



This month's issue features dairy's effects on cardiovascular disease risk, the microbiome of mother's milk, Holder pasteurization's effect on immune proteins in human milk, and noncow's milk's effect on children's height development.

## Dairy's Counterintuitive Effects on Cardiovascular Disease Risk

- **A new meta-analysis finds that there is a neutral association between dairy consumption and cardiovascular disease (CVD) risk.**
- **The study also suggests that greater consumption of fermented dairy products, including cheese and yogurt, may decrease the risk of CVD.**
- **The findings add to the body of evidence that despite being a major source of dietary saturated fats, dairy products may not be bad for heart health and may even be beneficial.**

Consuming fats, and particularly saturated fats, increases the odds of cardiovascular disease (CVD). CVD is the major cause of death and disability worldwide, and many public health guidelines recommend diets that are low in saturated fats [1,2]. Although dairy products are a major source of dietary saturated fats, they have been shown to have various beneficial effects on cardiovascular health, including a reduced risk of stroke and coronary heart disease [3–7].

“That was surprising, and to many people it is still surprising,” says Professor Ian Givens of the University of Reading. “To some it’s a bit of a paradox, that you don’t see a big increase in coronary heart disease risk in particular, from high consumption of dairy products,” he says. “One of our biggest challenges has been to explain to the public why we either see a reduced or neutral cardiovascular disease risk with most dairy products when in many people’s minds they would expect the risk to be increased.”

In a new meta-analysis that looked at 29 prospective cohort studies, Givens and his colleagues found a neutral association between dairy consumption and cardiovascular and all-cause mortality [8]. “We thought it’s been quite a while since there has been an up-to-date meta-analysis,” says Givens. “We end up with a kind of neutral outcome. In other words, increasing consumption isn’t associated with increased risk,” he says. The researchers also found that consuming more fermented dairy products, including cheese and yogurt, may decrease the risk of CVD.



Givens became interested in studying how dairy products affect CVD due to the topic’s important public health implications. “Dairy products are consumed by a very high proportion of the population,” he says. “If there is some issue with these foods, it would potentially have a big effect at a population level, and it would affect everyone from children to adults,” says Givens. If dairy products are not harmful or are even beneficial to heart health despite their saturated fat content, that’s good news for the large proportion of people who regularly consume dairy.

Givens started doing meta-analyses looking at dairy and CVD back in the mid-2000s. “The first thing we did was we simply compared high consumers versus low consumers with a range of outcomes, but focusing particularly on coronary heart disease, and expanding into cardiovascular diseases in general, which includes stroke,” he says.

“When I started, and to some extent I guess it’s still the same now, the public was uncertain whether dairy products were good or bad,” says Givens. “I think that was mainly due to the messages that had been coming out about saturated fats,” he says. “The evidence on saturated fats on their own I think is still quite strong,” says Givens. But what about when these saturated fats were present in dairy?

Givens and other researchers have conducted many studies, including several meta-analyses, to figure out how dairy products might affect CVD risk [9–11]. “The strength of these meta-analyses comes from bringing together evidence from studies done in different parts of the world,” says Givens. “If the tendency for all of these is in the same direction, it gives you quite a lot of confidence,” says Givens.

Most of these studies have found no evidence for a harmful association between dairy consumption and CVD risk. In fact, consuming dairy may even be beneficial, as there’s evidence that higher milk and cheese consumption may be associated with a slightly lower risk of stroke [6,11]. “There are two CVDs to be really worried about: stroke and coronary heart disease,” says Givens. “Fermented dairy products may play a role in decreasing the risk of both stroke and coronary heart disease,” he says.

“I think in some dairy products there is a kind of balancing effect, so that the saturated fat effects might be negative, but there are

other things there that are positive, which reduce risk, so overall you get a sort of neutral risk,” says Givens. “There’s also increasing evidence that the matrix of cheese prevents some of the fat in it from being digested and absorbed,” says Givens.

Researchers have made a lot of progress in studying the effects of dairy on CVDs, but doing these kinds of meta-analyses isn’t without its challenges. “The biggest challenge is really being able to identify studies of adequate quality that you can actually use them in meta-analyses,” he says. “If you look at the current meta-analysis, we’re still only talking about 29 studies, it’s not massive numbers,” says Givens. “The other big challenge is how you deal with confounding factors,” he says. “There’s no simple answer to that, you just try to include confounding factors that you think are important,” says Givens.

Differences between individual studies can also make them harder to analyze. “A lot of the studies have classified dairy products as high-fat or low-fat, but there’s been no consistent agreement between studies as to what counts as a low-fat or high-fat,” says Givens. “We’ve seen for example one study that had ordinary milk in the high-fat group, because none of the fats had been removed, and it was in the same group as cheese with about 34 to 35% fat,” he says. “That doesn’t seem sensible, and I think that’s one of the problems lurking through many of the studies that are out there.”

On the plus side, researchers have refined their study designs since Givens started doing these meta-analyses. “One of the things that has happened in the last year or two is the move away from simply comparing high consumers versus low consumers, because in doing that you inherently assume a linear response between a low consumer and a high consumer,” says Givens. “The move has been to do dose response meta-analyses, where you actually try to look at the progression from low consumption to high consumption and assess whether or not it is linear,” he says. “If you can demonstrate a dose response, it actually gives you a whole lot of confidence that you’re dealing with a real mechanism rather than some accidental outcome, so I think that’s helped a lot,” says Givens.

In addition to prospective studies, Givens and others have also conducted intervention studies looking at the effects of dairy products and proteins on markers of CVD risk, including blood pressure. “There is a lot of interest in intervention studies, they are in many ways the gold standard,” says Givens. “The problem with intervention studies is that they’re very expensive to do,” he says. “And they still have to rely on markers of risk rather than absolute disease outcomes,” says Givens. “In contrast, the benefit of prospective studies is that they last a long time, and you actually have real disease events as the outcome,” he says.

The good thing is that researchers continue to find new and better markers. “10 to 15 years ago in studies looking at the effects of dietary fats, you would really only see cholesterol, or triglycerides, or maybe blood pressure as markers,” says Givens. “Nowadays, we’re beginning to look at cholesterol subtypes, or arterial stiffness,” he says. “This probably tells you more about your future risk of CVD than just blood pressure,” he says. “So the markers are getting better, which means that randomized control trials are getting more helpful, but they are still expensive,” says Givens.

If there’s one takeaway from the recent meta-analyses, it’s that unlike most other products rich in saturated fats, dairy products may not be bad for heart health, and may even be beneficial. “I think we can say that dairy products are at least not harmful with respect to CVDs,” says Givens. “So I think this neutral association between dairy consumption and CVD risk is significant.”

1. GBD 2013 mortality and causes of death collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2015 Jan 10;385(9963):117-71.
2. Sacks F.M., Lichtenstein A.H., Wu J.H.Y., Appel L.J., Creager M.A., Kris-Etherton P.M., Miller M., Rimm E.B., Rudel L.L., Robinson J.G., Stone N.J., Van Horn L.V.; American Heart Association. Dietary fats and cardiovascular disease: A presidential advisory from the American Heart Association. *Circulation*. 2017 Jul 18;136(3):e1-e23.
3. Crichton G.E., Alkerwi A. Dairy food intake is positively associated with cardiovascular health: findings from Observation of Cardiovascular Risk Factors in Luxembourg study. *Nutr Res*. 2014 Dec;34(12):1036-44.
4. Markey O., Vasilopoulou D., Givens D.I., Lovegrove J.A. Dairy and cardiovascular health: Friend or foe? *Nutr Bull*. 2014 Jun;39(2):161-171.
5. Wang L., Manson J.E., Buring J.E., Lee I.M., Sesso H.D. Dietary intake of dairy products, calcium, and vitamin D and the risk of hypertension in middle-aged and older women. *Hypertension*. 2008 Apr;51(4):1073-9.
6. Petherick A. (2013). A Hearty Helping of Dairy. SPLASH! milk science update: February 2013. (<http://milkgenomics.org/article/a-hearty-helping-of-dairy/>).
7. Givens D.I., Soedamah-Muthu S.S. Dairy fat: does it increase or reduce the risk of cardiovascular disease? *Am J Clin Nutr*. 2016 Nov;104(5):1191-2.
8. Guo J., Astrup A., Lovegrove J.A., Gijssbers L., Givens D.I., Soedamah-Muthu S.S. Milk and dairy consumption and risk of cardiovascular diseases and all-cause mortality: dose-response meta-analysis of prospective cohort studies. *Eur J Epidemiol*. 2017 Apr;32(4):269-287.
9. Thorning T.K., Raben A., Tholstrup T., Soedamah-Muthu S.S., Givens I., Astrup A. Milk and dairy products: good or bad for human health? An assessment of the totality of scientific evidence. *Food Nutr Res*. 2016 Nov 22;60:32527.
10. Lovegrove J.A., Givens D.I. Dairy food products: good or bad for cardiometabolic disease? *Nutr Res Rev*. 2016 Dec;29(2):249-267.
11. de Goede J., Soedamah-Muthu S.S., Pan A., Gijssbers L., Geleijnse J.M. Dairy consumption and risk of stroke: A systematic review and updated dose-response meta-analysis of prospective cohort studies. *J Am Heart Assoc*. 2016 May 20;5(5). pii: e002787.

*Contributed by*

*Dr. Sandeep Ravindran*

*Freelance Science Writer*

*Sandeep.com*

## Mother's Milk Microbes

- A new study reports that 30% of the bacteria in infant's guts are associated with bacteria in breast milk.
- Breastfeeding frequency was positively associated with diversity of gut bacterial strains.
- Beneficial bacteria provided by breast milk may act as seeds in the infant gut, selecting for future generations of bacteria that regulate metabolism and enhance immune function.
- Having a diverse community of beneficial bacteria in the gastrointestinal tract is associated with positive health outcomes from infancy through adulthood.

Twenty years ago, breast milk was believed to be sterile—any bacteria present were assumed to originate from the infant's mouth or the mother's skin [1]. Fast-forward to 2017, and bacteria are not viewed as contaminants but as ubiquitous and possibly important ingredients of breast milk. Recent research suggests a link between the infant's gut bacterial community, or microbiome, and the adult microbiome; starting out with the right mix of beneficial bacteria in the gut influences health throughout the lifespan [2–4]. The infant's gut is initially colonized by bacteria that may come from different sources, such as breast milk, or worse, such as a hospital environment. Special carbohydrates in breast milk, such as free oligosaccharides or glycans attached to proteins, then selectively nurture the good bacteria.

What can a parent do to make sure their offspring's gastrointestinal tract has the most beneficial strains of bacteria? The results of a new study [2] out of UCLA and Children's Hospital Los Angeles demonstrate that breast milk-derived microbes make up almost one-third of all beneficial bacteria in the infant's gut. Far from contaminating, breast milk bacteria may be instrumental in getting the gut off to a good start.

### The Good Guys

Bacteria have been given a bum rap. Ask someone about bacteria in the gut and they are likely to think of infectious agents like *Escherichia coli*, C-diff (*Clostridium difficile*), and cholera. However, the overwhelming majority of bacteria living in the human gut (estimated to be in the trillions!) are commensal, meaning they have no negative impact on their host, or mutualistic, meaning they provide a benefit to the host [3–5]. In fact, these bacteria are considered so essential to metabolism and immune function that they are often referred to as “the forgotten organ” [5].



Take food digestion, for example. The stomach and the intestines get much of the credit, but beneficial gut bacteria help break down otherwise indigestible plant starches and fibers and enhance the absorption of nutrients [4–6]. In doing so, they also create useful metabolic byproducts, such as short-chain fatty acids that are important sources of energy for the host and also feed other beneficial bacteria [4,5].

The spleen, thymus, and lymph nodes are important immune organs but are also highly reliant on gut microbes to train the immune cells exactly what “bad” bacteria look like [4–6]. Those immune organs can also thank beneficial bacteria for making their jobs easier. By forming a protective barrier along the surface of the intestines, beneficial bacterial strains keep pathogens from ever reaching host tissues and stimulating an immune response.

But the intestinal microbiome does more than help out the host's immune system—it is actively involved in regulating that system. As a result, there is a strong link between the strains of bacteria that make up an individual's microbiome and their health [3]. For example, some bacterial strains enhance the anti-inflammatory response of lymphocytes. Animal studies demonstrate that when these strains are not well represented in the microbiome, the resulting pro-inflammatory bias can trigger diseases such as Chron's and inflammatory bowel disease [3,5]. The make-up of the gut microbiome has also been linked to obesity and the metabolic syndrome. The very same bacteria that help to extract nutrients and energy from foods have been linked to higher body mass index, obesity, and altered metabolism—having some of these bacteria is essential, but having too many is associated with negative health outcomes [2,5]. Thus, although there is not a one-size-fits-all for healthy guts, researchers have converged on the concept of diversity and balance of bacterial strains when trying to describe the optimal gut microbiome [2–6].

If an abnormal gut microbiome is a gut with too many of one strain of bacteria and too few of another, then what can be done to make sure not to tip the balance? Several lines of evidence demonstrate that building a balanced and diverse gut microbiome starts early...really early [1].

### Baby's First Microbes

Parents can recall all of baby's "firsts"—first steps, first word, and the first day of school are carefully recorded and photographed. But very few parents probably even consider the sources of their baby's first microbial exposure despite the overwhelming evidence suggesting the sources of exposure can influence health in infancy and throughout the lifespan [1–3].

Until recently, it was believed that infants start their life with a clean slate, born free of any bacterial colonization. However, evidence for bacterial strains in the meconium (stool produced from materials consumed while the infant is *in utero*) argues against this "sterile infant" perspective [7]. The [placenta may have its very own microbiome](#) and could potentially represent the first source of maternal-offspring microbial transfer [7,8]. The second exchange occurs during childbirth when the newborn is exposed to bacteria from the vagina, and potentially even maternal fecal bacteria. Both exchanges are viewed as adaptations, where mothers provide infants with a "microbial inoculum" [7] before exposure to any other environmental microbes. Disrupting these opportunities, either through antibiotic use during pregnancy or birth via C-section, can increase the risks of developing celiac disease, type 1 diabetes, asthma, and obesity [6–8].

And then comes breast milk. Milk does not simply provide more of the same maternal microbes as previous sources; bacterial communities provided by breast milk are unique from those transferred via placenta or through vaginal birth. Bacteria genera commonly isolated from breast milk samples include *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Ralstonia*, *Staphylococcus*, and *Streptococcus* [1,4,7]. The last two in that list may sound familiar, and not in a good way; both genera include strains of pathogens known to cause disease in humans. It seems surprising, then, that these are usually among the dominant genera in breast milk. However, not all species in these genera are infectious agents. For example, some strains may provide benefits to the hosts by preventing colonization of the gut from their more lethal cousins [1]. Another possibility is that disease-causing strains are passed from mother to offspring, but the presence of other beneficial bacteria or other antimicrobial agents in milk negates their actions.

These hypotheses deserve more investigation and studies on milk bacteria should employ techniques that allow for detection at the level of the species, rather than just the genera, because breastfeeding is considered one of the most critical postpartum factors influencing the programming of metabolism and the immune system [2,5]. As was true of prenatal disruptions in the transfer of microbes from mother to offspring, the use of formula in place of breast milk has been linked to increased risks of poor health outcomes, including autoimmune diseases, inflammatory diseases of the gastrointestinal tract, and metabolic syndrome [7].

The reason the effects of antibiotic use, C-section, and formula (each of which is a novel, cultural development) are often realized long after the colonizing events is because the earliest microbes select for their predecessors [2]. Just as you can send off your DNA-laden spit and get a report detailing your ancestral origins, researchers can analyze fecal bacteria and determine the first microbes to seed your gut [2,6]. For this reason, Pannaraj and colleagues [2] argue that the earliest stages of life, including the time spent *in utero* and during birth, are a critical window for building a healthy gut microbiome.

### Planting The Seeds

Despite such an important role in short- and long-term health outcomes, it may be surprising to learn that researchers are still trying to quantify how much of the beneficial bacteria in the gut is provided by breast milk and how much comes from other sources, such as maternal skin and the infant's environment. In the largest study to date to tackle this question, Pannaraj and colleagues [2] analyzed the bacterial composition of breast milk, maternal areolar skin, and infant stool in 107 mother-infant pairs. Importantly, not all 107 infants were exclusively breastfeeding when the milk and stool samples were collected. As such, the researchers were able to explore the influence of nursing duration, nursing frequency, and the introduction of solid foods on the composition of the infant microbiome.

The bacterial communities in the milk and the mother's skin were distinct, and each was found to make a different contribution to the infant's gut microbiome. In the first 30 days of lactation, in infants that were primarily breastfed (defined by the research team as receiving at least 75% or more of their milk from breast milk) nearly 28% of their gut bacteria matched those from mother's milk and just over 10% of bacteria matched those from areolar skin [2].

As all the infants aged, the contribution from milk and areolar skin to their gut microbiome decreased. However, the way in which the infants' gut microbiomes changed over time was associated with the percentage of daily breast milk intake in a dose-dependent manner [2]. Exclusively breast-fed infants had bacterial communities that were distinct from those that received both milk and formula, indicating that even small amounts of formula have the potential to shift the microbial communities in the gut away from the breastfeeding pattern [2]. Interestingly, these differences seemed to persist even after the introduction of solid foods, which represent a source of novel microbes. Pannaraj and colleagues found that that dramatic shift in bacterial communities that usually occurs when food-derived bacteria are introduced was suppressed in infants that continued to receive at least 75% of milk from the breast after the introduction of solid foods [2]. An early maturation (that is, the development of an adult-like microbiome) has been associated with several negative health outcomes, including asthma and obesity [2].

Taken together, the results of this study suggest that breast milk makes its largest contribution to the infant gut microbiome during the first month of life but continues to positively influence the diversity of microbes throughout lactation. This supports the current World Health Organization's recommendation to breastfeed exclusively for the first six months of life and to continue breastfeeding through at least the first year.

## Lingering Questions

The researchers accounted for the sources for 40% of the infant's beneficial gut bacteria, but where does the other 60% come from? Unfortunately, this study did not investigate alternative sources but acknowledges that exposure to vaginal and fecal bacteria during childbirth and the environment are major contributors. Additionally, other researchers have suggested the location of birth (i.e., at home vs. a hospital) may provide another important source (or lack thereof) of microbial exposure [4].

Even without quantifying the individual contribution of mode of delivery and early infant environment, it seems reasonable to assume that evolutionary novel events such as C-sections and hospital births may be associated with suboptimal microbial development [4]. However, because these events may not be avoidable (e.g., C-section due to maternal pre-eclampsia or infant distress), it would be worthwhile to investigate how much breastfeeding may restore the balance in the microbiome.

Finally, perhaps the largest question that remains unanswered from this and other studies of its kind is the source of the breast milk bacteria. Recall that the bacterial strains from the milk and the maternal skin were distinct communities [2]; this indicates that the skin of the mother's breast is not the source for all breast milk bacteria. But if it is not from the skin, then where is it coming from? One possible hypothesis is that there is a connection between the maternal gut microbiome and the mammary gland [1]. Maternal immune cells may be responsible for carrying bacterial strains on a special intracellular route referred to as the entero-mammary pathway [1,4]. This proposition is not without controversy, but it is supported through several lines of evidence. Several studies in mouse models support the ability of dendritic cells to carry bacteria to locations in the body outside the gut, in a process known as translocation [1]. Moreover, human and mice mothers fed specific strains of probiotics (not commonly found in milk) produced milk containing that specific probiotic [1]. Finally, Pannaraj et al. [2] found that infant's stool microbiome was closer to that of its own mother's microbiome compared to a random mother from the study. That is, although there are common bacteria genera passed on in milk from all mothers, microbial profiles are unique to mother-infant dyads.

It is important to note, however, that infant gut microbiomes are distinct from their mother's. Thus, cells are not just bringing any microbes from the mother's gut to the mammary glands; they have instructions to only bring specific strains. Another possible hypothesis, which would also be supported by the observations of shared strains of mother and baby, is a fecal-oral route of contamination. For most of human history, there hasn't been modern plumbing and even with modern plumbing, most babies are still born through an opening that is very near the fecal canal.

Despite these issues, studies like that from Pannaraj and colleagues [2] highlight the importance of breast milk in establishing a healthy gut. We might not know where the bacteria come from, but it is becoming clear that they have the potential to improve health outcomes throughout the lifespan.

1. Rodriguez J.M. 2014. The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? *Adv Nutr.* 5: 779–784.
2. Pannaraj P.S., Li F., Cerini C., Bender J.M., Yang S., Rollie A., Adisetiyo H., Zabih S., Lincez P.J., Bittinger K., Bailey A., Bushman F.D., Sleasman J.W., Aldrovandi G.M. 2017. Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatr.* doi: 10.1001/jamapediatrics.2017.0378
3. Hooper L.V., Littman D.R., Macpherson A.J. 2012. Interactions between the microbiota and the immune system. *Science* 336: 1268–1273.
4. Martin M.A., Sela D.A. 2013. Infant gut microbiota: developmental influences and health outcomes. In *Building babies*, pp. 233–256. Springer: New York.
5. Quigley E.M.M. 2013. Gut bacteria in health and disease. *Gastroenterology and Hepatology* 9: 560–566.
6. Cabrera-Rubio R., Collado M.C., Laitinen K., Salminen S., Isolauri E., Mira A. 2012. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr.* 96: 544–551.
7. Mueller N.T., Bakacs E., Combellick J., Grigoryan Z., Dominguez-Bello M.G. 2015. The infant microbiome development: mom matters. *Trends Mol Med.* 21: 109–117.
8. Aagaard K., Ma J., Antony K.M., Ganu R., Petrosino J., Versalovic J. 2014. The placenta harbors a unique microbiome. *Sci Transl Med.* 6:237ra65.

*Contributed by*

*Dr. Lauren Milligan Newmark*

*Research Associate*

*Smithsonian Institute*

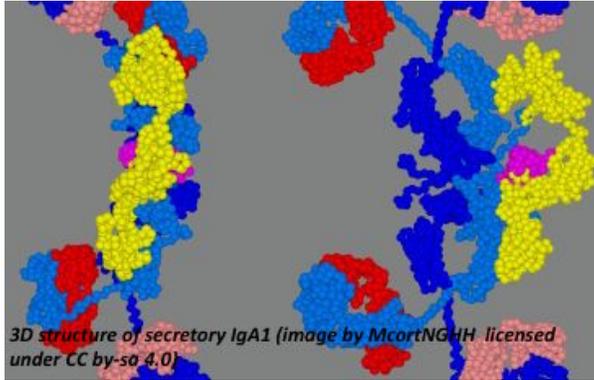
## Holder Pasteurization Damages Some Immune Proteins in Human Milk

- **When proteins are heated, they risk losing their biological function.**
- **Various studies have shown that the heat treatment involved in Holder pasteurization (HoP) reduces the activity of various proteins in human milk.**
- **A number of the human proteins that suffer a loss of activity after HoP are thought to have immunological functions in infants, among them antibodies and some cytokines.**

When you heat proteins, you very often ruin their function. This is because bonds that maintain a protein's final structure start to rupture—that is, segments that have folded and wrapped around themselves, forming a fiddly and exact arrangement in three

dimensions, come apart. “Denaturation,” as the process is called, is why Holder pasteurization (HoP)—heating milk to 62.5°C for 30 minutes—successfully removes the potential growth of germs such as bacteria when mother’s milk is stored, as in a milk bank. [But in defanging some microorganisms](#) through heating, HoP risks destroying the function of a suite of human proteins in milk that are thought to have beneficial effects on infants’ immunity. This third article in our series on HoP considers how well these proteins survive this type of pasteurization.

Probably the best-known biologically-active proteins in human milk are immunoglobulins, or antibodies, as they are commonly known. Newborn infants have immature immune systems, so to make up for a shortfall in pathogen defense, they arrive in the world with a dose of antibodies received through their mother’s placenta since the end of the second trimester [1]. After birth, human milk provides a boost, supplying many different kinds of antibodies, especially IgA (which, in its dimeric form, is by far the most common antibody in human milk), IgM and IgG.



Given that the specific details of antibodies’ three-dimensional structure are central to how they recognize whatever the immune system needs to rally against, it is unsurprising that HoP reduces their function. Recently, Brodie Daniels of the University of KwaZulu-Natal in South Africa, and her colleagues reported the retention of IgA after HoP as 78.9% [2]. Other investigators who have measured secretory IgA (the dimeric form), and IgA present in colostrum, the yellowish milk produced in the first few days after birth, have also found reductions [3]. These studies were performed using an assay known as an ELISA, which, by measuring how well antibodies bind to a specific target, effectively quantifies how well they are working, as opposed to their mere amounts in a nonfunctional state. As for

IgM and IgG, they too have been found to decrease after HoP [3], generally more so than IgA [3, 4].

Lysozyme—a bacteria-busting enzyme present in tears as well as human milk—also appears to be partially destroyed by HoP. Not all studies find this (Brodie Daniels and team being a case in point [2]). Those that do find extremely varied levels of lysozyme’s destruction. Some put its post-HoP functional concentration as low as 20%, whereas others report this to be as high as 85% [2].

Lactoferrin, another important antibacterial protein in human milk, appears to suffer a similar dent in its effectiveness after pasteurization, although the evidence is even more complex to interpret in this case. The protein in question fights bacterial infection through a range of mechanisms, and so researchers have attempted to measure how well it survives HoP in different ways. Some suggest that lactoferrin forms aggregations during pasteurization, rather than HoP fundamentally wrecking the protein’s structural integrity [3]. Other scholars point out that peptides such as lactoferricin, formed during an infant’s digestion of lactoferrin, extend the latter’s bacteria-fighting effects [3, 5]. They, therefore, suggest that lactoferrin’s downstream properties aren’t addressed by simple measures of how much is left functionally available in milk after pasteurization.

The immunological proteins in milk don’t end there—in fact, there’s a long list of additional components that modulate the infant immune response. Many of the cytokines in milk dampen down inflammation, while others ramp it up. Interleukin 10, for example, is present in human milk, and a case study of the former suggests that it inhibits the production of white blood cells called lymphocytes. However, so-called colony-stimulating factors (specifically, granulocyte-colony stimulating factor, macrophage-colony stimulating factor, and granulocyte-macrophage colony stimulating factor) were discovered in human milk relatively recently. As suggested by their titles, these proteins encourage the proliferation of different kinds of white blood cells [6].

In 2011, a team at the University of Alberta, in Canada, set about measuring a suite of these molecules in pooled samples of the milk of 34 women, and once again after the same samples were subjected to HoP [7]. Not all of the molecules they investigated were significantly reduced in the ELISA, although, for example, interferon-gamma, tumor necrosis factor-alpha, and the interleukins 1 beta and 10 all were.

Does that mean that HoP has a net impact on the overall immunomodulatory effect of milk? The answer, unfortunately, is unclear. Some of the affected cytokines tend to be of the type that attenuates the immune response, while others among those apparently destroyed by HoP are understood to have the opposite effect. Moreover, the roster of HoP-damaged cytokines appears to be different in different studies, with Espinosa Maeyos and colleagues in Madrid, Spain, reporting that macrophage inflammatory protein-1 beta and interleukin-7 were affected in samples of colostrum subjected to HoP—but not some of the other molecules tested by the Alberta team [8].

Despite the uncertainties in the details, the fact that HoP has been repeatedly found to alter the activity of various immune proteins in milk has motivated the search for more targeted methods of making human milk safe for storage. Ideally, these would be as effective against germs without the making the active human proteins in milk work less well. *SPLASH!*® will turn to that topic at the end of this series.

1. Edwards K. M. 2015. Maternal antibodies and infant immune responses to vaccines. *Vaccine* 33:6469–6472.
2. Daniels B., Schmidt S., King T., Israel-Ballard K., Amundson Mansen K. & Coutsooudis A. 2017. The effect of simulated flash-heat pasteurization on immune components of human milk. *Nutrients* 9:178.

3. Peila C., Moro G. E., Bertino E., Cavallarin L., Giribaldi M., Giuliani F., Cresi F. & Coscia A. 2016. The effect of Holder pasteurization on nutrients and biologically-active components in donor human milk: a review. *Nutrients* 8:477.
4. Sousa S. G., Delgadillo I. Saraiva & J. A. 2014. Effect of thermal pasteurisation and high-pressure processing on immunoglobulin content and lysozyme and lactoperoxidase activity in human colostrum. *Food Chemistry* 151:79–85.
5. Lonnerdal B. 2003. Nutritional and physiologic significance of human milk proteins. *American Journal of Clinical Nutrition* 77:1537S-1543S.
6. Garofalo, R. 2010. Cytokines in human milk. *Journal of Pediatrics*. 156(2). Suppl. 1. S36-S40.
7. Ewaschuk J. B., Unger S., O'Connor D.L., Stone D., Harvey S., Clandinin M.T. & Field C. J. 2011. Effect of pasteurization on selected immune components of donated human breast milk. *Journal of Perinatology* 31:593–598.
8. Espinosa-Martos I., Montilla A., de Segura A. G., Escuder D., Bustos G., Pallas C., Rodriguez J. M., Corzo N. & Fernandez L. 2013. Bacteriological, biochemical, and immunological modifications in human colostrum after Holder pasteurisation. *Journal of Pediatric Gastroenterology and Nutrition* 56:560–568.

*Contributed by*

*Anna Petherick*

*Professional science writer & editor*

*www.annapetherick.com*

## Plant-Based Milk Beverages Affect Children's Height

- **Children's height is used as an index of health and normal development.**
- **Children consuming noncow's milk beverages are shorter in height than those consuming cow's milk.**
- **Some plant-based milk substitutes may not adequately support the full growth and developmental needs of children.**

A recent large-scale scientific study concluded that children drinking plant-based substitutes for cow's milk were associated with slightly shorter height [1]. The authors speculate the study provides the first indication that the increasingly popular consumption of nondairy milk substitutes may not adequately support the full nutritional requirements of rapidly growing young children.

### What Determines Human Height?

Geneticists conclude that human height has a strong genetic component accounting for about 60–80% of the variation in height of a population, i.e. tall parents usually produce tall offspring [2,3]. The remaining 20–40% of the variation in height is due to factors such as nutrition and illness [2–4].

### Five Foot Tall and Rising (Johnny Cash)

The anthropologist Franz Boas identified the strong influence of environment on human height over one hundred years ago [5]. He noted that children of immigrants to the USA when adults were on average taller than their parents. Boas attributed this observation to better nutrition. Some individuals never reach their full genetic potential for height due to adverse environmental



influences such as illness and poor nutrition. Height is, therefore, a sensitive indicator health status.

Human height changes during development from an infant to an adult are easy and cheap to measure. Height is an important indicator of the health of a population and individuals within a population. This is particularly evident in malnourished populations of the developing world that consume insufficient dietary protein and are generally shorter than comparable populations receiving adequate nutrition [5,6]. Indeed, the World Health Organization recommends the widespread use of population-based growth curves covering all ages but particularly infants and children to allow monitoring of the populations by health authorities [7]. Individuals departing from the normal range of these growth curves may signify poor

health or poor nutrition, thus indicating the need for medical investigation or nutritional changes. These interventions are particularly important for children as inadequate nutrition or illness early in life can have lifelong consequences [8].

A previous article published in *SPLASH!*<sup>®</sup> and authored by Lauren Newmark highlighted the extensive evidence that adequate animal protein in the diet of humans is positively associated with human height and optimal growth [9]. Numerous investigations have shown that cow's milk is a rich dietary source of protein and essential amino acids. Milk consumption is also positively associated with human height and it promotes optimal growth and development of children [10,11]. Health authorities in many countries, often as part of national health initiatives, recommend milk consumption by children.

### The Times They Are a Changin' (Bob Dylan)

Recently, there has been increasing consumption of nondairy alternative milks, particularly plant-based beverages derived from soy,

rice, and almonds (summarized in [1]). However, little is known of the health benefits to young children from consumption of these alternative milks. A team of scientists led by Jonathon Maguire and based in Toronto at the University of Toronto, St Michael's Hospital and the Hospital for Sick Children has now investigated the association between child height and consumption of noncow's milk [1]. The landmark study was recently published in the *American Journal of Clinical Nutrition*.

### **Grand Designs (Rush)**

Good experimental design and analysis are essential in scientific research. The Toronto investigators analyzed an impressive data set from over 5,000 children ranging in age from 24 to 72 months [1]. The investigators recruited the children from the TARGetKids Toronto initiative but excluded a small number of children with known health, growth or developmental issues. The dataset contained information on the level of child consumption of cow's milk and noncow's milk beverages. About 92% of the group consumed cow's milk and 13% consumed noncow's milk, with some children consuming both milks. The investigators used a statistical score of child height for age in their analysis. Importantly, they adjusted their multiple statistical models for known factors that have potential to indirectly affect height. These confounding factors included age, sex, body mass index, maternal ethnicity, income and maternal height. Without consideration of these factors, the investigators would be *Walking on the Wild Side* (apologies to Lou Reed).

### **Key Results (Milk It – Nirvana)**

The investigators concluded that for the average child, each cup of noncow's milk consumed per day was associated with a height decrease of 0.4 cm [1]. The investigators also concluded that the effect of the noncow's milk beverages on height was not just due to the removal of the positive benefits of cow's milk from the diet, i.e. consumption noncow's milk was associated with the height loss. The height reduction at three years of age for the average child drinking three cups per day of noncow's milk compared with the average child drinking three cups of cow's milk was 1.5 cm.

Maguire and colleagues speculated that many noncow's milk beverages may have reduced protein content compared with cow's milk, which could explain the height decrease in the group consuming noncow's milk. Other studies additionally suggest that plant-based milk proteins, unlike animal proteins, often do not contain all the essential amino acids required for optimal human growth and development [12–14]. The investigators further suggested that consumption of noncow's milk by children may not induce increased levels of a natural growth promotant (insulin-like growth factor 1) as happens with the consumption of cow's milk.

### **Limitations of the Investigation**

The investigators noted some limitations of their study [1]. First, they could not determine the relative influences of the different types of noncow's milk beverages. Second, the primary analysis did not include paternal height as a potential confounding factor. However, the investigators performed an analysis of a subset of the whole dataset that additionally included paternal height, but there was no change of the major conclusions. Third, the investigators emphasized that statistical association in a large population study does not necessarily infer causation and therefore they proposed additional research to determine whether noncow's milk consumption by children caused their reduced height. Fifth, like most good large-scale studies, there is sometimes a minor issue. The dataset included some children that consumed goat's milk and these children were included in the noncow's milk group for the analysis. However, the number of these individuals was likely quite small and therefore unlikely to alter the major conclusions.

### **Long Tall Sally Could Be a Bit Shorter in The Future**

The experimental design used by Maguire and colleagues could not test for lifelong effects of consumption of noncow's milk by children nor whether only height may be affected in the future [1]. At present, these questions are unanswerable due to the very recent changes in the milk consumption habits of some Western countries. The investigators also suggested that some noncow's milk beverages, unlike commercial cow's milk, had poorly defined and often variable contents. They suggested that content standardization and content labeling "would help parents make informed choices about the appropriate choice of milk for their children."

The elephant in the room not addressed by the investigators was the commercial adoption of the word "milk" in the brand name of the plant-based milk substitutes. The European Court of Justice ruled that the word "milk" only applies to dairy products and not to plant-based products. Other countries have similar regulations, although often these are unenforced by the relevant authorities. Resolution of this commercial issue may be required in many countries.

1. Morency M.E., Birken C.S., Lebovic G., Chen Y., L'Abbé M., Lee G.J., et al. Association between noncow milk beverage consumption and childhood height. *Am J Clin Nutr.* 2017;106(2):597-602.

2. Visscher P.M., Macgregor S., Benyamin B., Zhu G., Gordon S., Medland S., et al. Genome partitioning of genetic variation for height from 11,214 sibling pairs. *Am J Hum Genet.* 2007;81(5):1104-10.
3. Visscher P.M. Sizing up human height variation. *Nat Genet.* 2008;40(5):489-90.
4. Lai C-Q. How much of human height is genetic and how much is due to nutrition? : *Scientific American*; 2017 [Available from: <https://www.scientificamerican.com/article/how-much-of-human-height/>].
5. Stocking G. *A Franz Boas Reader: The Shaping of American Anthropology, 1883-1911.* Chicago and London: The University of Chicago Press; 1974.
6. United Nations Children's Fund, World Health Organization, World Bank. UNICEF WHO-World Bank Joint Child Malnutrition Estimates 2017 [Available from: <http://www.who.int/nutgrowthdb/estimates/en/>].
7. WHO. Child growth standards: methods and development Geneva (Switzerland): World Health Organisation; 2006 [Available from: [http://www.who.int/childgrowth/standards/technical\\_report/en/](http://www.who.int/childgrowth/standards/technical_report/en/)].
8. Prendergast A.J., Humphrey J.H. The stunting syndrome in developing countries. *Paediatr Int Child Health.* 2014;34(4):250-65.
9. Milligan L. Milk protein in diet predicts human height. *SPLASH.* 2015. [Available from: <http://milkgenomics.org/article/milk-protein-diet-predicts-human-height/>].
10. Wiley AS. Consumption of milk, but not other dairy products, is associated with height among US preschool children in NHANES 1999-2002. *Ann Hum Biol.* 2009;36(2):125-38.
11. Wiley A.S. Does milk make children grow? Relationships between milk consumption and height in NHANES 1999-2002. *Am J Hum Biol.* 2005;17(4):425-41.
12. Boland M. *Global food supply: the world's need for protein New Zealand: Riddet Institute, Massey University, New Zealand; 2013* [Available from: <http://www.riddet.ac.nz/sites/default/files/content/2013%20Protein%20supply%20Mike%20Boland.pdf>].
13. Food and Agricultural Organization (FAO). *Dietary protein quality evaluation in human nutrition: report of an FAO expert consultation.* Rome, Italy: FAO; 2013 [Available from: <http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf>].
14. Ertl P., Knaus W., Zollitsch W. An approach to including protein quality when assessing the net contribution of livestock to human food supply. *Animal.* 2016;10(11):1883-9.

*Contributed by*

*Dr. Ross Tellam (AM)*

*Research Scientist*

*Brisbane, Australia*

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

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