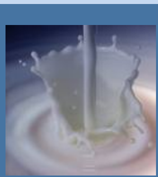
SPLASH! milk science update: August 2015 Issue



SPLASH! **milk science update**
August 2015 Issue

This month's issue features how mammary glands "remember," how dairy helps dieters maintain muscle, carotenoids in breast milk, and a comparison of milk banks around the world.

The Amazing Mammary Memory

- **Both humans and cows produce more milk after a second pregnancy compared to the first.**
- **The first pregnancy actually primes the mammary gland to respond during subsequent pregnancies.**
- **The DNA of mammary tissue shows epigenetic marks following the first pregnancy.**
- **Epigenetic changes result in changes in gene expression.**
- **The influence of these epigenetic changes have now emerged as a mechanism for increased milk production in subsequent pregnancies.**

Any dairy farmer or lactation consultant knows that first-time mothers don't produce as much milk. The peak daily production for a first calf heifer may be around 70 lbs of milk while the same animal on its second lactation can produce 90 lbs of milk daily. Somehow the mammary gland seems to remember how to make milk and does a better job the second time. Why is that?

The mammary gland has an extraordinary capacity for change during pregnancy and lactation. A first-time pregnancy triggers the release of the hormones, progesterone and estrogen, and along with other hormones, like Growth Hormone and Insulin, results in development of new mammary gland cells to form ducts, alveoli, and the capacity to produce milk. Immediately following birth, there is another change to initiate secretion of the milk, largely driven by the hormone, prolactin [1]. Once the need for milk production ceases, the milk producing cells rapidly die, and the mammary gland undergoes remodeling. These changes have been documented at many levels, including the characterization of the physiology, and cell and molecular biology.

What is clear from many lactation biology studies is that the mammary gland behaves differently during the first pregnancy compared with subsequent pregnancies. This is observed mostly in the amount of mammary tissue that develops and the volume of milk produced. In a recent study, Camila dos Santos et al. [2] set out to investigate differences in the DNA of mammary tissue between the first and second pregnancy.

The scientists hypothesized that, during the first pregnancy, the DNA in the mammary cells changed. They used laboratory mice as their model system. They divided their mice into two groups; in one group they mated on two consecutive occasions and allowed the animals to proceed through pregnancy and lactation until pups were weaned and the mammary glands regressed. The second group had not been previously mated, or gone through pregnancy. They also added another group of mice that were treated with hormone pellets containing estrogen and progesterone to simulate what normally happens during pregnancy.

The initial experiments checked for the changes in the mammary gland tissue by observation using microscopes. The scientists found showed that there was a marked difference in the response to pregnancy hormones in both mated mice and those treated with hormones. Having established that their experimental system was ready, they set about characterizing the DNA. They were particularly interested in measuring the changes that cause a chemical modification of the DNA, called methylation. We know that changes in the amount of DNA methylation has an effect on the activity of genes, which means it influences how much protein is produced. The proteins from these genes include milk proteins, and other proteins that regulate milk production. Because methylation patterns can be altered during the lifetime of an animal, it is a way to evaluate epigenetic or environmental effects on the genome [3-5].

The scientists dissected the mammary glands from the mice at post-mortem and divided the cells into the different cell types that make up the milk producing tissue. This required the careful use of a number of specific antibodies with fluorescent probes attached to them. The antibodies only recognized the relevant cells, so they automatically sort into different test tubes using a machine known as a fluorescence activated cell sorter. This machine checks each cell, one by one, for the antibody marker and then sorts the cells into positive and negative groupings.

Once they had collected the cells of interest, they extracted the DNA for each cell type. The DNA was then sequenced using a special technique that picks up the methylated DNA. They found between 40,000 and 70,000 sites where the methylation patterns were different in mammary cell types compared to other unrelated cell types, such as brain cells. They then looked at these sites and compared mammary cells between mice that had not been pregnant and those that had. They found that many sites changed their patterns and also that methylation had been diminished in the mammary cells of mice that had been through two pregnancies. Most changes (more than 800), happened in the cells that are directly responsible for producing milk.

What were these epigenetic changes doing? When they investigated the DNA sequence around these sites, a significant number were found to be places for binding to factors that regulate gene activation. The most notable was a site that binds a protein called Stat5. Stat5 has an important role in turning on a number of genes during pregnancy and lactation [6-12]. The genes under its control are crucial during early pregnancy, the initial growth response of the mammary gland, and then again when milk production and secretion is turned on. So, there is a direct link between the changes in methylation patterns during pregnancy, and the capacity of Stat5 to do its job. Importantly, when the scientists measured the activity of the genes that were under Stat5 control, they found that the changes were permanent. This indicates that the mammary gland cells were primed and therefore more responsive to further pregnancies.

These modifications to the DNA of mammary gland cells have a tremendous effect. For laboratory mice, they allow for a much larger litter size supported by an improved capacity of the mammary gland to produce milk. For dairy cattle, milk production is markedly increased. For women, it can often mean that breast feeding is much easier to manage after the first child. Now the question becomes: can we use this information to address important issues around lactation [13]?

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Milk Nutrients Augment Muscle Growth and Recovery

- **The most successful weight loss diets reduce body fat and maintain lean body mass.**
- **Dairy-derived whey protein has a high concentration of amino acids that help maintain muscle tissue while restricting calories and, along with calcium, may also influence the body's use of fat as an energy source.**
- **Overweight or obese subjects in randomized controlled trials receiving increased dairy or dairy-derived whey protein supplements lose more body fat and less lean muscle tissue than the control subjects.**
- **High protein, low carbohydrate diets that incorporate more dairy seem to positively influence changes in body composition, as well as facilitating more rapid weight loss.**

If you have ever been on a diet, chances are you own a scale. From contestants on popular weight loss reality shows to at-home dieters, the scale is used as an indispensible tool for measuring dieting success. Unfortunately, those changing numbers on the scale only tell part of the story. Successful weight loss is not just about losing body mass, but about losing fat mass while preserving lean muscle mass. So, what is the secret to success? Dieters eating more protein and fewer carbohydrates have been shown to maintain muscle while dropping fat (1), and a growing number of studies are finding that increased consumption of whey proteins from dairy promotes even greater fat loss and lean muscle preservation (2-5). If you also take into account calcium's positive effects on fat metabolism, there is great potential for dairy to really tip the scales in a dieter's favor.

Lose the fat, keep the muscle

The National Institutes of Health estimates that over 70% of American adults are overweight (having a body mass index or BMI of 25.0 – 29.9), or obese (BMI > 30.0). While much research has gone into understanding potential genetic factors that predispose individuals to obesity (6), solutions for this epidemic must certainly come from behavioral modifications, particularly with regard to diet.

Reducing energy (caloric) intake is obviously an important component of weight loss. After all, in order to lose weight you need to expend more energy than you take in. But simply cutting calories to get to an ideal number on the scale is a very shortsighted and ineffective approach. Instead, clinicians advise that weight loss should focus on reducing fat mass while preserving lean body mass (i.e., muscles) in order to improve the metabolic health of the individual (1-5, 7). Maintaining lean body mass while dropping fat results in an increased basal metabolic rate (BMR), because muscle tissue is more metabolically expensive than fat. A body that is more expensive to run can burn calories more efficiently and has a better chance of keeping the fat weight off, preventing the yo-yo effect that is so common with many diets. In addition, improving the proportion of lean to fat tissue in the body positively influences glucose metabolism and the regulation of blood sugars (1, 3, 7). While the numbers will not go down as quickly (muscle is heavier than fat), the end result is a more metabolically healthy individual.

When someone looking to lose weight reduces their energy intake without regard to macronutrient intake (the proportion of carbohydrates, protein, and fat), they run the risk of losing both muscle and fat. This is because the body responds to energy reduction by decreasing the rate of muscle protein synthesis—a very costly physiological process. Increasing the consumption of dietary protein is integral to keeping the muscle making machinery in the "on" position.

Diets that trade some carbohydrate calories for calories from protein are effective at maintaining lean tissue during weight loss (1). But not all dietary proteins are as successful in maintaining (or growing) lean body tissue. Amino acids, the chemical building blocks of protein, are biologically active and can differentially influence the rate of protein synthesis. For example, the branch chain amino acid leucine is known to act as a chemical signal directly on the nuclei inside of muscle fibers —promoting muscle growth and also preventing muscle breakdown more effectively than other amino acids. Keeping lean muscle, then, is not only about eating a sufficient amount of protein, but also about eating the right proteins.

Dairy's special ingredients

Although leucine is found in several dietary sources, including eggs, fish, beef, and chicken, the highest concentration comes from the whey fraction of milk. Whey protein has more than twice the leucine than other protein sources, and so it is no surprise that it has been a primary focus of dietary intervention studies on overweight and obese individuals (2-5, 7, 8).

But dairy's whey proteins do more than stimulate muscle protein synthesis. Leucine is hypothesized to inhibit fat cell production (lipogenesis) and facilitate fat cell breakdown (lipolysis). Currently, these two effects have been demonstrated conclusively only in in vitro studies (2). In addition, leucine and other amino acids found in whey influence hormones related to fat and muscle metabolism, as well as appetite. For example, whey protein lowers serum cortisol levels, which can augment the preservation of muscle tissue (4). Whey also acts on ghrelin, the so-called hunger hormone, produced

by the stomach when it is empty. Lower circulating levels of ghrelin act to enhance satiety (feeling full). Baer et al. (8) found that study subjects that are supplemented with whey protein had lower levels of circulating ghrelin compared to those supplemented with soy or carbohydrates. Therefore, while consuming proteins can make people feel more full in general, whey protein is believed to have an even greater influence on appetite, which can be especially important for individuals trying to stick to an calorie restricted diet.

In addition to whey proteins, dairy is one of the best dietary sources of calcium. Numerous lines of evidence support the hypothesis that increased dietary calcium positively influences fat loss. Increasing dietary calcium is believed to positively influence how dietary fat is absorbed and metabolized directly following a meal. In contrast, diets low in dietary calcium are associated with decreases in lipolysis and increases in lipogenesis (9). These findings suggest that calcium, like leucine, may help direct the body to burn up fat cells as opposed to lean tissue an energy source (reviewed in 5).

Putting dairy to the test

Despite these known functions of whey and calcium in muscle maintenance and fat metabolism, there are only a handful of studies that have directly investigated dairy's affect on body composition during weight loss. For example, in a 2012 meta-analysis, Abargouei et al. (5) found only 14 randomized control trials that looked at dairy and weight loss, and of these only six collected data on changes in lean body mass. The meta-analysis from the data on weight loss, which represented nearly 900 overweight or obese adults, found that increasing dairy intake to simply meet the recommended daily requirements (3 servings) while on a caloric-restricted diet was associated with significantly greater weight loss than conventional low calorie diets (5). Of the studies that considered lean body mass changes, the meta-analysis identified an average gain of 0.58 kg in lean mass in individuals randomized into the higher dairy groups. Interestingly, the effects of dairy were only significant when accompanied by energy restriction. The positive effects of dairy on fat loss and muscle preservation may be more pronounced when changes are made to daily caloric consumption (5).

One of the biggest issues identified by Abargouei et al.'s systematic review was the variation in experimental variables. Some studies increased dairy consumption, but not the proportion of calories coming from protein, while others did both. This makes it difficult to conclude with certainty whether the positive weight loss effects are from higher dairy intake alone, or higher dairy intake alongside increased protein consumption. Josse et al. (2) tackle this problem by randomizing their subjects (90 overweight or obese premenopausal women) into one of three groups: high protein/high dairy (30% of calories from protein, 6-7 serving of dairy per day), adequate protein, adequate dairy (15% of calories from protein, 3-4 servings of dairy), and adequate protein, low dairy (15% of calories from protein, 0-1 servings of dairy). In the adequate protein groups, 55% of the calories were carbohydrates and 30% fat; in the high protein group, carbohydrate energy was reduced to 40%. All groups underwent the same caloric restrictions (- 500 kcal/d from their pre-study diet) and daily exercise regimen (designed to burn off an additional 250 kcal/d). Using body composition data collected at weeks 0, 8, and 16 by duel-energy X-ray absorptiometry (DXA), Josse et al. (2) found the high dairy/high protein subjects had the greatest gains in lean tissue as well as the greatest fat loss. And while lean body mass was maintained in the adequate protein/adequate dairy group, the low dairy group actually lost lean mass. These findings suggest that even under conditions of adequate protein intake, caloric restriction accompanied by low dairy intake can result in muscle turnover rather than muscle preservation.

Josse et al. also utilized MRI scans to assess changes in visceral adipose tissue (VAT) over the course of the study period. Higher amounts of VAT, particularly that located in the trunk, are associated with several health risks, including cardiovascular disease and type 2 diabetes, and therefore many weight loss studies are particularly interested in identifying methods for targeting VAT. In all of the groups, changes in VAT correlated with intakes of protein and calcium; the more protein and calcium consumed, the greater the loss in VAT. Moreover, DXA scans showed greater changes in trunk fat in the high protein/high dairy group compared to the other two study groups. Not only did the increased protein and dairy intake favor fat over muscle as an energy source, it targeted the very fat stores that are most detrimental to the individual's metabolic health.

Got Vegetables?

- **Carotenoids are pigments found in fruits and vegetables and have various functions in the human body.**
- **The levels of different carotenoids in a woman's diet are reflected in her breast milk.**

- **A recent study comparing Chinese, Mexican, and American women's breast milk identified national differences in breast milk carotenoid composition, indicating national dietary tendencies.**

One of the marvels of breast milk is that its composition stays roughly the same, even when working mothers have lopsided diets, or mothers in poor places cannot find enough to eat. That much is at least true for overall levels of the major components—the fats, proteins and sugars in milk. However, recent research has suggested that chemicals called carotenoids—natural pigments found in fruits and vegetables—appear in breast milk according to how much of them a mother eats. And when compared across countries, by some measures, American moms do a fairly poor job of providing their suckling infants with them.

The research in question appears in a paper by Tristan Lipkie of Purdue University, in Indiana, and his colleagues. They set about collecting samples of breast milk from mothers living in Shanghai, Mexico City, and Cincinnati. On comparing the types and levels of carotenoids in these milk samples, the team first confirmed that no matter where a mother lives, different individuals produce very different amounts of carotenoids; if a mother is a relatively low carotenoid producer or a relatively high one, her levels tend to stay low or high throughout lactation. Providing that women are generally creatures of habit when it comes to food, this finding is consistent with the idea that mothers' dietary norms are crucial.

In all three countries, lutein was the carotenoid with the highest levels in breast milk, but mothers in China had far more of it than mothers in Mexico or the US—most likely because Chinese people tend to eat more green vegetables. The other common carotenoids in milk were in all cases β–carotene, β-cryptoxanthin, and lycopene. Of these, lycopene also showed a strong trend: it was typically found at relative high levels in the breast milk of the mothers from Cincinnati, probably because Americans consume quite a lot of tomatoes. Generally, the breast milk of the Mexican mothers had intermediate carotenoid content compared to the Chinese and American sample—although some had notably high levels of β-cryptoxanthin, which is found in papayas and oranges.

To confirm that carotenoids were coming from the diet, and not, say, made by the mammary gland, the researchers checked that the carotenoid levels found in the US mothers' blood plasma correlated with the levels in their breast milk, and that this also correlated with the amounts found in infants' blood plasma. Broadly speaking, they did correlate.

So, what does this all mean in terms of possible health impacts on Chinese, Mexican, and American infants? One approach to that question is to consider the roles of individual carotenoids. One form of lutein has been found in the retina, suggesting a role for it in eye development. Thus the retinas of Chinese infants appear to be better provided for than those of Mexican or American infants. Lycopene is known to play a role in immune system development, particularly in protection against inflammatory diseases; in this case, American infants seem to be better provisioned. But that is not to say that Chinese infants will suffer from inflammatory diseases or that US infants will develop eye problems. Even at the lower end of the scale, mothers' milk may provide enough of these compounds to furnish healthy development.

The issue of how much of the different carotenoids is required for proper development is intriguing, however, and warrants further investigation. Moreover, given that the authors found decreasing levels of carotenoids over the course of lactation, feeding wet-nursed or milk bank-supplied infants milk that is appropriate for their stage of development may come to be seen as increasingly important. Of course, that decrease might not mean that older babies are getting fewer carotenoids; they could actually get the same amount because they drink more milk. But whatever the situation, Lipkie's study is a reminder to all breastfeeding women to make sure they get enough fruits and vegetables—and for American women in particular, to eat more greens.

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Milk Banks Around the World

- **Milk banking is organized differently in different countries around the world.**
- **Brazil is widely acknowledged to have the most extensive and perhaps the best-organized system, with many lessons for other countries.**
- **Norway is unusual in that it does not pasteurize donor milk.**
- **In many countries, milk banking is still quite limited by a lack of political will, or due to religious concerns, or because mothers in populations with high rates of infectious disease do not fully trust the health system.**

As the benefits of breast milk have become better known and the world has become more connected, sharing or selling it has concomitantly become much more common. Milk banks are the most institutionalized method of milk sharing, and probably the safest, but different countries run them very differently. As the third item in SPLASH!'s series on the subject—the other pieces can be found here and here—this article explores those differences, and picks out lessons offered to the rest of the world.

Beautiful Brazil

Brazil is easily the global leader in milk banking. With a history of the practice dating back to the 1930s, the country also has a three decade-old public health law that stipulates all the steps required to operate a bank, based on advice from scientists at the respected research organization, FIOCRUZ. Today, Brazil has 217 milk banks, plus another 126 milk collection points, with at least one bank in each of the country's 26 states—from Amazonas to São Paulo. Last year, 166,848 Brazilian women donated breast milk; an even larger number of infants reaped the benefits [1].

This huge system is centrally organized; every state has a reference bank, with a national reference bank in Rio de Janeiro from which continuous monitoring of the whole system is managed [2]. An online portal called RedeBLH [3], which has won praise from foreigners [4], facilitates a vast data collection operation, and enables the public, as well as the government, to stay informed. FIOCRUZ's Fernandes Figueira Institute—where the national reference bank is kept—also disseminates information via a newsletter, conducts research, and runs undergraduate and graduate programs on policy and applied methodologies for milk banking [5]. In early July this year, it hosted milk-banking specialists from the European Milk Banking Association, Norway, and India, seeking to learn more about the Brazilian model [6].

But success is not about just about setting up a complex and responsive infrastructure; fundamentally, it requires getting mothers on board (and in Brazil, payment for donations, which might encourage them, is prohibited). Milk banks do a lot more than merely collecting, testing, pasteurizing, and providing milk. In Brazil, they run training programs for community peer counselors who promote and support breastfeeding in urban shanties and remote villages, and they are sites where mothers can access pediatricians, social workers, nutritionists, epidemiologists, and many other specialists. In fact, they are so well integrated into the country's public health system that new mothers are referred to a milk bank when discharged from a hospital maternity unit [5].

Over in Europe

No other country comes close to Brazil's level of success, but European nations tend to do a decent job. In Italy, milk banks have become much more common since the turn of the century. In a recent questionnaire sent out by the Italian Association of Donor Human Milk Banks, Italian milk banks recorded an upward trend in the total volume of donations between 2007 and 2012. However, they have problems relating to their typically small scale [4]: when the single pasteurizer or the only freezer breaks down in one of these banks, the whole operation grinds to a halt. Worryingly, the testing of mothers for lifestyle choices and infectious diseases that would make them unsuitable donors, although very widely conducted, was not found to be water-tight. Similarly, record keeping to facilitate the tracing of milk from donor to recipients was possible in only 88% of cases.

Norway stands out in the world of milk banking for choosing not to pasteurize donated milk. This would be unthinkable in most countries. But in Norway it is made possible primarily because breastfeeding rates are very high (99% breastfeeding initiation)—which is, in turn, because employment law minimizes difficult choices for mothers, and breastfeeding in public is entirely accepted. This means that demand can be met, even when screening measures for donors are extremely strict.

Unlike in most countries, Norway ensures that mothers don't face economic burdens from donating their milk. The bank at Rikshospitalet University Hospital in Oslo, for example, pays donors about USD $20 per liter (about 57 cents per ounce) to cover expenses like parking fees, and also offers the use of hospital-grade pumps. All infants who may need donated milk are provided it free of charge. And, occasionally, the hospital even makes milk deliveries to family homes—without asking for a prescription.

The question of whether it would be better to pasteurize the donated milk is often revisited [7]. So far, the balance of opinion in Norway has remained against it. The country's health system treats milk donation in a similar vein to blood donation, and thus elevates the importance of perfectly tracing milk from donor to consumer. The testing of donors is also regularly repeated and extremely rigorous. To date, no donor has failed any of the retesting procedures, and the population of 4.5 million generally harbors very low HIV and hepatitis rates, which makes the choice not to pasteurize a plausible option. The virtue of this choice is that the high temperatures involved in pasteurization, which are required to kill germs, also stop some of the active proteins in raw milk from working properly; these active proteins in human milk provide health benefits to infants.

Still finding their way

On the opposite side of the spectrum to the countries discussed thus far are those where milk banking is just getting off the ground. Surprisingly, not all are places where governments are strapped for cash, or where citizens have cultural quibbles with the principle of milk donation.

Milk banks are notably few and far between in Australia, for example. Over the whole country, there are only four established milk banks, and none in South Australia [8]. What's odd about this situation is that it was not always so. In the 1940s, it was commonplace for Australian women to donate excess milk for use in the local hospital. Scholars blame the decline on a number of factors—from excessive marketing from formula manufacturers to a lack of political will to promote breastfeeding [8]. These days, research suggests that mothers would be willing to provide what milk they could, but still sometimes come across medical professionals who don't seem supportive of breastfeeding, and, by implication, might create barriers to the donation and receipt of breast milk.

Cultural and religious complexities around donor milk do, of course, exist. Islamic law, for example, forbids marriage between a donor's offspring and the recipient of the donor's milk [9]. In a recent survey of Turkish women, just over 90% said they had never previously heard of the concept of breast milk banking, 36% stated that they had problems of a religious nature with the idea, and 29% thought it would lead to social and moral problems [10]. Elsewhere, populations appear to have become more accepting of milk banking given time. This is the case in Taiwan, where the first milk bank opened in 2005 [11].

In a different bracket, however, are places where citizens have genuine medical concerns over the safety of the milk that infants might receive. This is certainly the case in South Africa, as a study by Irene Coutsoudis and colleagues at the University of KwaZulu-Natal, makes clear [12]. In a country with a very high HIV rate, the province of KwaZulu-Natal has the highest burden of all—the rate among pregnant women in 2007 was 37.4%—in addition to South Africa's highest infant mortality.

These authors held focus groups to try to understand the attitudes and experiences associated with milk banking of both healthcare professionals and mothers. As expected, mistrust of screening standards was foremost in mothers' minds, and even some nurses held that concern. But the work also uncovered women's worry of being stigmatized if they accessed a milk bank, since infant feeding choices are understood in the community to be linked to HIV. Shortcomings in communication were also evident. One woman recounted being shown her baby for the first time when it was already receiving milk, and yet the concept of milk banking was completely unknown to her—and left unexplained.

As such, while the global advice might be to 'do as Brazil does,' or, if appropriate, 'as Norway does,' there are important differences that need to be taken into account to enable milk banking to work in different countries. Some of these differences, as described above, are already known thanks to researchers conducting surveys and trying to learn from women and doctors how to improve existing systems. Yet, in many places, reliable information is of as great a shortage as donor milk.

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