growth and development occurs during infancy when we are ingesting breast-milk or formula. Serpero and her co-authors revealed two important clues about the importance of these brain proteins for the human neonate. First, the concentration of both Activin A and S100B is higher in the mother's milk than in her bloodstream. This means the high concentration of these proteins in milk is *on purpose* rather than a byproduct of the mother's circulation and suggests Activin A and S100B are critically important for the human neonate to ingest. Secondly, the concentration of S100B in human breast milk is higher than in the milk of other domestic dairy animals. Differences in the concentration of the brain protein S100B in milk between human and non-human species likely reflect the greater post-natal neurodevelopment of the human neonate. Activin A may also be higher in humans, but data are not yet available.

Enhancing Milk Formula

Currently, the brain proteins Activin A and S100B are not detectable in commercial milk formula. The absence of Activin A and S100B could be a result of lower concentrations in cow's milk to start with, but the amount of these proteins may also be diminished during commercial food processing. For example, bioactive properties of S100B are retained through pasteurization, but are degraded by spray-drying. Identifying the presence and abundance of bioactive constituents in human breast-milk, and their function in the developing infant, provides opportunities to better engineer commercial infant formula. After all, there was also a time when commercial formula lacked supplemental long-chain polyunsaturated fatty acids like DHA. Improvement of milk formula composition will come from advances in formula preparation and from the make-up of the milk that dairy animals produce. Researchers can now insert human DNA into transgenic, cloned dairy

cows to express human-type lactoferrin and lysozyme in cow's milk. Lactoferrin and lysozyme are immune constituents with anti-bacterial properties, and similarly to S100B, they are found in higher concentrations in human breast milk than in cow's milk.

Conclusions

As a science, we still know very little about the mechanistic and functional outcomes of most milk constituents in isolation, not to mention their complex, synergistic interactions. However, we are clearly at the outset of significant advances in milk science because biochemical, microbiological, and genomic tools for investigating milk are exponentially expanding. With these tools, we can expect to better answer the questions, what exactly is milk, and how does it do a *baby* good?

Serpero LD, Frigiola A, Gazzolo D. 2012. Human milk and formulae: Neurotrophic and new biological factors. *Early Human Development*. 88:S9-S12.

Contributed by

Prof. Katie Hinde
Assistant Professor
Human Evolutionary Biology
Harvard University
Cambridge, Massachusetts, U.S.

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

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