SPLASH!® milk science update January 2017 Issue

This month's issue features recent findings about HIV drugs and human milk, a look at how genome technology may help dairy farmers, and new information about how dairy fats may relieve inflammation and boost calcium absorption.

The Beneficial Effects of Dairy Fats on Post-Meal Inflammation

- **Eating saturated fats is associated with a higher risk of cardiovascular disease and post-meal inflammation.**
- **Studies suggest that not all saturated fats are equally harmful, and dairy fats in particular may even have some beneficial effects on cardiovascular disease risk and inflammation.**
- **A new study found that a high-fat diet containing cheese lowered the levels of an inflammatory marker compared with a similar diet containing a vegan cheese alternative.**
- **Another study found that a high-fat meal containing a component of milk called milk fat globule membrane (MFGM) lowered post-meal inflammatory response compared with a high-fat meal without MFGM.**
- **These studies indicate that dairy fats may have some beneficial effects on post-meal inflammation.**

Fatty foods are known to have adverse effects on health, and saturated fats in particular have been linked to an increased risk for cardiovascular disease [1–3]. However, studies suggest that not all saturated fats are created equal, and the source of the fat may play a major role in determining its health risks or benefits. Dairy foods and certain components of dairy may even have beneficial effects on cardiovascular risk factors [4–8].

"Until very recently, we really have not done a good job of separating out what kinds of fats are contributing to these negative effects of high fat diets," says Angela Zivkovic of the University of California at Davis. "But now more and more people are recognizing that there's really a big difference based on what the specific food is that you eat," she says.

A pair of new studies led jointly by Zivkovic and UC Davis's Jennifer Smilowitz further supports the notion that dairy fats may not be as harmful as previously thought, and may even have some beneficial effects [9,10]. In the first study, the researchers found that when overweight individuals consumed a high-fat diet containing cheese, they had lower levels of a post-meal inflammatory marker than when they consumed a similar diet with a vegan cheese alternative. The second study found that when overweight individuals consumed a high-fat meal containing a component of human milk called milk fat globule membrane (MFGM), post-meal inflammation was lower than when they consumed a high-fat meal without MFGM.

"All these things are really kind of surprising because up until now the story has been that everyone should eat low-fat dairy because saturated fat is bad for you," says Zivkovic. "It's starting to look, not just because of our studies but also other studies that have been published, that even though dairy fat is enriched in saturated fat it does not seem to have the negative effects on cardiovascular risk that we assumed it would because of its saturated fat content," she says.

The researchers also suggest that rather than considering individual dietary components in isolation, it's important to view them in the context of the foods and meals in which they are consumed. "Diet composition, such as the breakdown of the fatty acid composition of our diet or meals, doesn't work alone to influence health," says Smilowitz. "The structure of food also determines how we will digest, absorb, metabolize and respond to food," she says.

The researchers focused on the effects of dairy fat on the post-meal, or postprandial, state, which is usually characterized by a brief increase in lipid and inflammation levels. "It's kind of an illustration of how your body is handling this particular meal that you're being challenged with," says Zivkovic. Meals rich in saturated fats, for example, have been shown to induce an increase in postprandial inflammation [11]. When elevated levels of lipids and inflammation persist for several hours, they can contribute to an increased risk of cardiovascular disease.

In their first study, Zivkovic and her colleagues compared the effects of dairy fats and non-dairy fats on postprandial inflammation [9]. "We're looking at the difference when you consume a grilled cheese sandwich that's made out of normal dairy cheese compared to one made out of vegan cheese substitute product that still had equivalent amount of saturated fat from palm oil," she says. The researchers assayed a number of cytokine and protein markers of inflammation, as well as blood lipids, glucose, and insulin.

"What we found was that it looks like neither cheese nor palm oil are particularly bad for you when you look at this postprandial state," says Zivkovic. "It's not what we expected," she says. "We were giving such a high amount of fats in one meal, we really were thinking that they would be more inflammatory than they ended up being," she says.

In addition, the researchers found that one particular marker of general inflammation, called C-reactive protein (CRP), was significantly lower in response to the cheese compared with the vegan alternative. "That the cheese was actually beneficial in terms of CRP was a pretty important finding," says Zivkovic.

In their second study, Zivkovic and her colleagues looked at the postprandial effects of one particular component of dairy, called milk fat globule membrane (MFGM) [10]. MFGM is a membrane that encases milk fats, and is rich in bioactive proteins and complex lipids. "Previous research has shown that MFGM has some potentially anti-inflammatory and other beneficial effects," says Zivkovic [8,12-14].

The researchers tested the effects of adding MFGM to a meal consisting of milkshakes rich in saturated fats from palm oil. "These were pretty high-fat challenges, so we certainly expected these to be pretty inflammatory," says Zivkovic. "So we were really interested to see if adding MFGM would actually have beneficial effects," she says.

The researchers found that adding MFGM to the meal seemed to have beneficial effects on the postprandial state. "The main finding was that the insulin response was lower when we added MFGM," says Zivkovic. "What's interesting is that subjects in the study were all pre-diabetic," she says. "So in these individuals, something that can actually decrease their insulin excursion in the postprandial state would be a very beneficial thing," says Zivkovic.

The researchers also examined how adding MFGM to the meal affected the levels of various cytokines. "What was surprising was that we didn't really find that the pro-inflammatory cytokine levels increased very much," says Zivkovic. "Instead what we found was that IL-10, an anti-inflammatory cytokine, was actually higher after MFGM meals," she says. "Essentially, instead of reducing the levels of pro-inflammatory cytokines, what it seems to have done is to increase the levels of anti-inflammatory cytokines," says Zivkovic.

The researchers plan to follow-up these experiments with a longer term study. "I'm really interested to see whether long-term supplementation with MFGM would have beneficial effects," says Zivkovic. The researchers are also interested in figuring out the mechanisms by which dairy fats influence postprandial inflammation and cardiovascular risk.

"We have this long nutritional history of blaming fat and saturated fat for all of our health problems," says Zivkovic. "We really need to do more research to understand better why dairy fat isn't as bad for you as we expect it to be, even though it has so much saturated fat," she says.

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Drugs to Prevent HIV Infection are Barely Present in Human Milk

- **The World Health Organization warns women against taking HIV prophylactic drugs while breastfeeding, due to the potential for side effects associated with these drugs to affect infants.**
- **The WHO recommendation was made because it was not known whether such drugs find their way into human milk in significant concentrations.**
- **Researchers have now measured the exact levels of two HIV prophylactics in human milk: tenofovir and emtricitabine, which are often taken together.**
- **Tenofovir and emtricitabine are present in such extremely low concentrations that it is impossible for side-effects to pose a risk to infants who consume milk from women taking them.**

The World Health Organization considers someone with more than a 3% chance of contracting HIV in the next year to have a "substantial" risk of infection. This is important because it is the cut-off for which the WHO recommends taking anti-retroviral drugs, like tenofovir, to reduce the odds of infection. Breastfeeding women in sub-Saharan Africa easily meet this risk threshold [1]. But medical professionals are discouraged from offering the drug to these women because of WHO warning labels about potential adverse reactions in nursing infants. That advice may now change, paving the way for many more at-risk women to receive HIV prophylaxis. A new study shows that when infants consume human milk from a woman taking tenofovir in combination with emtricitabine, they are exposed to extremely low levels of either drug. Therefore, the infants' likelihood of experiencing adverse reactions is virtually non-existent [2].

The usefulness of tenofovir to prevent HIV infection has been supported by many studies. A trial of intravenous drug users in Thailand, for example, recorded a 48.9% lower incidence of infection among participants who took it instead of a placebo [3]. A study of heterosexual people in Botswana found that tenofovir taken alongside emtricitabine (a combination given the acronym, FTC-TDF) was 62.2% effective at preventing HIV infection [4]. But these trials offered no insight into whether lactating women could take the drug without harming their breastfeeding offspring. This is because lactating women were excluded from the trials. Indeed, women were dropped from the trials as soon as they became pregnant [1].

As a result, there was a gap in medical knowledge about the drugs' usefulness. Without proof either way as to whether FTC-TDF circulating in a woman's bloodstream could get into her milk—and thus whether or not the drugs might affect the tiny body of a breastfeeding infant—the World Health Organization's official advice was to err on the side of caution.

Kenneth Mugwanya of University of Washington, Seattle, was all too aware of how many women's lives might be saved with more data. If indeed these drugs present minimal risk to nursing infants, women could continue to protect themselves against HIV while they breastfed. Prophylactic drugs are especially important for women in the fight against HIV/AIDS because individuals have full autonomy over the decision to protect themselves, which is not the case with some other methods, such as use of condoms, for protection against its sexual transmission. So Mugwanya and his colleagues set about designing a lactation trial.

The team, drawn from Uganda, Kenya, and the United States, managed to recruit 50 HIV-free women, who, along with each infant's father, consented to their infants being enrolled in the trial. These women were then given FTC-TDF for 10 days in a row. During this period, they breastfed their infants as usual.

The researchers sought to find out the extent to which both tenofovir and emtricitabine were excreted by the women's bodies into their milk. Using a technique called liquid chromatography-tandem mass spectrometry, the team measured the amounts of both drugs circulating in the mothers' bloodstreams, the concentrations found in their milk, and how much these concentrations varied throughout the day. The same measurement technique was then applied to a blood sample drawn from the breastfeeding infants after their mothers had taken FTC-TDF for a week.

The results were surprisingly reassuring. Infants in the trial received about 12,500 times less tenofovir via their mother's milk than the dose of this drug that has been proposed for prophylatic use in infants whose mothers are HIV positive. The amount of emtricitabine in human milk was also very low—more than 200 times less than the dose proposed for an infant prophylatic.

Therefore, both drugs were well within the medical community's rule-of-thumb for when a lactating woman can continue to take a medication without worrying about her child experiencing associated side effects: the concentration was less than 10% of the weight-adjusted recommended therapeutic dose of the drug for infants [5]. Tenofovir could not even be detected in 94% of the infants' blood samples, and the levels of emtricitabine in the infants' blood were also extremely low. Importantly, there was little variation in blood concentrations of the drug among the infants, nor among the milks produced by their mothers.

Mugwanya and his team have provided the evidence that a change is needed in the WHO's official advice. HIV-negative women at high risk of contracting the virus should not stop taking their prophylatic drugs because they are breastfeeding. This finding is hugely important given that 71% of new HIV infections in sub-Saharan Africa are among women and girls, and HIV prevalence in the region is now eight times higher among teenage girls from 15–19 years old, than it is among males of the same age [1].

In the past year, both Kenya [6] and South Africa [7] have approved the prophylactic use of FTC-TDF. As those countries roll out these new public health policies, they should ensure that medical professionals are fully aware of the findings of Mugwanya et al. [2].

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Dairy Foods Promote Calcium Absorption and Bone Mineralization

- **The bioavailability of calcium is a measure of how well dietary calcium is absorbed by the intestines and then transferred to the skeleton.**
- **Foods vary in their calcium bioavailability because some contain components that block its absorption, whereas others have components that promote its absorption.**
- **Milk contains no inhibitors to absorption but instead provides several nutrients that work together to boost calcium absorption.**
- **Most Americans are not meeting their recommended daily allowance (RDA) of calcium.**
- **Dairy foods are the best dietary source of calcium because of their high calcium concentration and bioavailability.**

Nutrition pop quiz: Which food provides the most calcium for an adult human body, 10 cups of spinach (containing 300 mg of calcium) or 1 cup of milk (also containing 300 mg of calcium)? This is not a trick question, but calcium is a tricky nutrient. Although both servings of spinach and milk contain the same amount of calcium, they differ dramatically in how much of the ingested calcium is eventually absorbed and deposited into bone. This is because spinach, milk, and other foods with calcium contain additional nutrients that promote or inhibit the movement of calcium from the digestive tract to the skeleton. Whereas spinach contains an acid that binds calcium and renders it almost completely indigestible, the ingredients in milk—and other dairy products—work synergistically to enhance calcium absorption and its subsequent deposition into bones in a manner not seen in any other dietary source of calcium. So if you are looking to get the most calcium per bite, the obvious choice is dairy. Care to take the pop quiz again?

The bone bank

Ninety-nine percent of the calcium in our body is stored in the skeleton, with the remaining 1% found either inside of cells or moving between them, helping with critical physiological processes such as muscle contraction, hormone secretion, and nerve conduction [1,2]. Calcium in the bloodstream is maintained at a constant concentration [3], which means that the skeleton acts as a bank for the body's calcium supply; when we eat foods containing calcium we make a deposit, and when our cells need calcium, we make a withdrawal.

And the human skeleton is a busy bank! It is constantly remodeling throughout the life course, building bone with newly acquired calcium and resorbing (or releasing) calcium due to physiological requirements. As we grow, the emphasis is on deposits and reaching the highest peak bone mass (i.e., the amount of bone tissue present when the growth period ends). Failure to reach an optimal peak bone mass can predispose individuals to poor bone health (including osteoporosis) as they age. This is because the aging process is, unfortunately, associated with an increase in calcium withdrawals. As soon as we reach peak bone mass, the process of bone loss begins, estimated to be approximately 0.3% of total bone mass each year [1]. Thus, while sufficient calcium intake is important across the lifespan, it is particularly critical for growing children (where bone formation exceeds bone loss) and older adults (where bone loss exceeds bone formation) [1,3].

The current U.S. dietary guidelines for calcium were designed to protect against negative calcium balance—losing more calcium (in urine, stool, and sweat) than the body absorbs—and reflect age-related differences in calcium needs. For children 9–18 and adults over 71, the recommended daily allowance (RDA) is 1200 mg, compared with 1000 mg for adults 19–71 years old (with the exception of females aged 50–71, who have a higher recommendation of 1200 mg due to bone loss changes related to menopause) [4]. Unfortunately, the recent Dietary Guidelines for Americans suggest that most Americans, particularly those over 50, are not meeting their RDA for calcium [5].

From the fork to the skeleton

It would seem the simple solution to our country's calcium problem would be to just eat more foods that provide calcium. However, getting the calcium from the food we eat to the bones in our body is quite a complicated process. One's diet could contain the RDA for calcium, and yet their body could still be in negative calcium balance because foods vary in the bioavailability of calcium—that is, how well the calcium present in that food is absorbed in the intestines and then deposited into the skeleton [1].

Variation in calcium bioavailability results from the mineral's very specific requirements for intestinal absorption. Calcium travels from the digestive tract into the cells of the small intestines by two routes: active transport (passing through the cells of the intestinal mucosa) and passive diffusion (passing between these cells). Whichever method is utilized, calcium must be in a soluble form and attached to an organic molecule. If it does not meet these requirements, the calcium just continues along the digestive tract, ending up as waste.

Many components of familiar foods are calcium blockers, that prevent intestinal calcium absorption through their influence on its solubility or blocking the appropriate binding molecules [1]. Take spinach, for example, where the bioavailability of calcium is so low (estimated around 5% absorption) because spinach also contains oxalic acid [6]. During digestion, this acid binds to the calcium and creates an insoluble salt, called oxalate. In the same way, phytic acid (found in seeds, grains, and legumes) binds calcium to create phytate, another insoluble salt [1]. Although the nutritional labels indicate these foods contain calcium, the amount on your fork may be quite different from the amount that is absorbed during digestion, and subsequently transferred to the skeleton.

Hitching a ride

In contrast to food components that prevent calcium absorption are those that work to enhance either active transport or passive diffusion of calcium in the small intestine. Perhaps the most well known is vitamin D. In its active form, vitamin D is a hormone (calcitriol) that influences the expression of a protein called calcium-binding protein (CaBP) [1]. As its name suggests, this protein binds calcium ions and takes them through the cells (via active transport) that line the upper portions of the small intestine. The more vitamin D in your body (either from the sun, diet, or supplements), the more CaBP your body makes, and the better able your body is at absorbing the calcium provided in your diet.

But CaBP is not the only protein that binds calcium. Twenty percent of the calcium in milk is bound to casein proteins [1]. Because the calcium is already bound to a protein, it does not need CaBP for transport across or between the cells of the intestines. Calcium in milk not bound to proteins can benefit from the presence of lactose, which facilitates the movement of calcium ions in the lower portions of the small intestine [1]. Indeed, calcium in milk has a high rate of absorption even in the absence of vitamin D, what Guéguen et al. [1] refer to as "ensured absorbability."

Hitching a ride with casein proteins gives calcium in milk another digestive advantage—time. Casein proteins in milk are present as small spheres called micelles, which then transform into solid curds when they encounter digestive enzymes. The structure of the curds makes them more difficult to digest, so they travel slowly through the stomach and intestines as enzymes work to break apart the individual amino acids. Because the calcium ions are part of the curd structure, they also spend more time in the digestive tract than they would as part of other food products or supplements, increasing the opportunities for absorption [1,7].

Calcium's packaging in milk and other dairy foods provides one final advantage. The same casein proteins that bind calcium also bind phosphorus, a mineral that promotes bone mineralization [1,6]. Bioavailability, after all, is not just a measure of how much calcium is absorbed but how much is utilized by the body. Simultaneous delivery of the two minerals during casein protein digestion greatly increases the chances that the absorbed calcium will end up in the skeleton [1,3].

Drink up

Milk is not the only food with a high bioavailability of calcium. Cabbage, bok choy, and broccoli also have a high absorption rate. However, because their total calcium content is lower than in milk, you would need to eat a lot more servings to reach the RDA for calcium. Dairy foods combine a high bioavailability (estimated at around 40%) with a high concentration of calcium, requiring fewer servings to meet daily requirements. The 2010–2015 Dietary Guidelines for Americans estimates that 80% of the U.S. population (> 9 years old) does not eat the recommended three servings per day of dairy. For those looking to make more deposits than withdrawals from their calcium bank, an easy dietary change may be to grab a glass of milk and drink up!

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Dairy Farmers Prefer Healthy Manageable Cows

- **Economic gain has been a focus for breeding dairy cows.**
- **New genome technologies may allow farmers to broaden selection criteria.**
- **Uptake of the technology and understanding farmer preferences are issues that impact successful herd management and production.**
- **Danish organic and conventional dairy farmers differ in their preferences.**
- **Genetic selection may help farmers address their specific herd management concerns, while also addressing the goals for national herds.**

Reaping the rewards of the genomic revolution in selective breeding in of dairy cows requires an informed and engaged dairy farmer response. The technology has moved rapidly in recent years, and the introduction of molecular breeding values has resulted in significant gains in production [1]. However, the rate of technology uptake for end users is a limiting factor for capitalizing on technology transfer. A study published in December 2016 from a Danish group of dairy scientists reports that farmers rank health and management qualities above production traits in their cows [2]. However, this ranking differs depending on whether the farmer is classified as organic or conventional.

Many countries have dairy herd improvement schemes. These schemes have been traditionally based on the continuous improvement in production and profitability of herds, and have focused on a few key economic traits. The value assigned to key traits varies from country to country but mostly focus on some combination of milk production, protein yield, and fat yield. There has been extensive investment in designing and implementing these schemes over decades, and they have contributed to major improvements in modern dairy. The schemes are based on a complex system of ranking the value of bulls following many years of progeny testing, in which the performance of the daughters of the bulls is assessed for on-farm performance. Dairy farmers are then able to select from the available bulls to inseminate cows to maintain and improve their herds as they are gradually replaced.

Recent advances in genetics and genomics have led to rapid developments in using detailed genomic information to apply in these herd improvement systems. The use of genomic technologies has the capacity to shorten the time taken to evaluate dairy animals and to capture the best quality genetics from a DNA test [3]. Genomics also has the capacity to increase the number of traits that can be accommodated within the selection process. Including additional traits is potentially advantageous to avoid inadvertent selection for unwanted production and health characteristics, and to possibly incorporate customized traits that may be specific to farming systems or identified issues within specific herds. The results of these programs can be very impressive, but returns on the enormous investment in developing these systems can only be realized if farmers are informed and make use of them.

Recent studies have investigated farmers' viewpoints about heard improvement and compared those to the breeding values that are currently in place. The most recent was a survey of Danish dairy farmers conducted by Slagboom et al. [2] from Aarhus University, which asked farmers about preferences for improvements in their cows. Denmark has a system for certifying organic farmers, and the survey covered both conventional and organic farmers. The farmers were asked to rank their preferences for ten traits, ranging from health welfare and milk production characteristics. When they analyzed the results, the researchers identified a number of groupings in the data according to farmers' preferences: Health and Fertility, Production and Udder Health, Survival, and Fertility and Production. Mostly, farmers were concerned with the challenges that were immediate to on-farm management, in preference to the higher-level goals of national herd improvement schemes. Interestingly, organic farmers had less of a focus on health and more on production traits. This was presumably because they indicated that their herds had lower incidence of disease than the conventional farm herds, but also had lower average milk production. This pattern was observed in other preferences; that is, farmers responded according to the areas that were of immediate concern to their herd management experiences.

The researchers compared their results to those of similar surveys carried out by other scientists in Sweden [4] and Australia [5]. In Sweden, organic farmers chose preferences that were the opposite of those in the Danish study. It is very difficult to unravel the reasons for these differences, but Slagboom et al. [2] suggest that the design of their survey may have resulted in a more objective response. Australian farmers were not so clearly grouped by farm characteristics, and there were no organic farmers identified in the study. Variation in their responses did not correlate with production systems and led to the conclusion that farmer preferences aligned with their intrinsic views [5]. Overall, these studies reinforce the view that farmers are mostly concerned with the specific challenges that they each face, and that these challenges vary across the farming population.

The bigger question is, can anything be done to address the concerns of farmer groups using genetic selection? This is certainly possible, with tailored target traits measured and made available to farmers, they can address specific issues, while the overall goals for the national herds also can be addressed.

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