



SPLASH! milk science update October 2015 Issue

This month's issue features using smartphones for milk protein analysis, what dolphins can teach us about dairy fats, the genetics of lactose tolerance, and producing human milk sugars for use in formula.

Smartphone Detectors for Milk Protein Analysis

- **Information technologies are bonding with biotechnologies.**
- **Smartphone attachments and applications have been developed for measuring biomarkers in a field setting.**
- **The Portable Protein Microarray measures concentrations of individual proteins in complex mixtures.**
- **A poof-of-concept study has shown that this portable tool can be applied to milk.**

Parallel advances in biology and information technologies are converging into hybrid devices with the potential for widespread impact. Among other areas, these hybrid technologies will revolutionize field measurements and on-site analytics. A recent study by Ludwig et al., described the development of a novel device that has capitalized on these technologies and, in a pilot project, demonstrated its use in milk analysis [1].



Measurements that can be made cheaply and simply on the farm, or in processing factories, can quickly provide crucial information for decision makers. Development of micro- and nano-scale, bio-analytical samplers has advanced considerably in recent years, and will fundamentally change our capacity to make complex biological measurements on site that currently require well-equipped laboratories (see e.g. [2-5]). One example of this emerging technology is the Portable Protein Microarray.

Proteins are the building blocks of cells, tissues, and whole organisms. They also provide the means for these cells and tissues to perform their physiological duties, e.g. muscles are packed with proteins that work together to contract and give us strength. We also recognize proteins for their nutritional value, and milk is a great example of a rich nutritional source of protein. Because proteins are so intimately involved in biology, they are also very useful for monitoring processes or detecting events, in which case we refer to them as biomarkers.

There are many laboratory assays that have been designed to detect protein biomarkers for a range of purposes. For example, we can measure a small amount of a single protein in the complex mixture represented in milk. We can also detect changes to proteins in milk that may be associated with mastitis, or a particular stage of lactation. However, accurate measurements of this type require costly laboratory processing and equipment. More recently, there is increasing interest in developing tools that will provide this information on-site and in real time. One of the tools that has tremendous promise for such a purpose is the protein microarray.

The protein microarray is an existing technology that is best described as a miniaturized tool for measuring individual proteins in a complex, and often unprocessed sample (see e.g. [6]). This is commonly achieved using tiny nano-scale amounts of a panel (or array) of antibodies. Each antibody is customized to recognize just one type of protein in the sample. Ludwig et al., [1] have now married a portable version of this technology with smartphone technology to produce a prototype of a system that does not need a laboratory and can deliver instantaneous results. The team that developed this innovative technology focused on demonstrating its use analyzing milk samples in the field. They named this new technology the Portable Protein Microarray and, for those who need to measure and interpret biological information quickly, it holds enormous potential.

The essence of the invention is the use of existing smartphone camera functions to capture images from a protein microarray. This provides an added potential to send results to any other phone or compatible device. Both the smartphone and the protein microarray are existing platforms, but what the scientists developed was an interface that would connect the two. Using a 3-D printer, they printed a microscope to fit onto the lens of the smartphone camera.

They then wanted a real-world test to demonstrate that the device would work. They chose milk because of its complex mixture of proteins and set the challenge of detecting minute quantities of an antibody generated by the immune system of dairy cows treated with recombinant bovine growth hormone (rbST), also known as somatotropin. Although there are issues associated with use of rbST in some parts of the world, the reason for choosing to detect this biomarker was entirely related to the technical challenge. They also looked for a protein called IGF1, to demonstrate that they could multiplex, that is, detect more than one protein simultaneously. For these assays, they used antibodies bound to a molecule that was excited by the LED light fitted into the microscope adapter. The scientists also developed a simple smartphone app for capturing and analyzing the signals from the microarray.

The study demonstrated that the prototype system was effective in measuring the targeted proteins. It proved that the Portable Protein Microarray system is viable for field applications. There are still avenues for further developments, but even if we consider only our interests in farming and the dairy supply chain, there are many other potential uses. On the farm, for example, it could quickly check for the presence of protein biomarkers that indicate health status of an individual cow using milk or other biological fluids, or even at herd level using milk from the vat. A rapid detection method at factory level may also be useful for estimating concentrations of valued proteins, perhaps the presence of different amounts of casein variants, like A2. Potentially, the technology may also be developed to identify protein fingerprints, or trace sources of unwanted contaminants.

One thing is certain, with the remarkable growth of precision farming and automated systems for farm management, nanotechnologies and information technologies are here to stay, and will continue to impress with innovative applications.

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A Tale of Fats, Fish, Dolphins, and Dairy

- **As reflected in most dietary recommendations, intake of saturated fats has long been linked to increased risk of cardiovascular and metabolic diseases.**
- **In contrast, new research indicates that some saturated fats, such as milk fats, reduce the risk of cardiometabolic diseases.**
- **A dietary intervention study in dolphins showed that a saturated fatty acid, heptadecanoic acid (C17:0), present in some fish types, as well as in dairy fat, could help reverse metabolic syndrome.**
- **An observational study in humans showed that blood levels of the saturated fatty acid pentadecanoic acid (C15:0) were inversely associated with the risk of type 2 diabetes.**
- **The use of C15:0 and C17:0 as biomarkers for intake of dairy fat is debated, and their possible role in conferring beneficial health effects is still unclear.**

For decades, we have been warned about the evils of saturated fats in our food. We have heard that this whole “family” of fats increases our “bad cholesterol,” and hence increases our risk of cardiovascular and metabolic diseases. Recently, however, this widely accepted mantra has been challenged by growing evidence that some saturated fats, such as milk fats, do the exact opposite: they appear to reduce our risk of many diseases, including type 2 diabetes. While scientists debate the mechanisms involved, the changing view on saturated fats is underpinned by a new study of some unexpected contenders: dolphins (1).



How many of us haven't dreamed (secretly or otherwise) of "swimming with dolphins"? This not entirely uncontroversial pastime has boosted happiness, as well as knowledge among us humans. But we can glean a lot more than behavioral science from these social, smart, and playful animals. It turns out that bottlenose dolphins can develop a metabolic syndrome similar to a condition called pre-diabetes in humans. In the US, it's estimated that one in every three adults has metabolic syndrome, raising their risk of not only diabetes, but also heart disease and stroke.

Venn-Watson and colleagues (1) made some interesting discoveries recently when they compared the blood levels of fatty acids—the building blocks of fats—in two dolphin populations.

They found that the levels of three particular fatty acids were lower in a group of dolphins in California than in a group of dolphins in Florida. What's more, the California dolphins had higher blood levels of insulin, triglycerides, and ferritin—common indicators of metabolic syndrome and diabetes. Linking the different blood levels of fatty acids in the two dolphin groups to the different types of fish each group fed on, the scientists then did a dietary intervention study. They started feeding pinfish and mullet, which contain relatively high amounts of the three fatty acids, to some of the California dolphins. Within a few months, the dolphins had not only raised blood levels of the fatty acids but also reduced levels of ferritin, and normalized levels of glucose, insulin, and triglycerides.

C17:0—a key player?

So, what does all this have to do with milk fats? The fatty acids targeted in the dolphin study are not only present in some fish types but also in other animals, including cows. And heptadecanoic acid—a saturated fatty acid (called C17:0 for short) also present in cow's milk—was identified in the dolphin study as an independent predictor of both insulin and ferritin levels. "Our study findings suggest that C17:0 may be a key player in the metabolic benefits of dairy and other dietary products containing C17:0," Venn-Watson and coauthors wrote in their report (1). "Humans' movement away from diets with potentially beneficial saturated fatty acid C17:0, including whole fat dairy products, could be a contributor to widespread low C17:0 levels, higher ferritin, and metabolic syndrome," they hypothesized.

Contrary to the familiar recommendations by health authorities to avoid foods rich in saturated fats, including full-fat dairy products, several recent studies have shown that dairy consumption is associated with lower risk of several cardiometabolic diseases (2). And nutritional scientists are increasingly taking an interest in C17:0, along with its slightly shorter cousin, pentadecanoic acid (C15:0).

Odds and evens

C15:0 and C17:0 are among a handful of so-called odd-chain fatty acids that can be detected in human blood. The vast majority of fatty acids in our blood have an even number (2-26) of carbon atoms in their carbon chain. Since the 1960s, scientists assumed that odd-chain fatty acids had little biological relevance. However, over the past decade or so, C15:0 and C17:0 have emerged as plausible biomarkers for how much dairy fat a person has consumed. As an objective measurement, this is an attractive analytical tool for researchers looking for links between diet and disease in observational studies. Until now, most such studies have relied on the participants' self-reported food intake, which is prone to errors.

Using C15:0 and C17:0 as well as trans-palmitoleic acid (trans-16:1n-7) as biomarkers, two separate research groups recently evaluated the risk of stroke (3) and diabetes (4) in relation to dairy fat intake. From the most comprehensive study of its kind, Yakoob and colleagues reported that none of the three biomarkers were associated with the risk of stroke—neither a higher nor a lower risk (3). Santaren and coworkers showed that increased blood levels of C15:0, however, were associated with a 27% lower risk of type 2 diabetes (4). The study was not able to uncover any biological mechanism responsible for the association, but the authors hypothesized it might be ascribed to a still unknown, direct effect of C15:0, or to other beneficial dairy components.

Controversy and cautions

Reported in the same journal issue, both studies proved controversial among other researchers, who raised their concerns in letters to the editor in a later journal issue. Lankinen and Schwab (5) contended that the scientific literature contains conflicting data on the correlation between C15:0 levels and the intake of dairy fat. They also pointed to research showing a strong correlation between C15:0 levels and fish intake in humans (as also shown in dolphins [1]). "We are a bit

concerned if these odd-chain fatty acids are considered to be a valid biomarker for dairy fat intake in populations who consume considerable amounts of fish,” they wrote, cautioning against misleading conclusions.

In a reply letter, Santaren and colleagues (6) emphasized that the fish intake among their studied cohort was very low and unlikely to contribute substantially to the C15:0 blood levels. They did, however, agree “it is possible that in other populations with higher intakes of fish, C15:0 may be associated with the consumption of foods other than dairy products.”

The presence of C15:0, C17:0 and trans-16:1n-7 in other foods was a sticking point for Ratnayake, another peer who questioned the two studies. “In particular, fat from beef, veal, lamb, and mutton also contains all of these fatty acids at amounts similar to those found in dairy fat,” he wrote in his letter to the editor (7). He cited studies indicating C15:0 and C17:0 are present in many common foods, although this is not widely known. Ratnayake further made the case that a fatty acid should not be used as a biomarker if it is endogenously produced in humans. “It is not known whether animals and humans have the capability to synthesize C15:0 and C17:0, but this should not be ruled out until it has been examined,” he wrote.

Evidence is stacking up

It’s well known that microbes living in the rumen of animals produce odd-chain fatty acids, which then make their way into the ruminant’s tissues, including milk fat. However, the long-held assumption that odd-chain fatty acids in human blood originate solely from ingested dairy fat is gradually eroding. One inconsistency is the ratio between C15:0 and C17:0, which is generally around 1:2 in human plasma and 2:1 in dairy fat. Experimental evidence also suggests that odd-chain fatty acids might be produced in humans through a metabolic process known as α -oxidation. Hence, “there is at the moment no decisive evidence for a direct relation between both C15:0 and C17:0 plasma concentrations reflecting just dietary consumptions,” Jenkins and coauthors wrote in their recent review (2). Even so, they agreed C15:0 and C17:0 can be used as “rough markers for dairy fat intake,” as supported by the majority of existing studies.

So, what to make of all these “ifs and buts”? One thing, at least, is certain: the biology at work here is incredibly complex, and scientists don’t yet understand all the mechanisms involved. As for the links between intake of saturated fats and the risk of cardiometabolic diseases, one opinion leader has urged the research community to start looking at the health effect of “whole foods” (8). Because fatty acids derived from different foods don’t have the same biological effects. Their effect is modified by the food matrix they are delivered in, such as different dairy products. And as the evidence for the health benefits of dairy products is stacking up, “there is no evidence left to support the existing public health advice to limit consumption of dairy to prevent cardiovascular disease and type 2 diabetes,” Astrup wrote in his editorial (8).

Any change of national dietary guidelines, though, should of course be based on a tide of conclusive evidence—and the researchers aren’t quite ready to recommend feasting on full-fat butter. More controlled feeding studies—like the dolphin study (1)—are needed in humans to ascertain the associations found in recent observational studies.

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Producing Human Milk Sugars for Use in Formula

- **Certain types of milk sugars, called oligosaccharides, form the third largest component of human milk.**
- **These human milk oligosaccharides (HMOs) have been shown to positively influence the gut microbiome and immunity.**

- **These sugars are structurally complex and diverse and, as a result, extracting or synthesizing them for use in formula has been challenging.**
- **Researchers are studying different ways to obtain these sugars, including extracting them from cow milk, chemically or enzymatically synthesizing them, or using microbes to produce them.**
- **At the moment, only a handful of simple HMOs have been produced at a large scale, but many others have been synthesized in smaller amounts.**
- **Extraction and synthesis techniques are continuing to improve, but we are still a long way from replicating the full diversity and complexity of sugars in human milk.**

It's well known that human milk is good for you (1-5). Sugars, called oligosaccharides, form the third largest component of human milk and have been associated with many beneficial effects. These human milk oligosaccharides (HMOs) have been shown to influence the composition of the gut microbiome, modulate the immune system, and help protect against pathogens (6-11, 22). HMOs act as prebiotics, promoting the growth of certain beneficial bacteria while suppressing the growth of other disease-causing bacteria (12-18). In addition, some HMOs have been found to mimic the attachment sites of harmful bacteria and thus block their ability to attach to and invade cells in the infant intestine (19, 20). HMOs may also be involved in the development of the infant gut, immune system, and brain (8-11).



Given the various benefits of HMOs, there has been a lot of interest in figuring out how to introduce HMOs into formula. However, more than 200 human milk oligosaccharides have been discovered so far, and their variety and complexity makes them challenging to synthesize (21-23). “Right now there are no formula where human milk oligosaccharides are being added,” says Geert-Jan Boons, Professor of Chemistry at the University of Georgia.

In an effort to deliver some of the benefits of HMOs, current dietary products sometimes include simpler oligosaccharides, often derived from plants (19, 24). Some of these simpler oligosaccharides have been reported to have prebiotic effects, but

they do not have the structural complexity and diversity of HMOs. The effects of HMOs are considered to be highly structure-dependent, so in order to better replicate their function researchers are trying to produce oligosaccharides more similar to those in human milk.

“The bottom-line is that the carbohydrates that are being added right now to formula are not the carbohydrates found in human breast milk,” says Boons.

Extracting HMOs from milk

One way to obtain the same oligosaccharides found in human milk would be to purify them directly from breast milk. About a year ago, a press release by startup Medolac Laboratories announced its ability to commercially purify large amounts of native HMOs from donor human milk (25). But the difficulty of obtaining large amounts of human milk for commercial HMO production means that the majority of efforts have focused on other approaches to obtaining these molecules.

One such approach involves concentrating and extracting HMOs from cow milk. The oligosaccharides in cow milk are structurally similar to those in human milk, but their concentration is much lower (24, 26). Cow milk is already the most common milk used for infant formula in the U.S., so oligosaccharides extracted from it would be expected to be safe for human consumption. Researchers are trying to use filtration techniques to remove most of the lactose and salts from cow milk and increase the concentration of oligosaccharides.

The University of California, Davis milk processing lab is developing methods to extract large quantities of both human and bovine milk oligosaccharides from cow milk, according to Daniela Barile, an Associate Professor of food science and technology at UC Davis. These sugars could be tested in animal studies to determine whether they provide the beneficial effects associated with HMOs. Cow milk, and in particular whey—the liquid part of milk that separates from the curd during cheese production—could thus potentially serve as a way to produce commercial oligosaccharides with similar benefits to those in human milk.

“The technology’s in place, so we should be able nowadays to isolate oligosaccharides from whey,” says Barile. “Whey is a great source, but there are still great challenges if you want to really reach good purity and have a reproducible process batch to batch,” she says.

Individual oligosaccharides from cow’s milk are not exactly identical to those in humans, but an advantage of this technique is its ability to replicate some of the oligosaccharide diversity found in human milk, says Barile. Other methods have so far only been able to produce a handful of these sugars, she says. “If you really want to say that you want to mimic human milk, instead of having just one or two oligosaccharides you want to have the full complement,” says Barile. “The synthesis approach has been making a lot of progress, and they can now make bigger quantities, but it’s not representing the very complex constellation of different structures that is found in human milk,” she says.

“Right now, the isolation process can yield a better diversity than synthesis gets. So you can have more structures, you can have more molecules, so it’s more similar to human milk,” says Barile. “But there are still many challenges,” she says. “There is not a single product in the market right now made of oligosaccharide isolated from whey, so it’s all in the future,” says Barile. “We are at the beginning of the process, there’s still a long way to go.”

Using chemistry

Oligosaccharides can be synthesized through a series of chemical reactions, and that’s another approach that researchers have been pursuing. “The challenge is, we do not have robust technology to make complex carbohydrates at this time,” says Geert-Jan Boons. Unlike the process by which DNA is used to produce RNA and RNA is used to produce proteins, carbohydrates are not biosynthesized through a template-mediated process, Boons says. “If DNA goes to RNA goes to protein, that gives exact copies. When carbohydrates are being biosynthesized, because it’s not a template, you create heterogeneity,” he says.

“There are laboratories that are trying to automate chemical oligosaccharide synthesis in the way a peptide can be synthesized on machines off a standardized protocol,” Boons says. “The protocols are still not very robust, but progress is being made,” he says.

Glycom is one company that is using chemical processes for HMO synthesis, although the company also uses other production methods. However, using chemical synthesis to create commercial quantities of HMOs without making them prohibitively expensive could be a challenge. “The beauty of chemical synthesis is, they can make any HMO,” says Yong-Su Jin, Associate Professor of Food Science and Human Nutrition at the University of Illinois. “However, the cost would be much, much higher,” he says.

Stefan Jennewein, Managing Director and cofounder of one of Glycom’s competitors, Jennewein Biotechnologie, believes there are multiple issues that make chemical synthesis impractical for commercial production of HMOs. “At the time we founded the company, several chemical processes were established relying on chemical synthesis, which however are based on the use of toxic reagents like pyridine and chloroform and other noxious chemicals,” says Jennewein. While these processes might work fine at a small scale with extensive purification, they have high costs and lack scalability, he says. “Many in the industry are convinced that these processes are not compatible with food production,” Jennewein says.

Harnessing microbes

Instead of chemical synthesis, Jennewein Biotechnologie and many other companies and researchers use genetically engineered microbes to produce HMOs. “Microbial production is a very stable and safe method,” says Yong-Su Jin. It’s similar to techniques already used for large-scale production of amino acids and vitamins, he says. “It is a very robust and safe way to mass-produce food quality material,” says Jin.

Jin genetically engineers microbes to introduce the enzymes necessary to produce HMOs. At the moment he is using either the bacteria *Escherichia coli*, which is often used to produce proteins and metabolites, or the yeast *Saccharomyces cerevisiae*, used in baking, winemaking and brewing.

“So far we are using these two microorganisms to produce human milk oligosaccharides,” says Jin. “In particular we are making 2’-Fucosyllactose (2-FL), which is one of the most abundant HMOs in human milk,” he says (27). “We did very subtle chemical analyses, and we are very confident that our 2-FL is the same as 2-FL in human milk,” says Jin.

“That’s one advantage of biological production,” Jin says. “With chemical synthesis, you may have some minor modification somewhere, but enzymatic or microbial production have high fidelity in the reaction,” he says.

Jin says he is able to use genetically engineered *E. coli* to make up to 2-3 gm/L of 2-FL in the medium, similar to its levels in milk. Even though the *E. coli* strain he is using is very different from the ones that cause food disease, Jin says that, due to negative public perceptions about *E. coli*, he is now switching to using yeast. "Because they drink wine and beer or eat bread everyday, people believe that this strain is safer, so we are trying to make 2-FL in yeast right now," he says. He says he is still optimizing 2-FL production in yeast to produce similar levels to that in *E. coli*.

Companies including Glycom, Jennewein Biotechnologie, and Glycosyn LLC are working on producing simple HMOs at a much larger scale for commercial use. "Several companies are currently developing formula containing 2'-fucosyllactose, but also other HMOs will soon enter the stage," says Stefan Jennewein. Jennewein Biotechnologie produces 2-FL at a commercial scale using genetically engineered *E. coli*, and has been seeking market approval for their products. "We were the very first who filed for a Novel Food application in the EU for a food ingredient originating from a recombinant bacterial process," says Stefan Jennewein. "In 2014 we obtained GRAS (Generally Recognized as Safe) status for Infant and Toddler Nutrition and General Nutrition in the US," he says. The company is filing for registration in other major markets, and has been building production capacity for large-scale production of HMOs. "We completed the world's first commercial multi-ton facility for HMOs in 2014, which is fully certified for food production," says Jennewein.

Although microbial production can produce HMOs at a large scale, it has so far only been used to make a few of the simpler HMOs. Researchers are still trying to figure out how to expand the repertoire of HMOs that can be produced using microbes. "The good news is, although we have these 180 or so HMOs, they are not random chemical structures," says Jin. "If we look at the basal structures, only 2-3 different sugars are connected with some rule," he says. "So it doesn't mean that we need to construct 180 strains with different biochemical pathways. Maybe if we make only 6 or 7 pathways, by mixing and matching combinations we will be able to create 180 different HMOs."

"It's like Lego blocks," says Jin. "If you have 3 Lego blocks, then you can create 20 or 50 different shapes," he says. "So in the future, we should be able to make any desired HMO by microbial production," says Jin. "But I think it's still very far off," he says.

An enzymatic approach

To create some of the more complex oligosaccharides, Geert-Jan Boons and others have been focusing on enzymatic methods. "We have also very complex oligosaccharides in milk, and it is our belief that they are actually the compounds that perform very specific biological functions," says Boons. "Those are not easily accessible right now," he says.

Boons says his research group has been able to express almost every mammalian enzyme involved in modifying complex sugars, and he is working on using these enzymes to produce almost every human milk oligosaccharide. "The caveat is, we can produce only small amounts," he says. Although the technology may not be able to produce commercial levels of HMOs, Boons says it will be very helpful for research purposes, to find out which HMOs are beneficial and what their functions are.

"I think that discovery, what these molecules actually do and which ones are the interesting ones, that will be done through chemical and enzymatic synthesis," says Boons. "So, we will go through a discovery phase, find out how these molecules actually perform their beneficial properties, and which are really the active components, and create a mixture that can make a big difference for humans," he says.

"Large scale production will be done through biotechnology, with cells that are engineered to produce these oligosaccharides," says Boons. "I think what will be done in the next couple of years is, the relatively simple oligosaccharides, which can now be produced at a relatively large scale, they will move into the clinic," he says. "Basic scientists like me, we will develop protocols to make the more complex ones, and they will be examined in cell culture and animal models, and when we begin to understand how they work, they will move into the clinic," says Boons. "So, a lot of exciting things are happening," he says.

However, it's going to take a while before scientists or companies are able to produce formula that contains all the oligosaccharides found in human milk. "We are still a long way from making an artificial human milk oligosaccharide composition," Boons says. "What we can do is begin to supplement cow milk with the main simple oligosaccharides found in human milk," he says. "To make the whole structural diversity found in human milk, that is still quite far away," says Boons.

Yong-Su Jin is confident that a combination of academia and industry will figure out ways to produce HMOs in the same manner they were able to achieve the production of many other oligosaccharides over the last five or 10 years. "The last 2-3 years have been very exciting," he says. "Before that, although we had publications about the beneficial effects of

HMOs, I didn't see that much commercial activity, but now I see more and more infant formula companies interested in adding HMO into their product," Jin says. "So, it's a very exciting time," he says. "I'm very optimistic, but we are still at a very early stage."

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Accounting for Lactase Mutants

- **About two-thirds of adult humans around the world are lactose intolerant.**
- **The ability to digest lactose after weaning occurs in many northern Europeans, but also in many populations in the Middle East and in Africa.**
- **Recently, researchers have started to use new data about the genes behind the ability to digest lactose to uncover new details about human migratory history.**

Back in the 50s and 60s, work on lactose intolerance was often published under cringeworthy and blunt racial titles. A Nature article from 1969 sums it up with ‘Can Asians Digest Milk?’ It was also probably a subliminal non-accident that ‘lactose intolerance’—which is the typical condition for adult humans—became common parlance for a trait for which those with northern European ancestry are the real mutants. Many decades on, the genetic basis of the ability to digest lactose has been largely pinned down. As it turns out, there are different genetic reasons for the mutants’ lactose tolerance in the various populations that drink milk without intestinal incident, and the gene that confers mutant power in Europeans is only part of the story. That research history is discussed below, along with recent work that has extended the field’s reach beyond genetics. Investigations of the transcontinental basis of lactose tolerance are now providing insights into mankind’s cultural, as well as biological evolution.

Lactose digestion mutants fail to ‘turn off’ a gene that lies on chromosome 2. Early in life, human beings need to digest the lactose in their mother’s milk. The enzyme required to break it down is called lactase (full name, lactase-phlorizin hydrolase), which is produced in cells that line the small intestine. Lactase extracts the simpler sugars, glucose and galactose, from a milky meal by breaking lactose in half, ready for absorption into the blood. Without it, lactose continues on towards the large intestine, where bacteria feast on it—and it is these bacteria’s waste products that deliver the symptoms of lactose intolerance.



While they were naïve in their categorizations, the correlations of the early studies threw up strong patterns; genetic ancestry does matter for lactose tolerance. The blunt answer to the question set out in that Nature article was “No, Asians Cannot Digest Milk.” A researcher called Welsh measured lactose intolerance among Native Americans [1]. Then, he set out to compare its frequency among, as he describes, American blacks, Africans, Asians, Greek Cypriots, Australian Aborigines and South American Indians [2].

All the while, explanations for lactose intolerance that focused on nurture—in this case, food preferences that aligned with racial categories—also seemed to be plausible reasons for lactose intolerance. After all, the lac operon model of gene regulation, proposed in 1961, inferred a use-it-or-lose-it logic to the functioning

of the lactase gene. The way to convince the field of the pre-eminence of inherited genetic differences was to find groups of people with differing racial profiles, and tell them what to eat. A study on incarcerated Americans—20 white, 20 black—demonstrated the link between intestinal lactase activity and milk intolerance [3]. Then, given that result, the way to figure out whether the gene responsible is dominant or recessive, was to test the lactose tolerance of a group of people with one lactose-tolerant parent and one lactose-intolerant parent; studies of mixed race people in the early 1970s [4] suggested that tolerance—the persistence into adulthood of the ability to digest lactose—is a dominant Mendelian trait.

Shoot forward to the era of the Human Genome Project, and all the new-fangled genome-scouring technologies that developed as a consequence. In 2002, researchers in Finland found a mutation in a region of the genome upstream of the lactase gene that was common to all lactose-digesting Finns in their study (almost all Finns can digest lactose) [5]. Termed ‘T-13910’, this single nucleotide mutation sitting in an intron (a non-protein coding section of the genome – specifically in this case, a transcription factor binding site), appeared to explain why some people, including the vast majority of northern Europeans, are able to digest milk as adults.

It seemed like an elegantly neat and simple answer. The problem was that it only worked for Europeans. Lactose-tolerant people whose ancestors hail from the Middle East and Africa, rarely share this mutation. Enter Sarah Tishkoff, a geneticist who studies ethnically diverse African populations and an expert in human evolution.

In 2006, Tishkoff and her team published a paper detailing three mutations that appeared to be the reason why their study participants from Kenya, Tanzania and Sudan happily consume milk [6]. To hammer home the point, they also found no link between T-13910 and the lactose digestion abilities of pastoralists in Sudan. The obvious challenge was then to make sense of how the known lactose tolerance mutations spread through human migrations over millennia.

Recently, the team sequenced the intron in which T-13910 lies, and another intron nearby, in over 800 people—from 63 African populations and 9 non-African populations (from Europe, Asia and the Middle East) [7]. Aside from adding to the list of mutations associated with lactose tolerance, these data enabled the researchers to date each of its various genetic causes and to map their population frequency (see Figure <http://tinyurl.com/q4nvzlm>).

Putting the data together with what is known about human migrations over the relevant period, led to a number of insights into human evolution. Among them, the team found that the !Xhosa, who live mainly in southeast South Africa, share the same lactose persistence mutation as Kenyan and Tanzanian populations. This, in turn, suggests that the gene somehow flowed south from eastern Africa prior to the Bantu expansion, which began about 3,000 years ago, when the Bantu family of languages is thought to have spread.

There were additional surprises. A few African populations—the Mozabite from Algeria, the Fulani from Cameroon, and the Bulala from Chad—for example, did indeed have the T-13910 mutation, suggesting it was introduced from outside, perhaps during key historical moments, such as the spread of the Roman Empire into North Africa. Another mutation that confers lactose tolerance was found in 47% of volunteers belonging to the Hazda, a Tanzanian hunter-gather group—even though they have no known history of dairy production. Being able to digest milk seems to be a side effect in this case. The lactase enzyme performs a second, different enzymatic job in breaking down phlorizin, a bitter plant product that occurs in bark and the stems of fruit trees of the Rosaceae family, and a known traditional remedy for treating malaria. It was likely this lesser-known job of lactase that promoted the spread of lactose tolerance among the Hazda.

By evolutionary genetics standards, the evolution of lactase tolerance is a high-speed thriller. Few other genes are known to have experienced such strong positive selection in human history. But there are still many unanswered questions. Tishkoff, for one, wants to integrate the genetic data with microbiome studies, to try to get a better handle on human history.

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