This month’s issue features gastric ulcers, immune cells in mother’s milk, almond milk, and probiotics and pregnancy.

Cow Milk Can Protect the Gut from Alcohol-Induced Gastric Ulcers

- Certain dairy products and bioactive components of milk have been shown to have a protective effect on the gastric mucosa, but the gastroprotective effects of cow milk are still unclear.
- A new study finds that pretreatment with cow milk can prevent the development of gastric ulcers caused by acid and alcohol in mice.
- The protective effects of cow milk appear to occur through the modulation of antioxidant enzymes and anti-inflammatory processes.

Ulcers can be a real pain in the gut, and they’re unfortunately quite common, affecting more than 10% of the world’s population [1]. Drinking alcohol, smoking, stress, and microbial infections are all known to exacerbate these ulcers [2].

Alcohol can be particularly damaging to the gastric mucosa [3,4]. Alcohol increases the generation of reactive oxygen species and suppresses the activity of antioxidants that play a crucial role in protecting the stomach [5,6].

Studies have shown that dairy products—such as yogurt—and certain bioactive components of milk can help protect the gut mucosa [7-10]. A couple of recent studies found that multiple milk components, including casein, whey protein concentrate, and hydrolysates from casein and whey protein, showed protective anti-ulcerative effects on the gastric mucosa in rodent models of ethanol-induced ulcer [11,12]. However, there have been few animal studies of the gastroprotective effects of cow milk itself, particularly against gastric damage induced by acid and alcohol.

In a new study, Professor Hae-Jeung Lee and her colleagues at Gachon University in South Korea hypothesized that cow milk may protect the gastric mucosa by modulating antioxidant enzymes and anti-inflammatory processes [13]. They tested the protective effects of fresh, commercially available cow whole milk against gastric ulcers induced by acidified alcohol in mice.

The researchers gave mice either 5, 10, or 20 mL/kg of body weight of commercial fresh cow milk once a day for 14 days. An hour after the 14th dose of milk, the mice were given a single dose of a hydrochloric acid-ethanol mixture intended to provoke severe gastric damage.

The treatment with acidified ethanol did indeed cause severe gastric damage in mice. However, pretreatment with all doses of the cow milk significantly prevented the alcohol-induced gastric damage and significantly suppressed the formation of lesions in the gastric mucosa.

Acidified ethanol greatly increased the stomach activity of myeloperoxidase, an enzyme that can serve as an indicator of injury to the gastric mucosa. Pretreatment with cow milk significantly ameliorated myeloperoxidase activity. Acidified ethanol also inhibited the antioxidant enzymes superoxide dismutase and catalase, and pretreatment with cow milk increased the activity of these enzymes.

Pretreatment with cow milk affected the activity of several protective gastric factors. For instance, cow milk increased the levels of gastric mucus, which plays an important role in protecting the gastric mucosa against stimulating substances such as acidified ethanol [14]. Cow milk pretreatment also increased levels of nitric oxide, which is known to mediate the protection of the gastric mucosa [15].

Alcohol is known to upregulate the levels of inflammatory cytokines, and mucosal inflammation is associated with the activation of the Nfkb1 gene that encodes the regulatory protein NF-κB [16,17]. The researchers found that acidified ethanol significantly increased the expression of gastric Nfkb1, as well as two NF-κB-regulated genes, Cox2 and iNOS. Pretreatment with cow milk significantly downregulated all
three of these genes, suggesting that cow milk’s anti-inflammatory effects might be mediated via the NF-κB signaling pathway.

These findings suggest that cow milk has a protective effect on the gastric mucosa and can prevent the development of gastric ulcers caused by acid and alcohol in mice. This protective effect appears to occur through the modulation of the antioxidant defense system and anti-inflammatory enzymes, possibly via the NF-κB signaling pathway. Understanding the mechanisms by which cow milk protects against gastric damage could potentially help find new ways to ameliorate gastric ulcers.

7. Uchida M., Kurakazu K. Yogurt containing Lactobacillus gasseri OLL2716 exerts gastroprotective action against acute gastric lesion and antral ulcer in rats. J Pharmcol Sci. 2004 Sep;96(1):84-90.

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

**Milk is Alive with Mom’s Cells**

- Breast feeding protects babies from disease.
- Several types of mom’s cells are present in milk.
- Fresh breast milk contains a new class of immune cells called innate lymphoid cells (ILCs).
- ILCs may take up temporary residence in a baby’s gut tissues where they potentially aid protection of the baby from infections and gut inflammation, help train the baby’s developing immune system, and regulate the establishment of the microbiome in the gut.
- ILCs in milk may protect the mother from infection in her baby and signal the need to change milk composition to aid fighting the infection.

Surprises upturn accepted routines and demonstrate how little we really know. A new class of immune cell type, innate lymphoid cells (ILCs), was recently and unexpectedly discovered in fresh breast milk [1], and it promises to radically alter scientists’ understanding of how milk protects babies from infections, and possibly much more. The ground-breaking scientific paper [1] describing this discovery was recently published in the prestigious *Journal of the American Medical Association – Pediatrics* by Babak Baban and three colleagues from Augusta University. The paper has the modest but revealing title “Presence and Profile of Innate Lymphoid Cells in Human Breast Milk.”
Numbers talk, sometimes loudly. More than 80 years ago, Grulke and colleagues undertook a large study of 20,000 mother-infant pairs and demonstrated that breast-fed infants compared with non-breast-fed infants had strikingly higher chances of survival from various infectious diseases [2]. Investigators in many subsequent studies confirmed these results and concluded that something in fresh breast milk protects babies and infants from infections [3-6]. This advantage of breast feeding is important as babies are burdened by increased risk of infectious diseases due to their poorly developed immune defense system. But how does milk protect the very young from disease? A number of big surprises are now emerging!

Milk is well recognized for its nutritional benefits to babies. Moreover, there is substantial evidence that milk’s molecular components help to protect babies from infectious diseases [3,7]. The latter characteristic of milk is a consensus view that has withstood the test of time, but it largely ignores the role played by maternal cells in milk.

Milk Contains Large Numbers of Different Cell Types

Many investigators over the last 60 years reported that fresh milk contains maternal cells, including immune cells (leukocytes), stem cells (cells that can give rise to multiple cell types), and mammary cells shed from breast tissue [7]. The leukocytes include the usual well-known suspects in the immune defense system i.e. neutrophils, macrophages and lymphocytes. Each has a different functional role, like the army, navy, and air force, but they act together to fight infections. Babak Baban and colleagues reported that newborn babies ingest about 100 million maternal cells in milk every day; most of these cells are leukocytes [1]. In the past, scientists assumed milk cells must have little biological importance in the baby because it was thought that these cells could not survive in the acidic environment in the infant’s stomach and upper region of the small intestine. These ideas are now being challenged by Baban and colleagues [1] and Cabinian and colleagues [8].

The high abundance of leukocytes in milk, about a million cells in a milliliter, is a strong clue that their presence in milk is biologically important [3]; nature usually does not condone waste. Cabinian and colleagues, using a mouse model, demonstrated that milk leukocytes unexpectedly survived in the guts of mouse pups for several days [8]. This was the first big surprise. The second surprise was that some of the ingested maternal leukocytes were localized in the pups to small tissue masses in the wall of the intestine called Peyer’s patches. Peyer’s patches are specialized immune tissues that monitor and regulate bacterial populations in the intestine and prevent growth of disease-causing bacteria and parasites [9]. The survival of the ingested maternal leukocytes may be due to the less acidic gut of the nursing pup compared with the adult gut and the ready acceptance of these maternal cells by the immature Peyer’s patches [3]. Something similar could also occur to human breast-fed babies. This is startling news as it indicates that a baby may acquire some functional maternal leukocytes at a very early stage in life.

Cabinian and colleagues concluded that some milk leukocytes may directly protect the mother’s breast from infection while also supplementing the baby’s immature immune defense capability and possibly regulating the developing immune system of the baby [1,8]. The latter function is particularly important as the immature immune system of a baby is confronted by an onslaught of microbes colonizing its gastrointestinal tract. Therefore, the baby’s immune defense system must very quickly learn to tolerate good gut microbes and eliminate others that are a disease threat but without generating too much inflammation. A little help from mom’s leukocytes in the training of the baby’s immune system in the gut could be very handy at this time. Notably, once the pups ceased being nursed by the mother, there was rapid loss of the maternal leukocytes from the pup’s Peyer’s patches. Mom’s additional cellular help did not persist in her absence.

A New Class of Immune Cells is Present in Milk

Baban and colleagues analyzed the different types of leukocytes present in fresh breast milk. They used a
A machine that sorts individual cells based on fluorescent antibodies that bind to specific proteins on the surface of cells [1]. Leukocytes contain specific cell surface proteins that can be used for their detection and separation from non-leukocytes. The well-known classes of leukocytes were then easy to spot as each has additional and characteristic protein markers on their cell surfaces. The recently discovered class of leukocytes called innate lymphoid cells (ILCs) are much harder to find and isolate because they do not carry any known cell surface markers that are characteristic of only ILCs. Baban and colleagues used the absence of cell surface markers characteristic of all of the well-known leukocyte classes to isolate ILCs away from other leukocytes using samples of fresh breast milk. This was the third big surprise for this area of research; a new class of leukocytes was present in milk. Moreover, all three subtypes of ILCs were present in fresh breast milk. The ILC subtypes were identified by their differing abilities to produce specific proteins that communicate with other leukocyte classes. Scientists have started to document some of the functions of ILCs, but it is early days yet.

All defensive forces require a general who efficiently coordinates the army, navy, and air force to ensure a rapid and effective response to an invasion. ILCs, unlike the other classes of leukocytes, usually do not get directly involved in the battle against disease, but they stay in the background and coordinate the defense, just like a general. Their job is to sense injured cells in infected tissue and then send out protein signals to rapidly attract the business end of the immune defense system, the macrophages, lymphocytes, and neutrophils. These three are deadly to microbes, but they need to be carefully controlled by ILCs to prevent too much inflammation at the site of the infection, which can cause collateral damage to cells. Milk ILCs have not been formally shown to take up residence in the intestine of the baby, but this is likely as the leukocytes (mainly cytotoxic T cells) shown to be present in the intestinal tissue require command and control signals to keep them in line.

ILCs have an impressive résumé of specialized biological roles, including resistance to infectious microbes and parasites, regulation of inflammation, involvement in tissue remodeling, surveillance of the body for tumors, and the maintenance of energy balance in the body [10-12]. This diverse expertise of ILCs and their ability to direct the activities of other immune cells makes them very useful. Conversely, dysfunction of ILCs can lead to infections, allergies, asthma, autoimmune diseases, and obesity [10-12].

One of the functions of the ILC3 subclass is the ability to modulate the intestinal microbiome [13, 14]. Baban and colleagues suggest that maternal ILCs in milk could regulate the baby’s innate immune system and shape the establishment of the intestinal microbiome, which is essential to initiate the digestive functions of the rapidly developing infant. Some investigators also argue that the microbiome is important for prevention of various diseases [15]. Baban and colleagues additionally speculated that ILCs may somehow signal to the mother that a baby has an infection, thereby triggering a change in the composition of her milk to better fight the infection and protect the mother—milk is certainly dynamic. This is biological finesse at its best.

**Implications**

Baban and colleagues indicated that ILCs in milk do not survive refrigeration [1]. This observation may help explain why breast-fed infants have a lower risk of infections. The many functions of ILCs in the context of maternal ILCs in ingested milk that potentially take up residence in a baby’s intestinal Peyer’s patches may be fertile ground for future scientific explorations. The growing realization by scientists that there is often an early life origin of many adult diseases of increasing incidence in modern populations emphasizes the urgent need for these explorations.

Almond “Milk”: A Case of Identify Theft?

- Non-dairy milks are gaining in popularity, and almond milk is the most widely consumed plant-based milk alternative.
- Almond milk is low in calories and high in vitamin E but has only 12% of the protein in cow milk.
- Consumers, most especially children and adolescents, that substitute almond milk for cow milk may have diets lacking in many key nutrients, including vitamin D, calcium, and high-quality protein.

Juliet Capulet famously asked, “What’s in a name? That which we call a rose by any other name would smell as sweet.” Juliet was able to look past “Montague” and love Romeo in spite of his surname. But when it comes to food and nutrition, names matter. Case in point—plant-based “milks.” Their placement in grocery stores in the dairy case and the use of “milk” on their packaging can give the false impression that they are nutritionally equivalent to cow milk. Although plant-based milk alternatives offer many nutritional benefits and are produced to have the same texture and appearance as milk, they are not a suitable nutritional substitute for cow milk, particularly for children and adolescents.

This is true even of almond milk, the highest selling plant-based milk alternative [1,2]. Almonds are known to be high in protein, vitamin E, and healthy fats, and many consumers believe that almond milk shares these health benefits. Almond milk may be a healthy beverage, but consumers are misled if they believe it offers identical health benefits to cow milk, or even identical health benefits to almonds. But would almond milk by any other name taste just as sweet?

Can You Milk an Almond?

The dairy case has become quite a crowded spot over the last 10 years. Plant-based milk alternatives that were once only found in the health food aisles of grocery stores have slowly started to claim equal real estate with cow milk in the dairy aisle. Worldwide, the sales of non-dairy milks—made from nuts, beans, oats, and even peas—doubled between 2009 and 2015 [1]. This trend is attributed to dietary restrictions, such as lactose intolerance or cow milk protein allergy, and consumer concerns about the health of animal-based foods, particularly saturated fats [1-5].

Almond milk, which can be purchased sweetened or unsweetened, and even flavored with vanilla or chocolate, is the most popular type of alternative milk beverage [1,2]. One reason for its popularity may be the well-known health benefits of almonds, particularly their high protein content (one cup of sliced almonds has around 20 grams of protein). However, many of the nutrients that make almonds so appealing to health-conscious consumers are actually lost during the milk-making process.

You can’t get blood from a turnip, and no matter how hard you try you can’t milk an almond. After almonds are soaked in water and ground up, the mixture is strained, so that the almond pulp remains behind and the almond water is collected (almost like making almond tea). This liquid is made more “milky” both in appearance and consistency by homogenization and, in some almond milk brands, the addition of thickening agents such as seaweed-derived carrageenan [1].

The good news is that because this process requires a lot of water, almond milk is low in calories relative to cow milk or other plant-based milk alternatives [1-4]. The bad news is that because the milk is made from the almond water and not the pulp, most of the nutritional benefits from the almonds are lost in the production process.
Almond Water vs. Milk

Almond milk has a nutty taste, but that may be one of the only things it shares with actual almonds. By straining the liquid and discarding the pulp, almond milk has only a fraction of the protein found in almonds. And at 1 gram of protein per 8 ounce serving, it also has only a fraction of the protein found in a serving of cow milk (8 grams of protein per serving) [1,2,5]. Considering that just one serving of cow milk provides nearly 60% of the recommended daily allowance (RDA) of protein for toddlers and just over 40% of the RDA for protein in young children [3], the discrepancy between the two beverages seems even more salient.

Toddlers and young children that replace cow milk with almond milk could be coming up short on dietary protein, as well as many micronutrients [3]. Almonds are known to be good sources of magnesium and copper, but both minerals are basically lost in the “milking” process [1,3]. And although a serving of almonds is nearly equivalent to a serving of cow milk in calcium content, the same cannot be said for almond milk. Thus, most (but not all) almond milks are fortified with calcium, many advertising that they offer even more calcium than cow milk.

But buyer (and drinker) beware—more calcium in the bottle does not necessarily mean more calcium in your body. Foods vary in how much of the ingested calcium is eventually absorbed and deposited into bone, referred to as calcium bioavailability. The bioavailability of calcium from cow milk is high because of the presence of casein proteins and lactose, both of which facilitate intestinal absorption, and phosphorus, which promotes the movement of calcium from the blood to the bones.

Vitamin D also promotes calcium absorption, and this hormone is added to both cow and almond milk. However, vitamin D fortification of almond and other plant-based milks is not regulated in the same manner as fortification of cow milk, meaning vitamin D levels are variable across alternative milk types and brands. A study [6] on children aged 1–6 years living in Toronto Canada found that children that consumed non-dairy milks, which included almond as well as soy and rice milk, were at a higher risk for having vitamin D deficiency than those that drank only cow milk. This suggests that despite fortification, non-dairy milks may come up short on meeting vitamin D requirements for young children.

Almond milk does have cow milk beat on one essential nutrient, however—vitamin E. Vitamin E is an antioxidant involved in preventing damage to tissues from free radicals, and may also play a role in immunity. One serving of almond milk can provide 50% of the RDA of vitamin E [1-3], without the added calories of fat that usually come with consuming vitamin E-rich foods such as nuts and nut oils.

The Name Game

The Europeans take names very seriously and prohibit the use of the term milk on anything not produced by a mammal [4]. Might it be time for the U.S. and other countries to follow suit? Almond milk is certainly used in the diet in the same ways as cow milk—poured over cereal, blended up in smoothies, and added to coffee. But the critical question is whether it is perceived by consumers to be a nutritional substitute? The addition of the name “milk” may mislead many who do not do a thorough side-by-side comparison of the nutritional labels. For those using it to make a more nutty-flavored latte, this may not be cause for concern. But without other dietary changes to fill in the missing gaps, growing children and adolescents may be missing out on key nutrients.


Contributed by
Dr. Lauren Milligan Newmark
Research Associate
Smithsonian Institute
Probiotic Milk Reduces Rate of Pre-eclampsia and Pre-term Birth

- A study of tens of thousands of Norwegian pregnancies suggests that consuming probiotic milk products can reduce the likelihood of developing pre-eclampsia and of giving birth spontaneously before 37 weeks’ gestation.
- Early-pregnancy consumption of probiotic milk products as important for reducing pre-term birth risk.
- Late-pregnancy consumption of probiotic milk products as important for lowering pre-eclampsia risk.
- In both cases, the mechanism of probiotic action is likely to be a modulation of the immune response, although probiotics may also cut pre-eclampsia risk by lowering blood pressure.

Probiotics are living microorganisms that improve health when they are administered in sufficient numbers. Often, administration means eating—in yogurt form or perhaps as a food supplement. Orally consumed probiotics can shift the composition of vaginal bacteria, making it harder for potentially pathogenic bacterial and yeast species to grow there [1]. Probiotics have also been shown to have anti-inflammatory effects [2,3]. And it is these reports that encouraged a team of Scandinavian scientists to investigate whether consuming probiotic milk products during pregnancy cuts the probability of developing pre-eclampsia or having a spontaneous, pre-term birth [4]. The results of their study are promising, not least because probiotic milk products are cheap and widely available. The next step will be to further investigate the mechanistic details of the effects.

The study involved a vast number of women, and is all the more convincing for that. The researchers plugged in to a prospective cohort study called MoBa (this stands for Norwegian Mother and Child Cohort Study), which followed more than 70,000 single-birth pregnancies. Because previous pregnancies can affect the frequency of the two outcomes of interest, the team studied only those women in the cohort who had never given birth before. This still left them around 35,000 pregnancies to analyze.

Both pre-eclampsia and pre-term birth have been linked to inflammation in somewhat different ways. Pre-eclampsia is where the placenta develops abnormally, because, it is thought, of problems relating to the laying down of endothelial cells. That process is influenced by the immune system, specifically by uterine or decidual “CD56bright” natural killer cells, and by peripheral “CD56dim” natural killer cells [5]. Spontaneous pre-term birth is linked to inflammation via several routes. The first step typically involves microbes getting into the amniotic cavity. This, for example, can prompt an increase in cytokines like IL-1, which in turn stimulates prostaglandin production by the lining of the uterus, and therefore leads to uterine contractions [6]. Another route links activation of the immune system to disruption of the extracellular matrix of fetal membranes [4].

Whatever the fine details of how the inflammatory response raises the odds of pre-eclampsia and pre-term birth, if probiotics can quench the response, that seemed to be worth investigating. The women in the MoBa study filled in questionnaires about their intake of probiotics, both early and late in pregnancy. Luckily, this was a fairly straightforward business in Norway during the years of the study because there were only two such products on the market—"Biola” and “Cultura,” both milk products—and both contained probiotic lactobacilli. Probiotic food supplements were very uncommon in Norway while the data were collected. Moreover, neither of the available probiotic milk products had vitamin D added. Because vitamin D may on its own reduce the risk of pre-eclampsia, its absence from the probiotic foods facilitated a cleaner evaluation of the effects under investigation.

The team identified two main results, which were recently published in the British Medical Journal’s open access journal [4]. In short, consumption of probiotic milk products during early pregnancy significantly reduced the likelihood of a spontaneous pre-term birth, but consumption in late pregnancy did not. Meanwhile, consumption during late pregnancy was significantly associated with a lower risk of pre-eclampsia, but consumption in early pregnancy was not. So, if a pregnant woman were to deploy these findings to her own benefit, she should regularly eat probiotic milk products throughout her pregnancy. The average amount consumed by women in the study was just over a cup-and-a-half per day, although the amount was not significant in driving the results—instead, it was the mere fact of regular consumption that mattered.

The researchers do not have enough information to pinpoint what is going on inside these women’s bodies, which could explain the reduction in risks during particular periods of pregnancy. There are clues in the literature, though. A randomized controlled trial of probiotic supplements given to pregnant women
demonstrated that *Lactobacillus rhamnosus* (in combination with *Bifidobacterium lactis*) changed the expression of toll-like receptor (TLR)-related genes in the placenta (these encode important innate immune system proteins) [6]. Other findings suggest that part of the reduction in pre-eclampsia risk may be indirect, deriving from probiotic effects on blood pressure. One component of a diagnosis of pre-eclampsia is high blood pressure. Clinical trials of probiotics of various lactobacilli species in non-pregnant individuals have shown changes in the gene expression of the gut lining, which have lowered blood pressure [3].

There is healthy skepticism about the benefits of probiotic products, and far from every test of their effects finds statistically significant health improvements [7]. The fact that the study found improvements in Norwegian women is especially impressive, however, since Scandinavian countries have among the lowest rates of both pre-eclampsia and pre-term birth in the world. When it comes to preterm birth, 9.6% of pregnancies in the United States lead to spontaneous delivery prior to 37 weeks’ gestation, compared with 5.6%–6.4% in Scandinavian countries. The results of this study suggest an easy and inexpensive way of reducing the risk of two relatively common problems for which modern obstetrics currently has no reliable treatments. That should be welcomed. The next step will be to develop a richer understanding of why probiotics work.


Contributed by
Anna Petherick
Professional Science Writer & Editor
www.annapetherick.com

Editorial Staff of SPLASH!*® milk science update:

Dr. Danielle Lemay, Executive Editor
Dr. Katie Rodger, Managing Editor
Anna Petherick, Associate Editor
Dr. Lauren Milligan Newmark, Associate Editor
Dr. Ross Tellam, Associate Editor
Dr. Sandeep Rovindran, Associate Editor
Prof. Peter Williamson, Associate Editor
Cora Morgan, Editorial Assistant
Tasslyn Gester, Copy Editor

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

The views and opinions expressed in this newsletter are those of the contributing authors and editors and do not necessarily represent the views of their employers or IMGC sponsors.