



SPLASH!® milk science update

December 2019 Issue



This month's issue features the IMGC Symposium, dairy and diabetes, and human milk fatty acids.

Highlights from the 2019 IMGC Symposium

The 2019 International Symposium on Milk Genomics and Human Health, the sixteenth in this series, was held in Aarhus, Denmark, home to Aarhus University and Arla Foods. The local organizing committee designed a diverse and engaging program and provided a warm welcome during a cool Danish November. There was a total of 28 speakers over three days of thought-provoking science, and as always with these meetings, there was a great blend of dairy food science, nutrition, animal science and, this year, the hot topic of sustainability.

Sustainability: meeting the demands of the future



Photo: Lars Kruse, AU Foto.

The first day opened with the traditional welcome and inspiration provided by Bruce German (UC Davis) who introduced the first session focused on sustainability. Troels Kristensen (Aarhus University) provided a systematic analysis of the environmental challenges of doubling global production to a half liter of milk per day per person for a future world population growing beyond 10 billion and in need of sustainable food security. He pointed out that to do so and simultaneously meet the challenge of reducing greenhouse gas emissions would require a concerted effort with a combination of new technologies, alternate cow management strategies and diversification of land use. Brad Riddout of CSIRO Agriculture and Food in Australia followed with his recent evaluation of an Australian population dietary survey in the context of environmental footprint. He found

that when the study population was divided into four groups based on both diet quality and emissions, those people who fell into the high-quality, low-emissions group were distinguished by the exclusion of discretionary foods and the inclusion of dairy consumption. The next speaker, Monika Zurek (Oxford University), presented an overview of the European collaborative SUSFANS project. Using a system of metrics and modelling, the program examined current and projected food systems to identify how Europe might achieve its goals of sustainable food production, and meet targets of reduced emissions, economic growth and improved health. An example was discussed using 16 metrics for performance in four categories: diet, equity, environment and EU competitiveness. The sustainability theme was wrapped up with a talk from Nina Aagaard Poulsen (Aarhus University) on how sustainable production practices can be employed in producing healthy dairy products. She presented data from a series of studies aimed at reducing the emissions footprint of dairy cows while maintaining milk quality. These studies demonstrate that strategies to alter on-farm emissions can be tolerated without compromising milk quality.

When milk and good bugs get together

The session on the microbiome and metagenome touched on many aspects of milk properties, milk production and human health. A keynote address by Christopher Stewart from Newcastle-upon-Tyne described studies from the TEDDY cohort, a long-term study of development from early childhood. Detailed analysis of gut microbiota characterized three phases: establishment, transition and stable presence of microflora. Breastfeeding was the dominant factor affecting the initial phase of establishing a healthy gut microbiome. Microbiome modelling of 1,000 pre-term infants showed a pattern of instability associated with necrotizing enteric colitis, a key cause of mortality in these babies.

Gut microbiota is also important in dairy cows. Rumen microflora has been studied for many years, but

intestinal microflora is not as well studied. This may be of particular relevance to immune development in ruminants, which rely on immunological tissue within the gut for developing antibody producing B-cells. Mikael Niku from Helsinki University discussed his studies of newborn calf microbiota. The talk from Guy Vergeres of Agroscope in Switzerland switched the focus to the metabolome of human subjects consuming dairy products, especially comparing the effects of fermented dairy as yogurt. In general, the studies involved a cross-over design and compared milk consumption to yogurt, and in some studies non-dairy plant-based drinks or foods were included. Whatever foods were ingested, serum or urine metabolite profiles generally reflected the differences.

Hot topics in milk science

Each year participants vote on the most valuable presentation (MVP) at the symposium. At this meeting, the 2018 MVP, Dr. Ishita Shah (UC Davis), presented an update on her work with human milk oligosaccharides. Gut health is affected by many factors that influence the capacity of the cells lining the intestines to provide a barrier to pathogens. This talk revealed how human milk oligosaccharides enhance barrier function and protect against pathogens.

Day one ended with the yearly round-up from Danielle Lemay of highlights from *SPLASH! milk science update*. The most viewed article of the year, with 90,000 hits, was an article on [breast milk stem cells found in infant tissues](#). Dr. Lemay foreshadowed the hot topics for the future, including artificial intelligence for the dairy industry in the context of data-driven decision making.

Structure and function of milk

Day two began with presentations on milk composition and functionality. Debashree Roy from the Riddet Institute in New Zealand presented studies on the comparative digestive properties of milk from different species, including cow, buffalo, sheep, goat and deer. There has been growing interest in alternative dairy products from these species. Some of the main differences in the properties of these milks include fat and protein composition, and differences in micelle and fat globule size. These influence the hardness and formation of a casein-derived curd in the stomach with varying digestibility and physical properties. Jarred Raynes (CSIRO) presented studies on the structure of micelle formation and focused on the effects of genetic variants in caseins. Variation in genes coding for milk proteins not only affects amino acid sequence but also affects post-translational modification, particularly glycosylation and phosphorylation, which can impact physical properties that are important to micelle formation and coagulation properties of the milk. Bernt Guldbbrandsten (Aarhus University) continued the theme of genetic variation of milk proteins but with a very different approach. He analyzed variants that were present in the amino acid coding sequence of data from the 1,000 bulls project. A large percentage of more than 3,000 variants identified were attributed to just five proteins. Variation in milk proteins can affect milk coagulation properties and therefore cheese production. Marie-Pierre Sanchez (INRA) presented a large-scale genetic analysis of milk composition with a focus on cheese-making properties. Over 100 functional gene categories were identified with strong links to factors that affect milk composition.

Physical properties and functionality of milk were highlighted in the keynote address by Thom Huppertz (Friesland Campina/Wageningen University). He focused attention on milk minerals and their relevance to micelle formation, and hence milk functional properties. Lotte Bach Larsen then presented an overview of milk proteases. Cathepsins are a particularly important class of proteases that affect digestion of milk proteins and properties of milk, and are also found in cheese. Next, Carolyn Slupsky (UC Davis) presented work on the effects of including milk fat globular membrane (MFGM) preparations in infant formula. MFGM have the capacity to enhance immunological properties of formula, and thereby affect the health and wellbeing of children. Dr. Slupsky was also interested to study whether the inclusion of MFGM affects brain development and cognition. With Swedish collaborators, she found that inclusion of MFGM increased brain development and cognition measurably by 12 months of age. Jan Trig Rasmussen (Aarhus University) presented details on a range of studies dealing with milk exosomes. Exosomes are extracellular vesicles that contain macromolecules, including proteins, DNA and RNA. There has been a particular interest in the RNA content, of which half are microRNA (miRNA). About 2,500 miRNAs were identified in this study, with 57% being similar in bovine and human milk.

Rounding out the session on inherent milk characteristics and functionalities, Daniela Jakubowicz (Tel Aviv Univ) presented the results of a study on the effects of whey protein feeding on post-prandial glycaemia. And Soren Drud-Heydary presented some early studies on characterizing glycosidases in milk. Despite the importance of oligosaccharides in milk, these related enzymes have not been well characterized.

The food matrix: a complex assembly of compounds

Day three began with a keynote address from Michelle McKinley (Queens University, Belfast) and her thorough review of the development and current state of research on the effects of food matrices on bioavailability of nutrients. With a focus on dairy foods, it was established that milk calcium availability is far greater than that of equivalent amounts of calcium in many foods. This is interpreted as an effect of the food matrix. Emma Feeney (UC Dublin) also presented studies on the food matrix effect in the context of the Irish diet and the Food for Health program in Ireland. Population level studies showed a reduce risk of cardiometabolic diseases associated with dairy intake. Ishita Shah (on behalf of Gulustan Oztruk) presented a study on the presence of xanthine oxidase in milk processing streams. The discussion panellists offered different perspectives on why the food matrix matters and the difficulty of conveying information about food structure to consumers.

Rising stars

The work of students is always a highlight of the IMGC symposium. This year there were many poster presentations that initiate a rigorous discussion facilitated by the delights of food and wine during the social mixer on the first evening. The tradition of highlighting students and their research efforts also continued, with five abstracts selected for travel awards and oral presentations in the main program.

Student award winners included Kajsa Nilson (Lund University), Vanessa Dunne-Castagna (University of California-Davis), Anne Vuhlom Sunds (Aarhus University), Yarden Golan (Technion Institute), and Pieter Dekker (Wageningen University). Kajsa Nilson examined the genetic variation contributing to poor coagulation properties in Swedish Red cattle milk. The study found significant effects of variation in kappa-casein and beta-casein variants. Vanessa Dunne-Castagna examined the effects of complexing human milk IgA with *Bifidobacterium longum* subsp *infantis* on invasion of pathogenic bacteria in gut epithelial cells. Anne Vuhlom Sunds studied BMO variation in eight Nordic cattle breeds, finding considerable variation in types and amounts. Yarden Golan found that transient zinc deficiency in breastfeeding infants results from a genetic disorder in mothers that is more common than previously believed. Pieter Dekker found that mothers with allergies have more non-human proteinaceous material in human milk.

This year's IMGC symposium was, once again, an invigorating and fascinating meeting.

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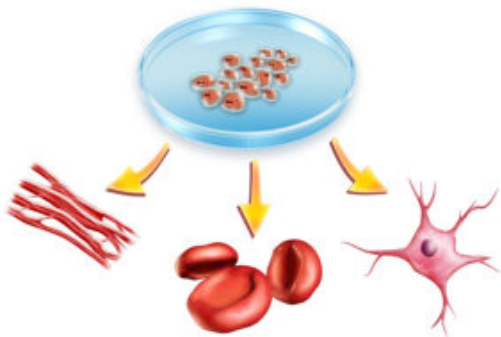
Stem Cells from Teeth Make Mammary Tissue

- **Mouse incisor teeth contain stem cells that produce the epithelial cells required for the growth of teeth.**
- **Dental stem cells transplanted into the mouse mammary fat pad generate epithelial cells that produce milk and structures like mammary gland ducts.**
- **Adult dental stem cells could be used in the future to regenerate diseased tissue, particularly mammary tissue.**

Sometimes science stuns. It unnervingly reminds us of how little we know but also how much it could change the future, and for the better. A recent publication [1] described how investigators isolated stem cells from adult mouse teeth and then transplanted these cells into mouse mammary fat tissue devoid of the highly specialized mammary epithelial cells that produce milk during late pregnancy and after birth. The stunning result was that mammary tissue was regenerated from the dental stem cells. Amazingly, the

new mammary tissue contained cells that produced milk proteins during pregnancy and formed structures somewhat like mammary tissue ducts. The certainty of established scientific ideas about cell fate is now much more fluid.

The Grand Enigma: What Is a Stem Cell?



Scientists report that stem cells, ideally, have the unique ability to generate all the different types of highly specialized cells that characterize each organ, like muscle cells, nerve cells, connective tissue cells (e.g., skin, tendons, and bronchial tubes in lungs), and epithelial cells (e.g., the surface of the gastrointestinal tract, mammary tissue, and dental pulp) [2]. No other cell type in the body has this enigmatic capability. Stem cells do not have any tissue-specific structures that perform specialized functions, i.e., they are unspecialized cells. This disguise hides their remarkable abilities. Under the right conditions, stem cells can also replicate by cell division and renew their population [2]. Highly specialized cells cannot replicate, and once these cells are lost or damaged, they need to

be replaced. That's when stem cells come to the fore. Stem cells reside in most adult tissues but only in small numbers, and they are usually in a dormant state. When stem cells receive specific molecular and cellular signals from their surrounding tissue, they awaken and generate the highly specialized cells required by the tissue.

The biological functions of stem cells are to maintain the cellular composition of a tissue and regenerate a tissue when it is damaged or diseased, or when the tissue loses cells through normal tissue activities [2]. In the latter case, some highly regenerative tissues, like the gastrointestinal tract and mammary tissue during the lactation cycle, have large requirements for new highly specialized cells. Hence, they strongly rely on resident stem cells for tissue regeneration. When scientists artificially grow stem cells in the laboratory, the cells become immortal, unlike every day specialized cells whose demise is as certain as the tide and taxes after a fixed number of cell divisions. Thus, scientists think that stem cells may also teach them a lot about the mechanism of aging and also the birth of cancer cells [3].

Embryonic stem cells are the most versatile stem cells as they can form all the major cell types [2]. Adult stem cells, in contrast, are much less versatile than embryonic stem cells. Adult stem cells are often restricted to only generating the specific specialized cells that characterize the organ in which the adult stem cells were originally found. For them, the acorn doesn't fall far from the tree. Thus, scientists conclude that adult stem cells may be less useful than embryonic stem cells for regenerating diseased tissue [2, 4]. Scientists have also taken a handful of genes and artificially activated them in highly specialized cells, like skin cells [2]. They explain that this procedure "*reprograms*" the specialized cells and makes them become unspecialized, and similar to embryonic stem cells, with the ability to generate a wide range of specialized cell types [2]. This idea was scientific heresy not so long ago, but science evolves, and this remarkable achievement of two pioneer scientists was awarded the Nobel Prize for Physiology or Medicine in 2012.

Dental Stem Cells Generate Mammary Tissue

Some highly specialized tissues, like teeth and mammary tissue, are considered by scientists as "*appendages*" with similar origins from the outer layer of cells in the very early embryo [1]. Thus, despite the enormous structural, functional, and anatomical differences of these mature tissues, they have a lot in common during their very early stages of development [1, 5, 6]. The similar early life developmental process involves embryonic stem cells; however, in later life, specific adult stem cells generate the highly specialized epithelial cells that characterize each tissue. The continually growing mouse incisor teeth contain adult stem cells that generate all the specialized cells required to support the growth of teeth [2]. However, scientists had not realized the potential of adult dental stem cells to regenerate unrelated tissues.

For the first time, investigators recently demonstrated that adult dental stem cells can generate a non-dental tissue, mammary tissue, and thereby they highlight an unexpected versatility of dental stem cells [1]. The investigators reported their research in the scientific journal *Cells*. The team of four investigators was based at the University of Zurich in Switzerland and the Iwate Medical University in Japan.

“Make the Green One Red” (W. Shakespeare)

Initially, the investigators isolated adult stem cells from mouse incisor teeth. This was not an easy task. These cells had identifying protein markers on their cell surface that characterized them as stem cells, but they also had markers of epithelial cell fate. The implication was that these adult stem cells were restricted to generating only dental epithelial cells. The dental stem cells were mixed with mouse mammary epithelial cells and then injected into mammary fat pads after the normally resident mammary epithelial cells in the tissue were completely removed.

The investigators highlighted their research finesse by using a sophisticated experimental technique that ensured any epithelial cells generated from the transplanted dental stem cells were colored fluorescent green, while the added mammary epithelial cells were colored fluorescent red. The investigators then used a special microscope to separately visualize new mammary epithelial cells derived from the transplanted dental stem cells and the added mammary epithelial cells at the transplantation site eight weeks later. About a third of the total number of mammary epithelial cells at the site was green and therefore derived from the transplanted dental stem cells. Some of the female mice with transplanted stem cells were then mated, and during the pregnancies, their green mammary epithelial cells at the transplantation site were shown by the investigators to produce milk proteins. Thus, the transplanted adult dental stem cells had potentially regenerated milk-producing mammary tissue.

The investigators then performed a similar experiment, but this time they did not transplant any mouse mammary epithelial cells with the dental stem cells. The green epithelial cells that were generated from the dental stem cells at the transplantation site now formed small duct-like structures and sometimes cysts. The newly formed ducts suggested a similarity with the ducts that normally collect milk in mammary tissue. The investigators concluded that the added mammary epithelial cells had somehow taught the dental stem cells to generate mammary epithelial cells that could produce milk at the transplantation site. The inference is that the dental stem cells had a very robust but private conversation with cells and molecules in the local environment of the transplantation site. A cell is changed by the company it keeps.

Stem Cell Therapies

Stem cells have the potential to regenerate diseased or injured human tissues [2]. It's a big promise for the future and with immense consequences. The most acclaimed example of the success of stem cell therapy is the medical treatment of blood cell disorders, typically cancer in children [2, 7]. Fifty years ago, the prospects for these children were dire. This outcome radically changed for the better when radiation or chemotherapy was used to kill the diseased blood cells and then bone marrow, containing adult stem cells, was transplanted into these individuals, which then regenerated normal blood cells [2, 7]. There are many other outstanding successes [2] but also challenges [2, 4, 8]. In many cases, it is still early days. Commercial stem cell therapies now abound for all manner of minor medical and cosmetic applications, however, the US Food and Drug Administration warns that some are unproven and unapproved [9]. *“A soothsayer tells you to beware”* (W. Shakespeare).

Implications

The investigators showed that adult dental stem cells transplanted into mammary fat pads regenerated new mammary tissue that produced milk proteins. This result demonstrated that adult stem cells can have more versatile cell fates than previously thought by scientists. The clinical implication from the investigator's research is that adult stem cells from teeth could, in the future, be used to regenerate some diseased or injured tissues containing epithelial cells, especially mammary tissue. Perhaps in the future, everyone will have a personalized bank of stem cells stored in the freezer. They could become very handy. *“All remedies oft in ourselves do lie”* (W. Shakespeare).

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Three Investigations Find Consuming Dairy Staves off Death or Cuts Diabetes Risk

- A study of Italian diets has found that a milky coffee per day reduces the risk of death.
- Another analysis of three large cohorts in the United States finds that replacing calories from dairy fat with calories from meat fat or refined sugars raises the risk of getting type 2 diabetes.
- Data from the same three large cohorts shows that switching from high-fat dairy products to low-fat dairy, such as low-fat milk and yogurt, cuts the odds of developing type 2 diabetes.

Diabetes is a major cause of death and morbidity around the world. The International Diabetes Federation estimates that about 9% of the global adult population has the type 2 form of the disease. Understanding dietary contributions to risk is therefore hugely important for global public health. Although genetic risk factors for type 2 diabetes do exist, the sheer rapidity of the rise in disease incidence over recent decades suggests that genetics is a minor part of the story. In a recent issue of the *American Journal of Clinical Nutrition*, three papers contribute further knowledge to the field [1]. They all describe prospective studies that followed one or several large cohorts of adults and noted how much dairy they consumed. Overall, these studies confirm that consuming dairy does not raise diabetes risk, nor the risk of other cardiovascular diseases, and if anything, that it may be protective.



The first paper looks at mortality. Valeria Pala and her colleagues from across various institutions in Italy describe how they analyzed dietary and health data from 45,000 Italians living in the cities of Varese and Turin in the north, from Florence in the center of the country, and Naples and Ragusa in the south [2]. These people enrolled in the mid-1990s as participants in a Europe-wide project known as EPIC, for European Prospective Investigation into Cancer and Nutrition. Almost 15 years later, more than 2,400 of the participants had died, mostly from cancer, but also—in 19% of cases—from cardiovascular disease. Statistically removing the contributions of other risks of death, such as age, sex (because in the West men die younger than women), smoking and body mass index, Pala and her team calculated how the diets of those who died during the 15-year period differed from those who did not—and specifically, whether their dairy consumption was unusual in any way.

The results are intriguing because they report that incorporating dairy in the diet reduces the likelihood of death, yet they also suggest that daily doses of milk corresponding to a typical Italian breakfast is enough to reap this benefit. According to the Italian EPIC data, consuming an amount of milk in the range of a

macchiato (50 g of milk per day), to a cup of cappuccino (160 g/day), to a latte (160–200 g/day), is associated with a lower risk of mortality compared with no dairy. But beyond this amount, the protective effect of milk does not increase any more. The authors suggest that this may be because milk has components like calcium, vitamin D, and conjugated linoleic acid, which protect against cancer or cardiovascular disease, as well as components like saturated fat that raise the risk of major causes of mortality. The health-promoting components of milk may be available in sufficient quantity to confer their effects in a single cup of milky coffee per day, and consuming more of them does not do much to reduce the risk of death further. Meanwhile, the saturated fat may contribute to the risk of mortality, and the rate of increase to the health risk does not abate with the amount swallowed.

The other two papers bring together three cohort studies in the United States. They both focus on how dairy affects the risk of developing type 2 diabetes, and they consider making changes to the diet.

Andres Ardisson Korat and his team of Boston-based medical and statistical experts considered different sources of energy in the diet [3]. They wanted to know whether replacing energy consumed in the form of dairy fat with the same number of calories from meat fat, refined carbohydrates, and whole-grain carbohydrates confers different probabilities of developing type 2 diabetes. To find out, they brought together over four million person-years of data from three large, long-running, prospective studies of medical professionals. These studies were similar in the sense that they all collected lifestyle information and medical history via detailed questionnaires at their outsets, which were updated every two years. Presumably because the participants were medical professionals, the dropout rate across the three studies was exceptionally low—it was less than 10%.

By studying the different diets of medical professionals, the researchers showed that getting one's energy from dairy fat is much healthier than getting it from meat fat or from refined carbohydrates. Indeed, replacing just 5% of calories from dairy fat with the same number of calories from meat fat conferred a 17% higher risk of developing type 2 diabetes. Replacing those dairy fat calories with the same number calories from refined carbohydrates conferred a 4% higher risk. However, eating roughage is good for you: whole-grains beat dairy. Swapping 5% of calories from dairy fat with energy from unrefined carbohydrates reduced the odds of getting diabetes; this was associated with a 7% lower risk. Other research has shown that eating whole grains improves glucose tolerance and insulin sensitivity [4].

The third paper used data from the same three large cohorts as the second, and some of the authors were involved in both papers. But instead of analyzing different kinds of energy sources in the diet, Jean-Philippe Drouin-Chartier and his colleagues looked at the ways in which the medical professionals enrolled in those cohort studies had actually changed their diets over time [5]. Their results propose some clear public health messages. For example, they found that people who had increased their yogurt consumption over the years lowered their diabetes risk, and that substituting cheese with yogurt or reduced-fat milk led to a lesser chance of developing the disease. However, they also found a few quirks in the data. For some reason—which may not stand up to the scrutiny of further research—ice cream got a good report. Decreasing ice-cream consumption appeared to increase the risk of diabetes. However, there is a chance that this pattern might be the result of reverse causation—of mainly people at higher risk of diabetes deciding that they should really not be eating so much ice cream, and so cutting back.

These three papers are far from the final word on the link between dairy consumption and the risk of diabetes and mortality. But they do bring together vast amounts of data, and perhaps the best sources of data that are currently available. The large cohort studies evaluated all relied on people very occasionally filling in questionnaires about their dietary habits, as best they could recall them. One can only imagine how, in the future, as information from mobile apps that collect dietary intake data, and more detailed genetic information about risk profiles are brought together, medicine's grasp on the link between dairy and diabetes risk will improve by leaps and bounds.

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Genes, Diet, Environment: A Host of Factors Influence Human Milk Fatty Acids

- A Canadian study of more than 1,000 mother-infant dyads examined milk fatty acid composition alongside data on diet, genetics, maternal education, and maternal weight.
- No two human milk fatty acid profiles are identical, but the study identified four broad milk fatty acid patterns.
- Diet, genetics, body mass index, and other socio-demographic factors only explained 25% of the variation in long-chain polyunsaturated fatty acids and only 10% of the variation in saturated fats across milks from the study population.
- Human milk fatty acids are the result of complex interactions between genetic and non-genetic factors, only a fraction of which has been identified.

Fatty acids are the most variable macronutrient in human milk. So variable, in fact, that researchers believe each mother produces her own unique milk fatty acid signature [1]. Unfortunately, not all fatty acid signatures are optimal for infant growth and development. Decades of research have demonstrated that docosahexaenoic acid (DHA), an omega-3 long chain polyunsaturated fatty acid (LCPUFA), is necessary to optimize the growth and development of infant neural functions [2]. DHA also happens to be one of the most variable fatty acids in human milk, which means many mothers produce milk with concentrations that might not meet infant developmental requirements.



The reason for all of this variability has to do with the source of milk fatty acids. The mammary gland can only manufacture fatty acids with 16 carbons or less (short- and medium-chain fatty acids). However, which fatty acids the mammary gland synthesizes *de novo* (and how much of these fatty acids it makes) is influenced by maternal diet [3]. Longer chain fatty acids (meaning longer than 16 carbons), including DHA and other polyunsaturated fatty acids, are pulled from circulating levels in the maternal bloodstream and maternal fat stores, which means they are also influenced by the mother's diet (both present and past) [1, 2]. To make things even more complicated, there are several known genetic variants that influence the concentration of milk LCPUFA, such as the *FADS1* and *FADS2* genes that code for enzymes responsible for polyunsaturated fatty acid metabolism [4].

A new study [1] from a team of Canadian researchers investigated *FADS* genes, maternal diet, and a multitude of other non-genetic factors with the goal of identifying the independent and combined impact of each factor on particular milk fatty acids. They argue that the only way to understand variability in milk fatty acids is to study the potential effects holistically and simultaneously, and included in their analysis many factors that have not received the same attention as maternal diet, such as whether the mother suffered from chronic health conditions (e.g., asthma, inflammatory bowel disease), maternal age and ethnicity, season of milk sampling, and whether the infant was exclusively breastfed [1].

The study population of 1,094 mother-infant dyads comes from four areas throughout Canada (Edmonton, Manitoba, Toronto, and Vancouver). Each mother provided one milk sample during a home visit between

the third and fourth month postpartum. Data on non-genetic factors was obtained either directly from medical records (such as mode of delivery) or from questionnaires administered to the mothers. Diet was estimated during pregnancy using a commonly used form called a Food Frequency Questionnaire (FFQ), with particular interest in any and all foods that contain LCPUFA (fatty cold-water fish, white fish, shellfish, eggs, nuts, fish oil supplements, and multivitamins). Blood samples taken during pregnancy were used to determine two genetic variants (called single nucleotide polymorphisms or SNPs) of interest in *FADS1* and *FADS2* genes.

The team identified four broad milk fatty acid composition patterns: (1) mono-unsaturated fatty acids and low saturated fatty acids, (2) high omega-6 PUFA, (3) high omega-3 PUFA, and (4) high medium-chain fatty acids (defined in this study as <14 carbons) [1]. Neither of the genetic variants examined was associated with milk DHA concentration but were associated with lower milk ARA and had higher scores on the medium-fatty acid chain pattern. Both fish oil supplementation and fish consumption were positively associated with the high omega-3 PUFA pattern. Fish oil supplementation either during pregnancy, during lactation, or both was associated with high omega-3 and DHA milk proportions. Moreover, the frequency of fatty cold-water fish consumption increased milk DHA concentration in a dose-dependent manner. Somewhat paradoxically, diets that received a higher Healthy Eating Index (HEI score)—a measure of how well the diet matches up with dietary recommendations from the Dietary Guidelines for America—was associated with higher total milk omega-6 fatty acids, including linoleic acid and arachidonic acid but not higher milk omega-3 or DHA [1]. One might assume that mother's with a higher dietary quality would produce milks with optimal milk DHA, and yet the study found that the majority (69%) of mothers in the study population produced milk with DHA proportions below the minimum proportion required in human milk substitutes (0.2% DHA) [1].

Perhaps the most interesting finding of the entire study was that diet, genetics, body mass index, and all other socio-demographic factors combined explained only 25% of the variation in LCPUFA concentrations and only 10% of the variation in saturated fats across milks from the study population. Diet and maternal BMI mainly contributed to the observed variation in saturated fatty acids, diet and socio-demographic factors (such as post-secondary education) contributed to variation in omega-3 PUFA, and *FADS* genetic variants contributed to variation in omega-6 PUFA [1].

Considering the sources of milk LCPUFA, it seems surprising that diet only accounts for 25% of the total variation in their concentration. The study did not identify any effect of infant gestational age, infant sex, maternal age, or smoking—what other factors might explain the remaining 75% of variation in LCPUFA variation? "I think the other 75% variation has a lot to do with the elements of diet and genetics that we did not capture in our study," explains corresponding author Dr. Meghan B. Azad, Assistant Professor in the Department of Pediatrics and Child Health, University of Manitoba and Research Scientist at Children's Hospital Research Institute of Manitoba. "If we had more precise data on dietary intake, fats especially, I am sure we would see even stronger associations and explain more of the variation." Dr. Azad also believes genetics are likely to play a larger role than the current study was able to detect. "We just looked at 2 SNPs in the *FADS* gene cluster. There are almost certainly other SNPs in that gene cluster that are important, and likely there are other relevant genes that also contribute to milk fatty acid levels that have not been identified."

The complex interaction between genes and diet (or genes and maternal body weight, or other non-genetic factors) suggests that simply telling mothers "eat more DHA-rich foods" might not be sufficient public health advice to increase milk DHA (How much? During pregnancy, lactation, or both? Are supplements just as good as DHA-rich foods?). But the very low levels of milk DHA identified in this study population certainly demonstrate the importance for evidence-based advice on diet and potentially other modifiable lifestyle factors.

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