SPLASH! milk science update: April 2014 issue

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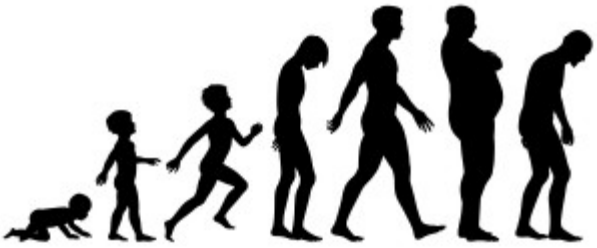
APRIL 2014 issue

This month's issue features articles about antibodies in breast milk, omega-3 fatty acids, the value of monkeys in milk research, and neonatal growth and the genome. Enjoy!

Breast Milk Antibody Promotes a Healthy Gut into Adulthood

- An antibody, SIgA, found in breast milk alters the kinds of bacteria that colonize the infant gut.
- The SIgA antibody also appears to change "turn on" and "turn off" genes involved in the rapid growth of the intestines that is normal in early life, as well as genes specifically linked to intestinal bowel disease.
- These findings suggest a potential new role for the antibody in treating bowel inflammation, even in adults.

Many pediatrics studies have shown that inflammatory bowel disease is more common in infants who are not breast fed than in those who are. But explaining why this is the case has been hard. Recently, Charlotte Kaetzel and her colleagues at the University of Kentucky, Lexington, went further in demonstrating a mechanistic link than any group has done before. They report1 that an antibody (SIgA) transmitted in breast milk from mom to babe alters the expression of genes in infants' gut epithelial cells. Not only are these genes associated with the development of irritable bowel syndrome, but the changes appear to last into adulthood.



The team conducted these experiments in mice, though the genes in question are very similar to those in humans. They started comparing the kinds of gut bacteria found in mice whose moms made breast milk containing the antibody, SIgA, with mice whose moms were mutants. These mutant moms made breast milk lacking the antibody. Unlike those that consumed SIgA, the mice without SIgA in their diets had draining lymph nodes stuffed with bacteria, including one disease-causing species called Ochrobactrum anthropi, which is often found in immunocompromised individuals.

A broader sweep of the kinds of gut bacteria of the two groups of mice drew many parallels to human patients suffering from inflammatory bowel disease (IBD). For example, just like infants with IBD, the mice without SIgA in their diets had many more bacteria from the families Pasteurellaceae and Lachnospiraceae than is the norm. Similarly, the relatively large numbers of bacteria from the phylum Proteobacteria in the guts of these mice are also typical of adults with IBD.

That a link between the gut bacteria promoted by not consuming SIgA as an infant and the gut bacteria of adult IBD patients could be found at all was suggestive that drinking breast milk during the first few months of life can cause lifelong changes to the gut. Kaetzel and her team probed this further by investigating gene expression in gut epithelial cells.

And this is where the research got really interesting. In all, the team noted 69 genes whose expression was altered in the infant mice that drank breast milk lacking the antibody. Some of these genes are involved in DNA copying and repair, as well as other processes necessary for growing gut tissue rapidly. And some were the very same genes for which the human equivalents have been pinpointed as being associated with an elevated risk of developing IBD.

Another experiment backed up the finding that breast milk regulates genes that have a helping hand in growing gut tissue rapidly. When Kaetzel and her team had mice consume a chemical known to induce damage to the gut epithelium, they found that subsequently feeding these mice SIgA went some way to repairing the problem. Presumably, this was because the SIgA turned on genes in healthy gutepithelial cells, which prompted these cells to divide more rapidly, replacing damaged tissue.

These results clearly make the case for testing novel infant formulas with purified SIgA added to the mix. Kaetzel and her coauthors go further, however, by proposing SIgA as a medicine for intestinal infections and inflammation in adults as well. That is an interesting idea. Either way, this work adds more weight to the broad recommendation that breast is best: it simply gives health professionals another reason as to why.

1Rogier EW, Frantz L, Bruno ME, Wedlund L, Cohen DA, Stromberg AJ, Kaetzel CS (2014). Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. PNAS 111:3074–3079.

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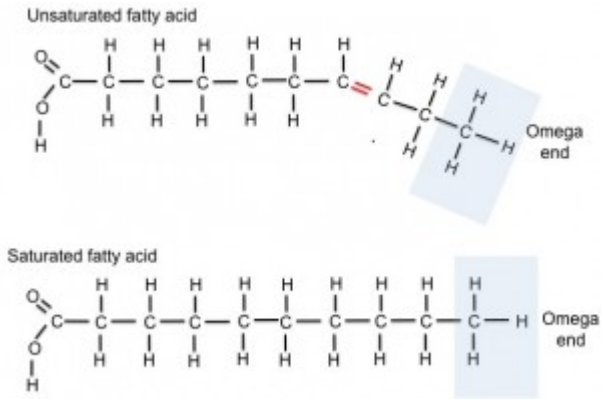
Getting More Omega-3 Fatty Acids from Milk

- Western diets contain insufficient amounts of omega-3 fatty acids, which are important for heart and brain health.
- One solution to this health issue is increasing the concentration of omega-3 fats in commonly consumed foods such as cow's milk.
- Dairy cattle supplemented with omega-3 fats produce milk with higher concentrations of omega-3 fats and fewer saturated fats.
- Cows from organic farms consume more grass and legumes and less corn, and produce milk with more omega-3 and fewer omega-6 fatty acids than conventional milk.
- The nutritional quality of cow's milk can be improved through dietary changes in dairy cattle.

In the early 20th century, vitamin D was added to commercial cow's milk in response to the rise in malnourished children and adults with insufficient amounts of this essential nutrient in their diets. Today, many Americans and other populations consuming a primarily Western diet face another nutritional challenge. Despite having plentiful amounts of fat, the Western diet is lacking in a specific group of fatty acids called omega-3s, touted for their benefits to heart and brain health. In an effort to increase omega-3 intake, food manufacturers have started fortifying commonly consumed foods, including breads, cereals and eggs, with these essential fatty acids. Cow's milk also is getting in on the act, in more ways than one. Although milk can be fortified with plant-based omega-3 fats, researchers are exploring the potential benefits of fortifying dairy cattle diets with omega-3s to influence the proportion of these healthy fats in milk. Do more omega-3s for cows mean more omega-3s for milk consumers?

The skinny on fatty acids



Mammalian milks contain over 400 different fatty acids, but each has the same basic structure: a chain of carbon atoms bonded to two hydrogen atoms, with an acidic "head" (CO_2H) and a methyl group (CH_3) "tail" (also called the omega end). The variation in fatty acid structure comes from the total number of carbons in the chain (<6 carbons = short-chain, 6–12 = medium-chain, >12 long-chain) and the presence of double bonds between the carbons. If the chain of carbons has only single bonds, the fatty acid is said to be saturated, whereas the presence of at least one double bond makes it an unsaturated fatty acid. Putting this all together, a fatty acid chain with more than 12 carbons and multiple double bonds earns the lengthy title of long-chain polyunsaturated fatty acid (LCPUFA).

But wait, there's more! LCPUFA are further grouped based on the location of the last double bond from the omega end of the molecule. Omega-3 fatty acids, such as alpha-linolenic acid (ALA) and docosahexaenoic acid (DHA), have their last double bond at the third carbon from the omega end of the chain. Accordingly, omega-6 fatty acids, such as linoleic acid (LA) and arachidonic acid (AA), have the double bond at the sixth carbon.

Getting the right ratio

Both omega-3 and omega-6 fatty acids are considered essential because humans, and other animals, cannot synthesize them and must instead get them from the diet. Western diets are plentiful in sources of omega-6 fatty acids (corn oil, palm oil and most nuts) but lacking in omega-3s (green leafy vegetables, flaxseed, olive oil, fish). To put the imbalance in perspective, it is estimated that Western diets contain between 10 and 15 times more omega-6 fats than omega-3 fatty acids. The "optimal" ratio of omega-6 to omega-3 is estimated to be closer to 2:1 (Benbrook et al., 2013).

The issue is not that omega-6 fatty acids are unhealthy; as LCPUFA they are considered one of the "good" fats and an important part of a healthy diet. But too many omega-6s can interfere with the way the body utilizes omega-3 fatty acids and thereby limit their numerous health benefits, such as reducing the risk of cardiovascular disease, diabetes and obesity. The prevalence of omega-6s in the Western diet may make it challenging for many people to reduce their consumption. But what if commonly consumed foods, such as milk, had higher concentrations of omega-3s?

If you give a cow some flaxseed

LCPUFA in milk are derived from LCPUFA in the mother's diet. As a result, mothers that eat more omega-3 fatty acids have higher concentrations of milk omega-3s (Brenna et al., 2007; Iverson and Oftedal, 1995; Milligan and Bazinet 2008; Yuhas et al., 2006). At least, that is how it works in humans and most other mammals. The relationship between diet and milk is a little more complicated in cows (and other ruminants). As cows digest the fats in their food, the microbial activity in their rumen converts the double bonds in the LCPUFA into single bonds, effectively turning them into saturated fatty acids (Benbrook et al., 2013).

Microbial conversion of double bonds in fats to single bonds presents a challenge for increasing cow milk PUFA concentration. For cows, it is not just about eating more omega-3s, but eating the right type or combination of omega-3s to maximize their transfer from the diet to milk. To this end, researchers have experimented with feeding cows different combinations and concentrations of various vegetable sources of omega-3 ALA, and the results are promising. For example, cows consuming flaxseed-supplemented forage produce milk with 60–100% more ALA than those fed a control diet (Neveu et al., 2013, 2014). Cows fed linseed (a derivative of flax), rapeseed (canola) and alfalfa also produce milk with higher concentrations of omega-3 fatty acids (Dang Van et al., 2011; Oeffner et al., 2013; Puppel et al., 2013).

There is an additional benefit to upping the contribution of omega-3s in milk. When the proportion of one fatty acid goes up, another (or others) must go down. A consistent finding of these supplement experiments is that when milk omega-3s go up, saturated fatty acids go down. The total fat content of the milk is not altered, only the relative contribution of saturated and unsaturated fatty acids. And fortunately for human consumers, the shift is in the direction of improved nutritional quality.

More pasture, more omega-3s

The evidence for omega-3 fatty acid consumption greatly improving the fat profiles of cow's milk is compelling, and receives further support from a new study that compares milk fatty acids from organic and conventional milk (Benbrook et al., 2013). Cows from organic milk producers are not purposely supplemented with omega-3s, but they consume more omega-3s and less omega-6s as a result of increased access to pasture (where they consume grasses and legumes) and decreased consumption of grains, particularly corn. As predicted, differences in the amount of omega-3s in the diet produce differences in milk omega-3s between organic and conventional dairy cattle. Organic milk has significantly higher proportions of omega-3s (62%) than conventional milk (Benbrook et al., 2013). Moreover, the ratio of omega-6 to omega-3 fatty acids also is significantly lower in organic milk (2.3 compared with 5.8). This study demonstrates what omega-3 ratios may be achievable on other farms around the globe that have increased access to pasture and reduced use of grains.

Fluid dynamics

Milk fat composition is dynamic, and we can use this physiological property to our advantage by increasing the contribution of good fats (omega-3s) and decreasing that of less desirable fats (saturated fats). Vitamin D fortification of milk had a profound impact on human nutrition nearly 100 years ago. It is intriguing to speculate how increasing the nutritional value of such a widely consumed food may influence human health today.

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Monkey Model of Milk and Lactation

- Lactation physiology in monkeys is similar to humans.
- Work with monkeys increases experimental control, avoids cultural complications.
- Availability of milk samples and new tools provide promising opportunities.

Like humans, monkeys generally give birth to one baby at a time and nurse them for extended periods during a time of infant and toddler-like development. Primates need this extended lactation period for social development. Just as humans need to learn interpersonal and societal rules, monkeys also need to learn how to find food and not kill each other. Thus, monkeys, like humans, produce a dilute milk to feed slow-growing young.

Monkey Milk Composition

To date rhesus monkey milk composition has been studied in hundreds of animals in multiple captive populations at research centers. Rhesus milk is slightly more energetically dense than human milk, but much less concentrated than milk from many other mammals (Hinde and Milligan 2011; Skibiel et al. 2013). Depending on timing during lactation, it is ~4–6% fat, 2% protein and ~7.5% carbohydrates. Other constituents, including minerals, oligosaccharides, fatty acids, bacteria and hormones, have been systematically investigated (Lönnerdal et al., 1984; Milligan and Bazinet, 2008; Osthoff et al., 2009; Goto et al., 2010; Jin et al., 2011; Sullivan et al., 2011; Tao et al., 2011; O'Sullivan et al., 2013; Hinde et al., 2013). In this way the "food" and "signal" aspects of milk (nutritive and signaling constituents) have been targeted for ongoing work. However, the "medicine" of milk—the immunofactors that protect and train the infant's developing immune system—have received relatively less effort.

Milk composition is not static among individuals, or even in the same individual. Studies in monkeys point to some sources of this variation. Let's use the total amount of milk fat produced as an example. Total milk fat varies by maternal body mass and parity (Hinde et al., 2009). Moreover the percentage of fat in milk produced for sons is much higher than in milk produced for daughters (Hinde, 2007a). Other less obvious factors can also influence milk fat. Monkey mothers with higher intestinal parasite loads produce lower fat concentrations in milk (Hinde, 2007b)! These inter-individual differences emerge even though animals consume a standardized diet. New tools will allow us to unpack rhesus macaque milk in new ways, in order to understand the causes of variation in milk composition and production.

Why Work with Monkeys

Tiny macaque breastfeeding

To tease out the environmental contributors to lactation, the monkey model enables the study of primate lactation physiology, in the absence of human cultural contexts. Monkeys in an outdoor breeding colony are all fed the same diets and live in the same environment. Human diets can't be easily controlled. The use of infant formula, scheduled feedings and pumping, clinical recommendations, and well-intentioned relatives, friends, and strangers influencing infant feeding decisions are absent in rhesus monkeys. With monkeys, we can study the biological variation in mother's milk, the sources of that variation, and the consequences for infant development.

On the flip side, monkeys are extremely costly to study, compared with rodents (Neville et al., 2012). They live longer lives, breed more slowly, and require more expensive food, housing, and medical care. Monkeys in an outdoor breeding colony also live in social groups and naturally experience higher or lower social rank, as they would in the wild. These variable life-stressors that could affect milk production can be observed, but are difficult to control. Thus, it is important to be aware of these factors when designing primate studies. But these costly aspects are precisely why monkeys are so valuable; rich social lives and complex behavioral dynamics more closely resemble those of humans.

New Tools for the Study of Monkey Milk

Unlike cows, monkeys produce small amounts of milk and haven't been studied extensively. New tools bring state-of-the-art 'omics techniques to extract large data sets from small volumes of monkey milk. Most recently, we demonstrated that RNA extracted from milk fractions is representative of RNA extracted from mammary tissue (Lemay et al., 2013). No biopsies needed! Just milk. This study also revealed sufficient similarities between the human and macaque transcriptome of milk-producing cells to further legitimize the macaque as an animal model of human lactation (Lemay et al., 2013). The monkey model has been further legitimized for microbial and metabolomics research. Milk microbes are similar in human and rhesus milk (O'Sullivan et al., 2013). Likewise, human and monkeys share a common set of metabolites in their urine and serum (O'Sullivan et al., 2013).

Other tools have been developed to identify scarce, but important, constituents of milk in studies involving monkeys. Researchers have standardized methods for hormonally inducing lactation in rhesus macaques, and for tracking viral loads and antiviral constituents of milk (Permar et al., 2008). Such tools then tracked the transmission of HIV from mother to offspring via milk (Fouda et al., 2013). Researchers can even use barium levels in teeth—yes, teeth—to determine when an extinct mammal was weaned (Austin et al., 2013). These new tools enable researchers to "see" the previously unobservable in milk.

Monkey Milk Samples Available

The most exciting research occurs at the intersections among disciplines. As such, 21st century science will require expanding networks of researchers. Many of the studies described above involved interdisciplinary collaborations among anthropologists, biochemists, microbiologists, food scientists, animal scientists, evolutionary biologists and psychologists. Moreover many of the samples analyzed in the above studies were made available via the Archive of Rhesus Macaque Milk Samples (ARMMS) Program. Since being established in 2005, we have collected and archived milk samples from nearly 300 rhesus macaques, often repeatedly across lactation, totaling >10,000 mL of milk. To maximize the knowledge that can be gained from this archive in consideration of the three principles of animal research—Replacement, Reduction and Refinement (three "Rs")—these milk samples, paired with detailed behavioral and morphometric data on monkey mothers and infants, are available for colleagues to assay for bioactive components that contribute to infant growth, development and health.

New Research Questions

What is the complete catalog of everything in milk? What are the genetic and environmental sources of variation in these milk constituents? What are the physical, intestinal, microbiological, neurobiological and behavioral developmental effects of milk on the consumer? We have an archive of precious samples, the tools to probe the samples and the research questions. Now we just need the funding. (Hint Hint).

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Picking Winners: How to Identify Genes Important for Neonatal Growth

- Neonatal growth is dependent on lactation sufficiency.
- Mice are an animal model of growth.
- Pre-genome: a broad region of the genome was mapped for neonatal growth.
- Post-genome: we can use integrated strategies to zoom into these regions.
- The study demonstrated how this strategy may be used to identify individual genes.

We all know that newborn babies need frequent and adequate nutrition to get a good start on life. Indeed, it is a particularly susceptible period when insufficient nutrition or complications can be life threatening. In recent years, it has also emerged that nutrition and the pattern of growth in this period can affect lifetime health and well being. By far the best source of nutrition during this period is mother's milk, which is tuned to the babies nutritional and growth requirements. So as you might expect, there is a close relationship between the volume and composition of milk consumed by babies and their rate of growth.

What makes some babies grow faster than others? A recent publication by my own group has looked into the rates of growth in mice and used an integrated genomics strategy to identify genes that are important in lactation and related to the differences that contribute to growth of neonates (1). This study was conducted by Palani Ramanathan and Jerry Wei when they were graduate students in my lab at the University of Sydney. They were interested in the maternal-neonatal interaction and how milk production contributes to growth across a series of mouse strains, where the rates of growth vary greatly and the capacity of the dam to sustain a litter depends on milk produced.

Before we had genome science, a small number of studies were conducted using traditional genetic approaches in cross-bred mice. These studies and similar approaches in other species, identified regions of the genome that had a statistical association with neonatal growth, but the sizes of the regions were very large and contained so many genes that it was impossible to predict how they were linked. In the post-genomic world, experimental tools have been developed to refine the process of discovery and improve its sensitivity and accuracy.

In this study, the daily weights of litters from inbred strains of lactating mice were measured for the first eight days of life. We previously had established that this period of growth was predictive for the full lactation period in mice and hence the amount of milk that was produced (2). Using inbred strains of mice has the advantage of negating genetic variation within the strain, so we could concentrate on the differences between the strains. The other advantage is that many inbred strains of mice are now fully genotyped; that is, we know their genetic makeup in great detail. By combining this level of detail with measures of genes that are switched on during lactation (3), we could reduce the number of potential genes that were driving this process considerably. This report focused on the regions that were most prominent in neonatal growth, referred to collectively as Neogq. When Palani and Jerry applied the algorithm to these regions, they narrowed down the areas under investigation to much smaller stretches of DNA that each contained only one gene that was switched on during lactation, e.g. in the region of greatest interest the gene Neo1 stood out.

We continue to apply this approach to other measures that are relevant to maternal performance (4-6), and along with colleagues that have approached similar questions in other studies, we now have an intricate picture of genes that are fundamentally important in lactation and how these are crucial to neonatal growth (e.g., (7-9)). The mouse remains an important and informative animal model for these studies and will continue to produce leads for investigation in other species of interest, most notably humans, in trying to elucidate the complexities of neonatal growth and well being (10).

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