



SPLASH! milk science update May 2015 Issue

This month's issue explores how dairy consumption may protect brain health, differences in milk protein levels between humans and monkeys, genetic links to milk fat composition, and how a cow's diet affects her milk fat.

Dairy Consumption Linked to Better Brain Health

- **Oxidative stress has been implicated in aging and several neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease.**
- **A new study finds that higher dairy consumption is associated with higher brain concentration of glutathione, a potent antioxidant that can help protect the brain from oxidative stress.**
- **A follow-up intervention trial could confirm whether increasing dairy intake could enhance the brain's antioxidant defenses and protect against aging and various neurodegenerative diseases.**

It's well known that milk and calcium are good for your bones, but it turns out that they might also be good for your brain. A new study suggests that dairy consumption could potentially play a role in protecting the brain from oxidative stress, a process that has been implicated both in normal aging and in many neurodegenerative conditions such as Alzheimer's disease or Parkinson's disease [1,2].

The study, led by Debra Sullivan and In-Young Choi from the University of Kansas Medical Center, found that higher dairy consumption was associated with higher brain concentrations of glutathione, a potent antioxidant [3]. Glutathione is part of the brain's antioxidant defenses, which help stave off oxidative stress. "When we have higher antioxidant defenses, that helps the brain to be protected against neurodegeneration and also preserve our cognitive function," says Choi, an associate professor of neurology.

Leading a healthy lifestyle with healthy food choices can boost our antioxidant defense system, says Choi. "Drinking milk could be one of the healthy choices people can make," she says. The study's results were consistent with a recent study in which individuals who consumed the Dietary Approaches to Stop Hypertension (DASH) diet, which is rich in dairy foods, had higher plasma glutathione concentration [4].

The new study relied on a technique developed by Choi to detect glutathione in the brain using magnetic resonance imaging [5,6,7]. When Sullivan, a professor of dietetics and nutrition, first found out about this technique, she realized its potential in examining the effects of diet and lifestyle on brain glutathione concentration. "We did an exploratory study, and based on that, what was significantly related with brain glutathione was dairy food, and calcium," she says.

The researchers were initially surprised at this association. Choi expected glutathione concentrations to be associated with "antioxidant-enriched vegetables like blueberry, pomegranate, and broccoli," but the only statistically significant association was with dairy. Choi and Sullivan decided to do a larger study focused on dairy to see if they could confirm the results of their unpublished pilot study.



In the new study, the researchers recruited 60 healthy older individuals covering a broad range of daily dairy consumption. "The biggest challenge that we had was actually finding older individuals that were consuming the recommended three servings of dairy a day," says Sullivan. The researchers assessed study participants' diets in the week before their brains were scanned, and then examined the link between dairy intake and brain glutathione concentration.

Choi and Sullivan found that higher brain glutathione concentration was associated with higher daily dairy intake, confirming the results of their exploratory study. In particular, there was a significant correlation between glutathione concentration and milk consumption. Sullivan cautions that further studies will be needed to find out whether the effect is specific to milk or applies to other forms of dairy.

It is still unclear how exactly dairy consumption leads to increased glutathione in the brain, and one of the next steps is to figure out "what exactly is it within dairy that's causing the effect," says Sullivan. "There are various different components

of dairy that could theoretically be responsible, and make sense based on what we know of the biochemistry and physiology,” she says.

One component of dairy that could play a role is the amino acid cysteine. “We know that dairy is high in cysteine, and cysteine would be the rate-limiting substrate that’s needed to make glutathione,” she says [8]. In addition, dairy is a significant source of calcium and riboflavin, both of which have been implicated in maintaining glutathione concentration [9, 10, 11, 12].

While the current study can tell us about the association between dairy consumption and brain glutathione concentration, it is not designed to uncover cause-and-effect relationships. Sullivan and Choi hope to do a follow-up intervention study that would look at whether increasing the dairy consumption of low-dairy consumers would also increase their brain glutathione concentration. “In order to prove this concept, we would have to have a randomized control trial, and intervention,” says Sullivan.

If dairy consumption is found to have a causal effect on brain glutathione and brain health, then this could be yet another reason to promote increased dairy consumption in aging populations. According to national surveys, only 23% of older adults in the United States appear to meet dietary recommendations for dairy [13]. “Sadly, the US population, whether they’re elderly or young adults or adolescents, don’t consume the recommended three servings of dairy a day,” says Sullivan. This study “is just the initial step, but it is very intriguing,” she says. “I think it sets the stage for a lot of future research and hopefully great health benefits if it holds true.”

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Milk Protein Comparison Unveils Nutritional Gems for Human Infants

- **The milk proteome is a detailed list of all of the proteins present in milk.**
- **In the first study of the macaque milk proteome, Beck et al. identified over 1600 proteins in human milk and 518 proteins in macaque milk.**
- **Compared with macaque milk, human milk had a higher abundance of proteins that assist with the digestion of lipids, slow the digestion of proteins, and potentially increase the absorption of iron, B-12, and vitamin-D.**
- **Proteins higher in human milk, compared with macaque, were associated with brain tissue, suggesting possible involvement in neurodevelopment.**

As any new parent can tell you, human newborns are very needy—and for good reason. Born at an earlier developmental stage than their primate relatives, human infants must accomplish a great deal of fetal development (some say the equivalent of nine more months!) outside of the womb. Responsibility for this “fourth trimester” falls squarely on the shoulders of human breast milk, and so it is no surprise that decades of research have searched for attributes in human milk that support the unique developmental needs of human infants. While much of the focus has gone to milk fats and

carbohydrates, a new study has turned the attention to milk proteins (1). Utilizing new methods in protein identification, Beck et al. present the most comprehensive human milk proteome (the list of the types and quantities of all proteins in milk) to date, as well as the first milk proteome for a nonhuman primate, the rhesus macaque. Despite having less total protein than rhesus macaque milk, Beck et al. found that human milk has more than three times the types of proteins in rhesus milk and higher quantities of nearly every milk protein that the two species share. Their findings demonstrate that from digestion, to immunity, to neurodevelopment, human infants get more of a boost from milk proteins than their primate cousins.

Taking it to the next level

At first glance, there does not appear to be anything remarkable about the protein content of human breast milk. With just over 1% total protein, which contributes only 10% of the total calories in milk, it might even be assumed that protein is not very important for human infants. But human milk protein deserves a second look—a much (much) closer look. The human milk proteome details all of the types and quantities of proteins present in human milk, from enzymes that aid in fat digestion to immunoglobulins that provide specific immunity for naïve infant immune systems. Although total protein content of human milk may be low, the story from the level of individual proteins is much different.

Previous studies on the human milk proteome have demonstrated that within the 1% of total protein there are, in fact, hundreds of different proteins. However, because no two studies identified the same proteins, there has been no consensus of just how many different proteins are actually present.

The inconsistency among results comes from the methods used to isolate and quantify proteins. Milk proteins are identified using a mass spectrometer, but this analysis requires using only the protein fraction of the milk. Previous methods all prepared their samples by taking whole milk and stripping all other components out until all that is left is protein. In addition to being complicated, this method has the potential for throwing the baby out with the bathwater by removing a lot of important proteins, such as lipid-bound hormones.

Beck et al. (1) developed a less complicated route and simply removed the protein from the whole milk sample. “The advantage to this method,” said Kristen Beck, lead author of the study and PhD candidate at the UC Davis Genome Center, “is that we were able to analyze whole milk samples, exactly what a human infant would be receiving.”

They also improve on previous methods by “cleaning up” the proteins before they are run on the mass spectrometer. Glycans are carbohydrates that sit on the surface of milk protein chains. These glycans are important for protecting proteins during digestion, but can also keep proteins hidden during analysis. Removing the glycans (in a process known as glycan cleavage) exposed more of the protein chain, which in turn increased the rate of protein detection, particularly of smaller proteins.

Their improved methods produced the most complete picture, thus far, of the human milk proteome, identifying 1606 different types of proteins, 524 of which were newly identified in human milk. These results surprised even the researchers themselves. “When we started out, we thought we might improve our list of human milk proteins by 10%, but 30% of our whole proteome were proteins that were unknown in milk,” said Beck.

Comparing proteomes

Because their interest was in human infant nutrition, Beck et al. (1) went beyond the descriptive and provided much-needed context for the human milk proteome. After perfecting their new methods on human milk, they applied them to rhesus macaque milk and produced the first ever rhesus macaque milk proteome.

The most obvious difference between the two proteomes is in the total number of proteins. Despite having half as much total protein (human milk 1.2%, rhesus milk, 2.1%), human milk has over three times the number of different proteins than macaque milk (1606 vs. 518). But perhaps the most interesting finding came from comparing the quantities of proteins between the two species. Among the shared proteins (called orthologs), 82 were found in higher quantities in human milk compared to six enriched proteins in macaque milk.



One protein found in higher concentrations in human milk compared to macaque milk is bile salt stimulated lipase (BSSL), an enzyme that helps human infants (who have low bile production) digest fat. Another is lactoferrin, a protein that binds iron in milk, presumably to help infants fight infection by keeping iron away from pathogens that use it for food. To determine what other enriched proteins might do for human infants, Beck et al. looked at which tissues in the body manufactured each protein—different tissues manufacture proteins at different rates and make

proteins that are unique to that tissue. Of the ten different tissues that passed the statistical test for significant enrichment of these proteins, five are part of the brain, suggesting a greater need for proteins that play a role in neurodevelopment.

“The higher levels of these proteins in human milk are consistent with the well-established perspective that human babies, compared to other primate infants, are born at a slightly earlier stage of development and require higher levels of specific proteins that will nurture them as they mature,” said principal investigator Danielle Lemay, a nutritional biologist at the UC Davis Genome Center. “These are specific proteins that are enriched in human milk,” added Beck. “It is truly [evolutionary] fine-tuning for the infant.”

Follow the recipe

It is intriguing to hypothesize that the enrichment of these specific proteins is unique to human milk compared to milks from primates and other mammals. The results from the macaque milk proteome certainly support this evolutionary hypothesis, but a better test of it will come from side by side comparisons with ape milk proteomes—particularly chimpanzees or bonobos who share the most recent common ancestor with humans.

But regardless of whether ape milks show similar protein enrichment, the 82 differently abundant proteins identified by Beck et al. (1) are clearly important for human infants and offer important insights about their nutritional, immunological, and neurodevelopmental needs. As Lemay explains, “Human milk provides a recipe for human nutrition during the neonatal period.” The more research we do on human milk macronutrients, micronutrients, and bioactive components, the more detailed this recipe becomes. Beck et al. (1) have added 82 potential ingredients to this list. These proteins, particularly those involved in neurodevelopment, represent excellent candidates for future research on improving infant formula composition. As Beck puts it, “If we can use this research to enhance infant formula, we can give those babies the helping hand that evolution has been working on for tens of millions of years.”

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The Fat Controllers: Dairy Cattle Genetics and Milk Fat Composition

- **Milk fat is made up of over 400 components—each with different effects on health and dairy food value.**
- **Different breeds of cows produce different amounts of the healthier components.**
- **The ability of a cow to balance the composition of milk fats in her milk is influenced by her genes.**
- **By combining selective breeding with specific diets, cows could produce healthier milk that is also better for making dairy foods.**

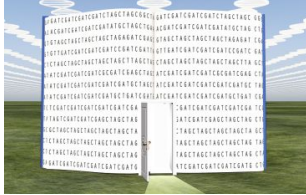
Milk is rich in each of the major nutrients: proteins, fats, and carbohydrates. We can easily recognize that milk contains fat from the cream that separates from milk and from butter and cheeses, which are rich in milk fats. But did you know that milk fat actually contains over 400 individual types of fat? These lipids (the technical term for fats) provide the fundamental ingredients that determine, for example, the cheese-making qualities and the taste and flavor. However, consumption of fats needs to be balanced in our diet so that they don't adversely affect our health. The current consensus amongst nutritionists is that too much fat in the diet is unhealthy, and that the type of fats or fatty acids that are consumed makes a big difference. Taking all of these factors into consideration, dairy scientists from the Netherlands and Denmark have been studying the genetic basis of milk fat composition.

Overall, fats can be divided into saturated and unsaturated categories. Nutritionists advocate that we eat less saturated fats, and increase the relative amount of unsaturated fats. The American Heart Association recommends that saturated fat should be no more than 7% of our total energy intake [1]. Although the role that dietary fats play in our health and wellbeing is not entirely clear, this recommendation has a marked effect on milk consumption. Milk fat is, on average, about 70% saturated fat, but the dairy industry has, for many years, produced a huge range of low fat dairy products through manufacturing processes. What the scientists wanted to know was, could the cows themselves produce milk with a healthier combination of fats?

The mixture of fats in milk fat varies a lot between dairy cow breeds, different farms, and even individual cows. Depending on what the cows eat and how long they have been milking, the percentage of fat in the milk will fluctuate. Furthermore,

we also know that there is a very strong genetic influence on the quantity of milk fat [2]. The Dutch, and more recently, the Danish dairy scientists, decided to dissect the milk fat into individual components, and measure the impact of the cow's genetic makeup on each component.

Led by Johann van Arendonk at Wageningen University, and supported through the Dutch Milk Genomics Initiative, the scientists began to collect data about ten years ago in collaboration with dairy farmers from across the Netherlands. They first set out to analyze the differences between farms, herds, and other factors that affect the composition of milk fat. Prior studies by dairy geneticists in 2002 identified a gene that codes for a naturally occurring enzyme found in cows, referred to as DGAT1, as a major determinant of milk fat percentage [3]. When van Arendonk's graduate student, Anke Schennink began the analysis, she found that, just like the percentage of milk fat, milk fat composition was indeed influenced by the genetic background of cows. In this initial study, Schennink et al., identified a number of genomic regions associated with either short, medium, or long-chain fatty acid content in milk [4]. In further analysis, they discovered that different genetic variants of DGAT1 affected the amount of specific fatty acids in the milk fat. Indeed, the DGAT1 accounted for about half



of the fat composition attributed to genetic variation that was present in the animals studied. Schennink then went further to look at the amount of unsaturated fat in the milk. They again found a large effect of the DGAT1 gene, and another lipid enzyme gene, SCD1, on unsaturated fatty acids. This success led them to extend their analysis to other prominent enzymes that synthesize or process fats. They found that specific mutations in some of these enzymes influenced the mix of fats in the milk [5,6].

Over the past ten years, there has been a lot of excitement amongst scientists about the development of genomic technologies for studying dairy genetics. The Dutch brought these new technologies into their dairy program to expand their search for stretches of dairy cow DNA that carry genes that influence milk fat composition, but which had not been previously identified. They identified four regions from amongst many thousands analyzed, that affected the composition of fats that are mostly made in the mammary gland [7], and an additional three regions that influenced the fats that mostly get into the milk via the diet [8]. When they took a closer look at the DNA using informatics (computer-based analysis of database information), they found a number of genes that are clearly involved in fat metabolism. The technologies continued to develop, permitting an ever-increasing sensitivity for finding mutations that cause the differences in fat composition. All up, 11 genes were candidates for causing the differences in milk fat composition [9]. Additionally, they found a novel effect from a mutation in DGAT1 and, for the first time, an effect from a gene called PGRMC2 [10].

Milk fat composition can change significantly between winter and summer, especially in regions where food sources for the cows depend on the seasons. When winter and summer milk fat compositions were compared by the Dutch scientists, there were clear differences in milk fats, but no substantial differences in how this was affected by the cow's genetic makeup [11,12]

Meanwhile, scientists from the Danish-Swedish Milk Genomics Initiative completed a study of milk fat composition in Danish Holsteins and also introduced a comparative analysis of the Danish Jersey breed [13]. The milk of Jersey cows is richer in fats compared to many other dairy breeds. Like the Dutch program, they were looking for genetic factors that influenced the healthier milk fats and that also controlled cheese-making qualities. They found similar effects of DGAT1 and SCD1 to add to the mounting evidence that these enzymes have the greatest influence on the type of fat found in milk. Furthermore, they also found some evidence for a new candidate gene called ACSS3 [14]. When they put all the regions together and analyzed them, they could discern a pattern of those genes that regulated fat digestion and absorption, and which had the most significant influence.

Thanks to these enormously detailed and seminal studies, we can say that, by combining specific types of diets for the cows, and by selecting cows for breeding based on their milk fat composition, milk could become a healthier choice for consumers, and top quality for producing dairy products at the same time.

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Foraging for Answers: Cow Feed and Milk Fat

- A diet that involves a lot of grazing has been linked to an increase of milk fat globules of one particular size, while slightly lowering the amount of total fat in milk.
- This hike in globules of size 3.3 μm goes along with increases in the concentrations of two fatty acids—capric and lauric acids.
- Noticing the globule size change has suggested to scientists how mammary cells in udders respond to changes in a cow's diet.

The reason that milk is opaque and white is that it is full of very small beads, or globules, of fat. What may surprise milk drinkers, though, is that these globules are highly structured, having a center composed of three-tailed fatty acids, surrounded by a membrane of quite different chemical structures. The size of these globules varies a great deal, in ways that matter for industrial processes like cheese making, and defining how much of the fat in milk is saturated. The latest research into milk fat globules has taken some bold steps: the aim is to feed cows differently, monitor how this changes the size of the globules in their milk, and work out what exactly is going on in their udders as a result.



The cows assisting in this pursuit are Israeli-Holsteins, living on an experimental farm not far from Tel Aviv. They have swapped between two diets. For a few weeks they have been eating what they normally do, what is known in the dairy business as 35% forage and 65% concentrate—meaning that that a minority of what their energy comes from grazing, and the majority comes from what is laid out for them in troughs by the farmer. Then for another few weeks, this balance was reversed—35% concentrate and 65% forage. The order of these diets has been different for different cows.

Meanwhile, scientists at the Hebrew University of Jerusalem have been measuring the size of the milk fat globules that have come out of the cows [1]. Milk fat globules can be as 'teeny tiny' as 200nm, and as 'merely tiny' as 15 μm , which is 75-fold difference in diameter. Often, when scientists monitor milk chemistry, they measure only the average globule size. But, an increase in the average wouldn't distinguish between slight upward shifts in the sizes of the range of globules and a singular hike in the relative amount of one quite-large kind of globule. Hence, Nurit Argov-Argaman and her colleagues took a more detailed approach.

It paid off. They noticed that when the cows were on the high-forage diet, the average size of the fat globules in their milk increased. Their milk contained many more globules with a diameter of 3.3 μm . A few other changes happened. The high-forage diet led to slightly lower levels of lactose and slightly lower concentrations of fat overall, while milk protein levels didn't budge. The new prominence of fat globules measuring 3.3 μm in diameter, was linked to changes in the relative abundances of particular fatty acids—specifically, to rises in the concentrations of capric and lauric acids and a reduction in the amount of oleic acid.

What does this say about how udders respond to changes in diet? How does it help cheese makers and those of us watching our cholesterol levels? When cows eat differently, the membranes of the mammary epithelial cells in their

udders—from which milk fat globule membranes are made—must change. What Argov-Argaman and the coauthors suspect occurs is an enrichment of these membranes with something called PhE (which has the clunky full name: 1,2-dioleoyl-sn-glycero-3 phosphoethanolamine), alters how readily nascent lipid droplets fuse together, and so influences their size. Cheese makers might wish to advise their farmers on cow feed if 3.3µm globules alter the physiochemical characteristics of their particular product. As for the rest of us, capric acid—unlike most saturated fats—should present no health bother [2] for people watching their cholesterol.

Of course, these findings are based on a comparison of milks resulting from just two cow diets. Across the pastures and among the herds of the world, there is a lot more variation than that. Argov-Argaman and her team, and any other research group that wishes to get in on the act, may yet have plenty more to say.

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