SPLASH!® milk science update April 2016 Issue

INTERNATIONAL MILK GENOMICS CONSORTIUM

This month's issue features combatting malnutrition with certain milk sugars, increasing breast milk production with herbs, suggestions from the recent edition of the *Dietary Guidelines for Americans*, and how *trans* fats from dairy may be healthier than other sources of *trans* fats.

Dairy Industry's Opportunity to Combat Malnutrition with Milk Sugars

- **Sugars found in breast milk are known to serve as prebiotics and help establish gut microbiota in infants.**
- **A new study finds that specific milk sugars that are sialylated are present at lower concentrations in the breast milk of mothers of undernourished children compared with mothers of healthy children.**
- **The researchers transferred gut microbes from the undernourished infants into germ-free mice and pigs and then fed these animals a diet supplemented with sialylated sugars extracted from cow's milk by the Hilmar Cheese Company.**
- **The mice and pigs that received sialylated milk sugars showed microbiota-dependent improvements in growth and many metabolic processes.**
- **Unlike the milk sugars, the plant-based fiber, inulin, did not rescue the effects of malnutrition.**
- **The results indicate that sialylated milk sugars could be a valuable food supplement to help combat malnutrition, and suggest an opportunity for the dairy industry to produce such sugars.**

Childhood malnutrition is a major global problem that can lead to severe growth stunting and causes more than 3 million deaths every year [1]. Efforts to develop therapeutic foods to counteract the effects of malnutrition have had limited success.

A new study, conducted by David Mills at the University of California at Davis and Jeffrey Gordon at Washington University School of Medicine and others, finds that diets supplemented with specific milk sugars helped to promote growth and metabolic processes in germ-free animals that were transplanted with microbes from malnourished children [2]. The growth improvements appeared to be mediated by the effects of sialylated sugars on gut microbiota.

Sialylated sugars are abundant in breast milk, and they have been associated with the development of gut microbiota and prevention of intestinal infections. The new study used sialylated sugars extracted from cow's milk, where they are found at much lower concentrations, and the researchers suggest that this provides an opportunity for the dairy industry to produce these sugars.

"I think it's a beautiful study that shows how milk components are key to growth, and also shows why breast feeding is so critical," says Mills. "That also creates opportunities because you can use those same fractions to try to help kids that can't get human milk," he says. "It also shows that the microbiota of the developing child is critical to that growth as well, so it's not just milk, it's milk and the microbiota," says Mills.

A crucial step will be to replicate the findings in humans. "Most importantly, we need to repeat these studies and move it to a human clinical trial," says Mills. "What we need to do is try to get these into foods, into clinical trials, first for healthy kids, and then hopefully to help produce some durable changes in the kids who are malnourished," he says.

Developing better foods for malnourished children

Previous studies have shown that the normal development of gut microbiota is disrupted in undernourished children [3]. Another new study led by Jeffrey Gordon also showed that impaired development of gut microbiota can have direct impacts on growth in a germ-free mouse model [4].

Sugars found in breast milk, called human milk oligosaccharides (HMOs) are known to function as prebiotics and help establish gut bacteria that provide various health benefits [5-9]. Mills had been working on milk oligosaccharides from both humans and cows for about 15 years, when in 2010 he received a $100,000 grant from the Bill and Melinda Gates Foundation to test whether oligosaccharides could help malnourished children.

Undernourished children are currently given therapeutic foods, "but they only work as long as the kids are eating the food," says Mills. The researchers hypothesized that the reason the effects didn't last was that the children's guts didn't have the appropriate microbes, perhaps because their foods didn't contain key elements present in breast milk.

In the new study, the researchers analyzed the composition of breast milk from Malawian mothers who had either healthy or undernourished children. "In general the oligosaccharides were lower in the milk of the mothers that nursed children who became stunted, and in particular, sialylated oligosaccharides were lower," says Mills.

To examine the effects of sialylated sugars, the researchers transferred a collection of microbes from the stunted infants into germ-free mice that lacked pre-existing gut microbes. When these mice were fed a typical Malawian diet supplemented with sialylated milk sugars "they had a dramatic increase in lean body mass," says Mills. "Then we went on to measure a variety of health measurements, bone and body size measurements, all of which were better just with this simple addition to the Malawian diet," he says. "That was amazing."

The researchers also repeated the experiment in germ-free pigs, whose digestive tracts are much more similar to the digestive tracts of humans. They found that the pigs experienced similar improvements in growth and metabolism when their diets were supplemented with sialylated sugars.

Mice that were fed an unsupplemented diet or one supplemented with a common plant-based prebiotic called inulin did not experience this growth effect. "As a matter of fact, not only did inulin not have that effect, but with inulin they gained fat body mass," says Mills.

Understanding the microbiota

The researchers found that the sialylated sugars appeared to promote growth through their effect on gut microbiota. The primary gut bacteria that responded to the milk sugars turned out to be *Bacteroides*, which are known to feed on these sugars.

"The only problem was, the way *Bacteroides* eats these sialylated milk oligosaccharides, is they sort of eat them outside of the cell, because they only want to take in the parts that they care about, and then the other parts flow downstream," says Mills. "Therein lies the double-edged sword of this study," he says.

"Because the oligosaccharides are being eaten by one population that's leaving some crumbs behind, those are cross-feeding another population that wasn't really involved in the health benefit," says Mills. In the study, the leftover material cross-fed an *Escherichia coli* population, and "you could imagine cross-feeding a not-so-nice population," he says.

Cross-feeding bacteria that are pathogenic could end up causing more harm than good. "The lesson here is we have to be very thoughtful when we come up with solutions like this, because you could end up hurting an unfortunate child if you happen to cross-feed the wrong population," says Mills. "You really need to understand the microbiota you're feeding, or you need to put organisms in that can consume these downstream products," he says.

In follow-up work, Mills plans to examine ways to prevent cross-feeding. In the current study, the researchers did not put the entire spectrum of microbiota from undernourished infants into germ-free animals. Instead they used a representative cocktail consisting of microbes that could be cultured.

The resulting gut microbiome was missing *Bifidobacterium longum* subsp. *infantis*, bacteria, which completely consume sialylated milk oligosaccharides. "It's a big vacuum cleaner for those kinds of milk oligosaccharides," says Mills. "I think there would be no cross-feeding of *E. coli* if you had *B. infantis*," says Mills.

An opportunity for the dairy industry

Plant-based oligosaccharides such as inulin are already added as prebiotics to formula, and sialylated milk sugars could eventually play a similar role. Companies are looking at ways to produce synthetic oligosaccharides, but although individual oligosaccharides can be chemically synthesized, the comprehensive mix of these oligosaccharides is really only present in animal milk [10].

Using sugars extracted from cow milk could also have advantages when it comes to translating this research to humans. "It could be that these oligosaccharides which are naturally found in bovine milk and concentrated by filtration could have an easier regulatory path," says co-author Daniela Barile at the University of California at Davis. Given that cow milk is already widely consumed and used to make infant formula, one would expect sugars extracted from it to be safe for human consumption.

Barile has been developing methods to extract milk oligosaccharides from cow milk, and specifically from whey, the liquid part of milk that separates from the curd during cheese production. Large quantities of whey are currently produced as a by-product of the cheese-making process, making it a useful source from which to purify milk sugars. Although milk oligosaccharides are present in much smaller quantities in cow milk compared with human milk, the researchers have been developing filtration techniques to increase the concentration of these sugars in whey [10].

Barile collaborated with the Hilmar Cheese Company to obtain the sialylated milk sugars used in the study. Given their much lower concentrations in bovine milk, the researchers had to concentrate them from large quantities of cheese whey. "It was really a big investment," says Barile. "They produced on the order of 10 kilograms at a time, which is really unheard of, because these molecules are low abundance in bovine milk," she says. "I think the take-home message for me is how low-abundance molecules in milk are very potent in their activity," says Barile.

"Without the industry believing in this project, there would be no result," says Barile. "Now more industries can read this paper and start investing in the technology to isolate oligosaccharides from bovine dairy streams, so I think this research is very meaningful and very timely," she says.

"My hope is that this kind of very complicated study that clearly shows the benefit of this for kids in Malawi, would prompt the dairy industry to realize that they should start figuring out how to make this stuff, and testing it in other children," says Mills. "If they have whey streams, even though this might be a very small percentage of a whey stream, if it's very valuable then it's worth going after," he says. "When I think of all the yoghurt, and all the cheese being made, and I think of all those whey streams, I now start thinking 'gee, I wonder how many oligosaccharides could we get from that, that are probably being thrown away,'" says Mills.

Mills says there might be parallels with the rise in production of whey protein. "Thirty to forty years ago dairies used to pay farmers to dump the whey protein on their fields," he says. "Some academics pointed out to them that there's a lot of good protein in there, and at the time, folks were resistant to spend money to go capture it because it was so dilute," says Mills. "Of course now, some big companies make as much or more money on whey than they do on cheese," he says. "So it's a very valuable byproduct, and I wonder if we're looking at the same thing again," says Mills.

1. Maternal and child undernutrition and overweight in low-income and middle-income countries. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, Ezzati M, Grantham-McGregor S, Katz J, Martorell R, Uauy R; Maternal and Child Nutrition Study Group. Lancet. 2013 Aug 3;382(9890):427-51. doi: 10.1016/S0140-6736(13)60937-X.
2. Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. Charbonneau MR, O'Donnell D, Blanton LV, Totten SM, Davis JC, Barratt MJ, Cheng J, Guruge J, Talcott M, Bain JR, Muehlbauer MJ, Ilkayeva O, Wu C, Struckmeyer T, Barile D, Mangani C, Jorgensen J, Fan YM, Maleta K, Dewey KG, Ashorn P, Newgard CB, Lebrilla C, Mills DA, Gordon JI. Cell. 2016 Feb 25;164(5):859-71. doi: 10.1016/j.cell.2016.01.024.
3. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA, Petri WA Jr, Ahmed T, Gordon JI. Nature. 2014 Jun 19;510(7505):417-21. doi: 10.1038/nature13421.
4. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Blanton LV, Charbonneau MR, Salih T, Barratt MJ, Venkatesh S, Ilkaveya O, Subramanian S, Manary MJ, Trehan I, Jorgensen JM, Fan YM, Henrissat B, Leyn SA, Rodionov DA, Osterman AL, Maleta KM, Newgard CB, Ashorn P, Dewey KG, Gordon JI. Science. 2016 Feb 19;351(6275). pii: aad3311. doi: 10.1126/science.aad3311.
5. Oligosaccharides in 4 different milk groups, *Bifidobacteria*, and *Ruminococcus obeum*. Coppa GV, Gabrielli O, Zampini L, Galeazzi T, Ficcadenti A, Padella L, Santoro L, Soldi S, Carlucci A, Bertino E, Morelli L. J Pediatr Gastroenterol Nutr. 2011 Jul;53(1):80-7. doi: 10.1097/MPG.0b013e3182073103.
6. Breast milk oligosaccharides: structure-function relationships in the neonate. Smilowitz JT, Lebrilla CB, Mills DA, German JB, Freeman SL. Annu Rev Nutr. 2014;34:143-69. doi: 10.1146/annurev-nutr-071813-105721.
7. Stool microbiota and vaccine responses of infants. Huda MN, Lewis Z, Kalanetra KM, Rashid M, Ahmad SM, Raqib R, Qadri F, Underwood MA, Mills DA, Stephensen CB. Pediatrics. 2014 Aug;134(2):e362-72. doi: 10.1542/peds.2013-3937.
8. Secreted bioactive factors from *Bifidobacterium infantis* enhance epithelial cell barrier function. Ewaschuk JB, Diaz H, Meddings L, Diederichs B, Dmytrash A, Backer J, Looijer-van Langen M, Madsen KL. Am J Physiol Gastrointest Liver Physiol. 2008 Nov;295(5):G1025-34. doi: 10.1152/ajpgi.90227.2008.
9. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, Tobe T, Clarke JM, Topping DL, Suzuki T, Taylor TD, Itoh K, Kikuchi J, Morita H, Hattori M, Ohno H. Nature. 2011 Jan 27;469(7331):543-7. doi: 10.1038/nature09646.
10. Ravindran, S. (2015). Producing human milk sugars for use in formula. *SPLASH! milk science update*: October 2015. (http://milkgenomics.org/article/producing-human-milk-sugars-for-use-in-formula/)

Contributed by
Dr. Sandeep Ravindran
Freelance Science Writer
Sandeepr.com

Herbs to Aid Breastfeeding

- **Herbal remedies known as galactogogues have been used for millennia with the intention of improving the production of breast milk in women who struggle to produce enough.**
- **Despite widespread use, there is limited reliable evidence on whether these herbal remedies work.**
- **One recent double-blind clinical trial did, however, find persuasive evidence that tea containing fenugreek seed increased the frequency of breastfeeding to the extent that, in one month, there was a significant acceleration in infant girls' weight gain and growth in head circumference.**

The benefits of breastfeeding are well known, yet however hard some women try, they struggle to produce sufficient milk.

For millennia, herbal remedies have been thought to fix this problem. Even Hippocrates—from whose name comes the "Hippocratic Oath"—is said to have advised, "If the milk should dry up give her to drink the fruit and roots of fennel" [1]. To this day, however, there is limited reliable data on whether herbal supplements actually work.

The formal name for substances that aid with milk production is a mouthful, and implies, incorrectly, that they are rulers of galaxies: they are called galactogogues. Many of the supposed herbal galactogogues can be found in a restaurant kitchen. Aside from Hippocrates' fennel seed, there's caraway seed, dill seed, alfalfa herb, and fenugreek, which is a spice often used in Indian and Middle Eastern cooking and also thought to aid digestion. Outside of the kitchen, the list includes goat's rue herb, torbangun, blessed thistle herb, and milk thistle seed. And it's not just plants that are galactogogues; in South Korea, many women who struggle with producing enough breastmilk eat pigs' feet in an effort to solve the problem.

Even though many cultures use herbal galactogogues, and there are studies on their efficacy, it's often hard to tell how reliable the findings are. This is because getting good data typically requires randomization—assigning some women in the study to taking an herb thought to act as a galactogogue, and others to placebo. Without random assignment, it's easy to imagine that a mother who seeks information on an herbal remedy, goes to the effort of buying it, and chooses to take it, might in general be trying more things to increase her milk supply than a mother who isn't consuming the herbal remedy. So it's difficult to tell whether the causal effect implied by the results is due to the herb or to something else.

The importance of randomization is illustrated by a study of Korean women. Eighty-two percent of the women who said they consumed pigs' feet to increase their breast milk volume also said that they experienced an increase in their milk production entirely due to the pigs' feet [2]. Is that real, or the power of suggestion, or due to something else that these women are more likely to do than other women?

Of six studies identified in a recent review to have some degree of causal rigor, five trials found an increase in breast milk production, although only one of the studies described a randomization procedure, and the review authors still pick holes in its method [3]. But perhaps the best research so far comes from Iran. In 2015, Vida Ghasemi of Tehran University of Medical Sciences, and her colleagues, published the results of a double-blind clinical trial. It tested whether herbal tea containing fenugreek seed had any impact on breastfeeding sufficiency among mothers with infant girls, who were aged between zero and four months [4]. Both the mothers in the group receiving the intervention and in the group receiving the placebo drank the tea three times per day. The placebo tea had no fenegreek seed and was mostly herbal. It also contained 3 g of black tea per serving to avoid a difference in taste from the intervention tea.

Before the trial started, these two groups of mothers and infants were statistically almost identical, although, by chance, the women in the placebo group breastfed slightly more frequently. After a month of tea consumption, however, the number of times that the women in the intervention group breastfed had increased from about 9 times per day to about 15. The women drinking placebo tea, meanwhile, saw no change; they breastfed about 11 times per day at both the beginning and the end of the study. There were differences in the infant girls' growth, too. The infants whose mothers drank the fenugreek tea gained more weight over the course of the trial—and their head circumference also expanded more—than the infants whose mothers drank the placebo tea.

These results imply not only that fenugreek seed increases the flow of milk but also that the mechanism by which it does so is not—or at least not entirely—psychological. Difficulties with breastfeeding are often associated with anxiety. Some studies such as one conducted in Perth, Australia, in which twenty breastfeeding women who use herbal galactagogues were interviewed at length, conclude that one of the chief benefits of these herbal remedies is a boost confidence and self-empowerment [5]. Thus, they imply that the effect of herbal remedies on milk flow may occur via the brain—by decreasing anxiety, some herbal remedies somehow help women to produce more milk.

Ghasemi et al.'s study doesn't offer any data on why the fenugreek tea works, but other researchers have shown that fenugreek affects metabolism via the insulin pathway [6]. In dairy cows, these kinds of metabolic shifts increase milk yields [7].

Whatever the case, far more high quality research into herbal galactogogues is needed. Almost every study in the field repeats that call. More work is needed not just to measure and understand efficacy, but also to check safety. These pleas are driven home by the following statistic: In the U.S., it is thought that 15% of breastfeeding women already use herbal galactogogues [2].

1. Budzynska, K., et al. 2013. Complementary, holistic, and integrative medicine: advice for clinicians on herbs and breastfeeding. Pediatrics in Review 34(8), 343-353.
2. Kim M. K., et al. 2013. The effects of pigs' feet consumption on lactation. Ecology of Food and Nutrition 52, 223–238.
3. Mortel, M. & Mehta M. D. 2013. Systematic review of the efficacy of herbal galactogogues. Journal of Human Lactation 29(2), 154–162.

4. Ghasemi, V., et al. 2015. The effect of herbal tea containing fenugreek seed on the signs of breast milk sufficiency in iranian girl infants. Iran Red Crescent Med J. 17(8), e21848.
5. Sim T. F. et al. 2015. The use, perceived effectiveness and safety of herbal galactagogues during breastfeeding: a qualitative study. Int. J. Environ. Res. Public Health 12, 11050-11071
6. Neelakantan, N., et al. 2014. Effect of fenugreek (Trigonella foenum-graecum L.) intake on glycemia: a meta-analysis of clinical trials. Nutr. J. 13(7). doi: 10.1186/1475-2891-13-7.
7. Kawashima, C., et al. 2016. Relationship between the degree of insulin resistance during late gestation and postpartum performance in dairy cows and factors that affect growth and metabolic status of their calves. J. Vet Med Sci. http://www.ncbi.nlm.nih.gov/pubmed/26781705

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

New Dietary Guidelines Emphasize Dairy for Better Health

- **The U.S. government just released the 2015-2020 Dietary Guidelines for Americans, including recommended eating patterns to improve health and reduce the risk of diet-related chronic diseases.**
- **Emphasizing that there are many paths to healthy eating, this year's edition focuses on three different diet patterns: Healthy U.S.-Style Eating Pattern, Healthy Mediterranean-Style Eating Pattern, and Healthy Vegetarian Eating Pattern.**
- **Dairy is a key component of each eating pattern, both because it is a nutrient-dense food and because it has a unique combination of nutrients not available in other foods.**
- **For adults, consuming the 3-cup per day recommended intake of dairy may be the only way to meet daily requirements for essential nutrients such as calcium, vitamin D, zinc, and potassium.**

Americans just received their dietary report card, and are doing below average in almost every category [1]. With one in two American adults suffering from a preventable chronic disease [1], we must find ways to improve our marks in nutrition. Every five years, the U.S. Department of Health and Human Services (HHS) and the U.S. Department of Agriculture (USDA) provide help in a nutritional study guide, the *Dietary Guidelines for Americans* (DGA). The recently announced 2015-2020 DGA tackles many of the same dietary issues highlighted in previous editions (e.g., too much saturated fat, too few vegetables, not enough calcium), but offers a new approach to reaching the recommended healthy diet. Rather than a complete dietary overhaul, Americans are instead encouraged to think about making small shifts in their current diet choices; high-calorie, low-nutritional-value foods and drinks should be substituted with those that are nutrient-dense. Leading DGA's list of nutrient-dense choices are low- and fat-free dairy foods, including milk, yogurt, and cheese. And because dairy foods offer a set of nutrients (including calcium, protein, potassium, phosphorus, and zinc) not all together found in any other recommended nutrient-dense food, meeting the daily-recommended intake for dairy may be the only way to earn passing grades in all nutritional categories.

From the scientific literature to the dinner table

The goal of the DGA is to "help Americans make healthy choices for themselves and their families" [1]. To determine what "healthy" is for Americans from age 1 to 100, the HHS and USDA enlist top researchers in the fields of nutrition, health, and medicine to scour the scientific literature and make evidence-based recommendations. For example, the recommended three cups per day of dairy for adults is not randomly selected, but grounded in support from numerous studies, including dietary intervention studies with dairy foods (e.g., potential benefits from adding one serving of dairy to bone health in children and adults) as well as those that evaluate at what level more servings would add no additional benefit.

Because science never sleeps, a new edition of the DGA is produced every five years, with the most recent edition (2015-2020) released in December 2015. But not to worry if you have not yet read through the 146-page document to figure out what you should (and should not) be eating. The intended audience is not the American public but nutritional policymakers (e.g., USDA's National School Breakfast and Lunch Programs) and health care professionals (e.g., pediatricians, nutritionists, primary care physicians).

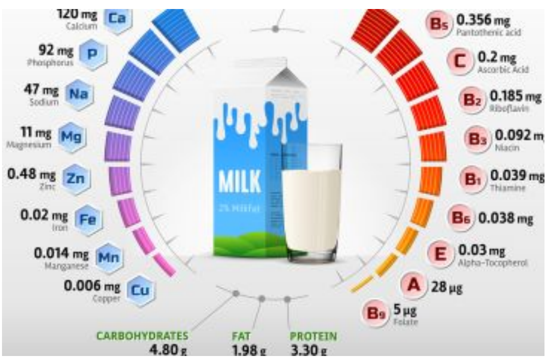
But in between the many facts and figures is one overarching theme that Americans can easily adopt into their own lives: the need to think about healthy eating in terms of a dietary pattern rather than the inclusion of particular nutrients or foods. After all, when was the last time you heard someone say "I'm really craving some potassium right now?" Although humans have specific nutrient requirements, we consume nutrients in food, and foods as part of a meal, usually comprised of many different food groups. By emphasizing patterns over nutrients, the DGA hopes to make healthy eating something that people of all cultural backgrounds can attain without having to abandon to their current diet [1].

Time to make the shift

The 2015-2020 DGA proposes that there are many ways to reach a healthy diet, not simply a one-diet-fits-all mindset that many Americans may find difficult to achieve. But even with dietary flexibility, the DGA emphasizes that all healthy diets need to include fruits, vegetables, protein, dairy, grains, and oils while limiting sodium, added sugars, and saturated and *trans* fats (for more on *trans* fats click here) [1]. The number of servings of each of these food types varies depending on which healthy diet pattern you choose to follow. This edition offers three choices: Healthy U.S.-Style Eating Pattern, Healthy Mediterranean-Style Eating Pattern, and Healthy Vegetarian Eating Pattern. The latter two are modifications of the Healthy U.S.-Style Eating Pattern; the Mediterranean-style eating pattern contains more fruits and seafood and less dairy, while the Vegetarian-style lacks meats, poultry, and seafood but includes more beans and peas, nuts and seeds, whole grains, and soy products [1].

The DGA argues that these eating patterns are “attainable and relevant in the U.S. population” because they are based off data on the types and proportions of foods that Americans are currently eating [1]. Thus, small modifications or shifts in diet (as opposed to changing the diet as a whole) allow individuals to move closer to one of these patterns. The key is to replace foods (and drinks) with little to no nutrition with those considered nutrient-dense. Nutrient-dense foods provide vitamins, minerals, and other substances such as protein or healthy polyunsaturated fatty acids, but little or no solid fats or added sugars and sodium. Examples include: lean meats, vegetables, fruits, nuts and seeds, and low- and fat-free dairy products.

Dairy foods and healthy eating patterns



Lest you think the DGA aren’t intended for you, here is a startling fact: 75% of the U.S. population currently has an eating pattern that is low in vegetables, fruits, dairy, and oils and exceeds the recommended intake of saturated fats, sugars, and sodium [1]. And if that statistic alone isn’t enough to make you want to give up your fast food lunch, consider the links between diet and health. Currently, 50% of all American adults have one or more diet-related chronic disease, such as type 2 diabetes, high blood pressure, or poor bone health [1]. But there is a silver lining to all of this doom and gloom—these diseases are preventable and can be improved with changes in diet and lifestyle.

For example, the scientific literature offers strong support for prevention and improvement in insulin sensitivity, cardiovascular health, and bone mineral density through dietary changes. Indeed, one food group – dairy – has been shown to have positive effects on each of these health issues. One reason dairy is related to so many health improvements is because it is unique among the nutrient-dense foods in the combination of nutrients it provides: calcium, phosphorus, zinc, potassium, riboflavin, magnesium, selenium, choline, vitamin B12, vitamin A, vitamin D (if fortified), and high-quality protein [1]. In fact, it is such a good source of calcium, potassium, and vitamin D that diet patterns that do not meet the recommended daily intake of dairy are lacking in these essential nutrients [1]. Both the Healthy U.S.-Style Eating Pattern and the Healthy Vegetarian Eating Pattern recommend three servings (cups) of low or fat-free dairy per day, but the Mediterranean diet only recommends two. The guidelines acknowledge that those who follow the Mediterranean diet will have lower daily intakes of calcium and vitamin D than currently recommended, leaving them vulnerable to deficiencies should they not get these nutrients elsewhere (e.g. calcium from green leafy vegetables, vitamin D from sun exposure).

Improving our nutritional GPA

Right now, on the dietary report card, dairy consumption is one of America’s worst nutritional subjects. The DGA estimates that 80% of Americans (age 9 and up) do not meet the recommended daily intake of three servings (serving = 1 cup) (In fact, the only other “subject” for which we have a lower grade is vegetable intake). So when the DGA talks about making shifts, nearly all Americans will benefit from shifts that incorporate more low- or fat-free dairy products. Small substitutions, such as a glass of low-fat milk instead of soda, have the potential of making big impacts on current health and the prevention of chronic disease.

1. U.S. Department of Health and Human Services and U.S. Department of Agriculture. *2015-2020 Dietary Guidelines for Americans*. 8th Edition. December 2015. Available at: http://health.gov/dietaryguidelines/2015/guidelines/

Contributed by
Dr. Lauren Milligan
Research Associate
Smithsonian Institute

Dairy's *Trans* Fats Associated with Health Benefits

- ***Trans*** **fatty acids found in partially hydrogenated oils are associated with poor health outcomes, particularly in cardiovascular health.**
- **Dairy foods provide a source of natural *trans* fats, including vaccenic acid and conjugated linoleic acid.**
- **Research in animal models suggest natural *trans* fats may have multiple health benefits, including enhancing immune function, decreasing body fat mass, and preventing or slowing of tumor formation in several types of cancer.**
- **Human studies with natural *trans* fats from dairy foods are less conclusive than animal studies, but they highlight the need to distinguish between natural and industrially produced *trans* fats when making dietary recommendations.**

In 2007, they were banned from all restaurants in New York City. In 2013, the Food and Drug Administration (FDA) claimed that they were no longer "generally recognized as safe" for use in human food. And in 2015, the FDA went one step further and gave the food industry three years to remove them from all human food products [1]. What are these evil and soon to be illegal food items?—*trans* fatty acids (TFA).

Legislation about TFA stems from the poor health outcomes associated with their consumption, particularly in cardiovascular health [2, 3]. It should come as no surprise, then, to learn that the recently released *Dietary Guidelines for Americans, 2015-2020* (DGA) recommends all Americans limit TFA intake [3]. But what this recommendation ignores is the fact that not all TFA are created equally. Whereas industrially created TFA (commonly referred to as partially hydrogenated oils) may increase cholesterol and plaque in the arteries, naturally occurring TFA found in dairy may actually provide health benefits, from preventing tumor formation to decreasing body fat composition [2, 3, 5, 6]. Recommendations for avoiding all TFA may be throwing the potentially healthy baby out with the unhealthy bathwater.

Making a *trans* fat

Fatty acids are strings of carbon and hydrogen atoms. Precisely how the carbons and hydrogens bond determines whether or not the fatty acid is classified as saturated or unsaturated. Saturated fatty acids have single bonds between all of the carbons and unsaturated fatty acids have at least one double bond between the carbon atoms (Figure 1). Single-bonded carbon chains are "saturated" because they have the maximum number of bonds (two) to hydrogen atoms. Adding a double bond between carbons removes one of the hydrogen atoms, thus these fats are "unsaturated."

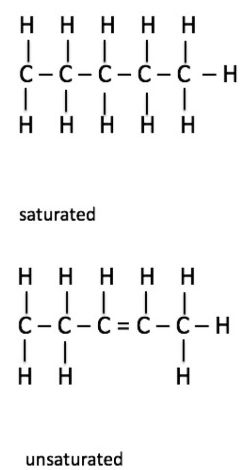


Figure 1

These seemingly small differences in molecular arrangement are associated with large differences in how the fatty acids are metabolized and how they function within the body [3]. After all, saturated fats have become known as the "bad" fats due to their association with high cholesterol and cardiovascular disease whereas unsaturated fats, particularly polyunsaturated fats (unsaturated fats with more than one double bond), are touted for their health benefits. Differences in molecular structure also influence properties of the foods these fats are found in. For example, saturated fats are packed together more tightly and as a result have a higher melting point than unsaturated fats. Thus, foods high in saturated fat are more likely to be solid at room temperature than those with unsaturated fats.

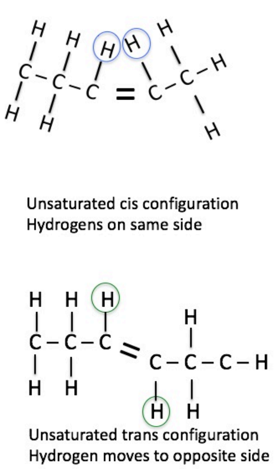


Figure 2

The term *trans* comes into play when an unsaturated fatty acid loses one (or more) of its double bonds by the addition of hydrogen atoms. The hydrogenation causes the molecule to twist so that the hydrogen atoms end up on different sides of the carbon chain (Figure 2). The resulting Z-shaped fatty acid molecule is referred to as *trans* because the hydrogen atoms are now across from one another (as opposed to *cis* where the hydrogen atoms remain on the same side of the carbon chain).

Industrial (or artificial) TFA are made by partial hydrogenation of vegetable oils; hydrogen is added to unsaturated fats, which in addition to changing the configuration of the carbon chain, also makes the fatty acid more saturated by removing double bonds. Why would anyone go to the trouble of doing this? TFA, like saturated fats, have a higher melting point and have the advantage of being solid at room temperature. When they are added to prepared foods such as baked goods, frozen pizza crusts, and crackers, they can significantly extend shelf life because they take longer to go rancid than unsaturated fats in the *cis* configuration. They are also regularly used in restaurants to fry foods because the oil requires less frequent replacement [4]. From a financial point of view, then, TFA offer significant cost savings on the part of the food providers and manufacturers.

It turns out that bacteria that live in the first stomach chamber (or rumen) of ruminants (e.g., cows, sheep) can also reconfigure an unsaturated fatty acid from the *cis* to *trans* structure. As polyunsaturated fats from the grass diet pass through the first chamber, particular bacterial species metabolize some of these fats by adding hydrogen atoms. These newly manufactured TFA eventually make their way into body fat stores and, in lactating females, to the milk. Thus, cuts of meat or dairy products that contain fat will have a small proportion of natural (or ruminant) TFA, including vaccenic acid (VA) and conjugated linoleic acid (CLA).

Banning the bad TFA

Using the umbrella term TFA to refer to both industrial and natural TFA can be problematic, as it suggests that TFA are the same regardless of how they are made. The most recent edition of the DGA does distinguish between artificial and natural (or ruminant) TFA, but only to indicate that TFA have two potential dietary sources. They recommend all Americans limit dietary TFA, and advise people to "consum[e] fat-free or low-fat dairy products and lean meats and poultry [to] reduce the intake of natural *trans* fats from these foods" [4]. Any consumer reading this would be led to believe that all TFA cause adverse health effects.

However, TFA are not a homogenous group; those found in partially hydrogenated oils have different metabolic effects in humans and other animals than those produced by bacteria. Most significantly, artificial TFA are associated with increasing the "bad" cholesterol (low density lipoprotein, LDL) and total triglycerides. As such, these TFA increase the risk of cardiovascular disease [2-4]. Concern for the heart health for Americans led the FDA to deem TFA as unsafe for human consumption, and prompted the World Health Organization (WHO) to call for a global elimination of these fats from the food supply [7]. Even consumers who do not know what a TFA is know they should not eat them; food labels proudly stating "No *Trans* Fats!" clearly communicate that these types of fats are unhealthy and should be avoided.

Can *trans* fats be healthy?

In stark contrast to global campaigns to eliminate artificial TFA from our diet are nearly 30 years of scientific research that suggest natural TFA are associated with improvements in health in both animal and human models [2, 3, 6-8]. The first health benefits of natural *trans* fats were identified in the mid 1980s by Michael Pariza and colleagues who found that an extract from grass-fed ground beef actually had an anticarcinogenic effect [9]. This extract was later identified as CLA, a unique fatty acid because the two double bonds are located on adjacent carbons (hence the name conjugated), as opposed to being separated by one or more carbon atoms. One double bond remains in the usual **cis** position while the other is *trans*. The location of the double bonds varies across CLA molecules, and the different positions of the double bond are referred to as CLA isomers. There are almost 30 different CLA isomers, which may explain the diversity of metabolic and physiological effects of this fatty acid [3, 6, 9, 10].

Since the original discovery by Pariza and colleagues, CLA isomers (which includes VA) have demonstrated numerous anti-cancer effects in animal models, including inhibiting the development of tumors and preventing metastasis [reviewed in 3, 6, 10]. There have been multiple explanations of the mechanisms of CLA that produce these outcomes, including interfering with tumor cell signaling, inhibiting DNA synthesis, and enhancing tumor cell apoptosis (aka cellular suicide) [3]. With these promising findings, it is disappointing that evidence for CLA anti-carcinogenic activity in humans is inconclusive. For example, Park [3] cites one study that found a preventative effect of CLA on breast cancer risk only to then discuss several more that failed to identify any correlation with breast cancer development.

The same discrepancy between animal and human studies is found when considering the effect of CLA on body mass. In animal models, CLA has been found to positively effect body composition by decreasing fat mass and preserving or even increasing lean body mass. CLA is believed to influence body fat by direct mechanisms (e.g. reduction of fat accumulation in adipose stores) and possibly indirectly, by influencing hormones involved with lipid metabolism (e.g. leptin, adiponectin) [3, 9]. Results of clinical trials with humans are mixed, but many support the role of CLA in decreasing fat mass [9]. However, the effect of CLA on human fat mass was not as strong as that identified in rodent models [3].

Why might CLA behave one way in animals and another in humans? One possible reason for the conflicting study results is the variation in methodology utilized in each study. Rodent subjects are often fed diets with much higher proportions of CLA than human subjects and are fed for much longer durations [3]. Dose and dose duration could significantly alter results [9]. For example, in human studies on CLA and body fat mass, greater effects of CLA were identified when the study period exceeded six months [3]. Subject differences also account for different results. Body fat mass reductions were observed in rodent models during a period of growth (or positive energy balance), whereas most human studies involved subjects on low energy (i.e. weight loss) diets. However, human subjects in positive energy balance responded to CLA in the same manner as rodents, with a decrease in fat mass [3, 9].

To understand the role of natural TFA in human health necessitates comparing apples to apples, and that is nearly impossible with much of the current literature. One topic that seems to show some consensus across studies, however, is the potential role of CLA as an immune modulator. And importantly, the mechanism by which CLA influences immune function could help elucidate its influence on cancer, body fat, and even cardiovascular health [3, 5]. Specifically, CLA stimulates the production of anti-inflammatory chemical signals (called cytokines) while also decreasing the production of signals for inflammation [5]. Importantly, this effect was identified *in vitro*, in animal models, and also in human subjects [studies reviewed in 5]. For example, in both rats and humans dietary CLA supplementation was associated with increased production of immunoglobulin A (IgA) and IgM (antibodies associated with an anti-inflammatory response) but decreased production of the allergy-related (aka, inflammatory) IgE [5]. Driving the immune system in the direction of an anti-inflammatory as compared with an inflammatory response can explain why CLA may play a role in cancer, weight gain, and atherosclerosis, because each involves inflammation.

To eat or not to eat

There are several review articles on the influence of dietary CLA on health outcomes and each ends with the same message: more research is needed in order to make conclusive statements about the benefits of natural TFA [3, 5, 8, 9]. Currently, the strongest evidence for their role in cancer, weight gain, or immunity comes from *in vitro* and animal studies. These results should not be overlooked or discounted as they have provided an understanding of the mechanisms by which natural TFA interact with immune, cancer, and numerous other types of cells and chemical signals in the body. Indeed, this information is critical as it suggests natural TFA are metabolized very differently than their industrially manufactured cousins.

While there may currently be insufficient evidence to recommend that humans consume more natural TFA in their diet, the breadth of research on their health benefits suggests the need to distinguish them from industrial TFA by more than just the use of “natural”. After all, people are accustomed to the idea of animal-derived fat providing a health benefit; simply look at the shelves of fish oils in health food stores for evidence of this. It may be harder to convince consumers that not all *trans* fats should be banned.

1. FDA 2013. FDA takes step to further reduce *trans* fats in processed foods. http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm373939.htm
2. Kleber ME, Delgado GE, Lorkowski S, März W, von Schacky C. 2015. *Trans* fatty acids and mortality in patients referred for coronary angiography: the Ludwigshafen Risk and Cardiovascular Health Study. Europ. Heart J. doi:10.1093/eurheartj/ehv446
3. Park Y. 2009. Conjugated linoleic acid (CLA): good or bad *trans* fat? J. Food Compos. Analysis 22: S4-S12
4. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015-2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at: http://health.gov/dietaryguidelines/2015/guidelines/
5. O’Shea, M, J. Bassaganya-Riera, and I. C. M. Mohede. 2004. Immunomodulatory properties of conjugated linoleic acid. Am. J. Clin. Nutr. 79:1199S-1206S.
6. MacDonald HB. 2000. Conjugated linoleic acid and disease prevention: A review of current knowledge. J. Am. Coll. Nutr. 19:111S-118S.
7. http://www.who.int/bulletin/releases/NFM0413/en/
8. Field CJ, Blewett HH, Proctor S, Vine D. Human health benefits of vaccenic acid. 2009. Appl. Physiol. Nutr. Metab. 34(5):979-991. Review.
9. Pariza MW. 2004. Perspective on the safety and effectiveness of conjugated linoleic acid. Am. J. Clin. Nutr. 79:1132S-1136S.
10. Pariza MW, Park Y, Cook ME. 2001. Review: The biologically active isomers of conjugated linoleic acid. Prog. Lipid Res. 40: 283-298

Contributed by
Dr. Lauren Milligan
Research Associate
Smithsonian Institute

Funding provided by California Dairy Research Foundation and the International Milk Genomics Consortium