Drinking More Milk Associated with a Lower Risk of Cognitive Disorders

- In a new study, researchers conducted a systematic review and meta-analysis of previous research on the relationship between milk consumption and cognitive disorders.
- The researchers analyzed seven articles published between 2006 and 2015 and found that higher milk intake was significantly associated with a decreased risk of cognitive disorders.
- The study could not rule out the influence of other confounding factors on the association between milk intake and cognitive disorders, and follow-up prospective studies are needed to further explore this association.

Increased age brings with it a greater risk of cognitive decline and disorders such as dementia and Alzheimer’s disease. The lack of effective treatments for these cognitive disorders has spurred the search for factors that can prevent or slow cognitive decline. One of the factors that has attracted a lot of interest is nutrition, and it turns out many of the things we eat or drink could play a role in preventing cognitive decline [1-4].

Given the widespread popularity of milk and its many beneficial health effects, several recent studies have investigated the association between milk intake and cognitive disorders [5-11]. However, so far these studies have come to contradictory conclusions. Some studies report that an increased risk of cognitive disorders is significantly associated with a lower intake of milk [5-8]. Other studies do not find this inverse association [9-11].

A new study tries to resolve these contradictory findings by conducting a systematic review and meta-analysis of previous observational studies that investigated the relationship between milk consumption and the risk of cognitive disorders [12]. The study was conducted by Lei Wu of the Chinese People’s Liberation Army General Hospital and Dali Sun of the Houston Methodist Research Institute and expanded on a 2010 systematic review that showed an inverse association between dairy intake and cognitive functioning [13]. The new study finds that higher milk intake is significantly associated with a decreased risk of cognitive disorders.

The researchers searched the Pubmed and Embase databases for observational studies that reported the association between milk consumption and cognitive disorders or cognitive decline. They identified seven articles published between 2006 and 2015 that fit the selection criteria for their analysis. The sample size of the studies ranged from 601 to 4809, for a total of 10,941 healthy participants.

The combined analysis of all seven studies showed that higher milk intake was significantly associated with a decreased risk of cognitive disorders and Alzheimer’s disease. The risk of cognitive disorders was reduced by 28% at the highest level of milk consumption compared with the lowest level of milk consumption.

The researchers note that these results were based on a limited number of studies and that there was significant heterogeneity in the association between milk intake and cognitive disorders. They conducted subgroup analyses based on factors such as race, gender, and type of dairy intake, and found that none of these explained the heterogeneity in their pooled analysis.

Although the study did not explore the mechanisms underlying milk’s preventive effects on cognitive disorders, the researchers suggest that they could be a result of milk’s beneficial effects on type 2 diabetes, hypertension, and obesity, all of which are associated with an increased risk of age-related cognitive impairment [14-16]. Another possible explanation is that the effects are due to some of the nutritional components of dairy, such as calcium and vitamin B12, which are known to have some effects on cognition [17].

Previous studies have also indicated that full-fat and low-fat dairy products could have different effects on cognitive function [18,19]. This wasn’t something the meta-analysis addressed, as the studies it evaluated did not describe the fat content of the milk. The researchers suggest that follow-up studies could investigate the role of high-fat or low-fat dairy on cognitive decline.

The researchers conclude that their systematic review and meta-analysis of previous observational studies showed an inverse association between milk consumption and cognitive disorders. However, due to the limitations of the study design, the meta-
Analysis was unable to rule out the influence of other confounding factors on the association between milk intake and cognitive disorders. The researchers suggest that large follow-up prospective studies are needed to further explore this association.


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

Ancient DNA Provides the Clue to Modern Cattle

- DNA from remains of the auroch, the ancestor of modern cattle, provides clues to breed selection.
- Genes regulated by miRNA have changed over time.
- Ensuring that the ancient gene pool is preserved will provide greater options for developing healthier productive cattle in the future.

Modern dairy cows are as elite as Olympic athletes. They are champion milk producers and enable humans to turn fodder into dairy food with incredible efficiency. Underlying this performance is thousands of years of selection and improved management practices. Initially, the selection process was farmer driven and resulted in the development of many cattle breeds, but since the mid 20th century, when coordinated efforts by farmer groups and the dairy industry focused attention on the best methods to achieve improvements in production, the gain in efficiency through genetic selection has been remarkable.

Nowadays this process is benefiting from the revolution in genomics, which has led to the development of a huge amount of knowledge on the DNA sequence of cattle. Scientists are using these data in many ways, including detailed analysis of those parts of the genome that show signatures related to the selection process, and how it compares with the original domesticated cattle, aurochs [1]. We reported on a seminal study on this topic in 2015 [2], and now scientists have developed a computational method to dig deep into the DNA sequence to identify how selection has affected very specialized areas of the genome that contain microRNA (miRNA) [3].
Today there are a few favored cattle breeds for milk production, and similar trends have occurred for beef cattle. However, there are still approximately 800 breeds in the world and others that are now extinct. All of these breeds arose from a common ancestral stock of aurochs—ancient cattle—originally domesticated in the Near East perhaps as long as 10,000 years ago. Aurochs spread throughout the ancient world as people and farming moved west towards Europe and east towards Asia. DNA from the remains of one of these animals was sequenced in 2015 and has been the source of some very interesting discoveries in the development of modern cattle breeds [1].

DNA contains genes that encode proteins, but what has become clear is that the genes encoding the proteins only partially account for individual differences and breed traits. Significant changes to the composition of a protein can have major effects, but subtle differences in the amount of protein that is produced at any one time is also critical. This is influenced in a number of ways, including regulating the amount of RNAs, which are the molecules that carry the DNA code to the site of protein production inside cells. The RNA world is complex and has become even more so in recent times. For many years we thought that there was a simple relationship between DNA, RNA, and protein; however, in 1993 a discovery was made in worms that was at first thought to be a curiosity [4,5], but it soon became clear that it was a major biological advance [6,7]. That discovery was the identification of miRNA. These molecules play a significant role in regulating the activity of genes by dampening the process that leads to the production of functional proteins within cells. Hence, they are very important in determining traits, like milk production in dairy cows. The recent study of aurochs focused attention on this aspect of the genomic differences in modern cattle [3].

The scientists had to develop a way to find and compare the miRNA sequence amongst the billions of coded letters that are each of the chromosomes. They began by concentrating not on the miRNA sequences but on all the known genes in both modern cattle and aurochs. This left just under 20,000 DNA sequences for further analysis. The scientists were able to use information previously determined about specific regions within the genes that the miRNAs recognize. Once they had collated these data, they developed a computer program to compare the same regions in the auroch DNA with genomic sequences derived from representative animals from Holstein, Jersey, Angus, Limosin, Romagnola, Fleckvieh and N'Dama cattle breeds. The computations made the enormous task of filtering this information possible, and as a result, they discovered 2,634 differences between modern cattle and the auroch.

Differences in miRNA recognition sequences can result from a loss or gain of just one or two letters in the DNA code. In this case, 892 of the sites lost and 885 gained miRNA binding sites. The simple explanation of these changes is that loss of a site may increase the level of protein products generated from that gene and any gain may reduce that level. The net effects contribute to shaping the characteristics of modern cattle breeds.

What traits might these changes in miRNA affect? To address this issue, the scientists focused on the 1,620 genes where the changes were found. Knowing that genes behave in a coordinated manner to control biological processes, the genes were grouped according to their respective biological activities. The five areas that stood out were metabolism (related to food and energy), immunity (probably related to disease response), reproduction (especially fertility and lactation), development (affecting size and stature) and general physiological capacity.

These data are important for two reasons. They help to define the genetic detail of what makes a good dairy cow or beef animal, and they also help to understand what has been lost from the gene pool. The tendency to focus on specialized cattle breeds in modern agriculture, and even on a few lines within the breeds, has limited breed genetic diversity, and overall cattle diversity. Having that genetic variation available should it be required for future selective breeding is an important consideration. Despite the fact that there are still around 800 cattle breeds in the world today, there is a real threat that the rare breeds will become extinct. Efforts to preserve diversity through conservation of rare breeds is gaining attention [8], and the studies of the auroch genome are a vital clue to what may be most important.


Contributed by
Professor Peter Williamson
Associate Professor, Physiology and Genomics
University of Sydney, Australia
Holder Pasteurization Holds Up Well Against Most Germs

- Holder Pasteurization of human milk involves heating it to 62.5°C for half an hour.
- The method effectively inactivates the vast majority of bacteria and viruses, including HIV and Ebola; however, evidence thus far suggests it is not perfect at eliminating hepatitis B and *Bacillus cereus*.
- Since Holder Pasteurization is so broadly effective, and because milk banks in many countries conduct a thorough screening of donors’ blood before accepting donations, women can feel confident in the safety of donor milk.

There is nothing particularly surprising or complicated about the most common method of making stored milk safer than it would otherwise be. Holder Pasteurization, or HoP, aims to rid milk of potentially harmful germs by heating it to 62.5°C (145°F) for half an hour, and then cooling it back down to room temperature. This method is used by all of the Human Milk Banking Association of North America (HMBANA) milk banks and differs from the high-temperature, short-time (HTST) pasteurization used in the dairy industry. But these days HoP has some newfangled competitors—potential alternatives to the tried and tested method—suggesting that the time is ripe for a full evaluation of HoP’s performance. This article is the first in a series of five on the topic. Starting at the beginning of this in-depth look, how good is HoP at its core mission: keeping pathogens at bay that could, in theory, find their way into milk and make it their home?

A few years ago, Susan Landers and Kim Updegrove, both in Austin, Texas, set about asking this most basic of questions [1]. They cultured a vast number of human milk samples on agar plates and incubated them for 48 hours to give whatever bacteria were in the milk a chance to proliferate. For every sample of milk—810 individual samples and 303 samples that were mixtures of several women’s milk—two cultures were created, one before and one after the sample was pasteurized. Only 7% of the pasteurized milk samples grew any kind of bacterial culture. But 87% of the unpasteurized samples grew colonies of *Staphylococcus*. Many other bacterial species were found in these cultures, although none were as common as Staph. (Notably, only 4% of the unpasteurized cultures contained *Staphylococcus aureus*, however, which is what causes most “staph infections.”) A related study by Spanish researchers looked at HoP’s effectiveness in a much smaller number of colostrum samples [2]. They report that the method does indeed destroy the bacteria present in the samples before pasteurization.

But what of the 7%—those rare occasions when some bacteria survive HoP? This was the preoccupation of Aránzazu Gómez de Segura and others in the research group, who set about answering the question [3]. Specifically, they were worried that in being so effective at destroying almost all bacteria, HoP might remove the competition that was keeping the surviving bacteria at bay. In other words, those that remained in a sample after it underwent HoP might grow faster than they could in raw milk, and for that very reason pose a health risk.

To test this, de Segura and the Madrid team took 21 human milk samples and pasteurized them using HoP. Of the 21, only three contained surviving bacteria, always the species *Bacillus cereus*. The researchers then set about genetically analyzing these *B. cereus* cultures to find out if they contained particular genes that must be present for the bacteria to produce toxins. These toxins, such as one called cereulide, are the reason *B. cereus* is sometimes linked to cases of food poisoning. In the end, there was no cause for concern. De Segura and her team report that the *B. cereus* that survived HoP did not have a high virulence potential.

So HoP does a good job at zapping bacteria, but what about viruses? For many of the really scary ones, it does a surprisingly brilliant job. Take HIV [4] or cytomegalovirus [5], for example, both of which HoP completely destroys. Oncogenic human papillomaviruses were recently added to that list by Manuela Donaliso and her colleagues in Torino, Italy [6]. More recent, prominent threats within the US (but ongoing threats in some African countries), Ebola and Marburg, were tested by a group of Texas-based researchers, with milk from the Mother’s Milk Bank of North Texas, and viruses from Galveston National Laboratory that they added to the milk. Their study, which is published in the current issue of the *Journal of Human Lactation*, shows that HoP is also successful at inactivating these viruses [7] (specifically, the Angola strain of Marburg and the Zaire strain of Ebola).

The data on hepatitis B virus are less emphatically reassuring, however. Indeed, one study by Patricia Ribeiro de Oliveira at the University of São Paulo in Brazil, and her coauthors, suggests that HoP hardly dents the probability of detecting hepatitis B surface antigens, nor hepatitis B DNA, in human milk [8]. It’s not known exactly why this is—and the authors themselves point out that the possibility of laboratory contamination cannot be discarded. But there is another, more concerning, alternative. Among a small number of women whose blood tested positive for hepatitis B antigens but who had no evidence of viral DNA in their blood, both viral markers could be found in their milk. The authors write that this combination of facts points to the possibility that hepatitis B might replicate in the mammary gland.

If you are feeding your offspring milk from a HMBANA milk bank, there is no reason to be alarmed, however. The organization—like many others—performs blood tests on all of its donors and checks whether there is any trace evidence that a mother has hepatitis B before she can begin to donate. Even so, the broadly good performance of HoP against a range of bacteria and viruses should also be
reassuring to women receiving donor milk from sources that do not screen donors as rigorously as HMBANA. As long as the milk is heated to 62.5°C (145°F) for half an hour and then cooled, and stored in properly cleaned containers, almost all germs of note will have been eliminated. Similarly, there’s no need to worry excessively about donor milk that has been thawed and refrigerated for the full 24-hour limit that is recommended before it should be discarded. In 2012, Ronald Cohen and his colleagues of Stanford University analyzed milk that had been pasteurized by HoP, frozen, and then left thawed for slightly more than the recommended time. Their 18 bottles of “expired” human milk did not show any bacterial growth [9].

Overall, then, HoP does a good job and eliminating the vast majority of the infectious agents that can, in theory, contaminate milk. Check back in coming months for summaries about how HoP influences human milk’s nutrient content, its immunological components, its digestibility for infants—and for an analysis of the alternative pasteurization methods out there.


Contributed by
Anna Petherick
Professional science writer & editor
www.annapetherick.com

**Milk-Derived Exosomes Enhance Drug Effectiveness**

- Milk naturally contains small lipid vesicles called exosomes that deliver biochemical packages from the mother to her offspring.
- Bovine milk-derived exosomes have many physical properties that may help improve the targeting of human therapeutic drugs to diseased tissues.
- Exosomes derived from cow’s milk, loaded with a cargo of different types of therapeutic drugs and delivered into mice, improved drug effectiveness.

Delivering parcels around the world is a tough business. The company must deliver an individual parcel to the correct address, on time and without damage. A problem in any one of these areas results in a very unhappy customer. Ideally, the delivery of a therapeutic drug to a specific diseased tissue within a person has similar stringent requirements to the delivery of a parcel.

Many drugs, however, often end up in the wrong tissues (incorrect addresses) in addition to the desired diseased tissue, and this can cause unwanted side effects of the drugs. Scientists speculate that lower drug doses could be used in humans if there was greater accuracy in the delivery of the drug to the diseased tissue. This would also reduce drug side effects. Moreover, the body modifies many drugs, often damaging them before they reach the targeted diseased tissue. These changes can reduce drug effectiveness and adversely alter the timing of drug bioavailability to the diseased tissue.

Luckily, nature has already mastered the intricacies of accurately delivering chemical parcels between the tissues of an individual. Using nature for inspiration, a research group led by Ramesh Gupta from the University of Louisville recently highlighted a new approach for improving therapeutic drug effectiveness by artificially packaging drugs in milk nanoparticles called exosomes [1-4]. The group also may have identified a future business opportunity for the dairy industry. Their principal research was published in Cancer Letters in 2016 [1].
Milk Exosomes

Gupta and many others have demonstrated that milk, like most body fluids, naturally contains secreted exosomes that enable biological communication between tissues within an individual [1, 4-14]. An exosome is a very small secreted particle, typically measuring less than 100 billionths of a meter in diameter, i.e., about one-thousandth the diameter of a human hair and only a small fraction of the diameter of a cell. An exosome is structurally characterized by a phospholipid coat wrapped around a biochemical cargo consisting of an assortment of molecules that change the functions of target cells. Exosomes release their cargos into target cells in other tissues, sometimes in a highly specific way through the joint action of specialist proteins embedded in the phospholipid coat of the exosome and partner proteins on the target cell surface. Scientists are still discovering the natural functions of milk exosomes, but there is consensus that they probably provide maternal help to enhance offspring immune defense systems and accelerate maturation of offspring gastrointestinal tissues.

Gupta and colleagues initially identified a highly efficient process for purifying exosomes from cow’s milk [1]. They noted that the process is potentially scalable to a larger exosome production system, which is essential for any commercial application. In contrast, the investigators highlighted the limited availability and lack of scalability of alternative body fluids or fluids harvested from cells grown in the laboratory [1]. After providing comprehensive and convincing evidence that the purified material from milk did indeed have the known physical characteristics of exosomes, the investigators showed that the exosomes were stable when frozen, another commercially relevant attribute [1]. Subsequently, Gupta and colleagues artificially loaded cow milk-derived exosomes with a cargo of fluorescent dye and demonstrated that exosomes delivered the dye into human lung cancer cells grown in the laboratory, i.e., the purified bovine milk exosomes delivered their cargos into cells and were therefore functional.

The investigators also administered fluorescently labeled exosomes either orally or intravenously into mice [1]. Both routes of administration delivered the exosome fluorescent dye into multiple tissues. However, the intravenous administration predominantly stained the liver, whereas the oral administration uniformly stained many tissues. Thus, the route of administration was important as it changed the tissue destination of the exosomes. A similar experiment undertaken by the Gupta team established that the isolated cow milk-derived exosomes were not toxic and did not induce an inflammatory response after oral administration to rats, i.e., the cow milk-derived exosomes were safe [1].

Improved Action of Therapeutic Drugs Packaged Within Milk Exosomes

Gupta and colleagues then loaded cow milk-derived exosomes with different types of chemotherapeutic drugs used to fight cancer and tested the abilities of these drugs to slow the growth of human lung cancer cells grown in the laboratory [1]. They demonstrated that those drugs, when delivered by exosomes to the cancer cells, had enhanced effectiveness compared with a control. Research often turns up the unexpected. The cow milk-derived exosomes by themselves (the control for the experiment) also decreased the growth of the cancer cells but to a lesser extent than the drug loaded exosomes [1]. This result alone is intriguing and it invites further investigations into the natural cargos of the milk exosomes. Finally, the investigators transplanted human lung cancer cells into mice susceptible to developing cancer from these cells and showed that the injected cow milk-derived exosomes loaded with a cargo of an antitumor drug inhibited tumor growth in the mice to a greater extent than did the drug administered alone [1]. How this happened is unclear, but the investigators suggest that the exosomes protected the drug from damage within the body of the mouse and may have preferentially targeted the developing cancer cells.

The investigators’ strategy of using exosomes to improve the effectiveness of drugs not only worked for anticancer drugs. Gupta and colleagues also demonstrated improved effectiveness of anti-inflammatory drugs packaged into cow milk-derived exosomes [1]. Thus, the overall strategy may have broad applications.

Conclusions

Improving the effectiveness of drugs whilst minimizing their side effects is a major aim of the pharmaceutical industry. Gupta and colleagues demonstrated that delivery of drugs packaged into cow milk-derived exosomes was more effective than direct administration of the drugs. This research is preliminary but promising. They also demonstrated the safety of using cow milk-derived exosomes. Gupta noted that the pharmaceutical industry still needs substantial additional research before commercial applications can be available for routine human use. This will take time. However, if the additional research is successful, the dairy industry may have a new commercial opportunity—the production of cow milk-derived exosomes for use in the human pharmaceutical industry.


Contributed by
Dr. Ross Tellam (AM)
Research Scientist
Brisbane, Australia

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