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This month's issue features probiotics and mother's milk, and milk casein fibers.

Maternal Probiotic Consumption Affects the Oligosaccharides in Mother's Milk

- **Until recently, the various oligosaccharides found in human milk were thought to be entirely genetically determined.**
- **A new study finds that women who consumed probiotics during late-stage pregnancy had higher levels of some oligosaccharides and lower levels of others, than women who took a placebo.**
- **The identities of the sugars whose levels appear to be affected by probiotic consumption suggest that probiotic supplements influenced the amount or activity of enzymes operating in specific biological pathways.**

These days, the health-giving properties of human milk oligosaccharides, or HMOs, are much appreciated. The medium-length sugars, which are the most common component of human milk after water, lactose, and lipids, are not metabolized by infants. Instead, they have diverse non-nutritive roles, such as protecting infants against invading microbes, and encouraging the proper development of the growing gut. It is well established that different women secrete different collections of HMOs in their milk, and until very recently, genetics was understood to hold complete sway over this, dictating the various types and relative amounts of the various HMOs that a woman produces. In a recent issue of *JAMA Pediatrics*, however, Antti E. Seppo of the University of Rochester Medical Center, in New York, and his colleagues, report that women who consume probiotics have altered blends of HMOs in their milk [1].

There are four main groups of women classified by the HMOs that they make. Women may be "secretors" [2] (meaning that they have a functional FUT2 gene, and can consequently produce enzymes called fucosyltransferase-2), or "non-secretors" (who lack functional FUT2, and thus also fucosyltransferase-2). Possessing fucosyltransferase-2 endows secretors with the ability to produce HMOs in which a fucose subunit links to a lactose part via an alpha-1-2 linkage. Another gene called FUT3 makes possible the synthesis of other types of links between sugar subunits. The combination of functionality of a woman's FUT2 and a FUT3 genes determines into which of the four main groups she falls.

The recent study undercuts the dominance of the genetic perspective. To be sure, genetics determines the biochemical raw materials available for reactions to be feasible in the human body, but it now seems that environment—in the form of maternal diet—can affect the relative abundance of the different HMOs among those that her genetics permits her to produce.

In the study, Seppo and his team defrosted samples of colostrum that were put into storage during a prospective probiotics trial in Finland. (The original trial was concerned with the question of whether probiotics consumed during pregnancy influenced childhood allergy status.) These defrosted samples were then assessed for their concentrations of 19 different HMOs, using a method called high-performance liquid chromatography. In total, colostrum was analyzed from 51 women who had taken probiotics between week 36 of pregnancy and giving birth, alongside colostrum from 30 women who had taken a placebo over the same period.

Two HMOs occurred at much higher levels in the women who had received the probiotic supplementation. These were 3-fucosyllactose and 3′-sialyllactose. Nonetheless, the overall level of HMOs was lower among women who had taken probiotics compared with placebo. The much longer list of HMO structures that occurred at lower concentrations in the treatment group include difucosyllacto-N-hexaose, lacto-N-tetraose, lacto-N-fucopentaose I, and 6′-sialyllactose.

The intriguing point about these lists is that they give clues as to the mechanisms involved. The HMOs that occurred at higher levels are both synthesized via one biochemical pathway, and those that were reduced in amount via another. This implies that the probiotics used in the study—specifically, capsules of *Lactobacillus rhamnosus* GG, *Lactobacillus rhamnosus* LC705, *Bifidobacterium breve* Bb99, and *Propionibacterium freudenreichii*subspecies *shermanii*—were in some way affecting the amounts or activity of the enzymes in those pathways in opposite ways. Although how exactly that is happening is unclear.
The finding that probiotics can alter the HMO component of human milk marks a sea change in how applied scientists understand the options available to them as they seek to make available to infants HMOs that they lack. Until now, the focus has been on synthesizing simple HMOs and adding these structures to infant formula, an area of research that has made significant strides [3] lately, even though there is a long way to go before these synthetic structures functionally imitate natural HMOs. Providing gestational probiotics could offer a potentially cheaper alternative. Indeed, 3′-sialyllactose—one of the two sugars for which probiotics appear to stimulate production—lowers the rate of binding of *Escherichia coli*onto intestinal epithelial cell surfaces [4], reducing the probability of infection. As such, one day in the not-so-distant future, late-pregnancy probiotics could be prescribed or withheld to usefully influence HMO composition and quantity.

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Dairy Battles Bad Bacteria

- **A new study demonstrates that milk fat globules can counteract *Escherichia coli.***
- **Milk fat globules directly bind *E. coli* and reduce its adherence to intestinal cells.**
- **Mice fed high-milk fat globule cheese had lower levels of *E. coli* colonization compared with mice fed low-milk fat globule cheese.**
- **More research is needed to understand how milk processing affects these properties of milk fat globules.**

It seems that more and more frequently, the news reports on outbreaks of pathogens like *Escherichia coli*, which can result in food poisoning. The common approach to treatment is antibiotic therapy, but sometimes this treatment is not effective, or the bacteria are resistant to the antibiotic. Scientists are working hard to find ways to control bacteria without antibiotics. A study by Douëllu et al. [1] has shown how a component of milk—milk fat globules (MFG)—may counter effects of *E. coli*.

Milk is a complex fluid containing proteins, carbohydrates, and lipids. The lipids, also known as fat, are largely organized into MFG. Lipids naturally form droplets when mixed with water. These droplets are usually hollow spheres (or micelles) with the lipids arranged so that parts of the molecules repelled by water molecules are buried inside the droplet. However, MFG are not just hollow vesicles, rather they are a complex mixture of lipid droplets coated with layers of lipid and a range of proteins. Some of these proteins are coated with sugars to varying degrees. These glycosylated proteins may provide the key to the ability of MFG to bind to and interfere with bacterial infection.

The MFG develops from lipid droplets that form in epithelial cells that line the tiny, milk-producing alveoli of the mammary gland. When the lipid droplet migrates from inside the epithelial cells through the cell membrane and into the lumen of the alveoli, they become coated with parts of the epithelial cell membrane complexes and thereby capture proteins that are associated with these membranes. As a result; the MFG are approximately 50% protein and 50% lipid [2]. MFG have been studied extensively in recent years and have become a focus of research concerning infant formulations. Traditionally, infant formula had lipids substituted by vegetable oils, and little attention had been paid to MFG content. However, the research with MFG-supplemented formula points to a potential benefit of providing MFG for neonatal gut health and microbiome establishment [3].

The range of proteins detected in MFG varies according to concentration [4-8], but prominent proteins are responsible for some of its most effective bioactive properties. One of the most abundant proteins is a member of the mucin family, MUC1. This is one of the most highly glycosylated proteins known. Its structure includes multiple repeated sequences of amino acids. This extends the length of the protein so it protrudes beyond the surface of the MFG and potentially provides a protective barrier. This is complemented by the multiple molecules of attached oligosaccharides or sugars that coat the protein, adding to the barrier effect, but also providing binding sites for bacteria. Bacteria have their own set of molecules which seek out oligosaccharides as a natural link to adhere to cells and tissues.

With these MFG properties in mind, Douëllu et al. [1] set out to research the potential for MFGs to combat infection by dangerous bacteria that are commonly identified as a food-borne pathogen. The potential dangers associated with the bacterium known as *E. coli* is well known. This organism is ubiquitous in our environment, including in the gut of humans and animals. There are many strains of *E. coli*, some are harmless and some of are known to cause disease by producing Shiga toxin [9,10]. These enterohemorrhagic strains can cause sickness, from simple gastrointestinal upsets to violent haemorrhage, kidney failure, and death. The Centers for Disease Control estimates that over 200,000 infections occur each year in the USA alone. In a recent event in 2018, five people died and over 200 were infected by the O157:H7 strain following distribution of contaminated vegetables. In Europe, over 4,000 cases were recorded due to contamination of fenugreek sprouts by the O104:H4 strain in 2011. A number of outbreaks have been linked to contamination of raw milk products [11].

The first stage in infection is adherence whereby molecules on the surface of the bacteria bind to cells of the intestinal lining. Once bound, the bacteria can invade the tissue and cause infection. Douëllu et al. [1] tested the ability of MFG to influence adherence of *E. coli* strains in three different ways. First, they wanted to examine whether the bacteria bound directly to MFG. They grew bacteria in the laboratory and mixed them at different doses with MFG preparations. They stained the bacteria so that they could observe the response under a microscope. They found that regardless of the strain of bacteria used, they were bound by the MFG. Secondly, they established sterile cultures in the laboratory using cells that simulate those found lining the intestinal walls of humans. Using this system, they were able to measure bacterial interactions. When bacteria were introduced to these cultures, they found that added MFG hampered bacteria-cell interaction, and as a result reduced the numbers of bacteria that adhered to the cells. Thirdly, they fed laboratory mice cheese containing low or high levels of MFG. Some mice were given cheese contaminated with different bacterial strains, and others were given cheese followed by a dose of bacteria. When they examined the intestines of mice, they found that the contaminated cheese containing the higher levels of MFG reduced bacterial colonization of the intestinal surface and altered the spread of this contamination. The cheese was less effective against direct bacterial inoculation, although this seemed to be related to the relatively high dose administered.

This study adds important information to the growing evidence for anti-microbial properties of milk and dairy foods [1]. The anti-microbial properties are characterized by a number of highly developed approaches that include passive neutralization, passive inhibition, and sequestering or bioactive molecules. The study highlights the need for a detailed understanding of the impact of processing on these properties of milk, and further investigation of relevant bioactivities of milk-derived molecules and micro-structures. As stated by the authors of the study, it also adds to the prospect that milk-derived bioactive molecules may be useful as preventative or competitive treatments in cases of food poisoning.

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Immune Factors in Human Milk Shaped by Mother's Environment

- **Human milk contains antibodies and other immune factors that reflect the mother's pathogen experience.**
- **Human cultural practices, including mode of subsistence, influence the types of pathogens mothers are exposed to and the frequency of exposure over their lifetime.**
- **A new study found that the quantity of human milk immune proteins varied with respect to subsistence strategy across populations.**

Human milk may be a complex biological fluid, but many of the ingredients that make it so complex are influenced by culture. Milk fatty acids reflect the fat content of the mother's diet, and milk microbes have been linked to the mother's diet, antibiotic use, and psychological stress. Now, a new study [1] reports that a mother's subsistence strategy—that is, the way that mother's community makes a living—affects the quantity of immune proteins in her milk. Whereas maternal antibiotic use is a novel cultural influence on milk composition, subsistence strategies have influenced the maternal pathogen experience, and likely shaped milk immune factors, throughout human evolutionary history.

Pass It On

Photo credit: Jhangchuk Sangmo and Nyima Sangmo

Passive immunity is one of evolution's most ingenious inventions. Baby mammals are born with immature and naïve immune systems; they are incapable of manufacturing large numbers of antibodies or other immune factors (like T cells), and their lack of exposure to pathogens means their immune responses are slow and less efficient. And yet, these infants are not entirely vulnerable to infection. While their own immune systems develop and get acquainted with the local pathogen population, infants are protected against infection by their mother's immune factors, passed on either via the placenta or milk.

Many of the passively transferred immune components are part of the innate immune system, which is not pathogen-specific but nevertheless is integral in recognizing viruses, parasites, or bacteria as "not self" and mounting an immune response. But the real adaptive value of passive immunity comes from the transfer of the mother's acquired immunity to her offspring. Infants may be immunologically naïve, but their mothers have had a lifetime of exposure that they share in the form of pathogen-specific immunoglobulins (antibodies) and white blood cells.

For such a system of shared immunity to be effective, it must be able to predict the types of pathogens the infant will encounter during their first months of life. In other words, milk immune factors should be correlated with both the maternal and infant environment. Despite this important assumption of passive immunity, little is known about how specific environmental factors shape milk immune composition [1].

Climate clearly plays an important role in how pathogen exposure varies globally; people living in tropical environments come in contact with a greater diversity of pathogens than those living in more temperate environments [1]. But a mother's environment is more than simply climate; tropical populations can live in high-rise apartments in urban Singapore or in more rural agricultural villages in the Philippines. How does variation in human cultural practices shape the types and quantities of immune factors passed from mother to infant in milk?

These Are the Microbes in Your Neighborhood

To tackle this question, a team of researchers studying biocultural aspects of lactation in populations across the globe collaborated to quantify milk immune factors [1]. Seven global populations were grouped into one of the following subsistence strategies: urban-industrialism (n = 1 population), rural-shop (n = 3), horticulturalist-forager (n = 1), or agro-pastoralist (n = 2). These groups differ in population size (which relates to crowd diseases such as tuberculosis), level of interaction with soil and animals (which relates to parasitic and zoonotic diseases), and public health initiatives (e.g., access to clean drinking water and waste removal). Klein and colleagues predicted that the differences in disease ecology across these four modes of subsistence would be correlated with milk immune proteins, particularly those associated with acquired immunity [1].

The study analyzed 164 milk samples. In order to control for potential methodological errors, all samples (save for nine collected from an earlier research period) used the same, standardized collection procedure [1]. Moreover, all samples were analyzed in the same lab (UC Davis) to reduce measurement error between different assays and labs.

Populations differed more (in head-to-head comparisons, known as pair-wise comparisons) in the composition of acquired immune proteins (the quantity of sIgA, IgG, and IgM), which reflect individual interactions with pathogens compared with innate immune proteins (lactalbumin, lysozyme, and lactoferrin), which are more conserved, evolutionarily speaking. Higher milk immunoglobulin concentrations were positively associated with populations from countries with high levels of childhood mortality or higher childhood infection rates [1]. The lowest levels of milk immunoglobulins came from Boston mothers, the only urban-industrial population in the study. This supports previous research demonstrating that milk antibody concentration, particularly that of sIgA, is positively associated with the pathogen load in the environment [1].

The immunoglobulin composition of milk was different in 17 of 21 pair-wise comparisons across the seven populations. As predicted, those populations that were not significantly different shared their mode of subsistence, despite being geographically distinct (e.g., the Himba of Namibia and ethnic Tibetans in the Nubri Valley). This suggests that the mode of subsistence may be a more bioculturally meaningful comparison than country (or region) of origin, and the results reflect this: five of the six pair-wise comparisons were significantly different across the four different subsistence strategies [1]. The only exception was rural-shop (Cebu, Philippines; rural Polish; and Qom of Argentina) vs. horticultural-foragers (Tsimane, Bolivia). It may be that despite the fact that rural-shop populations purchase food from local groceries and have less dietary diversity than the Tsimane horticulturalists, other similarities in lifestyle, including exposure to livestock and agricultural activities (Cebu and Polish) and household environments, such as a lack of indoor plumbing (Qom), resulted in convergence in pathogen load [1].

Tailored Milk

This study is the first to examine the relationship between milk immune components and the mother's subsistence strategy. And like any good study, it creates more questions than it answers. Subsistence strategies have varying influences on the types of pathogens and the level of exposure, but which aspects have the greatest impact on maternal immunoglobulin production—Interaction with livestock? Clean water? Exposure to helminthes (parasites)? In that same vein, which aspects of the disease ecology have the strongest influence on the maternal immune response—Pathogen virulence? Frequency of pathogen exposure? Rates of pathogen transmission? Klein et al. acknowledge that their study design does not allow them to distinguish between greater antibody responses to similar exposures or similar antibody responses to different exposures [1]. But the influence of maternal environment on milk immune factors seems clear, and will hopefully motivate future research to investigate the specific ways in which human cultural practices shape passive immunity.

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Back to the Future? Milk Fibers in the 21st Century

- **Milk fibers are produced from casein proteins and were popular in the 1940s–50s as alternatives to wool.**
- **It takes approximately 100 pounds of milk to produce 3 pounds of milk fiber.**
- **Milk fiber clothing and textiles are regaining some interest by manufacturers and consumers looking for environmentally-friendly alternatives to less sustainable fabrics.**

A cow, a milkmaid, and a chemist walk into a bar… or a laboratory? Not quite the typical start to a joke, but this is how a 1950s brochure described a then-popular—and somewhat revolutionary—milk fiber textile called Aralac. Between World Wars I and II, wool was scarce and this milk fiber-blended fabric was becoming a go-to substitute for shirts, ties, and other accessories and clothing in the U.S. and Europe. For a moment in time, it seemed that the future of fabrics was milk-based. So why are we not wearing—and maybe not even aware of the existence of—milk fiber clothing now? The answer lies where economics and science intersected in the mid-twentieth century. But like so many things, it seems that history is bringing us full-circle, and the interest in milk fibers has been rekindled in recent years.

Futuristic Fiber in the Past

Milk fiber weaving by Liz Moncrief. Photograph by George Boe. Courtesy of Handwoven magazine.

Milk fiber was originally created by Germans during World War I, when the hard film left by dried milk was mixed into solutions and then spun to form fibers. But these first fibers, and the textiles made from them, were brittle and hard and so were of little practical use. But the idea of using milk to make fiber had been sparked, and in the 1930s, Italian chemist Antonio Ferretti tried to improve upon the first design. His fibers were much softer, and had a wool-like texture and quality, and he sold his patents to an Italian company that produced milk-based fibers under the trade name of Lanital [1].

In the U.S., production of milk fibers was developed further by Aralac, Inc., a company affiliated with the National Dairy Products Foundation, which later became Kraftco [2]. It seems that making fibers and fabrics was a kind of side project for the growing dairy company, but as wool fiber production declined during World War II, Aralac wisely found an economic niche for its milk fiber fabrics and textiles. A promotional brochure produced by the company itself in the 1950s compares the product to wool and other “natural” fibers, claiming “Aralac is the registered trade mark [sic] for the first chemically stabilized protein fibre to be produced by man” [3]. Advertisements found in Life Magazine in 1944–45 chronicle the use of Aralac in blended fabrics, including “gabardines, twills, flannels, serges, challis and fleeces, as well as in crisp, cool, summer-weight materials in apparel for men, women and children” [4]. The company emphasized both the physical qualities of the product (e.g. its texture and versatility) as well as its novelty: “Aralac is one of the wonders of the future… the promise of a richer, more wonderful tomorrow… but here today for your enjoyment!” [5]. Yet by 1960, Aralac had virtually disappeared from the market, unable to compete with cheap and easy-to-produce synthetics like nylon.

The Cow, the Milkmaid, and the Chemist

Producing milk fiber can be, perhaps paradoxically, both resource-intensive and ecologically-friendly. In its own brochure mentioned above, Aralac, Inc. described the production of it as the collaboration of “the cow, the milkmaid and the chemist … Out of this understanding grew the research and experiments which enabled the technician to change the casein of skimmed milk into a modern textile fibre" [3]. Caseins are among the most prevalent proteins in cow milk—comprising about 80% of its protein content—and they provide essential amino acids to millions of global consumers via dairy products. Other surprising and historical uses for casein include as adhesives and bases for paint, and more recently in edible films for food.

Making casein-based fiber begins with skimmed milk, which is often a non-consumable by-product in dairy production [1,3]. The milk is treated with acid, which results in a coagulated curd that is then washed, dried, and ground into a fine powder. Thirty-five liters of skimmed milk produce 1 kilogram of casein [6]. The casein is dissolved in caustic soda, ripened, and then filtered and de-aerated, which results in the casein becoming a kind of linear macromolecule structure [7]. The fiber is produced through a wet spinning technique, which includes pumping the casein solution at high pressure through a spinnaret—a kind of metal filter that may look a bit like a showerhead—with thousands of tiny holes [1,6,7]. The solution, streaming through these holes, is immersed in an acid and water solution. The acid neutralizes the alkali that dissolved the casein and the small fibers are then stretched and spun [1,6]. Additional processing to remove impurities, strengthen, bleach, and soften the fibers may be done with various additives and chemicals. It is also possible to blend casein fiber with natural fibers like wool, cotton, and silk.

Milk fiber contains as many as 18 amino acids, and has a pH of 6.8, which is the same as the pH of human skin. Some claim that the "natural protein humectant factor" of casein is preserved in milk fiber fabric, and so wearing it is actually good for the health of skin [8]. It is known to be comfortable and anti-bacterial; it is moisture-absorbent, permeable, and heat-resistant. It feels lightweight and silky and is biodegradable [1]. But it takes about 100 pounds of milk to make 3 pounds of milk fiber, which leads some to question whether it is truly an efficient enough product to be considered truly "eco-friendly."

Back to the Future

Today, milk fiber is (re)gaining interest and popularity as manufacturers and consumers look for alternatives to synthetic materials for clothing and accessories. Recent studies reveal that many mass-produced fabrics contain microplastics that can be released into watersheds and ocean waters during production and washing [9]. So various companies around the world are turning to milk as a potential source for making fibers and textiles that may not leave such a hazardous mark when produced and used. But whether milk fiber products will find enough of a market share to make an environmental impact remains to be seen.

Consumers like the idea of milk fiber clothing, and companies like QMILK in Germany and Swicofil in Switzerland continue to innovate and refine their production techniques. Yet clothing produced from milk fiber is said to lack durability and is highly susceptible to wrinkles. It is also costly to manufacture, and thus is not (yet) an option for most mainstream shoppers [1]. But like most products, demand and supply may help with cost and availability over time, and it may not be too long before we find ourselves milk fiber-based clothing.

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