This month's issue features global trends in milk consumption, protective effects of breastfeeding against cancer, and two studies showing how dairy may ameliorate arthritis.

### Milk Consumption Around the World

- The first quantitative global estimates of the consumption of milk and other non-alcoholic beverages have been calculated.
- Unsurprisingly, milk drinking—and calcium consumption—correlates closely with national per capita income.
- The global average calcium consumption is far below the level recommended for adults by the US National Institutes of Health.
- Perhaps more concerning, young adults appear to be drinking relatively little milk and far larger volumes of sugary drinks, which may bode ill for their bone health later in life.

The most extensive and sophisticated attempt to quantify the consumption of milk by people over the age of 20, living in all corners of the globe, was recently published [1]. Its key implication is that average milk intake—and the average consumption of calcium—by both sexes, living in high, middle, and low-income regions of the world, is less than commonly cited recommended levels.

The work’s other main finding is that milk consumption is much higher among older than younger adults in every one of 21 geographic subdivisions of the globe. On the one hand, this suggests that the elderly may be responding to nutritional advice to improve bone health, and protecting themselves against diseases like osteoporosis. On the other hand, however, it may reflect shifting habits. Since younger adults are also consuming far more fruit juice and sugar-sweetened beverages than older generations, less healthy drinks like sodas may be replacing milk in their diets, a tendency that may—worryingly—follow them into old age and leave them deficient in the raw materials of strong and healthy bones.

The research, which formed part of a wider effort to better understand the role of diet in the global distribution of disease burden, called NutriCoDE, was recently published in the journal, Plos One [1]. The raw milk consumption data came from 75 global surveys conducted between 1990 and 2010, involving almost 700,000 people. This raw data was subjected to complex statistical techniques that take into account differences in the way it was collected—allowing for the comparison of information from places with plenty of available measures of milk consumption, with information from places like Oceania and much of sub-Saharan Africa, where individual-level milk intake data is sparse.

According to the study authors, Gitanjali Singh of Tufts University, Boston, and her colleagues, the results offer "the first quantitative estimates of non-alcoholic beverage consumption in 187 countries of the world, and provide information that can inform several areas of global health.”

In 2010, adults over the age of 20 consumed on average 0.57 of an 8-oz serving of milk per day, which is about 135 ml. The global average daily consumption of calcium was 629 mg, which, although country-specific recommendations should vary with typical levels of sun exposure and other considerations, is nonetheless far below the 1,000 to 1,300 mg that is recommended by the US National Institutes of Health (NIH).

Generally, differences in milk consumption between men and women were slight. But the rich have far more milk in their diets than the poor. In high-income countries taken together, people drank about 170 ml of milk per day (5.8 oz) on average, whilst in low-income countries, the mean was 71 ml (2.4 oz). Similarly, mean calcium intake in high-income countries was 782 mg, but just 456 mg in low-income nations.

These broad categories of milk consumption mask wide variations among individual countries, though. The world’s most enthusiastic milk consumers in 2010 were Swedish and Icelandic women over 80 years old. They drank about 568 ml (19 oz) of milk per day. By comparison, men in North Korea—a country in which many hundreds of thousands of people are estimated to have died from famine in the 1990s, and where food shortages continue to this day—are thought to have consumed an average of only about 14 ml (or less than half an ounce) of milk. Such geographic patterns most likely reflect various influences, from milk availability, the commonality of alternatives (such as soy-based products), and the...
Interestingly, the protective effects of breastfeeding appear to be stronger the longer a woman breastfeeds her first child, especially for premenopausal women, but also persists for postmenopausal women. This protection appears to be greater in countries with high breastfeeding rates, such as Japan, Korea, and other countries. Various studies have shown that breastfeeding for longer periods reduces the risk of breast cancer in women. The World Health Organization (WHO) recommends that mothers breastfeed exclusively for the first six months, with breastfeeding to continue for two years or beyond, as the protective effect of prolonged breastfeeding against breast cancer in women has been shown globally amongst many ethnicities. Breastfeeding has also been shown to reduce the risks of other cancers, including ovarian cancer.

Breastfeeding has numerous benefits for babies. Research is showing that benefits extend also to the mother. Studies show that the longer a woman breastfeeds, the greater her protection against breast cancer. The protective role of breastfeeding is cumulative and significantly increases for women who have breastfed for more than two years. Further research is examining the biological explanation of the protection against breast cancer, which likely lies with changes occurring in the breast cells during lactation. Some studies also show that breastfeeding protects the mother against ovarian cancer.

Since the 70s, investigators have been trying to ascertain whether or not, and how, breastfeeding protects the mother against cancer, with the focus being largely on cancers of the breast and, to a lesser extent, of the ovaries. In earlier years, breastfeeding was shown to moderately protect women against breast cancer [1,2]. This is because some studies had shown significant protection, while others very small or no protection. This inconsistency was attributed to methodological discrepancies amongst studies and, also to the fact that other factors that influence our cancer risk were not always taken into consideration. These factors include age at first pregnancy, family history of cancer, how many children a woman has had, and smoking.

More recent research is showing that the secret probably lies more so with the duration of breastfeeding for each child, as well as the lifetime duration of lactation [3]. However, smaller protective effects have also been shown for women who have even breastfed for very short periods [4].

Breastfeeding of one to two years or more protects women not only against breast cancer, but also ovarian cancer [5-8] and potentially other cancers that have not yet been studied in detail [1]. And this protection increases even more when women breastfeed beyond the first two years after the birth of their child. Indeed, the World Health Organization (WHO) recommends that mothers breastfeed exclusively for the first six months, with breastfeeding to continue for two years or beyond [http://www.who.int/nutrition/topics/exclusive_breastfeeding/en/]; and the evolutionary norm is considered to be even longer than this. So, not only do the numerous benefits of breastfeeding apply to both the baby and the mother, but they are also dose-dependent for both—in other words, the longer a woman breastfeeds her child, the more benefits for both.

The protective effect of prolonged breastfeeding against breast cancer in women has been shown globally amongst many ethnicities, further supporting its significance. Numerous studies in the United States, Mexico, Greece, Germany, China, Japan, Korea, and other countries have reported a consistent reduction in breast cancer risk for women who breastfeed for long periods. This protection appears to be greater for premenopausal women, but also persists for postmenopausal women even after 50 years since the first lactation [3,4,7-14].

Interestingly, the protective effects of breastfeeding appear to be stronger the longer a woman breastfeeds her first child,
as well as cumulative, such that increased lifetime duration of lactation over multiple children [4,5,10] confers greater protection against cancer.

In a German study, protection of women against breast cancer appeared to be more pronounced for mothers who had full-term pregnancies, had a family history of breast cancer, or were older (>25 years old) when they first breastfed their child [5]. This is in contrast to an earlier study that reported that women <20 years of age at first lactation had greater protection [10], whilst a study of Japanese women showed no association with the age of the mother at first full pregnancy [12]. Therefore, the question of whether the age of the mother at first lactation is related to breast cancer risk remains controversial and warrants further study. Nevertheless, the increased protection of breastfeeding for those women with a family history of breast cancer remains consistent [15].

More recently, Kotsopoulos and colleagues [16] further examined the protective role of breastfeeding in women who had a higher risk of breast cancer due to the presence of certain mutations in their genes. Breastfeeding for at least one year reduced breast cancer risk by 32% for women with one of these mutations. In keeping with the dose-dependent protective effects of breastfeeding against breast cancer, this protection became even greater when breastfeeding occurred for two or more years, with each year of breastfeeding adding a 19% reduction in breast cancer risk [16]. These results emphasize the importance of family history and the genetic status of a woman when investigating the relationship between breastfeeding and cancer risk.

The dose-dependent protection of breastfeeding against breast cancer has also been reported for ovarian cancer; however, further studies are required to confirm this in different populations. In a Chinese study, 12 or more months of lactation significantly reduced the risk of ovarian cancer [6]. A subsequent multinational study conducted across seven countries (Australia, Chile, China, Israel, Mexico, the Philippines, and Thailand) also showed a reduction in ovarian cancer risk [17].

Although the association between the duration of breastfeeding/lactation and breast cancer risk is well established, perhaps less so for ovarian cancer, the biological explanation of this phenomenon is still unclear. Some investigators have suggested that breastfeeding facilitates removal from the breast of estrogens and/or carcinogens [18], yet no conclusive studies on these factors currently exist. More recent analyses of the changes occurring in the breast during lactation have revealed a pathway that potentially explains this protection.

Cells of the breast, isolated non-invasively from breast milk and analyzed, have shown changes in the cellular composition of the breast as lactation progresses [19,20]. These changes do not seem to influence milk supply during established lactation, yet they result in reduction of the numbers of those breast cells that may be sensitive to becoming cancerous. This suggests that the longer a woman breastfeeds/lactates, her breast contains less and less of the cells that have the potential to turn into cancer cells, potentially explaining the reduced breast cancer risk associated with prolonged breastfeeding [7,9,12,19].

Although many factors contribute to breast and ovarian cancer risk in women, evidence accumulated over many decades, and across many countries and ethnicities supports the protective long-lasting effects of breastfeeding for long periods against breast and, potentially, ovarian cancer. This is particularly important for women with a family history of breast cancer and who can practice breastfeeding not only as a means of providing nourishment to their baby, but also as a way of decreasing the risk of breast cancer. Breastfeeding is the physiological norm for the mother and the baby; therefore, it is not surprising that both reap significant benefits in the short- and long-term.

Creating Therapeutic Yogurt for Treatment of Arthritis

- Rheumatoid arthritis is a chronic inflammatory disorder affecting about 1% of the US population, and current therapies have many limitations.
- Previous work found that a non-pathogenic Salmonella strain engineered to express the Escherichia coli protein, Colonization factor antigen I (CFA/I) ameliorated arthritis symptoms in a mouse model.
- In a new study, researchers genetically modified the probiotic bacteria Lactococcus lactis to express CFA/I.
- The new study showed that arthritic mice fed these bacteria experienced a dramatic reduction in arthritis symptoms, and the effect appeared to be mediated by a suppression of inflammatory responses.
- Mice fed milk fermented with L. lactis also experienced a dramatic reduction in arthritis symptoms.
- The findings suggest that milk fermented with CFA/I-expressing L. lactis could be developed as a therapy against arthritis and other autoimmune or inflammatory diseases.

Consuming dairy products, such as milk or yogurt, is known to be good for general health [1]. New research may make dairy products even more beneficial by enabling them to treat certain autoimmune diseases such as arthritis.

In a study conducted by David Pascual at the University of Florida, arthritic mice were fed genetically-modified probiotic bacteria, or a yogurt-like product created using the same bacteria. In both treatments, the mice experienced a dramatic reduction in their arthritis symptoms [2]. “We believe that a yogurt-like therapeutic may have a tremendous appeal to a segment of consumers who are less willing to use ‘pills,’” writes Pascual in an email.

The same approach could be used to develop therapeutics against other autoimmune and inflammatory diseases as well. “What really makes our approach unique is that the very same ‘tool’ can be adapted to different autoimmune diseases, like a sort of panacea,” writes Pascual.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that affects about 1% of the US population [3,4]. Physical and nutritional therapies are unable to stop the disease’s progression, and pharmacological therapies typically rely on anti-inflammatory drugs, which have harmful side-effects and often increase the risk of other infections [5,6].

To find other ways to modulate harmful autoimmune and inflammatory reactions, researchers have looked to pathogenic bacteria for inspiration. “One of the ideas we pursued is that pathogens attach to mucosal tissues in such a way as to silence the immune response, avoiding detection,” writes Pascual. To attach to the intestinal mucosa and colonize it, certain pathogenic Escherichia coli express proteins known as “colonization factor antigens.” In previous work, the researchers genetically modified a non-pathogenic strain of Salmonella typhimurium to express one of the E. coli proteins, Colonization factor antigen I (CFA/I). They found that the modified bacteria could serve as an oral vaccine with anti-inflammatory properties, and could help treat autoimmune diseases [7,8]. “Our data showed that the Salmonella-CFA/I remarkably ameliorated RA symptoms in an experimental mouse model,” writes Pascual.

“Because S. typhimurium is intrinsically proinflammatory, as well as due to regulatory concerns about its therapeutic application, we decided to switch to a food-grade organism,” writes Pascual. “The logical choice was lactic acid bacteria and specifically, Lactococcus,” he writes. Lactococcus lactis is commonly used in fermented dairy products and has been approved for human use [9,10].

The researchers engineered Lactococcus to express CFA/I and tested their therapeutic properties in a mouse model of arthritis. “Following two doses, our recombinant Lactococcus expressing CFA/I showed a stunning capability to ameliorate RA in mice,” writes Pascual. “In contrast, untreated mice or mice given Lactococcus (without CFA/I) only were still susceptible to RA,” he writes. The therapeutic effect appeared to be mediated by the suppression of pro-inflammatory signaling molecules.
The researchers then fermented milk using the CFA/I-expressing bacteria, creating "a dairy product whose texture and olfactory features are indistinguishable from yogurt," writes Pascual. When the mice were fed this yogurt-like preparation, their arthritic symptoms reduced dramatically.

The researchers are currently testing their approach in a mouse model of type I diabetes, as well as in large animal models of human disease. "Once we can demonstrate efficacy in these larger animals, we can pursue testing in humans," writes Pascual. "While the final application in humans may not be exactly around the corner, we are ecstatic at the flexibility of our system," he writes.


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**Milk Vesicles Offer New Hope for Arthritis**

- **Milk contains vesicles that are known to contain molecules called microRNAs.**
- **These particular tiny RNAs are predicted to influence immune pathways, which in turn have a role in the development of arthritis.**
- **In recent research, milk vesicles added to water delayed the onset and improved the symptoms of arthritis in two mouse models.**

Tiny, bubble-like structures found in cow’s milk appear to slow the development of arthritis in mice. The structures, called vesicles, were originally thought to be little more than the waste products of cellular processes. But in recent years, such vesicles have been shown to contain molecules called microRNAs, which in some contexts perform important biological functions. Although not fully demonstrated, the working hypothesis of lead investigator Fons van de Loo is that the RNA molecules in milk vesicles are absorbed in the intestines and modulate local mucosa activity, thereby influencing the body’s innate immune system.

Van de Loo, of Radboud University Medical Center, Nijmegen, in The Netherlands, began work in the field when he noticed parallels between the main subject of his research, gene therapy, and the vesicles reported by other scientists to be bobbing around freely in cow’s milk. Gene therapists study viruses such as lentiviruses, viewing them primarily as encapsulating structures for the delivery of variable genetic material. “If you look at how lentiviruses are produced, [you see] they are made in a similar way to the microvesicles,” van de Loo says. “They are formed within the cell and then released with genetic information—just like the extracellular microvesicles. And the genetic information is RNAs.” The more van de Loo read about the RNA molecules found in milk vesicles, the more he wondered whether those RNA molecules might be performing unrecognized functions.

He was also inspired by his family history. During the Second World War, van de Loo’s father worked painting houses, a profession that was accorded more milk stamps than most because milk was thought to block the health effects of heavy metals in paint. His father suffered from arthritis, which led van de Loo to wonder about a link with high milk consumption, although initially he thought that the vesicles might aggravate disease. “Those two reasons came together to make a new project. I’m a gene therapist who looks at vesicles. And we have similar vesicles in milk,” he summarizes. “So what are they doing in milk?”

To date, van de Loo’s group has tested the effects of milk vesicles on two mouse models of arthritis. In a paper recently
published in the journal Molecular Nutrition & Food Research [1], van de Loo and his team reported research in which they isolated vesicles from semi-skimmed cow’s milk, and examined the RNA contents. They found that some of those milk-vesicle RNAs are already known to influence important immune pathways involving Toll-like receptors—pathways that are implicated in the development of arthritis in humans [2].

The next stage of the research looked at how the vesicles in milk were taken up by the digestive system. “These vesicles are very stable in the harsh acidic conditions of the stomach,” explains van de Loo. Knowing this, the team fluorescently labeled the vesicles, and demonstrated that they were taken up by intestinal cells, and notably also by cells called splenocytes, which are white blood cells located in the spleen.

At this stage, van de Loo had demonstrated two important points: that functionally relevant RNAs are found in cow’s milk vesicles, and that the vesicles are picked up by intestinal cells. Next, he wanted to see if the milk vesicles altered the development of arthritis in an animal model, so he fed the vesicles to laboratory mice. In the first of his mouse models, the mice had a mutation that predisposes them to spontaneously develop polyarticular arthritis (meaning that the disease afflicts many joints at once) at about five to eight weeks of age. In the second model, van de Loo’s team induced the disease by immunizing mice with bovine collagen type II. Mice with both versions of arthritis consumed vesicles extracted from cow’s milk via their drinking water.

The results were impressive—in both models, rather than accentuating the symptoms of arthritis, the vesicles appeared to alleviate them [1]. The milk vesicle-containing water was associated with a delayed onset of arthritis in the mutant mice. Under the microscope, the cartilage around their joints showed less deterioration, and their bone marrow less inflammation, than mice that didn’t drink the milk vesicles.

Despite these exciting early results, van de Loo is struggling to renew his funding for the project. This initial research was funded by a special grant for “serendipitous” work, and his findings nudge him out of that category. Government funders have suggested he turn to the dairy industry, but in The Netherlands, at least, industry seems uninterested. “If you take something out of milk, or add to it, you fall within the therapeutic regulations,” he explains.

Perhaps someone outside of his home country might push the work on.


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