



SPLASH! milk science update DECEMBER 2012 issue

This month, many of our articles make links between human health and milk. We explore a [glycan found in human milk](#) that may help curb metabolic disease, how [milk helps oral health](#), and how [milk lymphocytes help protect infants](#) from HIV. Additionally, did you know [mammals aren't the only ones](#) that produce milk for their young? Read on to learn about these fascinating milk findings.

Enjoy!

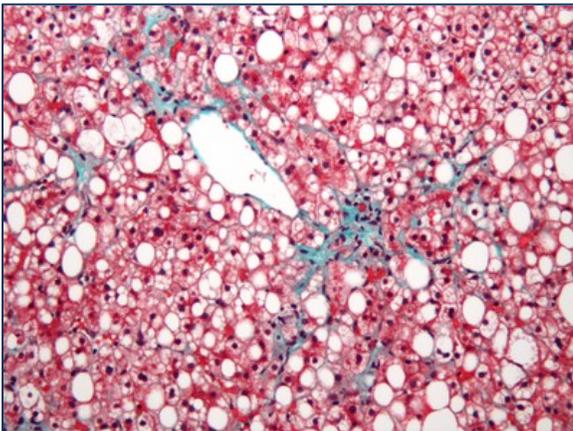
Milk glycan cures metabolic disease (in mice)

- **An unusual glycan found in human milk--LNFPIII--improves insulin resistance in mice.**
- **When cells detect LNFPIII, they produce an anti-inflammatory signal (IL-10).**
- **This anti-inflammatory signal upregulates insulin receptors, thereby increasing insulin sensitivity.**
- **LNFPIII causes the liver to burn more, and make less, fat.**

What do human milk and parasitic worms have in common? It sounds like an opening to a joke, but the answer reported in *Nature Medicine* is seriously cool. Both human milk and parasitic worms contain an unusual glycan that improves insulin resistance and reverses fatty liver disease in a mouse model (Bhargava et al., 2012).

This wonder glycan is called “lacto-N-fucopentaose III,” or “LNFPIII” for short. To understand what’s so cool about LNFPIII, let’s first review how the body handles energy from food and what happens when people chronically eat way more food than they should. Yes, they get fat, but other less visible, yet nefarious things happen, too.

Glucose and fat: The currencies of energy



Non-alcoholic fatty liver disease. Copyright © 2009 Nephron (<http://commons.wikimedia.org/wiki/User:Nephron>). Permission is granted to copy, distribute and/or modify this image under the terms of the GNU Free Documentation License Version 1.2 (<http://www.gnu.org/copyleft/fdl.html>) or any later version published by the Free Software Foundation.

Glucose is a convenient fuel for the body, readily used by all tissues, while triglycerides (fats) are more compactly stored. The liver acts as a Currency Exchange booth, converting fat to glucose or glucose to fat, depending on whether the fuel is to be burned or stored.

After eating, glucose rises in the blood, and the pancreas releases insulin, which is like a messenger that goes around telling all of the cells, “Hey, get your glucose.” If a person has lots of glucose in their blood all the time (like if a person never stops eating), the cells stop listening to the insulin messenger. They think, “Oh, that guy again,” if they even hear him at all. This is called “insulin resistance,” and it’s a growing epidemic among people who eat too much and move too little.

Amazingly, LNFPIII convinces fat cells to respond to insulin again. In other words, it improves “insulin sensitivity,” at least in mice that became obese as a result of a high-fat diet. Until now, the only natural cure for insulin resistance was the dreaded E-word: *exercise*.

Negotiating inflammation

Just how does LNFPIII convince fat cells to respond to insulin? Ah, the plot thickens (read: gets muddier). Interestingly, it involves resident immune cells (macrophages) that stand guard in every tissue in the body, waiting for a pathogen to appear. There are two types of macrophages: M1 and M2. M1 macrophages are like warriors. When they sense a foreign invader, they spew pro-inflammatory signals somewhat indiscriminately, often causing collateral damage to innocent neighbors. The M2 macrophages are like “peace keepers.” They repair the damage caused by the warrior M1s.

In obese individuals, fat tissue is infiltrated with warrior M1s. This causes low-grade, chronic inflammation.

When cells detect LNFPIII, they produce an M2-like, pacifying signal (IL-10) to dampen the effects of the warriors. In addition to this anti-inflammatory effect, the pacifying signal also causes neighboring fat cells to upregulate insulin receptors and their friends. With more receptors to detect insulin, the cells can “hear” the message to consume glucose again. “Insulin resistant” transforms into “insulin sensitive.”

Foie gras, not so tasty after all

When a person chronically overeats, their liver goes into fat-making overdrive. Instead of packaging the fat to be sent to other tissues, so much fat is produced that it gets stuck in the liver. This is called nonalcoholic fatty liver disease, or hepatosteatosis. Or “foie gras,” in the case of a force-fed duck.

LNFPIII transforms the liver: fat production slows, fat destruction quickens. And this is not merely a nice side-effect of the improved insulin sensitivity of distant tissues. LNFPIII exerts these effects locally as well as systemically.

Milk is the drug

No pharmaceutical can deliver such multi-modal positive health effects. Someone, somewhere, must be productizing LNFPIII. In the meantime, the choice is parasitic worms or human milk.

Milk wins again.

Bhargava P, Li C, Stanya KJ, Jacobi D, Dai L, Liu S, Gangl MR et al. (2012) Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. *Nat Med.* 18: 1665-1672.

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Milk promotes oral health

- **Lactoferrin, a milk protein, reduces the ability of gum disease-causing bacteria to stick together in biofilms.**
- **Osteopontin, another milk protein, makes bacterial biofilms easier to break up.**
- **Compared to cow's milk, a common mouth bacteria produces 4-5 times as much cavity-creating acidity in the presence of soy milk.**
- **Compared to formula-fed infants, breastfed infants have more mouth-friendly microbes.**

In almost every European language, milk has lent its name to the first teeth that people develop. But the association between milk and teeth is much more than linguistic, as a series of papers published this year demonstrates. Researchers from Denmark and Australia have reported that cow's milk helps to reduce the impact of bacterial species known to contribute to the development of cavities and gum disease. Another group, in Sweden, has found breast milk to have similar properties.

The bacteria in question tend to form carpets of cells called biofilms, which most of us recognize as dental plaque. Over time, when a biofilm on the surface of a tooth becomes populated by more acid-producing species, such as *Streptococcus mutans*, decay sets in—and more rapidly when those bacteria are well-supplied with sugar. When a biofilm extends into the gum, it slowly destroys the tissues that keep teeth in place and also increases the risk of several cancers elsewhere in the body. The species, *Porphyromonas gingivalis*, is often implicated in chronic gum disease.

So how does milk fight biofilms? By chemical warfare.

At least two proteins in cow's milk—lactoferrin and osteopontin—seem to be rather good at fighting biofilms. Lactoferrin was already known to make it harder for micro-organisms to find initial footholds on saliva-covered surfaces. In a recent study, Stuart Dashper at the University of Melbourne, in Australia, and his colleagues added to this information: they showed that lactoferrin works by impeding two enzymes positioned on the exterior of *P. gingivalis* [1]. In doing so, it



greatly reduces the cells' ability to stick together in biofilms, but without harming the rate of *P. gingivalis* cell division in a suspension. That's okay, because in a soup, rather than an invasive carpet, *P. gingivalis* isn't so bad for gums.

Like lactoferrin, osteopontin is a protein with lots of carbohydrate segments. In August, a Danish team led by Sebastian Schlafer, of Aarhus University, reported that osteopontin makes biofilms easier to break up by altering their species composition [2]. Osteopontin seems to make life particularly tough for another bacterium, *Streptococcus mitis*, to make headway in populating the biofilm. But the details of exactly how this protein destabilizes biofilms aren't known. Intriguingly, osteopontin even worked its magic on biofilms that had started to form 12 hours before it was applied, suggesting it might one day be a useful ingredient in a twice-a-day tooth-brushing regime.

How does milk's chemical warfare measure up against the alternatives? Dashper and colleagues have considered this question by studying the amount of acid released by cavity-causing *S. mutans* when it bobs about in soy milk as opposed to cow's milk [3]. Meanwhile, Pernilla Lif Holgerson's group in Umeå University, Sweden, have compared the oral bacteria of infants fed breast milk to those fed infant formula [4]. In both cases, real milk comes out on top.

In fact, in Dashper's experiments, *S. mutans* made five to six times more acid when it grew in an environment containing soy milk at pH 6.5 compared to one containing cow's milk at the same starting acidity. And even as the environment became more acidic, this harmful bacterium did better in soy milk: at pH 5.5, it produced three to five times as much acid as it did when swimming in cow's milk. These results indicate that even though soy milk is often marketed as a health product, it is probably not doing great things for mouths around the world. One reason for this is that the calcium in soy milk is less available to act as a buffer.

To compare breast milk to formula, Lif Holgerson's team took samples from the mouths of three month-old Swedes, and tried to culture the bacteria in those samples in their lab. They knew that the earlier in life humans pick up *S. mutans*, the higher their chances of developing caries. And that other bacteria can inhibit *S. mutans*'s growth--notably, those of the genus lactobacilli.

The team failed to grow a single lactobacilli colony from the samples that came from formula-fed infants. Conversely, the samples from infants fed breast milk either exclusively or part of the time gave rise to obvious lactobacilli populations in 27.8% of cases—not an impressive proportion, but better than nothing. Overall, the biofilms from the formula-fed infants' samples contained more anaerobic species of bacteria, which are associated with gum inflammation. And the lactobacilli that the researchers grew from the breastfed infant samples did indeed inhibit *S. mutans*, as well as its friendlier cousin, *Streptococcus sanguinis*.

Taken together, these four papers suggest that drinking milk is the healthy option for teeth and gums. They also point to the possibility of adding the useful components of milk to oral health products. For that, much more testing will, of course, be necessary. But make no mistake: the future of mouthwash may be milky.

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3. Dashper SG, Saion BN, Stacey MA, Manton DJ, Cochrane NJ, Stanton DP, Yuan Y, Reynolds EC. (2012) Acidogenic potential of soy and bovine milk beverages. *J Dent.* **40**:736-741

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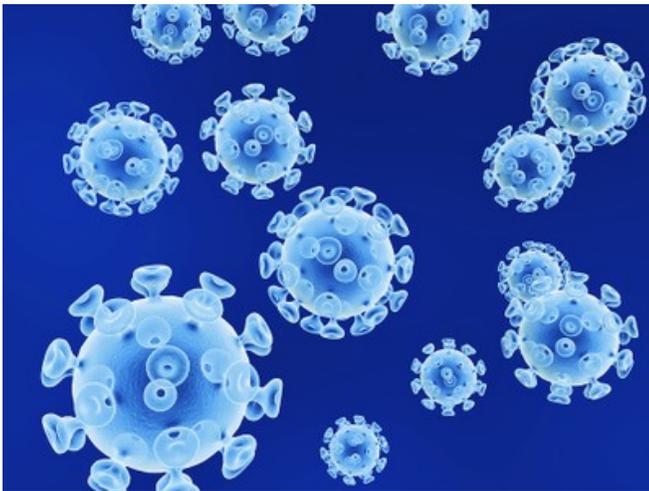
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Milk lymphocytes battle HIV

- **Transmission from mother to child accounts for ~30-50% of new HIV infections when anti-AIDS therapies are not used.**
- **Most breastfed infants of HIV-infected mothers do not acquire the virus, despite prolonged exposure.**
- **Milk from HIV-infected moms contains immune cells (lymphocytes) that respond to HIV.**

Contagious viral diseases have been the scourge of mammals ever since mammals first emerged some two hundred million years ago. There has been an arms race ever since, with viruses evolving new mechanisms to invade mammals, and in response, mammals evolve new counteractive defense strategies. The success of these evolutionary countermeasures is evidenced by our current existence, but the conflict continues.



The process of evolutionary change in response to disease threats has armed humans with a myriad of defenses, not least being those protecting the vulnerable young. Milk is likely to be front and center in this defensive arsenal.

Milk has three main functions in the newborn infant – to supply nutrients and water, promote growth and development, and protect against disease. The biological mechanisms underlying milk's ability to protect against disease are largely still being unravelled.

HIV transmission between mother and infant

New research by Lohman-Payne and colleagues¹ has concluded that immune cells present in human breast milk are influential in decreasing the mother-to-child transmission of viruses, and in particular HIV, the virus that causes acquired immunodeficiency syndrome, or AIDS.

The type of disease transmission familiar to most of us is typified by the flu, where there is person-to-person transmission. This is technically known as horizontal transmission. In contrast, the passing of a communicable disease agent from an infected mother to her offspring is called vertical transmission, as the agent is transferred across generations.

A large percentage of new HIV infections in many areas of the world are due to vertical transmission of the virus. In communities not receiving anti-HIV treatments, vertical transmission accounts for 30-50% of new infections. In this instance, infection of the fetus or newborn could have occurred *in utero*, during the birth process, or acquired during the postnatal period, especially while the newborn was breast-fed.

What is amazing, though, is that most breast-fed infants of HIV-infected mothers do not pick up the virus despite their prolonged exposure to it. There must be something protecting the infant from the virus.

Lymphocytes in milk

Milk contains many molecules that help fight disease. These molecules include antibodies, specific sugars and lipids, and a range of proteins. Some of these molecules, like antibodies, are highly specific for particular disease agents; other molecules have broad specificities. The latter typically recognise and bind to many different types of disease agents, often not killing them, but preventing the disease agent from gaining entry into cells lining the gastrointestinal tract. The problem is that viruses, unlike most bacteria, are usually hidden within cells of the host, where these milk molecules cannot gain access.

Milk also contains white blood cells, or lymphocytes, that are the mainstay of an individual's defense against viral infection within the body. These cells learn to recognise a specific virus, and then they activate the body's immune defense system, which seeks out and kills virus-infected cells. Some of the lymphocytes, called cytotoxic T-cells, can directly kill cells infected with a virus. To complicate things more, HIV hides out in some lymphocytes.

The researchers examined whether lymphocytes in the milk from HIV-infected mothers could help protect sucking infants from infection. This was a large study, conducted under strict ethical guidelines, and involved 510 pregnant Kenyan mothers who had evidence of HIV infection. A total of 474 infants were born to these women and 348 (73%) of the women breast-fed their infants.

The study first showed that the HIV ‘virus load’ in milk was related to the amount of virus circulating in the mother’s blood, but at a lower level. Importantly, milk from HIV-infected mothers was shown to contain specific lymphocytes that could be activated by synthetic HIV viral protein fragments. This result indicated these specific lymphocytes were able to recognise and respond to the presence of HIV.

Does milk protect suckling infants from HIV infection?

Lohman-Payne and colleagues also measured the viral load in the infants at birth and one month of age. The virus was undetectable in the infants at birth, but was detected in some infants at one month of age. Thus, transmission rates *in utero* were low. The researchers concluded the presence of HIV-activated lymphocytes in mother’s milk was associated with approximately 70% reduction in infant HIV infection at one month of age, suggesting a cause and effect relationship. Whether or not these lymphocytes could directly kill virus-infected cells is still unclear. Nor is it clear where the ingested milk lymphocytes could have conferred protection in the infants.

There was one important finding requiring more research. The researchers showed that the better the lymphocytes recognised HIV proteins (i.e., the ‘breadth’ of the response), the more likely there was a higher level of protection against infection of the infants. Perhaps artificially improving the breadth of this response in lymphocytes present in milk may be very beneficial.

In the face of the high-exposure risk of these infants, their surprisingly low infection rate is remarkable. The study potentially explains why most infants feeding on milk from HIV-infected mothers did not become infected.

We’ve heard it all before, but there is something very special about milk!

¹Lohman-Payne B, Slyker JA, Moore S, Maleche-Obimbo E, Wamalwa DC, Richardson BA, Rowland-Jones S et al. (2012) Breast milk cellular HIV-specific interferon γ responses are associated with protection from peripartum HIV transmission. *AIDS*. 26: 2007-2016.

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Milk: Not just for moms, not just for mammals

- **Pigeons, doves, flamingoes, and penguins produce crop “milk.”**
- **Pigeon milk shares functional properties with mammalian milk.**
- **Pigeon milk influences immune-modulating gene transcription.**
- **Pigeon milk contributes to bacterial colonization of the chick gut.**

Last month was Movember, during which men grow facial hair to raise awareness of men’s health. I started thinking about milk moustaches and realized you can’t have a milk moustache if you don’t have lips. I guess we won’t be seeing pigeons in any upcoming dairy ad campaigns- even though they make “milk,” and it functions like the milk of mammals.

“Pigeon milk” was first systematically described in the 1930s and continues to intrigue dairy scientists through today. Pigeon chicks hatch in a relatively undeveloped state, but during the first days after hatching, they show accelerated development. During this time, female **and male** pigeons shed “milk” from the epithelial cells in their crop, an enlarged compartment in the gullet in which food is stored prior to digestion. This chunky substance is rich in fats and proteins and is regurgitated to provision the chicks (Davies, 1939). Pigeon “lactation,” as well as parenting behavior, is hormonally regulated by prolactin, as is the case in mammals (reviewed in Horseman and Buntin, 1996). The time course of pigeon lactation also parallels many mammals. There is a period of exclusive milk feeding of chicks, and an increase in milk “volume” until mid-lactation is reached, after which production volume subsequently decreases (Vandeputte-Poma, 1980). Like mammals, the duration of lactation varies considerably between individuals. Moreover, just as in mammals, pigeon milk does not just contain macronutrients, but also immunofactors and bacteria (Goudswaard et al., 1979; Shetty et al., 1990).



Recently, Gillespie and colleagues reported an experimental model that demonstrated new functional properties of pigeon milk in the gut of chicks (2012). The gut associated lymphoid tissue (GALT) is a critical interface between the immune system and the gut bacteria. Additionally, the GALT produces a significant portion of immunoglobulin A (IgA) in humans, about 60% of total IgA. Feeding pigeon milk to chickens significantly altered gene regulation in the GALT, upregulating >1500 genes and downregulating ~600 genes. Of note, upregulation was detected in immune-specific genes for cytokine production and B cell activation and proliferation. As a functional outcome, feeding chickens the pigeon milk resulted in significantly greater IgA expression in the ileum compared to controls (Gillespie et al., 2012).

Moreover, intestinal microbial composition differed between chickens fed pigeon milk compared to controls. Chickens fed pigeon milk had a greater diversity of bacteria, determined using 16S amplification, at the levels of phylum, class, order, family, and genus compared to control subjects. Lactic acid bacteria, important for inhibiting the growth of pathogenic bacteria (Jin et al., 2011), were more diverse in the pigeon milk-fed chickens. Sixteen different species were present compared to 12 in the control chickens. Pigeon milk may contain prebiotics that support the colonization of beneficial gut bacteria, although the authors did not directly investigate such milk constituents. However, the researchers did examine the vertical transmission of bacteria through milk. The presence of bacteria in the pigeon milk was correlated with the presence of the same bacteria in the intestine of the chickens that were fed the pigeon milk, showing that bacterial inoculation occurs through milk feeding as in mammalian species (Martin and Sela, 2012).

Importantly, Gillespie and colleagues used chickens rather than pigeons because crop milk is obligatory for pigeon chick survival, and there are seemingly no options at this time for a “control” comparison group that receives a milk replacement formula. There is no more compelling evidence, to my mind, that crop milk serves critical functions in the newly hatched pigeon chick. Further experimental studies in other bird models will illuminate the mechanisms by which milk causes chicks to thrive beyond immune function and bacterial colonization, such as hormones digested through milk. One very interesting avenue is that in placental mammalian milks, prebiotic constituents are complex sugars (oligosaccharides), but pigeon milk does not seem to contain carbohydrates (Davies, 1939). However, technological advances in detecting and quantifying milk constituents may change our understanding of pigeon milk composition.

These results from pigeons are similar to findings in mammals. Among mammals, mother’s milk influences intestinal immune development and influences microbial diversity directly through both the bacteria present in milk and containing constituents preferentially consumed by beneficial bacteria (Donovan et al., 2012; Neville et al., 2012; Martin and Sela, 2012). Taken together, the results from pigeon milk reveal what is known as “convergent evolution” between avian and mammalian milks. The production of milk independently arose after the divergence of avian and mammalian lineages over 300 million years ago. However, these milks seemingly serve the same function: body-nourishing, bacteria-innoculating, immune-programming substances produced by parents specifically to support offspring development.

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