



SPLASH! milk science update

September 2014 Issue

This month's issue features articles about cultured milk and healthy skin, new tools to customize milk for premature babies, diabetes and breastfeeding, and the logistics of delivering dairy.

Cultured Milk- a Means to Healthy Skin?

- **Several studies indicate that regular intake of milk products cultured with probiotic bacteria can help keep the skin hydrated, robust, and healthy looking.**
- **The effects of cultured milk products on skin health are thought to involve crosstalk between the gastrointestinal and immune systems.**
- **Proposed mechanisms for the effects include suppression of gut phenols, release of bioactive milk peptides and stabilization of skin sebum.**



Since the times of ancient Egypt, women have used milk in various ways to attain beautiful, glowing skin. Today, Cleopatra's alleged penchant for bathing in milk from 700 donkeys may seem a tad rich, however there is growing evidence that consuming milk products cultured with probiotic bacteria can help you to not only get a healthy gut, but also healthy skin.

In fact, scientists have long suspected that skin health and gut health are two sides of the same coin. After all, the lining of the gastrointestinal tract is—sort of—an extension of the skin. These inside and outside protective barriers are thought to 'talk' with each other along a proposed 'gut-brain-skin axis', tightly interwoven with the immune system (1). This complex conversation becomes all the more fascinating by the fact that it involves the

millions of bacteria living on the skin and gut surfaces.

If the balance between the bacterial communities in the gut is disturbed, such as after an infection or other illness, it has long-range effects linked to, for instance, the immune system. By the same token, encouraging a balanced gut flora can stimulate the immune system and promote overall health.

One way to maintain intestinal health is boosting the so-called probiotics or 'good bacteria'. Natural inhabitants of the gut, probiotics help to keep 'bad bugs' in check. Some probiotic bacteria, for instance lactobacilli and bifidobacteria, are also used in the food industry, typically to make cultured milk products such as yogurt.

Not surprisingly, the manufacturers of such products do a lot of research, not only to improve flavor, texture, and other qualities related to the culturing or fermentation process, but also to study the products' potential health effects to expand their market range and value.

Fighting the gut phenols

Yakult, the Japanese pioneer behind the now world-known probiotic milk drink of the same name, recently reported several studies demonstrating the beneficial effects on skin health of a test product containing *Bifidobacterium*-fermented milk (2).

In a clinical trial, Yakult researchers compared the effects of the fermented milk and non-fermented placebo milk (3). After giving each drink to two groups of healthy Japanese women, they measured various parameters in the women's blood and urine as well as the hydration level of their skin. After four weeks, the women who drank the placebo milk had significantly drier skin than the women who drank the fermented milk.

Could drinking fermented milk really help keep the skin moisturized? Well, Yakult's research indicated as much, as well as provided some clues as to how. It showed that the women who drank fermented milk had lower blood and urine levels of phenols—a group of toxic by-products produced by some gut bacteria during digestion of proteins. Phenols are to some extent absorbed from the gut, circulated via the bloodstream and excreted from the body in the urine. But as the phenols paddle along with the blood, they may build up in the skin—at least in the skin of mice, as shown by Yakult researchers. In further tests on laboratory-grown human skin cells, they showed that phenols disturbed the cells' production of a major skin protein, keratin 10, which helps to strengthen the barrier function of the skin.

High levels of phenols are typically seen in people with a disturbed gut environment and are influenced by, among other things, the frequency of bowel movements. While a survey among Japanese women highlighted a popular belief that constipation is linked to various skin problems, including dry skin, there is so far not enough experimental data to prove this link. But based on the studies in humans, mice and skin cells mentioned above, Yakult researchers concluded that excess phenols produced by certain gut bacteria cause skin problems such as dryness. As an antidote, they suggested daily intake of fermented milk containing probiotics, which could help maintain healthy skin by promoting a favorable gut environment that suppresses the production of phenols (2).

Unlocking bioactive milk peptides

Japan has a long tradition for producing fermented milk drinks. Calpis, another manufacturer, recently examined various skin properties in laboratory mice given a diet containing fermented milk whey or non-fermented milk whey (4). They found that the fermented whey, to a modest extent, could reduce water loss from the skin and alleviate dermatitis, an inflammation of the skin. Based on these results, the researchers suggested the fermented whey might strengthen the barrier function of the skin.

As the fermented drink used in this study was milk whey—the liquid remaining after the probiotic bacteria were removed by centrifugation—the bacteria could not have exerted a direct effect in the mice's gut. Instead, the study's authors ascribed the effect to biologically active peptides produced during the fermentation process, in which the lactobacilli chomp certain milk proteins into peptides.

Typically, the power of bioactive milk peptides lies hidden inside the protein until it is unlocked by an 'encryption key' in the form of chemicals, enzymes or microbes. Probiotic bacteria are known to unlock milk peptides with a number of health-promoting properties. Some peptides promote a healthy gut ecosystem, for instance by binding toxins, inhibiting the binding of harmful microbes to gut cells, boosting the growth of 'good bacteria' or regulating immune responses (5). These activities are bound to register on the 'gut-brain-skin axis'.

Stabilizing the skin sebum

No doubt owing to the long-standing national tradition for sipping fermented milk beverages, such products are researched intensely in Japan—and not only by the companies producing them. Recently, scientists from various universities and a national research organization reported that the intake of fermented milk, compared with the intake of conventional yogurt, increased the skin hydration levels in young women (6). The study attributed the effect to increased levels of sebum. It also indicated that the effect depended on the women's age and hormone levels, which influence sebum production.

Now, isn't sebum the oily stuff that gives you pimples? Yes and no. In just the right amount, this cocktail of lipids helps protect the skin. Too little can give you dry, cracked skin, but too much can cause acne. And it seems milk could help alleviate the latter as well.

A Korean study (7) reported that daily consumption of fermented milk enriched with [lactoferrin—a powerful milk protein \(8\)](#)—had a therapeutic effect on acne in men and women. The over-production of sebum decreased in the study participants who drank lactoferrin-enriched, fermented milk and also, to a lesser degree, in those who drank 'plain' fermented milk. But only those in the lactoferrin group had significant improvement of their acne. So while some of the serum-stabilizing effect appeared to be due to the probiotic bacteria, lactoferrin provided the greatest efficacy. Lactoferrin's power—probably 'unlocked' by the probiotics as explained above—lay in its ability to reduce both skin sebum content and inflammation.

Inducing a 'glow of health'

As mentioned, anti-inflammatory effects may emerge by modulation of the immune system via the gut-brain-skin axis. Recently, a US-led team of university researchers proposed an interesting, sex-related explanation for the ability of probiotics to trigger this mechanism (9). Feeding probiotic yogurt to aged mice, the researchers found that the probiotic bacteria recreated the thick lustrous fur and 'glow of health' typical of young mice. (Fur luster was measured by an instrument as well as evaluated by human panelists unaware of which animals had received the probiotic yogurt or a placebo.) Linking this glow to various signs of youth and fertility, including increased sebum production, acidic skin pH, and anti-inflammatory mechanisms, the researchers suggested the effect was part of an evolutionary mechanism to ensure the reproductive success of both the symbiotic bacteria and their hosts. In other words, radiant skin and shiny hair signal good reproductive health and are therefore inherently attractive to prospective mates.

So what to make of all these studies seemingly suggesting different mechanisms for various beautifying effects of fermented milk products? The common thread among the probiotic-induced effects is the crosstalk between the gastrointestinal and immune systems, probably also involving the endocrine and nervous systems. The proposed mechanisms, whether related to suppressing gut phenols, unlocking bioactive milk peptides, stabilizing skin sebum or inducing a glow of health, are most likely closely related. But a lot more fermented milk needs to be drunk—by women, mice and men—for researchers to gather more evidence and figure out exactly how it works.

As for exactly how much fermented milk you need to drink to get that 'glow of health', the evidence isn't clear-cut either. In Yakult's study, the women consumed a daily dose of more than 100 billion of probiotic bacteria in total, which is far above the roughly 6.5 billion in a single-serve Yakult bottle (3). Most of the other studies also used higher doses than those typically consumed in actual foods and drinks. So fermented milk products are hardly going to be a magic bullet, especially considering their relatively un-dramatic effects. Perhaps the fermented milk products of the future will include higher doses of probiotics. In the meantime, the science suggests fermented milk can be one ingredient in a healthy lifestyle and for promoting beautiful skin.

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New Tool Helps Clinicians Customize Milk for Premature Babies

- **Premature babies require more protein than term infants and thus many need additional protein added to breast milk to achieve optimal growth.**
- **Human breast milk varies in protein concentration, making it difficult for clinicians to determine how much protein fortification each infant needs.**
- **A new paper demonstrates high accuracy for milk protein analysis from an instrument that easily fits in a NICU and produces nearly instantaneous results.**
- **The ability to measure each mother's milk and customize protein fortification could greatly improve premature infant growth outcomes.**

Mothers that deliver prematurely (< 37 weeks gestation), produce milk that differs in composition from mothers of term babies, including higher protein content. Premature babies have higher protein requirements compared with term babies, especially those that are very low birth weight (VLBW, ≤ 1500 g or approximately 3.3 lbs), yet this extra protein is still not enough to support their rapid body growth. To keep these babies growing at the appropriate rate, their mother's breast milk needs to be fortified with additional protein. Sounds simple enough, but determining just how much protein fortifier should be added is quite complicated due to the high variation in milk protein concentration across (and even within) human mothers. To take the guesswork out of it, clinicians need a method of quantifying the protein concentration in every mother's milk that is quick, reliable, and suitable for use in the setting of a Neonatal Intensive Care Unit (NICU). Could a tool long-used by the dairy industry to analyze cow's milk provide the solution they have been looking for?



The right instrument for the job

A new paper by Smilowitz et al. (1) tests an instrument called the Fourier Transform mid-Infrared spectroscope (FT mid-IR) against gold standard reference methods for analyzing milk protein composition, as well as fat, lactose, and total milk energy. Unlike reference methods for milk macronutrients that involve the use of large and specialized equipment, fume hoods, and numerous hazardous chemicals, the FT mid-IR is the size of a standard copy machine and does not require the addition of anything to the milk, except perhaps de-ionized water for sample dilution.

But its ability to fit in a NICU was only one of the reasons Smilowitz et al. believed it to be an impressive candidate for the job. The FT mid-IR works by measuring how molecules absorb IR energy. Molecules will differ in the wavelength at which they absorb IR, allowing for their identification and quantification in a given sample. It just so happens that many milk components, including protein, fat, and lactose absorb wavelengths in the mid-IR spectrum, which is why this technique is the standard method of analysis for cow's milk. What Smilowitz et al. hoped to demonstrate that it could be equally accurate when applied to human milk.

How does it measure up?

To put their instrument to the test, Smilowitz et al. (1) divided 116 samples (32 from preterm milk, 84 from term milk) into two equal aliquots, one to run on the FT mid-IR, and the other to run using gold standard methods of milk analysis. There was a high positive correlation between protein data from FT mid-IR and the reference method, but this statistical test alone does not indicate whether the FT mid-IR is accurate. What is important when it comes to fortifying preterm milk is how close the FT mid-IR is to the true value of the sample. To determine this, Smilowitz et al. ran a statistical test called the limits of agreement. This test measures how much the value from the new method (FT mid-IR) is likely to differ from the old (or reference) method (2).

Their results are very promising. Values for human milk protein calculated by the FT mid-IR were between 0.22% below to 0.06% above the reference method. This is a very narrow range and the authors believe that these small differences are unlikely to be clinically relevant (1). Moreover, these results suggest that the FT mid-IR is rarely overestimating the true milk protein concentration, which would be the most problematic from a clinical perspective. Additionally, the group analyzed ten separate milk samples on the instrument multiple times to determine the reproducibility (or reliability) of the instrument. The data were impressive with very high reproducibility (higher than 97%).

The right protein for each infant

Why is it so important to get the protein concentration just right for premature infants, but not for those born at term? Premature infants have to achieve fetal growth rates from nutrition provided in breast milk rather than the placenta, but their protein (and energy) requirements exceed that available in term and even preterm milk. To put this in perspective, Dr. Mark Underwood, the corresponding author on the FT mid-IR study and the Chief of Pediatric Neonatology at UC Davis Children's Hospital, describes the metabolism of a premature infant as being analogous to that of an Olympic swimmer. "Michael Phelps is reported to have consumed 10,000 calories per day when he was training intensively, which means he was consuming about 116 calories per kilogram per day," says Underwood. "Very small premature babies...often need to take in up to 130 calories per kilogram per day just to maintain adequate growth."

Human mature milk protein concentration ranges from approximately 0.8 % – 1.2% (g/100 ml), with higher values (2 – 2.5%) produced during the first several days of lactation (colostrum). Mothers that deliver prematurely produce milk with approximately 30% more protein (and thus, higher energy) than term milk (3), but these values also can be highly variable as well (1.8 – 3%) (4). How do you fortify a mother's milk with protein when you do not know how much protein it has to begin with?

"It is pretty astounding if you think about it," says Dr. Jennifer Smilowitz, Associate Director for Human Studies Research at UC Davis' Food for Health Institute and lead author of the FT mid-IR study. "We know what is in every food item consumed by an adult, and yet we have no idea what these infants are eating."

Simply adding the same amount of fortification to each milk can leave many infants undernourished. Indeed, this practice has been associated with many negative outcomes including poor weight gain, issues with long-term growth, and neurodevelopment delays (1, 4). And although formula allows clinicians to control for the amount of protein (and other nutrients) the infant is ingesting, the negatives outweigh the benefits in growth. "When premature infants get formula, their growth is better, but they have more infections and their brain and nerve development is not as good," says Underwood.

Next stop NICU?

The goal of Smilowitz et al.'s study was to demonstrate that the FT mid-IR had good agreement with reference methods of milk analysis, and at this they succeeded. Their next set of experiments will further assess the accuracy and the reliability of this instrument by measuring the protein concentration of a milk sample before and after fortification. If it can accurately measure a known quantity of human milk protein, the FT mid-IR may soon become a permanent fixture in the NICU. For clinicians, like Dr. Underwood, that have waited decades for this type of technology to develop, this is most certainly good news. "Coming up with a way to easily measure the nutritional value of human milk samples for the most premature babies and then adding the right amount of fortifiers before feeding will be a big step forward."

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Diabetes and Breastfeeding

- **Gestational diabetes may or may not delay the onset of milk appearing in the breast after giving birth because different studies tell a different story.**
- **Gestational diabetes does seem to reduce how long women breastfeed.**
- **Breastfeeding improves mothers' metabolic health. Breastfeeding for two years or more is best.**



When it comes to understanding the links between breastfeeding and diabetes, causation runs both ways. Diabetes can influence if and for how long a new mother breastfeeds. On the other hand, developing diabetes during pregnancy and lactation can affect mother's metabolic health later in life. As more studies in these fields generate results, a complex picture is emerging of interacting risk factors.

The metabolic gearshifts involved in growing and nurturing another human being are substantial. Not only does a pregnant woman's body mass change relatively rapidly over time, but in the United States, the Centers for Disease Control in Atlanta has recorded 8.8% of pregnant women developing gestational diabetes, even though just 1.7% of these women were diabetic before they became pregnant.

Although the reasons for this uptick are yet to be fully understood, it is partially because pregnancy hormones can make the body more resistant to insulin, which explains why diabetes disappears again after a woman has given birth in most cases. At that stage, a new mother's body spends about 500 calories more than it normally would on a daily basis if she breastfeeds. And yet, despite these dramatic changes, a recent paper by Ted Jost of ETH Zurich and his colleagues shows that the composition of bacterial species in the gut remains fairly stable throughout (1).

But how might diabetes influence breastfeeding? For one, getting things up and running seems to be more difficult for women with a history of diabetes prior to becoming pregnant. This has been demonstrated by a trawl of the CDC's data on the 73,000-odd women who became pregnant in 30 states of the US between 2009 and 2011. Using this information, a team of researchers from Ohio and West Virginia has found that even though pre-pregnancy diabetes makes initiating breast-feeding harder, starting to lactate appears to be no more difficult for women who develop gestational diabetes compared to diabetes-free mothers (2).

But other studies indicate the opposite. One of them examines data from northern Californian women who enrolled in a cohort called SWIFT(3). It looked at the more immediate factors for delayed onset of breastfeeding among women with gestational diabetes. As many as 33% of these women had to wait for more than 72 hours after giving birth to develop the fullness of the breast that indicates the arrival of milk. The same statistic for new mothers in general (diabetic or not) is 22% (4). According to the SWIFT results, the additional factors that appear to prompt a delay in lactation are obesity and older age. But the underlying mechanisms for these effects still need some attention.

There are, however, clearer answers to the question of how diabetes is associated with breastfeeding duration. The same CDC data trawl on women who become mothers from 2009 to 2011 found that having diabetes before pregnancy as well as developing it during pregnancy are both linked to a shorter breastfeeding period. Breastfeeding duration is important because, for example, it affects the odds that the infant will develop potential health problems when they are older. All things being equal, people who were breastfed struggle less with their weight and cholesterol levels (5).

Mother's metabolic health is also influenced by her reproductive decisions (6). Indeed, women who develop gestational diabetes are more likely than those who do not (and have no medical history of diabetes) to become diabetic in the years that follow giving birth. Perhaps the most insightful recent examination of this point comes from Iran, where 98% of women breastfeed, at least for a while. The Holy Quran suggests that new mothers should breastfeed for 2 years- so, as a result, many Iranian mothers lactate for much longer than the World Health Organization's recommendation of 6 months exclusive breastfeeding.

The Tehran Lipid and Glucose Study is noteworthy because it found a dose-dependent effect (7). Women in the study who breastfed for as least as long as the Quran recommends were less likely than those who breastfed for less than this period to develop metabolic syndrome. So, when mothers have both infants and young children feeding from them, they are, without realizing it, investing in their own long-term health.

Interestingly, for women who have previously had gestational diabetes, the reoccurrence of this problem with another pregnancy does not worsen their metabolic health down the line. This was recently demonstrated by Winhofer et al, who measured outcomes such as insulin sensitivity, secretion, and cardiovascular risk factors (8). It is worth mentioning because the results indicate that women who experienced diabetes during one pregnancy should not be put off a second pregnancy out of concerns for their own well being.

This field is particularly relevant in a context where diabetes is on the rise in many developing, as well as developed nations. Perhaps these results could provide a new means of promoting breastfeeding where it is uncommon, by, in essence, appealing to some women's more selfish instincts? That would be good for infants, too. It's worth a shot.

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Delivering Your Daily Dairy

- **Fresh milk continues to be delivered quickly to households.**
- **Dairy has established complex infrastructure for collection, processing and distribution.**
- **Milk moves quickly through pasteurization and homogenization processing.**
- **Despite its status as a highly nutritious staple, milk is remarkably low cost.**

Delivering milk to the table of households across the world is a triumph of logistics that has developed over many decades. In some countries, such as India, traditional approaches still predominate, albeit with steady changes. For the others, it engages a sophisticated organizational infrastructure on a regional, national or even international scale.

In 2012, 620 billion liters of milk were produced worldwide. This continues to grow incrementally with increasing demand. Domestic markets consume most of this, leaving about 5-7% available for trade. Dairy farmers and farm systems have boosted productivity because they've become more efficient in their production. For example, larger herd sizes, selective breeding programs, and improved cow nutrition have helped meet the increased demand while lowering production inputs. The proportion consumed as drinking milk varies between countries, but the challenge of delivering fresh milk to the consumer is ubiquitous. Drinking milk arrives on the supermarket shelves in enormous quantities, usually within 24 hours from the cow being milked. The certainty of supply and nutritional contribution of milk provides a staple for many households.



Essential to both the farmer, as well as the entire dairy system, is the reliability of milk collection. On most farms, milking occurs twice daily and the collected milk is then stored on the farm in large refrigerated vats. Milking times are coordinated with collection times, hence the early morning rising times that we associate with dairy farming. Each day, an armada of milk-collection tankers owned or commissioned by farm cooperatives or dairy companies finds its way to every farm to collect the stored milk and deliver it to the processing plants. One of the recent developments is an increasing uptake of automated milking systems. This has had a major impact on the lives of farmers and cows. The most notable

change is to milking times and frequencies, and although this initially raised concerns over timely on-farm milking relative to collection, it has not proved to be a problem. So, the farmers get to enjoy a reasonable night of sleep, and the cows don't seem to mind what time of the day or night they wander up to the dairy for milking.

The packaged milk that arrives on supermarket shelves is largely unchanged from the milk collected from the farm, except for two processes: milk homogenization and anti-microbial treatment. We generally know anti-microbial treatments as pasteurization (named for Louis Pasteur, who pioneered the process). The procedures used for pasteurization vary, but traditionally involve heating the milk to 63°C (145°F) for 30 minutes. This process was introduced to prevent the milk from spoiling due to microbial contaminants, as well as to prevent transferring potential pathogenic microorganisms to consumers. This process lengthens the shelf life of milk to approximately 2 weeks, if refrigerated. Nowadays, the same effect is achieved via a short period of higher temperature treatment, 73°C (163.4°F) for 15 seconds. Prior to packaging, heat is applied to the milk as it passes through stainless steel piping in the processing plant, which ensures even temperature distribution. Another alternative treatment that has grown in popularity uses a very short, very high temperature. It requires a temperature above 135°C (275°F) for about 2 seconds. This process, known as ultra high temperature treatment (UHT), achieves a greater level of microbial ablation and effectively sterilizes the milk. UHT treatment increases milk's shelf life for extended periods, approximately nine months. This has the added advantage of not requiring refrigeration, and is well suited for transporting milk over long distances, and supplying milk to remote locations.

Homogenization, as the name suggests, is a process that produces a homogeneous or standardized milk. Many may remember un-homogenized milk, which was previously the common form of drinking milk. The milk fat, or cream layer, would rise to the top and leave more watery milk below. Family members would favor the cream, or not, but in most instances the milk bottle would need to be given a thorough shake to mix the layers before opening. This was a manual process of homogenization, which is now automated and completed at the plant. This is achieved by passing the milk through a small valve or bore under pressure, which mixes the milk components and disperses the fat content of the milk, providing a uniform appearance, taste and texture. The milk is then packaged in cartons or bottles, not the expensive glass bottles of the past, but biodegradable cardboard or plastic recyclables.

Once the processing is complete, the milk is loaded onto delivery trucks that travel to the myriad of outlets: from the supermarkets that sell in large volumes, to the corner store or coffee shop that provides for everyday needs. The speed, efficiency, coordination, and regularity of this incredibly complex logistical achievement are the backbone of a successful dairy industry. Given the complexity of the infrastructure and the quality of product, drinking milk is remarkably, some say ridiculously, low cost.

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