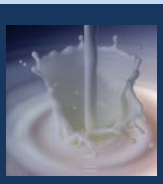
SPLASH! milk science update: August 2012 issue



SPLASH! milk science update
AUGUST 2012 issue

This month, guest writer Dr. Lauren Milligan reports on fats found in human milk. Our dedicated contributors deliver articles on perpetual lactation, how bacteria digest milk sugars, and a vision for the future.

Enjoy!

Which fats should be in infant formula?

- **A mother's diet affects her milk fatty acid composition**
- **Tsimane women produce milk with more DHA and fewer fatty acids from processed foods than American mothers.**
- **A mother's body composition, independent of diet, may also affect milk fat composition**

Human milk fat is made up of over 150 different types of fatty acids. While the mammary gland is able to synthesize many of these fatty acids, others must be supplied by fats in the mother's diet. As human mothers are not consuming identical diets, it is not surprising that human milk fatty acid profiles vary widely among populations. The variation in the types of fat in human milk has long been a concern of nutritionists, pediatricians, and more recently, anthropologists. Of special interest is the amount of DHA (docosahexaeonic acid), an omega-3 fatty acid implicated in neurodevelopment. Breastfed infants receive a wide range of DHA (between 0.06% to 1.4% of total fatty acids)[1] and the concentration of DHA in formula is approximately 0.3% (based on milk from women consuming a Western diet). But what is the optimal concentration of DHA for human infants?

A new paper by Martin et al.[2] addresses this question using an evolutionary perspective. They argue the extreme variation in the types of dietary strategies we see today (e.g., veganism, vegetarians, the Western diet) is novel. For the majority of human evolution, diets lacked domesticated meats, dairy, processed foods, and hydrogenated oils. Human milk fatty acid synthesis and infant postnatal patterns of growth and development evolved during a time when mothers consumed pre-agricultural diets, so the best model for human milk may come from human populations consuming a traditional diet.

Martin et al. compared milk samples from US women and the Tsimane of Bolivia, forager-horticulturalists who consume a largely plant-based diet while also eating wild game and fresh-water fish (both sources of omega-3 fatty acids, including DHA). Compared to milk from US women, Tsimane milk has significantly higher proportions of DHA – four times more, to be exact (0.69% vs. 0.16% of total fatty acids). Other significant differences include higher proportions of linoleic acid in US milk (a fatty acid found in vegetable oils and processed foods) and higher amounts of arachidonic acid in Tsimane milk (a long-chain polyunsaturated omega-6 fatty acid found in animal protein). Although not necessarily the optimal values for infant growth and neurodevelopment, Martin et al. propose that the proportions of various fatty acids in milk from Tsimane women are a better model for human milk fatty acid composition than those provided by women on a Western diet or other diets far removed from traditional dietary practices.

There are other novel aspects of Westernized populations that may also influence milk fatty acid composition. Take, for example, the current obesity epidemic. It is unlikely that pre-agricultural females attained a body mass index (BMI) greater than 25 (the general cut-off between normal and overweight), so it seems plausible that milk synthesis would be affected by such dramatic changes in body composition. In a recent study, Mäkelä et al.[3] found higher

proportions of saturated fats, lower proportions of unsaturated omega-3 fats (especially DHA), and a lower ratio of unsaturated to saturated fats in the milk of overweight as compared to normal weight women (categories based on pre-pregnancy measures of BMI). Importantly, these differences were not explained by dietary differences between the groups; after adjusting for estimated omega-3 fat consumption, normal weight mothers still produced higher mean values of DHA. The authors do not provide an explanation for this finding, but do indicate a previous study found the opposite pattern: higher DHA (and other polyunsaturated fatty acids) in milk from overweight women. Although contradictory, the results of both studies suggest body composition influences milk fatty acid composition in some way, even when controlling for dietary fat intake. Hopefully, further research on this topic will identify just what this influence is, and more importantly, how it may affect infant growth and development.

Taken together, these studies demonstrate that modern dietary practices have modified milk fatty acid composition. Beyond just being an interesting factoid, this finding has direct application to human infant formula – after all, the current model for formula fatty acid supplementation is milk from women consuming Western diets. The more we understand the impact of our cultural practices on milk synthesis, the better able we are to develop formulas that optimize milk fatty acid composition.

1. Brenna, JT. Varamini, B. Jensen, RG, Diersen-Schade DA, Boettcher, JA, Arterburn LM. (2007) Docosahexaenoic acid and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr **85**: 1457–1464.

2. Martin MA, Lassek WD, Gaulin SJC, Evans RW, Woo JG, Geraghty ST, Davidson BS et al. (2012) Fatty acid composition in the mature milk of Bolivian forager-horticulturalists: controlled comparisons with a US sample. Matern Child Nutr. **8**:404-418.

3. Mäkelä J, Linderborg K, Niinikoski H, Yang B, Lagstrom H. (2012) Breast milk fatty acid composition differs between overweight and normal weight women: the STEPS Study. Eur J Nutr. [Epub ahead of print].

Contributed by
Prof. Lauren Milligan
Visiting Assistant Professor
Dept. of Anthropology
University of California, Berkeley

The cow that keeps on giving

- **Scientists are looking to bovine mammary stem cells to extend a cow's lactation period.**
- **By either decreasing the rate of mammary epithelial cell death or increasing their renewal, a cow could produce milk beyond its normal lactation period.**

Imagine a dairy cow without a lactational dry period. Most people would say it's science fiction, but the discovery of bovine mammary stem cells perhaps brings this possibility a little closer to a reality.

A recent review by Capuco et al.[1] highlights the roles of bovine mammary stem cells in lactation and their potential to be used by the dairy industry to increase productivity and efficiency.

Increasing lifetime milk production

There are specific periods during a cow's lifetime when it is not producing milk or not optimally producing milk. Increasing lactation performance during these time intervals could markedly improve financial returns for the dairy farmer.

The dairy industry is already rearing heifers rapidly to bring them into early puberty, early breeding, and early lactation. This shortens the overall period of 'non-performance' in a cow's total lifetime. However, the non-lactating period, or dry period, between successive lactations still collectively corresponds to a significant proportion of the lifetime, and this period does not generate income for the dairy farmer. Scientists are now looking to stem cells as a means to extend the lactation period.

Shortening, or even eliminating, the dry period would substantially increase the productivity of each cow. This period corresponds with the biological and hormonally driven imperative linking lactation with reproduction. In humans, lactation simply follows as a consequence of reproduction, i.e. gestation and lactation are usually separated in time. However, in the dairy cow, there is typically substantial overlap in the lactational and pregnant states because the cow typically becomes pregnant about four months after the previous calving, and is therefore still lactating.

During the later stages of pregnancy, the cow diverts precious energy away from making milk and puts it into growing a fetus. Lactation during this period declines, eventually ceasing in the dry-off period. After giving birth the cow takes about a month before peak lactation is reached again.

Extending the lactation period by decreasing the dry period would not only increase milk production, but it would also reduce the total amount of time a cow spends in the perinatal period in its overall lifetime. The perinatal period is linked to increased health risks and additional costs.

Birth and death of milk-producing cells

Mammary epithelial cells (MECs) are the milk production factories in the mammary gland. Milk production is dependent on both the number and the milk secretory capacity of MECs within mammary tissue. Both of these factors, particularly the latter, increase during the approach to peak lactation. The number of MECs within the tissue is dependent on the rate of cell renewal and cell death. Amazingly, this race between cell renewal and cell death results in the replacement of the majority of MECs during each lactation cycle.

After peak lactation, the steady decline in milk production is caused by both the decreasing milk secreting activity of aging MECs and declining numbers of these cells. This loss of MECs during the later stages of lactation is caused by decreased renewal of MECs and a natural programmed cycle of cell death that eliminates MECs. Scientists therefore expect that increasing the MEC renewal process or decreasing cell death would significantly extend the lactation period.

Induced renewal of milk-producing cells

The complete cycle of lactation is characterised by substantial cellular restructuring of mammary tissue. The repeated cycle of tissue remodelling in the mammary gland during multiple lactations indicates the existence of a cell population with tissue renewal ability resident within the mammary gland.

Stem cells are critical for the normal growth and maintenance of the mammary gland. These cells have the capacity to renew themselves indefinitely and, under the right circumstances, they can produce a variety of different types of specialist cells characteristic of different tissues. Fetal stem cells are the most plastic and can generate all of the different tissue types that make up a living organism.

Adult stem cells are found at very low frequencies in all tissues of an adult animal, but their ability to make specialist cells is more limited than fetal stem cells. Adult stem cells usually have the potential to generate all cell types normally found within a tissue in which they reside but not cell types found in other tissues. Adult stem cells are usually inactive, or asleep, within a tissue, but once activated by specific molecular signals, they can generate many more cells in the tissue.

Mammary stem cells have been well characterised in the mouse and human mammary glands. Indeed, transplantation of a single mammary stem cell into a fat pad in a mouse can generate mammary tissue which produces milk.

Adult bovine mammary stem cells have been identified. It is possible these cells could be activated within mammary tissue to increase the number of MEC precursor cells that, in turn, would produce more MEC and therefore more milk, especially after peak lactation. As these new MEC would be young cells, they would also be expected to be very efficient at secreting milk. This strategy could markedly extend the lactation period, and in an extreme possibility, completely replace the dry period.

The challenges to implementation of this strategy are to find practical and acceptable means to increase the number of stem cells in lactating mammary tissue, to specifically activate the production of MEC precursors, and induce self-renewal of the stem cells. The industry may unknowingly be already inducing some of these changes through practices such as increased daily milking frequency and the administration of somatotrophin. There may also be unidentified and fundamental differences in mammary stem cell populations in different breeds of dairy cattle that could create a challenge in determining the most effective methods of increasing milk production in cows.

Caveat emptor

In biology there is usually a physiological cost for every commercial benefit. Some evidence exists that the dry period may be important for maximising milk production in the succeeding lactation. Consequently, any implementation of a strategy designed to reduce the extent of the dry period will need to be carefully assessed for unexpected physiological side effects and untoward impacts on the welfare of the cow.

The eventual elimination of the dry period may be possible, but it is only an embryonic ambition at this time. Still, one day the dairy industry may use a cow that keeps on giving.

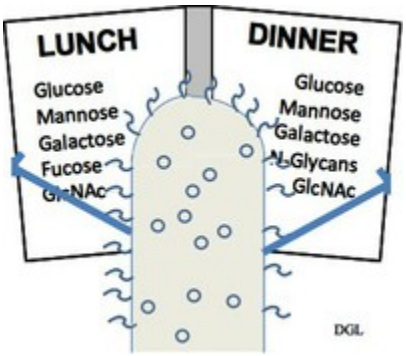
1. Capuco AV, Choudhary RK, Daniels KM, Li RW, Evock-Clover CM. (2012) Bovine mammary stem cells: cell biology meets production agriculture. Animal **6**:382-393.

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

Milk-fed bacteria's secret weapon

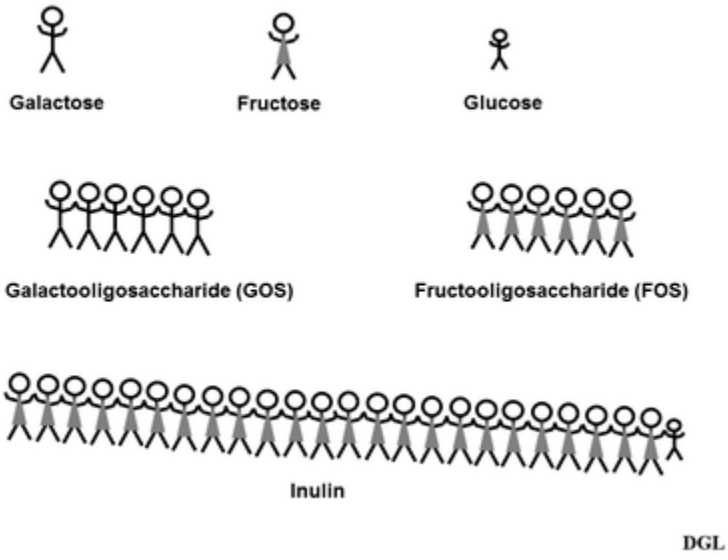
- **Milk contains several unique sugars indigestible to most bacteria.**
- **A special bacteria, *B. infantis*, has enzymes that allow it to digest the complex sugars in milk.**
- **Additionally, *B. infantis* contains the enzyme EndoBI-1, which cleaves these sugars from proteins, even in conditions that would render most enzymes helpless.**

If a bacterium walks into a Bar & Grill, what does he order to eat?



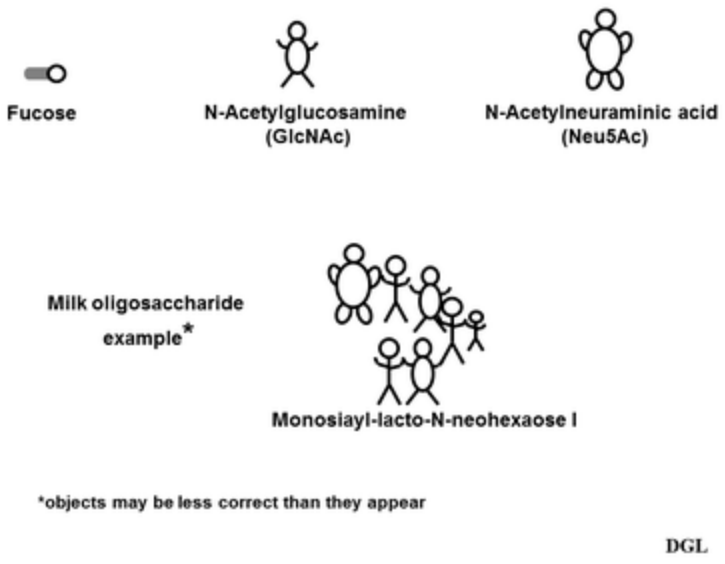
If there are any simple sugar molecules on the menu, such as glucose, galactose, mannose, then he'll order a plate of them. But given that these simple sugars are the first choice of pretty much any type of bacteria, the Bacteria's Bar & Grill is likely to run out quickly. Then what?

Some bacteria can eat linear polysaccharides (strings of simple sugar molecules) like fructooligosaccharides (FOS), galactooligosaccharides (GOS), and inulin. If the simple sugar galactose was drawn as a Stick Man, then GOS would be several Stick Men holding hands. Likewise, if fructose is a Stick Woman, then FOS is several of them holding hands. Inulin is a lot like FOS, except there are a lot more fructose molecules—20 to 1000s—and there is usually one glucose molecule at the end. So, in our Stick Person Chemistry world, if we assign glucose to be a Stick Child, then inulin would be a string of hundreds of Stick Women holding hands with one Stick Child holding the last woman's hand.



While we people eat FOS, GOS, and inulin, they are indigestible by our own digestive enzymes, leaving them available in our guts for bacteria to digest and absorb. However, not all bacteria can digest polysaccharides. Bacteria that consume only simple sugars like glucose would generally starve in the human colon because such sugars are completely absorbed much earlier in the digestive tract.

Even fewer bacteria can digest the complex sugars in milk. These milk sugars (milk oligosaccharides) contain a greater diversity of simple building blocks, so our Stick Person Chemistry is about to get more complicated. In addition to glucose and galactose, we now include fucose, GlcNAc, and Neu5Ac. Fucose is a simple sugar like glucose, but missing a hydroxyl group. In other words, fucose is like glucose but smaller, so let's say that fucose is a Stick Baby. Another milk sugar building block is N-Acetylglucosamine (GlcNAc), which is something like a glucose molecule plus a nitrogen and an acetyl group (two carbons and an oxygen). It is different from glucose, our Stick Child, so let's represent large GlcNAc with a Fat Stick Man. The final milk sugar building block we'll introduce is N-Acetylneuraminic acid (Neu5Ac, a sialic acid), which is even more complex than the bulky GlcNAc we just met, carrying several extra carbons and hydroxyl groups (OHs). Neu5Ac is Morbidly Obese Tall Stick Man who is too big to even find clothes at the Big & Tall store.

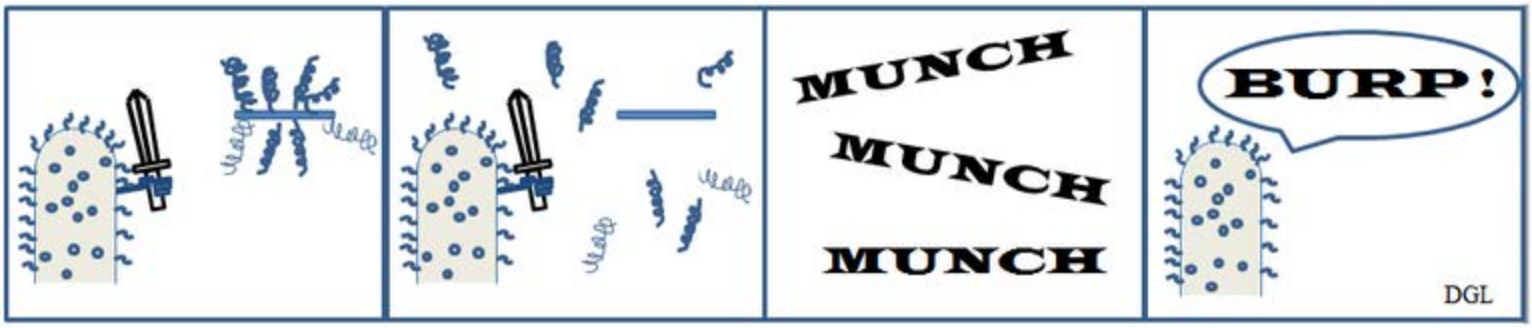


So what do milk oligosaccharides—those special sugars unique to milk—actually look like? Well, they're really complicated: picture a jumble of Stick Men, Stick Women, Stick Children, Stick Babies, Fat Stick Men and Morbidly Obese Tall Stick Men all joined together in myriad ways: some by hands, some by feet, some by the arm, some at the hip. Despite the complication, there appear to be less than 200 unique oligosaccharides in human milk [1]. So, while thousands of these Stick People Jumbles are theoretically possible, hardly any of them actually exist in nature.

Very few types of bacteria that walk into the Bacteria Bar & Grill would order milk oligosaccharides because they can't digest them. Digestion of these milk sugars requires an arsenal of special enzymes—fucosidases, sialidases, etc.—to break apart the unique bonds (joined hands, feet, arms, hips) in the Stick People Jumbles. *Bifidobacterium longum* subsp. *infantis* (*B. infantis*), which populates the guts of breastfeeding infants, is able to digest milk oligosaccharides [2]. In 2008, David Sela and colleagues discovered the particular arsenal of enzymes in *B. infantis* that enables this special bacterium to digest complex sugars that are unique to milk [3].

Last month, we explored how most proteins have sugars attached to them. Yet the sugars we have discussed so far—Stick Men, Women, etc. and even the Stick People Jumbles—are all floating free of proteins. While Sela et al. found that *B. infantis* can eat Stick People Jumbles, can it eat them even when they are attached to proteins?

Before bacteria can eat sugars attached to proteins, they first have to be able to separate the sugars from the proteins. This requires—you guessed it—more special enzymes. Last month, Daniel Garrido and colleagues published the secret weapon of *B. infantis*, a special enzyme called EndoBI-1 (an endo-beta-N-acetylglucosaminidase) that cleaves a special type of sugar from proteins [4]. Most proteins in milk have sugars that are attached very strongly to the Nitrogen of specific amino acids in the protein (usually the amino acid asparagine), and they are therefore called N-linked. These N-linked sugars contain various types of Stick People Jumbles that are hard to reach and cleave even for "professional" (i.e. store bought) enzymes, especially when the Morbidly Obese Tall Stick Man and the Stick Baby are present. And yet, astoundingly, *B. infantis* can chop any of these Stick People Jumbles off of the protein. So now we have this amazing bacterial species that can liberate any complex N-linked sugars from milk proteins and can easily consume them in their own privacy, away from the other bacteria at the bar.



If this secret weapon wasn't potent enough, there's one more thing. EndoBI-1 works in every condition. Most enzymes will unravel in hot water and become incapable of performing their normal functions. Even after 5 minutes in boiling hot water, EndoBI-1 can still cleave Stick People Jumbles. As a weapon, EndoBI-1 is not just a sword, it is Excalibur—the sword that makes *B. infantis* the King of Bacteria.

The bottom line is this: when *B. infantis* walks into the Milk Bar & Grill, it can order pretty much anything on the menu.

1. Wu S, Tao N, German JB, Grimm R, Lebrilla CB. (2010) Development of an annotated library of neutral human milk oligosaccharides. J Proteome Res. **9**: 4138-4151.

2. Ward RE, Ninonuevo M, Mills DA, Lebrilla CB, German JB. In vitro fermentation of breast milk oligosaccharides by Bifidobacterium infantis and Lactobacillus gasseri. Appl Environ Microbiol. **72**:4497-4499.

3. Sela DA, Chapman J, Adeuya A, Kim JH, Chen F, Whitehead TR, Lapidus A et al. (2008) The genome sequence of Bifidobacterium longum subsp. infantis reveals adaptations for milk utilization within the infant microbiome. Proc Natl Acad Sci U S A. **105**:18964-18969.

4. Garrido D, Nwosu C, Ruiz-Moyano S, Aldredge D, German JB, Lebrilla CB, Mills DA. Endo-β-N-acetylglucosaminidases from infant-gut associated bifidobacteria release complex N-glycans from human milk glycoproteins. Mol Cell Proteomics. [Epub ahead of print].

Contributed by
Dr. Danielle Lemay & Prof. Daniela Barile
Genome Center & Dept. of Food Science and Technology
University of California, Davis

What comes next

- **Upon retiring, Dr. Peggy Neville secured the future of human milk and lactation research by organizing leaders of the field and delivering a research plan for the coming decades**
- **Four key research areas:**
  - **1) milk composition and effects on neonatal health,**
  - **2) effects of milk consumption on cognitive development and behavioral phenotype of offspring**
  - **3) the genetic and environmental contributors to lactation success**
  - **4) the unique needs of vulnerable populations**
- **It's time for funding agencies and young researchers to carry the torch into the future.**

Keeping funding agencies and researchers properly in the loop, Peggy Neville, who recently retired from the University of Colorado, Denver, and colleagues have published a paper entitled "Lactation and milk: Defining and refining the critical questions" which serves to bring together discussions stemming from a conference held in Denver last January. In the review, she and her coauthors run through four key research priorities in the field: how the components of breast milk affect an infant's growth and health, how they impact an infant's brain and behavior, some key issues concerning regulation of mammary gland function and finally, how milk research can help infants born pre-term or to obese or undernourished moms. The intention of the project, says Neville, is to help streamline future efforts in a field with limited money and manpower.


Dr. Peggy Neville

So what are the cherry-picked priorities? First up, although a lot of recent work has looked at milk's components, such as sugars called human milk oligosaccarides, some classes of ingredients have been largely left untapped. For example, milk fat globules contain small crescent-shaped packages of cytoplasm--samples of the mammary alveolar cell whence they came. By comparing the RNA contents of these crescents, researchers might reveal important differences in the effect of obesity and other stresses on mammary cell function, says Neville.

Moreover, little is known about the sequence of changes in the composition of colostrum. Considering colostrum contains various molecules that suppress inflammatory responses, it probably also plays an important role in intestinal development. Similarly, there are obvious holes in current knowledge regarding how microbial communities affect the development of gut associated lymphoid tissue and how milk molecules that can bind to immune cells probably protect against allergies later in life. On the subject of milk and medications, lactating moms can draw on thorough advice if they pop pills for rheumatic diseases, but those who take anti-depressants have scant material on how those drugs may affect a breastfeeding baby's behaviour. Someone should get on that.

Then there's milk's effects on a babe's developing brain. Here, research is extremely limited, largely for lack of means to test hypotheses in humans. What is certain, however, is that human milk has curiously high concentrations of molecules known to foster brain development. Differences in the amount of cortisol in macaque monkeys' milk impart a lifelong behavioural trajectory on the infants nourished by it, but at present, there are only hints of this effect in humans from a much shorter study. The study in humans reported that consuming more cortisol as a neonate makes for a more labile human infant.

The list of opportunities in lactation and milk research goes on. What of the lactating mom? Neville and colleagues stress that a mechanistic understanding of the factors and signaling pathways regulating mammary gland differentiation during pregnancy, birth, and lactation, is sorely needed. Once such mechanisms are established, scientists can better

understand what can go awry. To be sure, the field has long-established that prolactin and oxytocin play key roles, but also, it appears, does cortisol.

Various other hormones are relevant--insulin, progesterone, and glucocorticoids among them--suggesting stress (acting via glucocorticoid levels), obesity, and gestational diabetes (both acting via insulin) may alter mammary gland differentiation. Perhaps, for example, the additional insulin circulating in an obese mom causes premature involution?

In addition to understanding mom's hormones, it makes sense to consider her genes. Little is known about genetic and epigenetic underpinnings of milk production, or about what moms can eat or do to improve the flow and composition of their milk.

Of course, those most likely to benefit from a renewed effort in lactation research are babies with unusually high odds of death and disease: preemies, those born in places of poor sanitation, or to obese or diabetic moms. These research topics are priorities. Preemies have nutrient demands that outstrip what breast milk can pack into their tiny tummies, and their moms sometimes aren't biologically primed to produce enough milk. More should be done to find out how to help both. Moreover, the babes of obese and diabetic moms are more likely to suffer from metabolic disease. And infants that remain stunted from under nutrition through their third birthday often remain cognitively restricted for life. Scientists currently conjecture that breast milk might abrogate this undesirable fetal programming. But does it really?

As always, a big new push requires someone to push and money for support. Lactation biologists are dwindling in number, and while organizations like the Bill & Melina Gates Foundation are waking up to the field's potential, more needs to be done to encourage national research agencies to step up. Lactation is a field where the basic biology is still up for grabs and new findings have great potential to help millions of infants around the world. It seems only smart that the new generation of researchers and financial supporters take heed of Neville's review.

Neville MC, Anderson SM, McManaman JL, Badger TM, Bunik M, Contractor N, Crume T et al. (2012) Lactation and neonatal nutrition: defining the refining the critical questions. *J Mammary Gland Biol Neoplasia.* [Epub ahead of print].

Contributed by
Anna Petherick
Professional science writer and editor
www.annapetherick.com